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GENRE *TIMARCHA* (*COLEOPTERA, CHRYSOMELIDAE*) EN ROUMANIE

AURELIAN LEONARDO ILIE

In this work are presented taxonomical, biological studies and the general distribution of the species of genus *Timarcha* in Romania.

1. INTRODUCTION

Quelques données faunistiques concernant les espèces de ce genre dans les diverses provinces de la Roumanie, ont été publiées dans les travaux près d'autres espèces chrysomélidés ou des coléoptères et parfois d'autres ordres d'insectes.

Une synthèse plus ample sur ce genre au niveau national n'a pas été réalisée jusqu'à présent.

2. MATÉRIEL ET MÉTHODES

Le travail comprend les données des espèces qui se trouvent dans les collections du Musée d'Olténie, celles provenant des collectes personnelles et de la littérature de spécialité.

On a utilisé la nomenclature existante [16] mais en ce qui concerne la dispersion des chrysomélides dans la région paléarctique on a consulté l'oeuvre de A. Winkler [31].

3. RÉSULTATS

En Roumanie le genre *Timarcha* Dej. compte 6 espèces. On présente ces espèces en mentionnant les endroits de collectes et en parenthèses les auteurs qui ont publié les espèces et les années des publications.

Genre *Timarcha* Dejean, 1821
Sous – genre *Timarcha* Dejean, 1821
Timarcha (*Timarcha*) *pratensis* Duftschmidt, 1825
Dispersion générale: Europe centrale, Péninsule Balkanique, Asie mineure.
Dispersion en Roumanie: Cheile Turului (Crişan, Teodor, 1995), Hagieni (Negru, Roşca, 1967), Bucharest, Constanţa (Fleck, 1904), Rimetea (Kutasi et colab.,

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1999), Mangalia et alentours (Negru, 1957), Haţeg, Făgăraş, Mediaş, Reghin (Bielz, 1887), Turnu Roşu, Gherla, Aiud (Petri, 1912), Tismana (Marcu, 1928), Cheile Sohodol, Craiova (Ilie, Chimişliu, 2000).
Notes biologiques: Espèce mésophylle. Les plantes – hôte appartiennent au genre *Galium* L.

Timarcha (*Timarcha*) *rugulosa* Herrich-Schaffer, 1838
Dispersion générale: Europe centrale et estique
Dispersion en Roumanie: Cheile Turzii (Crişan, 1992), Cheile Turului (Crişan, Teodor, 1995), Harghita (Rozner, 1997), Răcătau, Saschiz, Baia Mare, mont. Gutâi, Izvoara (Szel et colab., 1994), Prahova, Bucovina (Fleck, 1904), Prahova, Sinaia (idem) – *ab. lomnickii* Miller, Cernăuţi, Rădăuţi, Sucevita, Siret (Marcu, 1928) – *ab. lomnickii* Miller, Deva, Câmpulung, Cernăuţi (Hormuzachi, 1903), Rimetea (Kutasi et colab., 1999), Valea Sohodolului (Bobârnac, 1974), Tismana (Marcu, 1928), Bucovăţ, mont Căpăţânii et Mehedinţi (Ilie, 2002) – *ab. lomnickii* Miller, Bocicoiul Mare (Kuthy, 1897) mont. Rodna (Csiki, 1951), Broşteni, Azuga (Montadon, 1906) – *ab. lomnickii* Miller, mont Făgăraş, Braşov, Tesla, Pasu Buzăului, Aiud, Sighişoara, Turnu Roşu, Petroşani, Haţeg (Petri, 1912), Turnu Roşu, Sibiu, Cheile Turzii, Sighişoara (idem) – *ab. lomnickii* Miller, Porumbacu, Suru, Braşov (Bielz, 1887) – *ab. lomnickii* Miller.
Notes biologiques: La même situation que pour l'espèce précédente.

Timarcha (Timarcha) tenebricosa moravica Bechyné, 1945
Dispersion générale: Europe
Dispersion en Roumanie: Basin Carpatique, (Warchalowski, 1993; Kaszab, 1962). La présence de cette espèce en Roumanie est possible.
Notes biologiques: les plantes – hôte appartiennent à la famille *Rubiaceae* (genre *Gallium L.*)

Sous – genre *Timarchostoma* Motschulsky, 1860
Timarcha (Timarchostoma) goettingensis Linnaeus, 1758
Dispersion générale: Europe
Dispersion en Roumanie: Cheile Turzii (Crişan, 1992), Cheile Turului (Crişan, Teodor, 1995), Nemira, Valea Budului, Tociloasa (Tărăbuţă et colab., 1999) Harghita (Rozner, 1997), Gilău, Huedin (Marcu, 1957) et ab. *aerea* Fairmaire, Mangalia (Negru, Roşca, 1967) – ab. *rugosa* Duftschimdt, Meledic, Mangalia, Bucovina (Fleck, 1904), Rimetea (Kutasi et colab., 1999), Cheile Sohodol (Bobârnac, 1974; Ilie, Chimişliu, 2000), Gura Văii (Marcu, 1928), Buru (Ieniştea, 1932), Comana (Montandon, 1906), Buzău (Jacquet, 1904), mont. Mehedinţi (Ilie, 2002).
Notes biologiques: les plantes – hôte sont *Asperula odorata* L. et celles du genre *Gallium* L., espèce mésophylle.

Timarcha (Timarchostoma) montana Fairmaire, 1825
Dispersion générale: Macédonie, Bosnie – Herzégovine.
Dispersion en Roumanie: Prahova (Fleck, 1904)
Remarques: la présence de cette espèce sur notre territoire nationale est exclue, probablement c'est une détermination erronée.

Sous – genre *Metallotimarcha* Motschulsky, 1860
Timarcha (Metallotimarcha) metallica Laicharting , 1781
Dispersion générale: Europe Centrale, Péninsule Balkanique.
Dispersion en Roumanie: Bucovina, Azuga, Vf. Omu, Prahova (Fleck, 1904), Harghita (Rozner, 1997), Harghita Băi, Borşa, Lacul Bâlea (Szel et colab., 1995), Baziaş (Ieniştea, 1975), Bucovina (Marcu, 1928), mont Ineu (Csiki, 1951), mont. Maramureş (Frivaldszky, 1871), Transilvania (Teodoreanu, 1988), Broşteni (Montandon, 1906), Retezat (Ieniştea, 1932), Valea Răstoşnei (Ieniştea, 1931), Retezat, mont Cibin, Şura Mare, Braşov, mont. Făgăraş, Piatra Mare, Tesla, Pasu Buzăului, Aiud, Sighişoara, mont. Bălanului (Petri, 1912), Cheile Sohodol (Ilie, Chimişliu, 2000).
Notes biologiques: les plantes – hôte: *Vaccinium myrtillus* L., espèce mésophylle.

Timarcha (Metallotimarcha) gibba Hagenbach, 1825
Dispersion générale: Péninsule Balkanique, Basin Carpatique.
Dispersion en Roumanie: Cheile Someşului Cald (Crişan, 1999), mont. Făgăraş (Bielz, 1887), Bihor, Banat (Kaszab, 1962).
Notes biologiques: les plantes – hôte: inconnues, espèce mésophylle.

4. DISCUSSIONS

Parmi les espèces signalées dans la faune roumaine, une espèce constitue une présence possible, la présence d'une autre est exclue tandis que quatre espèces sont communes et une qui on peut considérer comme en étant rare.

5. CONCLUSIONS

1. Sur le territoire de la Roumanie le genre *Timarcha* Dej. compte 6 espèces.
2. *Timarcha montana* Fairm. n'appartient pas à la faune roumaine.
3. Toutes les espèces sont mésophylles.

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THE DYNAMICS OF FOREST SURFACES INFESTED WITH *TORTRIX VIRIDANA* AND GEOMETRIDAE SPECIES, BETWEEN 1986–2001

IRINA TEODORESCU, ADAM SIMIONESCU

In this work was analyzed the decreasing of the total surface of forests infested with defoliator lepidopters in Romania, between 1986 and 2001, to almost a half of the surface registered in 1986. The most of forests exhibited very low, low or medium infestation levels, caused by *Tortrix viridana* (85 % of the total infested surface) and Geometridae species (96 %). A severe decreasing (about 13 times) in forest surfaces with *Lymantria dispar* infestation registered.

The geographic distribution of forests infested with *Tortrix viridana* and Geometridae species in Romania is balanced. *Tortrix* species slightly predominates in Muntenia, Oltenia, Moldova, while Geometridae, in Transylvania, Banat and Dobrogea.

The defoliator populations control was performed by using especially biological (bacterial, viral, hormonal) control means, inhibitors of insect metamorphosis.

Key words: Tortrix viridana, Geometridae, *Lymantria dispar*.

1. INTRODUCTION

Lymantria dispar, *Tortrix viridana*, together with Geometridae species constitutes important defoliator lepidopters of *Quercus* forests, in Romania (Simionescu, Teodorescu, 1990, Teodorescu, Simionescu, 1987, 1988, 1989, 1991, 1994, Teodorescu, Simionescu, Ciornei, 1990, Teodorescu, Vădineanu, Simionescu, 2001). Although polyphagous, *Lymantria dispar* and Geometridae species have a marked preference to *Quercus* species.

2. RESULTS AND DISCUSSION

In the last decades these defoliators developed some outbreaks, instead of the generally low and very low infestation level. Their adverse effect have been probably enhanced by the simultaneous pest infestations, due to favorable forest composition and climate conditions in 1986–2001 period. Thus, their harmful effect on forests was enhanced.

Tortrix viridana feed only *Quercus* species, especially *Q. pedunculiflora* Koch., *Q. frainetto* Ten., *Q. robur* L., *Q. pubescens* Wild.

The Geometridae species exhibited wide spectrum polyphagy, as they were found in pure *Quercus* forests as well as in mixed forest, with *Quercus*, *Fagus*, *Carpinus* and others tree species.

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THE DISTRIBUTION OF FOREST SURFACES INFESTED BY TORTRIX VIRIDANA AND GEOMETRIDAE, IN ROMANIA

A relatively balanced proportion of forest surfaces infested by *Tortrix viridana* and Geometridae species was registered in all areas. The forest surfaces attacked by *Tortrix viridana* were slightly larger in the area of Muntenia and Oltenia Subcarpathian hills, of South Romanian plain, of Moldavian plateau and hills, while surfaces attacked by Geometridae were larger on Transylvania, Banat and Dobrogea hills (Table 1).

Table 1

The distribution of forest surfaces infested by *Tortrix* and Geometridae

Area	Infested surfaces (%)	
	Tortrix viridana	Geometridae
Muntenia and Oltenia Subcarpathian hills	35 – 40	33 – 36
South Romanian plain	24 – 26	22 – 23
Moldavian plateau and hills	19 – 21	17 – 20
Transylvania plateau and hills	9 – 11	11 – 16
Dobrogea	6 – 7	9
Banat	1 – 2	1 – 3

THE DYNAMICS OF FOREST SURFACES INFESTED BY MAIN DEFOLIATOR LEPIDOPTERS SPECIES

The total surface of infested forests by main defoliator lepidopters decreased during the 1986–2001 period, to almost a half of the surface registered in 1986. This was due especially to the reduction of *Lymantria dispar* attack (the surface infested with this species decreased about 13 times, from 3,149,000 ha to 119,000 ha).

The surfaces infested with *Tortrix viridana* and Geometridae species constitute the great majority of total surface infested with defoliator lepidopters (Table 2), even the infested surfaces diminished.

The percentage of forest surfaces attacked by these two defoliator categories was 69 % in 1986–1991 period and 80 % in 1996–2001. The highest value was registered in 2001 (92 %). Infested surfaces decreased, especially those infested with *T. viridana* (from 5,205,000 to 3,354,000 ha).

Table 2

The dynamics of forest surfaces infested with main defoliator lepidopters

Defoliators	1986–1991		1996–2001		2001	
	1000 ha / year	%	1000 ha / year	%	1000 ha / year	%
Tortrix viridana	520.5	45.4	358.9	50.0	335.4	56.6
Geometridae	267.0	23.3	211.2	29.5	210.0	35.4
Lymantria dispar	314.9	27.5	114.2	15.9	11.9	2.0
Total defoliators	**1146.0**	–	**716.7**	–	**592.5**	–

THE DYNAMICS OF FOREST SURFACES WITH DIFFERENT INFESTATION LEVELS

In the last years, low and very low infestations level predominated for all defoliator species, being found on 85 % of total infested surface for *Tortrix viridana* and on 96 % for Geometridae. High and very high infestations levels represented only 1 % for Geometridae and in the case of *Tortrix viridana* they decreased from 19 % to 5 % of the total infested surface.

The *Lymantria dispar* infestation degree was also evaluated in the purpose to explain the relative changes in proportion of *Tortrix viridana* and Geometridae infested surfaces. We found that forest surfaces with high and very high infestation levels of *Lymantria*, drastically decreased from 43 % in 1986–1991 to 0.4 % in 1996–2001 period. The severe reduction of surfaces infested with *Lymantria* could explain the relative increasing of forest surfaces infested with *Tortrix* and Geometridae species (Table 3).

THE CONTROL OF DEFOLIATOR DYNAMIC POPULATIONS

Forest supervision with feromonal substances is useful in detecting infested areas in which control measures must be applied.

In correlation with infestation level, biological and chemical control methods were applied on limited surfaces, with exceeding 50 % defoliation. Control methods were also applied, as protective measure, on limited forest surfaces, exhibiting 25 % defoliation degree.

The significant decrease of the surfaces infested by defoliator lepidopters and the preponderance of the low intensity infestations were due to the appropriate prevention and control strategies applied for the defoliator populations. After 1980, the organo-clorined insecticides use was stopped, because they have a long remanence and therefore, a negative impact on forest biocenoses. They were replaced by organophosphorous pesticides (with high toxicity, but low remanence), applied on limited surfaces. The synthesis piretrinoids, especially Decis, were used in 1991–1996 period, on 43–88 % of total chemical treated surface.

Table 3

The dynamics of forest surfaces with different infestation levels

Intensity of infestation	1986–1991		1996–2001		2001	
	thousand ha / year	%	thousand ha / year	%	thousand ha / year	%
Tortrix viridana						
low and very low	312.3	60	306.2	85	290.7	87
medium	106.8	21	35.0	10	32.9	10
high and very high	101.4	19	17,7	5	11.8	3
Total	**520.5**	**45.4**	**358.9**	**50.0**	**335.4**	**56.6**

Table 3
(continued)

Geometridae						
low and very low	242.7	91	201.8	96	194.5	92
medium	18.4	7	7.4	3	12.0	6
high and very high	5.9	2	2.0	1	3.5	2
Total	**267.0**	**23.3**	**211.2**	**29.5**	**210.0**	**35.4**
Lymantria dispar						
low and very low	134.2	43	70.0	61	11.4	96
medium	46.2	14	11.7	10	0.1	1
high and very high	134.5	43	32.5	29	0.4	3
Total	**314.9**	**27.5**	**114.2**	**15.9**	**11.9**	**2.0**

The use of dimiloid group of insecticides acting as inhibitors of insect metamorphosis, was gradually increased after 1997. These inhibitors of metamorphosis exhibited low negative effect in comparison with other chemical pesticides, but they have the disadvantages of affecting all invertebrates groups with chitine in tegument composition (inclusively predators and parasitoids). The Tebufenozide (Mimic 240 LV) insecticide groups which accelerate the moult was applied in last years.

Good results in control of *Tortrix viridana* were obtained with bacterial products (Dipel 8 L), chemical insecticides of the Dimiloin (Dimilin, Rimon) and Tebufenozide (Mimic 240 LV) substances.

The forest surfaces treated by biological control methods increased from 26 % in 1994, to 54 %–63 % in 1998 and 1999 respectively.

Drastic reduction of surfaces infested by *Lymantria dispar* could be explained by the use of viral products.

The maintaining and increasing of the useful forests entomofauna populations densities were favourised by the replacing of chemical by biological (bacterial, viral) control methods (Teodorescu, Vădineanu, 1999).

3. CONCLUSIONS

The total surface of forests infested by defoliator lepidopters decreased during the 1986–2001 period.

The most of the surfaces had low, very low, and medium infestation levels, caused by *Tortrix viridiana* and Geometridae species.

The relative increasing of surfaces infested with *T. viridana* and Geometridae species was correlated with the accentuate decreasing of surfaces infested with *L. dispar*.

The geographic distribution of infested forests by *Tortrix viridana* and Geometridae species in Romania was relatively balanced. *Tortrix* exhibited a slight dominance in Muntenia, Oltenia, Moldova, while Geometridae in Transylvania, Banat and Dobrogea.

Although the surfaces of forests affected by these pests is large, the populations outbreak could naturally extinct. In this way, the forest biocenoses, severe affected in the past by pesticides, could recover, becoming more resistant against to a possible attack of different pests.

The positive recovering of the forest biocenosis diversity, must be maintained by the preponderantly use of biological preparations. In this way, biocenosis stability would increase and could counteract some abiotic and biotic impact.

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THE MATING FLYING PHENOLOGY OF THE *TORTRIX VIRIDANA* AND GEOMETRIDAE SPECIES LEPIDOPTERA

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This study presents the results of using pheromon method with Atravir in the case of *Tortrix viridana* and glue rings placed on tree trunks, in the case of Geometridae species, to establish flying start, flying period, flying dynamics, mean flying duration, high values of flying period, period and duration of maxime flying, autumn-winter flying and winter-spring flying Geometridae species, outbreak, probable defoliating percent.

In *Tortrix viridana*, the flying period lasted 20–25 days, from early May to last June. Period of maxime flying were about fifteen days, in last May. The intensive flying occures especially in the first 1–6 days of flying period. The flying dynamics was influenced by the temperature values and precipitations cantities. The flying start was delayed and it lasted longer in the North forests, with a colder climate. In southern zones was early, but cold precipitations prolonged the flying period. *Tortrix viridana* develop some outbreaks in the forests of Muntenia and Oltenia Subcarpathian hills and plain, Moldova plateau and hills. The defoliation percent was estimated to be between 36–70 % in 29 % of the investigated zones.

Among Geometridae with autumn-winter flying (46 days in mean, especially in October-November, with maximum fly in 15–30 November), dominant species was *Operophthera brumata*. Period of maxime flying was 10–15 days, in November. Among Geometridae with winter-spring flying (28 days in mean, especially in February-March, with maximum fly in the day in which the flying starts and in the following 5 days), dominant species was *Erannis leucophaearia*.

Key words: phenology, *Tortrix viridana*, Geometridae, winter-spring flying Geometridae, autumn-winter flying Geometridae.

1. INTRODUCTION

The study of mating flying phenology in *Tortrix* and Geometridae species could lead to the evaluation of the influence of different environmental factors on this process.

Dissescu Gabriela *et al.* (1967) presented in detail the phenology of the Geometridae development. The authors found that *Operophtera brumata* L. had the highest proportion in Geometridae group (about 80 %) and registered it in forests from all geographical zones. This species was followed by *Erannis* genera species, among which the highest frequencies exhibited *Erannis defoliaria* Cl., *Er. aurantiaria* Hb. and *Er. leucophaearia* Schiff. In some zones were presented also *Alsophila aescularia* Schiff, *Erannis marginaria* F., *Colotois pennaria* L., *Phigalia pedaria* F. species.

In the present paper we underline only some aspects observed in the last decade.

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2. MATERIAL AND METHODS

The flying dynamics of *Tortrix viridana* was observed in *Quercus* fo
mainly in hill zones of Romania, but also in plain area. The dominating spec
forest composition was the common oak (*Quercus robor* L.), and in a l
extent, *Quercus robur* L. and *Q. frainetto* Ten. The majority of these forests has a
mature age, the rest being of middle age.

In the case of *Tortrix viridana* the captures were performed by phero
method with Atravir. The number of males captured with pheromonal traps sh
a wide variation range, due to the differences in climatic conditions during the
research interval (1988–2002).

In the case of Geometridae species, with aptere females, the capture me
with glue rings, placed on tree trunks was used. The winged males, whic
active earlier than females, were also captured. Generally, the sex ratio w
Because females are difficult to be captured, we considered one female to 6 ma

3. RESULTS AND DISCUSSIONS

Tortrix viridana develops outbreaks in the forests of Muntenia and Ol
Subcarpathian hills and plain, Moldova plateau and hills. The Geometridae sp
develop in oak forests, but were signaled also in mixed forests (*Quercus*
Fagus, Tilia, Carpinus and others species). The forests of Muntenia and Ol
Subcarpathian hills, were usually prefered, and in a low extent in south Roma
plain, Moldavia, Transylvania, Banat and Dobrogea (Teodorescu, Simion
1994, 1997, 1999, Teodorescu, Vădineanu, Simionescu, 2001)

The mating flying phenology of the Tortrix viridana (Table 1)

The flying period lasted 20–25 days, from early May to last June. **Pe**
and duration of maxime flying (55 % of population) were between 16 an
May (about fifteen days). The intensive flying usually occures in the first pa
flying period. Thus, 40 % of captures were registered in the first three days,
70 % in the first six days. **The flying dynamics** was influenced by the
temperature values and precipitations cantities. **The flying start** was delayed a
lasted longer in the North of the country, with a colder climate. The periods of
precipitations prolonged the flying period in the southern zones.

The reduced moths number captured by traps, in numerous investigated z
(43 %), enabled to give an optimistic prognosis of possible defoliation. In 37 %
the investigated zones, the defoliation percent was estimated to be between 21
35 %. In 29 % of investigated locations, the moths number was higher,
consequently the defoliation prognosis rised to 36–70 % (Table 2).

Table 1

Tortrix viridana flying dynamics established by feromonal traps with Atravir

Forest perimeter	Forests	Forest composition / Age	Altitude (m)	Years	Traps number	Moths number / trap	Flying period	Maximum flying period	
								Interval	%
1	2	3	4	5	6	7	8	9	10
Vâlcea Drăgăşani	Amaraşti, Izvoarele, Guguiana, Izvoraşu, Suteşti	*Q. robur* 24 – 60	240 – 300	1988	153	554	18.05 - 2.06	18 – 22.05	69
				1989	81	230	1.06 – 20.06	3 – 5 .06	54
				1990	66	267	14.05 – 26.06	19 – 22.05	70
Lunca Stăneşti	Mamu, Căprioara, Milovanu, Frumuşica, Dobrasa, Gliganu	*Q. robur*, *Q. frainetto* 40 – 115		1989	9	122	18.05 – 4.06	20 – 22.05	65
				1990	300	297	13.05– 22.05	13 – 16 .05	62
				1992	64	114	20.05 – 12.06	26 – 30.05	49
Bălceşti	Bălceşti, Măciuca, Făureşti, Valea Mare, Zătreni	*Q. robur*, *Q. frainetto* 40 – 100		1988	102	441	4.06 – 19.06	4 – 7.06	68
				1990	297	130	17.05 – 3.06	20 – 25.05	65
				1992	313	6	28.05 – 25.06	1 – 7.06	47
Stoiceni	Stoiceni, Dos, Topolog, Trepteni, Dărniceni	*Q. robur*, *Q. frainetto* 30 – 80	220 – 430	1988	45	41	20.06 – 10.07	26.05- 2.07	53
				1989	90	80	20.05 – 30.05	22 – 24.05	64
				1990	90	96	20.05 – 4.06	30.05 – 1.06	62
				1992	84	41	2.06 – 20.06	4 – 6.06	66
Argeş Topoloveni	Râncaciov, Priboieni, Negreşti, Leordeni	*Q. robur*, few other *Quercus* 30 – 120	340 – 450	1989	272	155	10.05 – 15.06	25.05 – 28.05	41
Poiana Lacului	Rogozea, Negrea, Zârna, Arnota, Ruginoasa	*Q. robur* 30 – 100	290–420	1989	197	344	18.05 – 20.06	21 – 27.05	62
Piteşti	Trivale, Dobrogotea, Micesti, Valea Mare	*Q. robur* 30 – 120	320 –580	1989	67	62	16.05 – 10.06	22 – 28.05	77
Bucureşti Braneşti	Cernica, Căldăraru, Cucu, Pasărea, Găneasa, Ieftimiu	*Q. frainetto*, *Q. pedunculiflora*		1990	33	52	13.05 –27.05	17 – 21.05	79
				1993	36	31	24.05 – 7.06	27 – 29.05	46
				1994	57	34	16.05 – 28.05	21 –24.05	66

Table 1

(continued)

1	2	3	4	5	6	7	8	9	10
Bucureşti	Mogoşoaia, Băneasa, Râioasa, Valea Mocanului, Socola, Tunari			1988	37	51	28.05 – 13.06	6 – 8.06.	51
				1990	20	36	14.05 – 20.06	21 – 24.05	40
				1991	46	42	28.05 - 30.06	9 – 13.06	34
Snagov	Vlădiceasca, Ghermăneşti, Balta Neagră, Buruiaşu, Ciofliceni	*Quercus* species		1990	28	65	12.05 – 10.06	14 – 22.05	53
				1994	12	66	15.05 – 25.05	21 –25.05	54
Dolj Amaradia	Balota, Viişoara, Boanta, Pârşani	*Q. frainetto* 40 - 70		1990	99	258	23.05 - 7. 06	29.05 – 2.06	73
Dâmboviţa Voineşti	Aninoasa, Barbulet, Măneşti	*Q. robur*	350- 550	1991	28	89	19.05 – 6.07.	1 – 11.06.	38
Neamţ Tg. Neamţ	II, V Dumbrava, Plăieşu, Coverca, Bulubeşti, Fageţel	*Q. robur* 40 - 160	310 -450	1988	30	98	23.06 – 5.07.	23 – 26.06.	79
				1989	36	29	2.06 – 23.06.	6 – 9.06.	54
				1990	33	34	28.05 – 21.06.	4 – 11.06.	67
				1991	45	20	4.06 – 28.06.	11 – 21.06.	73
				1992	39	31	29.05 – 30.06.	9 – 12.06.	47
				1993	39	12	27.05 – 28.06.	27 .05.– 3.06.	50
				2000	39	15	16.05 – 30.06.	30.05 – 9.06.	48
				2001	38	8	15.05 – 10.06.	22.05 – 8.06.	69
				2002	39	10	17.05 – 25.06.	24.05 – 4.06.	44

Table 2

Prediction of infestation intensity and defoliating percentages based on analysis of pheromon captures number

Number of sites with pheromon traps (%)	**Number of moths / trap**	**Probable defoliating percent**
43	< 50	10 – 20
14	51 – 75	21 – 25
14	76 – 120	26 – 35
9	121 – 200	36 – 57
20	> 200	57 – 70

A lower number of captured moths was registered in forests situated in proximity of Târgu Neamţ, Brăneşti, Bucureşti, Snagov, Piteşti and Stoiceni localities. Thus, the forests near Târgu Neamţ and Stoiceni forest perimeters from Vâlcea district offer unfavorable conditions for *Tortrix viridana* outbreak.

A high number of captured moths was registered in Drăgăşani, Lunca Stănești, Bălceşti, Poiana Lacului, Amaradia, Topoloveni forest perimeters.

Although the moths number was low in the analysed period, *Tortrix viridana* registered outbreak in some years in Brăneşti, Snagov, Bucureşti, Poiana Lacului and Piteşti forest perimeters.

The biological or chemical control methods were applied in some cases, decision being made on the layed number of *Tortrix* eggs.

The mating flying phenology of the Geometridae *species* (Table 3)

Some Geometridae species have **autumn-winter flying** (October–November, sometimes December), others **winter-spring flying** (February–March, sometimes December).

Among the Geometridae species with autumn-winter flying, *Operophtera brumata* was dominant, followed by *Erannis defoliaria, Er. aurantiaria, Colotois pennaria.* Flying period was in October end, and November begining. Flying duration varied between 30 and 55 days, with a mean value of 46 days. Period of maxime flying was 10–15 days, in November (Table 4).

Table 3

Flying dynamics of Geometridae species

Forests perimeter	Years	Forests altitude (m)	Forests composition / Age	Number of captured moths			Flying period	Maximum flying period	
				Males	Females	M/ F		Interval	%
1	2	3	4	5	6	7	8	9	10
Bucureşti Snagov	1993	Snagov Parc	80–120 *Quercus* species	1611	797	2	6.11.1993 15.01.1994	15.12 - 30.12	61
	2000	Vlădiceasa	*Quercus* species 60–100	922	44	21	8.11 – 13.12	14.11 – 24.11	45
Buzău Tisău	1997	IV 59-64; 94-107	*Q, robur* 40–90	9838	827	11,9	1.11 – 16.12	16.11 – 28.11	69
	1998	VII 95-98		1639	402	4,1	25.02 - 1.03	25.02- 27.02	57
		VIII 1-7; 8-13		1816	160	11,4	5.11 – 24.12	5.11 – 16.11	73
		V 1- 17		2375	636	3,7	5.11 – 24.12	5.11 – 16.11	56
		VII – VIII St. Gheorghe		564	470	1,2	9.11 – 18.12	11.11 – 27.11	80
	1999	Hales		1397	355	3,9	21.02 – 17.03	21.02 – 25.02	68
		St. Gheorghe		455	165	2,8	21.02 – 19.03	21.02 – 25.02	44
		VII, VIII		1852	520	3,6	21.02 – 19.03	21.02 – 25-02	62
		V , VII, VIII		7084	1329	5,3	2.11 –28.12	22.11 – 4.12	61
	2000	VIII 37- 43		1507	294	5,1	24.02 – 23.03	24.02 – 1.03	86
		V, VII, VIII		8380	519	16,1	4.11 – 30.12	24.11 – 4 12	54
	2001	VIII		195	34	5,7	31.01- 2.03	18.02 – 22.02	67

Table 3

(continued)

Botoşani Darabani	1994	Floroi, Comăneşti, Teioasa	*Quercus* species	12564	2233	5,6	16.10 – 10.12	13.11 – 21.11	63
Mihai Eminescu		Sarafineşti, Pintilia, Dogaria		3559	256	13,9	15.11 – 20.12	15.11 – 27.11	65
Truseşti		Călăraşi, Guranda, Ioneşti		–	–	–	26.10 – 21.12	26.10 – 5.11 10.12 – 16.12	23 26
Argeş Topoloveni	1990	Leordeni, Negreşti, Cârcinov, Priboieni	*Q, robur Quercus* species 20 - 100	6552	987	6,6	26.10 – 27.12	11.11 – 26.11	60
Dâmboviţa Găieşti		Cobia	*Quercus* species 40 - 100	3198	143	22,4	12.11 – 26.12	20.11 – 24.11	65
	2001	Catane	*Quercus* species 40 - 80	578	36	160	26.11 – 14.12	4.12 – 14.12	65

Table 4

Characteristics of autumn-winter flying period by Geometridae species

Parameters	Obtained data
Flying period	21 % between 16 and 26 October
	57 % between 1 and 9 November
Mean flying duration 46 days	43 % between 30 and 38 days
	29 % between 45 and 55 days
	28 % over 55 days
High values of flying period	64 days in forest perimeter of Topoloveni (Argeş)
	57 days in forest perimeters of Dărăbani, Truseşti (Botoşani)
Period of maximal flying	Usually in 15-30 November
	In few cases by the end of October and beginning of November or in December
Duration of maximal flying	10 days (concentrate flying)

The winter-spring flying was investigated only in Tisău locality, during 1998–2001 period, in forests of risk to development of Geometridae outbreak. The forests of this zone have in their composition *Quercus robor*, mixed with *Tilia*, *Fagus*, *Carpinus* etc., of 40–90 years old, the II–III class of production. Among the Geometridae species with winter-spring flying, *Erannis leucophaearia* was dominant and in a lower proportion, the species *Alsophila aescularia*, *Erannis marginaria*, *Phigalia pedaria.*

The flying period was in February. Mean value of flying duration was 28 days. Period of maxime flying was especially in the day in which the flying starts and in the following 5 days (Table 5).

In Tisău locality, autumn-winter flying Geometridae species flying in October-November represent 60–65 %, while winter-spring flying in February-March represent 35–40 %. In 1998 and 1999, in this locality was observed Geometridae outbreak, and the bacterial product Dipel 8 L was applied as biological control measure.

Of course, in zones where Geometridae infestation was frequently registered in autumn, their presence was observed also in winter, but the population density was very low.

Table 5

Characteristics of winter-spring flying period by Geometridae species

Parameters	Obtained data
Flying period	50 % in 21 February
	20 % in 24 and 25 February and 31 January
Mean flying duration	28 days
Period of maximal flying	The day in which the flying starts
	In few cases by the end of October and beginning of November or in December
Maximal duration of flying	64 % of moths in 5 days (concentrate fly)

4. CONCLUSIONS

Tortrix viridana and Geometridae are defoliator species which infest together the oak forests. The Geometridae are also found in mixed forests, with *Quercus*, *Tilia*, *Fagus*, *Carpinus* and other tree species. Their associated attack enhances the defoliation of *Quercus* forests.

By using pheromon method with Atravir for *Tortrix viridana* and glue rings placed on tree trunks, for Geometridae species, we established flying start, flying period, flying dynamics, mean flying duration, high values of flying period, period and duration of maximal flying, outbreak, probable defoliating percent, autumn-winter flying and winter-spring flying species.

Among species with autumn-winter flying (46 days in mean, especially in October–November, with maximum fly in 15–30 November), dominant species was *Operophtera brumata.*

Among species with winter-spring flying (28 days in mean, especially in February–March, with maximum fly in the day in which the flying starts), dominant species was *Erannis leucophaearia.*

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STRUCTURAL INDEXES OF GASTROPODS POPULATIONS WITHIN SMALL RESERVOIRS FROM THE OLTENIA PLAIN

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The researches made within some small reservoirs located in the Oltenia Plain emphasized the characteristics of the biocoenosis, an important role being played by the Gastropods populations.The structure of the Gastropods populations acquires certain peculiarities characteristic to each reservoir and reflected by the structural parameters of the populations belonging to this group.Thus, there have been calculated and established the following parameters: frequency, constancy, relative abundance, affinity, diversity and equitability.

Key words: Gastropods, reservoirs, the Preajba Valley, structural parameters.

1. INTRODUCTION

The ecological researches made within the small reservoirs located in the Oltenia Plain emphasized the characteristics of the specific biocoenosis and the important role played by the Gastropods populations (4, 5).

The reservoirs belong to the category of eutrophic ecosystems and are characterized by a high biological production and productivity (1, 3, 6).

The Gastropods ensure the highest biomass quantity. This situation is also emphasized by the biocoenosis structural parameters (2).

The structure of the Gastropods populations acquires certain peculiarities specific to each reservoir (7, 9).

Thus, there have been calculated and established the following parameters: frequency, constancy, relative abundance, affinity, diversity and equitability.

2. MATERIAL AND METHODS

In order to emphasize the qualitative and quantitative structures of the Gastropods populations, they have been sampled each season.

The quantitative samples have been taken with Wan-Wene bodengrapher, while the qualitative ones with the limnologic net.

The laboratory works included: taxonomic determinations, distribution within the habitat, structure according to age, dynamics of the populations' increasing number and of the structural parameters, determination of the production.

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The structural parameters have been established on the basis of statistical calculation.

3. RESULTS AND DISCUSSIONS

The distribution of the Gastropods populations depends on the location of the reservoirs along the schemed river named the Preajba Valley. There are two groups of reservoirs: the first one located along the upper course of the river and the second one along its middle and lower sectors.

Thus, for the first category of reservoirs, the populations *Physa acuta* followed by *Radix ovata* register the highest frequencies: up to 72.22% in the first case and 50–55% in the second case.

At the same time, it is worth mentioning that the diversity parameter is also the highest one. This increased parameter is directly related to the diversity of the habitat types: areas with paludous and aquatic macrophytes near the banks, areas with detritus and sandy facies.

The highest frequency of the *Physa acuta* species is mainly due to the presence of the macrophytes, habitat preferred by this species.

The presence of the *Succinea elegans* species, a species characteristic to rivers but identified within the first reservoir located along the upper course of the river, as well can be explained by the fact that the stream has quite a high speed here. The *Succinea elegans* populates especially the tributary streams of the Preajba Valley.

Besides the *Physa acuta, Radix ovata* and *Aplexa hypnorum* that can be considered dominant species for this area and not only, the other species (*Radix auricularia, Segmentina nitida, Anisus spirorbis*) are accessory or accidental species as they prefer the oozy areas rich in organic detritus.

As regards the second group of reservoirs, the highest frequency parameter is registered by two species: *Viviparus acerosus* and *Viviparus viviparus*. Both of them are stagnant species that prefer especially eutrophic lake ecosystems. The reservoirs built along the lower sector of the river belong to this category. The two main species as well as the others find here abundant food resources as they populate the border area rich in macrophytes; there develops a rich periphyton that can be also noticed on the submerged sides of the concrete plates that pave the banks of the river.

The appearance of the *Valvata piscinalis* species for the first time is a characteristic of this area (its frequency index is 34.61%); this species is considered to be oligotope.

Taking into account their frequency and distribution within the reservoirs, the species can be grouped as follows:

- eurytope species (*Physa acuta, Radix ovata, Radix auricularia*) that are present within all the reservoirs; oligotope species among which we mention *Aplexa hypnorum, Segmentina nitida, Radix peregra, Fogotia acicularis, Planorbis planorbis, Viviparus acerosus, Viviparus viviparus*; they are present in 4 –7 reservoirs;
- stenotope species (*Physa fontinalis, Valvata piscinalis, Fagotia esperi, Stagnicola corvus, Stagnicola palustris, Anisus spirorbis* and *Succinea elegans*).

On the basis of the calculations made on 4,000 samples belonging to the 17 species identified within the studied reservoirs, we established the relative abundance. Thus, the *Radix ovata, Physa acuta, Aplexa hypnorum* species are dominant in the first category of reservoirs. The *Viviparus acerosus, Viviparus viviparus* species present a high relative abundance, their highest rate being registered within the second category of reservoirs. Due to their numerical and biomass density, the two species represent the populations with the most important role in the functioning of the benthic biocoenosis; they ensure a biomass production that is several times higher than the one of the other populations.

The two species together with the *Physa acuta, Radix ovata* and *Aplexa hypnorum* species represent over 74% of the total number of samples, the other 12 species representing only 25.9% (Fig. 1).

In order to establish **the affinity** parameter, the species have been arranged according to the size of the ecological significance parameter.

Calculating the affinity parameter according to the Jaccard's formula ($q = \frac{c}{a+b-c}.100$) in order to establish to what degree the different Gastropods species are characteristic for the biocoenosis they populate, we noticed that there is a certain link or association degree among the species.

Thus, the maximum affinities that oscillate between 70.1 and 100% have been registered to a reduced number of species: *Physa fontinalis + Radix peregra, Physa fontinalis + Physa acuta, Radix peregra + Physa acuta; Viviparus acerosus + Viviparus viviparus, Viviparus acerosus + Radix ovata; Physa acuta + Stagnicola corvus.*

It is known that the affinity between species does not always directly depend on each other, but also on the environment conditions (8, 11).

This is the case of the two species *Viviparus acerosus* and *Viviparus viviparus* that have similar life conditions and thus, their affinity oscillates between 70 and 100%. In fact, these two species, due to the high numerical and biomass density, play an important role in the functioning of the biocoenosis they populate, in transferring the matter and energy.

A much lower affinity can be noticed between *Physa acuta* that populates all the reservoirs as it finds optimum development conditions and *Succinea elegans* that rarely appeared; the affinity between them is only 7.7 %. If the first species can be considered eurytope, as it is present within all the reservoirs, the other is an accidental species as it was identified within only one reservoir.

We can conclude that the affinity has increased values among the Gastropods species that are well established within the reservoirs and present a maximum number of individuals; this is the case of *Viviparus acerosus, Viviparus viviparus, Physa acuta, Aplexa hypnorum*. In the case of the species that are poorly consolidated and do not have an increased number of individuals, there are low or null affinities.

The calculation of the **diversity and equitability parameters** indicates the causes that induced the differences of diversity at the Gastropods populations located within the reservoirs. The diversity index varied between 1.68 and 2.49 (Table 1).

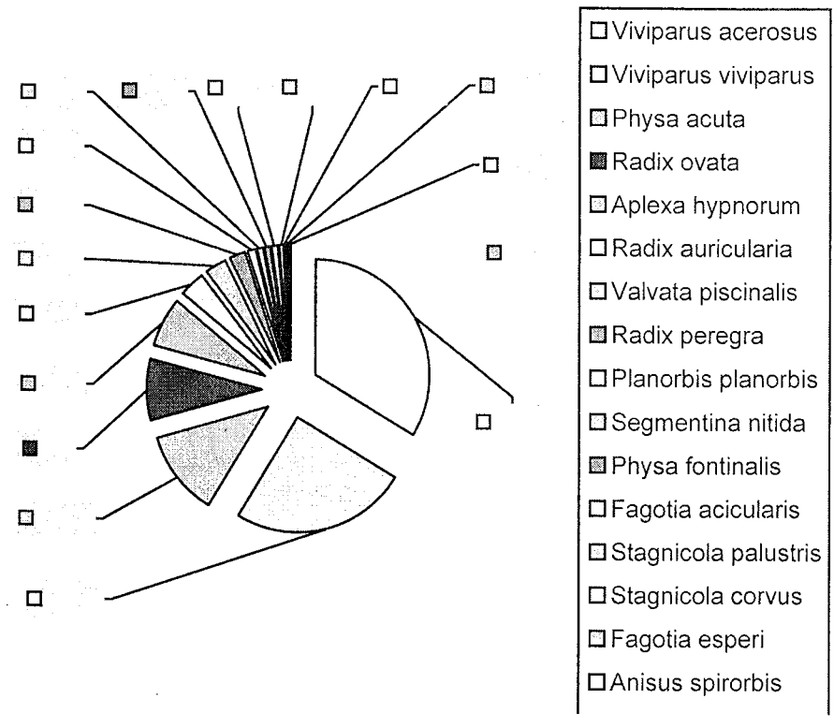

Fig. 1 – The numerical abundance of the Gastropods within the 5th–13th reservoirs (1996–1998).

Table 1

The diversity of Gastropods species within the 5th–13th reservoirs

REAL DIVERSITY								
RESERVOIRS								
V	VI	VII	VIII	IX	X	XI	XII	XIII
2.49	2.04	2.45	2.02	2.36	2.33	1.81	1.98	1.68

According to a series of parameters and we mention here the pollution, trophy degree and the preponderance of a certain type of substratum the reservoirs have been separated into two groups. The first group – upstream, along the upper sector of the river presents a higher diversity of habitats and ecological niches (12), while the second one, downstream has a more uniform structure of the environment conditions that are decisive for the development of the Gastropods populations.

On the basis of these factors we can explain the diversity indices. In the first group of reservoirs (large areas of macrophytes where the rheophyle character can be still noticed and the structure of the benthic biotope is more varied), the diversity indices that express the diversity of species is quite high. These species find here proper development conditions, specific to the ecological necessities of each species.

In the second group of reservoirs, most part of the bottom is evenly covered by sapropel silt rich in organic substance, with an increased level of trophy and the diversity is limited, the dominant species being *Viviparus viviparus, Viviparus acerosus, Radix ovata*; thus, the reservoirs can be considered typical ecosystems for the development of these species.

For the Gastropods populations the **equitability** presents values that oscillate between 0.59 and 0.88 (Table 2), the lowest value being registered within certain reservoirs located upstream, affected by man's actions.

Table 2

The equitability of the Gastropods species

EQUITABILITY								
RESERVOIRS								
V	VI	VII	VIII	IX	X	XI	XII	XIII
0.83	0.88	0.82	0.87	0.82	0.83	0.65	0.66	0.59

4. CONCLUSIONS

1. The distribution of the Gastropods populations depends on the location of the reservoirs along the schemed course of the Preajba Valley in two groups: the reservoirs located on the upper sector of the river and the ones located along its middle and lower sectors.

2. According to the analysis of the populations' structural parameters, there resulted that *Physa acuta, Radix ovata* and *Aplexa hypnorum* species register the highest frequencies, as they are constant species; *Radix auricularia, Segmentina nitida* and *Anisus spirorbis* are accidental species. *Viviparus acerosus* and *Viviparus viviparus* are the species that register the highest frequency parameter within the second group of reservoirs. They represent constant populations and present the most increased abundance.

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THE PLANKTON BIOMASS AND PRODUCTIVITY IN DANUBE DELTA LAKES BEING IN ECOLOGICAL SUCCESSION

VICTOR ZINEVICI, DOINA IONICĂ, LAURA PARPALĂ, CRISTINA SANDU, NICOLAE NICOLESCU

The researches were carried out in 1999–2001 period, in two Danube Delta shallow lakes being in different succession stages: Roşu and Tătaru. The first one is among the youngest lakes (from ecological succession point of view), the second one is in the small pool stage. The energy equivalent mean of organic matter was 179.53 kcal/m3. 56.41% of this value was represented by DOM (dissolved organic matter) and 43.59% by POM (particled organic matter). Of 78.25 kcal/m3, which represented the POM equivalent, 78.82% was tripton and 21.28% plankton. So, the plankton represented only 9.27% of the energy equivalent of water organic matter. The phytoplankton represented 87.10% of the biomass caloric equivalent of plankton trophic pyramid, while the zooplankton represented 6.56% and the bacterioplankton 6.34%. The phytoplankton represented 49.41%, the zooplankton only 0.90% and bacterioplankton 49.69% of total productivity. The phytoplankton proportion in caloric equivalent of plankton biomass and productivity decreased in the succession process of Danube Delta lacustrian ecosystems (91.50% → 78.36%, respectively 50.55% → 47.50%), in inverse correlation with the zooplankton proportion (5.90% → 7.88%, respectively 0.69% → 1.25%) and bacterioplankton one (2.60% → 13.76%, respectively 48.76% → 51.25%).

Key words: ecological succession, plankton, biomass, productivity, shallow lakes, Danube Delta.

1. INTRODUCTION

The ecological succession, one of the recent concept in ecology has some still unclear, even controversed, aspects. Used for the first time by Clements at the beginning of XXth century, this concept referred to the vegetation of terrestrial ecosystems. Further researches in aquatic ecosystems, have revealed some controversies against the terrestrial ecosystem analyses (1). Only few researches were carried out in shallow lakes (as Danube Delta lakes) (6), (8), and they evidenced significant differences towards deep lakes regarding the ecological succession process.

2. MATERIAL AND METHODS

The researches were carried out in 1999–2001 period, in two shallow lakes representative for Danube Delta: Roşu and Tătaru. The first one is among the youngest lakes (from ecological succession point of view). It has a large surface

(1450 ha) and it is less silted than other Danube Delta lakes. Its depth varies between 1.7–2.7 m. The Tătaru lake is in an advanced succession stage – of small pool – with a small surface (approx. 50 ha) and a low depth (0.7–1.5 m).

The samples were collected seasonally (spring, summer, autumn), in 3 points of every ecosystem, with a Patalas device of 5 l water. Total organic matter (TOM) was determined by oxidation method, using $K_2Cr_2O_7$ (4). The dissolved organic matter (DOM) was determined on water samples filtered through a polycarbonate filter (Millipore, 0.2 μm). The phytoplankton biomass was determined by the volumetric method. The standard weights, peculiar to species, sex, developmental stages and size groups were used for the zooplankton biomass calculation. The zooplankton net mesh had 65 μm and 50 l of water were filtered for each sample.

The bacterioplankton biomass was determined according to Findlay (3), by the phosphorus amount evaluation in phospholipids.

The primary productivity was determined by Winkler's method. The bacterioplankton productivity was calculated on biomass and generation time data. The zooplankton productivity was calculated according to Ilkowska and Stankzykowska, Galkowskaja, Winberg, Pečen and Suşkina (2).

For biomass and productivity transformation in energy equivalents were used biomass-calories conversion factors (7).

3. RESULTS

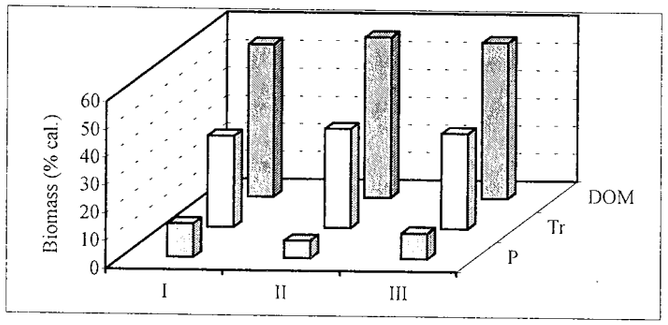
The Danube Delta lakes, which are eutrophic ecosystems, are characterized by the presence of an important amount of organic matter. Its major part will fall in sediments but another significant part will remain in water. The energy equivalent mean of organic matter was of 179.53 kcal/m^3 in 1999–2001 period in Roşu and Tătaru lacustrian ecosystems (Table 1). Its major part was represented by the dissolved organic matter (DOM) (56.41%) (Fig. 1) towards the particled one (POM) (43.49%). These figures were of the same range to the values characterizing the plankton organic matter in eutrophic lakes (5).

The particled organic matter mean of the two ecosystems was of 78.25 kcal/m^3, in 1999–2001 period. In its structure two components may be identified: the detritus particled matter, generated by the dead aquatic organism decomposition (tripton) and the living particled matter (plankton). The first one represented 78.72% of POM, while the second one only 21.28%. Consequently the plankton communities' populations represented only 9.27% of caloric equivalent of organic matter from water body. These figures are also in the range of eutrophic lacustrian ecosystem values (5).

Table 1

The gravimetric structure of water organic matter in Roşu and Tătaru lakes, and their mean values (Xa 1999–2001)

Ecosystem	Energy equivalent of water organic matter (kcal./m^3)			
	Plankton	Tripton	DOM	POM + DOM
Roşu shallow lake	22.160	60.130	100.699	182.989
Tătaru small pond	11.146	63.071	101.861	176.078
Xa	16.653	61.6005	101.280	179.5335



P = Plankton; Tr = Tripton; DOM = dissolved organic matter

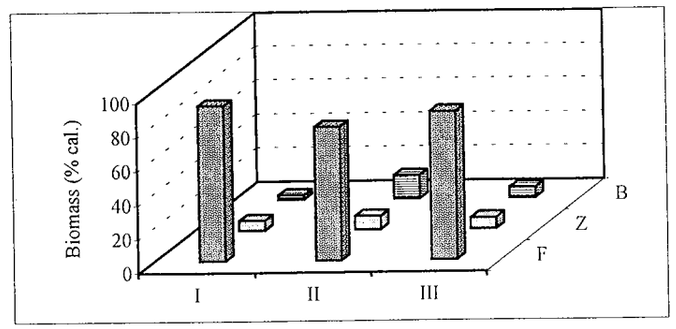
Fig. 1 – The structure of particled and dissolved organic matter in Roşu (I) and Tătaru (II) lacustrian ecosystems and their mean values (III) (Xa 1999–2001) (% cal).

The trophic pyramid of plankton communities from Roşu and Tătaru lakes showed the huge proportion of phytoplankton primary producers. Their caloric equivalent of biomass (14.49 kcal./m^3, Table 2) represented 87.10% of total plankton, while zooplankton consumers and bacterial decomposers represented only 6.56%, respectively 6.34% (Fig. 2).

Table 2

The plankton biomass structure differentiated on communities in Roşu and Tătaru lakes, and their mean values (Xa 1999–2001) (kcal/m^3)

Ecosystem	Energy equivalent of plankton biomass (kcal./m^3)		
	Phytoplankton	Zooplankton	Bacterioplankton
Roşu shallow lake	20.270	1.306	0.578
Tătaru small pond	8.727	0.879	1.533
Xa	14.4985	1.0925	1.0555



Ph = Phytoplankton; Z = zooplankton; B = bacterioplankton

Fig. 2 – The plankton biomass structure, on communities in Roşu (I) and Tătaru (II) lacustrian ecosystems, and their mean values (III) (Xa 1999–2001) (% cal).

It is important to notice that, in the conditions of significant increasing of ecosystems trophic level, plankton / tripton ratio and phytoplankton / zooplankton / bacterioplankton, biomass ratio differ significantly from our values (6).

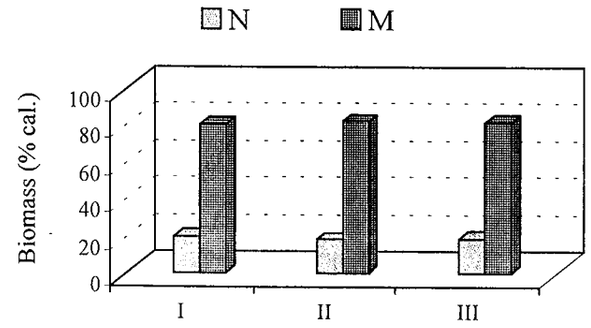
A different picture of the above-mentioned communities was shown by the productivity analysis (Table 3). The bacterioplankton, characterised by a remarkable reproduction potential, became the main producer, representing 49.9% of the total plankton production. The phytoplankton took the second place, but very close to the bacterioplankton (49.41%). The zooplankton productivity had insignificant values (0.97%), being on the last place (Fig. 3).

Table 3

The plankton productivity structure differentiated on communities in Roşu and Tătaru lakes and their mean values (Xa 1999–2001)

Ecosystem	Energy equivalent of plankton productivity (kcal./m^3)			
	Phytoplankton	Zooplankton	Bacterioplankton	Σ
Roşu shallow lake	8.838	0.1212	8.525	17.4842
Tătaru small pond	4.9562	0.1305	5.3482	10.4349
Xa	6.8971	0.1258	6.9366	13.9595

The Danube Delta lakes while aging, pass from the shallow lake stage to the small pond one. This process means significant changes, both in organic matter gravimetric structure and also in the plankton biomass and productivity. Consequently, the total water organic matter decreased (182.99 → 176.08 kcal/m^3), due to the similar evolution of living particled matter (plankton) (22.16 → 11.15 kcal/m^3). In an inverse correlation, the dissolved organic matter increased (100.70 → 101.86 kcal/m^3) as the detritus particled matter (tripton) (60.13 → 63.07 kcal/m^3) (Table 1).



N = Nanophytoplankton; M = Microphytoplankton

Fig. 3 – The relative structure of productivity, on communities in Roşu (I) and Tătaru (II) ecosystems, and their mean values (III) (Xa 2000–2001) (% cal).

The phytoplankton contribution to the plankton biomass and productivity decreased in the succession process (91.50% → 78.36%, respectively 50.55% → 47.50%) due to the raise of macrophyte primary producer proportion. In an inverse correlation, the zooplankton increased (5.90% → 7.88%, respectively 0.69% → 1.25%) as the bacterioplankton (2.60% → 13.76%, respectively 48.76 → 51.25%) (Figs. 2, 3).

4. CONCLUSIONS

– The plankton communities represented only 9.27% of caloric equivalent of total water organic matter.

– The phytoplankton represented 87.10% of biomass caloric equivalent of plankton trophic pyramid of Roşu and Tătaru lacustrian ecosystems, while the zooplankton – 6.56% and bacterioplankton – 6.34%.

– The phytoplankton represented, from productivity (caloric equivalent) point of view, 49.41% of total plankton productivity, while zooplankton represented only 0.90% and bacterioplankton 49.69%.

– The proportion of phytoplankton in plankton biomass and productivity (as caloric equivalent) decreased in succession process of Danube Delta lacustrian ecosystems (91.50% → 78.36%, respectively 50.55% → 47.50%), in inverse correlation to zooplankton (5.90% → 7.88%, respectively 0.69% → 1.25%) and bacterioplankton proportion (2.60% → 13.76%, respectively 48.76% → 51.25%).

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OLIGOCHAETA IN THE LOWER RIVER DANUBE

GETA RÎŞNOVEANU

Percentage of the Oligochaeta community within the benthic fauna, its species richness, composition and abundances in the lower Danube River were studied during 1992–1993. Among the 13 species of Oligochaeta identified three to four tubificid species represent over 90% of both total number and biomass of the benthic fauna. The other species were rare having occurrence frequencies less than 20% and abundances frequently less than 50 individuals m^{-2}.

1. INTRODUCTION

The structure of the benthic communities is considered to be an excellent tool for assessing tendencies in the trophic state of the aquatic ecosystems and the level of their pollution [2]. This due to the fact that benthic communities are relatively stable and their dynamics are influenced to varying degrees by different factors that can modify species composition, the population's life cycles and their abundances. Therefore, benthic communities integrate in their structure the effects of environmental pressure over long period of time. Changes in the structure of benthic communities could be assessed based on the presence/absence of the indicator species or, according to principles of the Systems Ecology, based on the species richness, composition and abundances. The data on the benthic communities in the Lower River Danube (LRD) are scarce and mainly focused on species composition in relation to specific habitats [3, 5, 6, 7, 10].

The present paper belongs to a series of papers concerning the structure and functions of the benthic Oligochaeta populations under hypertrophic conditions in the LRD. It aims to assess the size and the structure of the Oligochaeta community.

2. MATERIAL AND METHODS

Three benthic sample units were randomly taken from each riverbank at each of four sites dispersed along the LRD: S_1 – downstream the industrial area of Brăila and confluence with Măcin arm, S_2 – downstream Galaţi and confluence with River Prut, S_3 - downstream Tulcea but upstream the split of Chilia and Tulcea arms, S_4 – on the Tulcea arm, upstream the split of St. George and Sulina arms. This series was repeated 11 times from March 1992 to October 1993. During 1992 samples were taken at intervals of latest two months. During 1993 the samples were taken monthly

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between May and October. The samples were collected with a 50 cm^2 corer, washed through a sieve (mesh size 230 μm), and the retained sediments and fauna were preserved in 4% formaldehyde. All of the faunal specimens were hand sorted from sediments under a Zeiss stereomicroscope and transferred to separate containers filled with 70° alcohol. The fauna was subsequently identified to group level except for Oligochaeta that were identified to species level. The number of specimens of each species was countered. Data processing included the occurrence frequencies (F) and proportions of Oligochaeta to total benthic fauna and of each species to total Oligochaeta community assessed both as number (A1) and biomass (A2).

The animals in each sample unit were washed to remove preservative than dried for 24 hours at 60 ^{0}C and then weighed to five places of decimals using an OHAUS electro microbalance with a sensitivity of 1×10^{-6}g.

3. DESCRIPTION OF THE STUDY SITE

The River Danube is the second largest river in Europe. Its lower sector stretch over 1080 Km along the south part of Romania and have a drainage basin of 218,660 Km^2 from which 75% cover 90% of the Romania surface [8]. The present research focused on the maritime sector of the river located downstream Brăila. Along this sector the river flow through a unique channel with many turns. Several authors describe the peculiarities of hydrogeomorphic unit and biodiversity changes along this sector [1, 8, 9]. Multiplication of the point and diffuse sources of nutrients all over the catchments area as well as drainage of the former flooding areas are the main causes of the accelerated increase of nutrient load (from 7–63 μg P l^{-1} in 1980 to 42–330 μg P l^{-1} at the beginning of 1990) and deterioration of water quality in this river sector.

4. RESULTS AND DISCUSSION

Six groups of benthic animals (Oligochaeta, Chironomidae, Amphipoda, Ceratopogonidae, Polichaeta and Trichoptera) were identified. In each station, at each sampling time, the benthic community consists in one to three taxa. Among them Oligochaeta are constant (occurrence frequencies of 100%) and predominant in all stations excepting S_3 (Fig. 1) were Amphipoda dominates the benthic fauna and represents between 43% and 97% of the total biomass of benthic invertebrates. In S_1 and S_2 Chironomidae were frequent (F > 90%) but represent less than 10% of the total biomass of benthic fauna. In stations S_3 and S_4 they were scarce. All the other identified taxa were very scarce representing less than 5% of the total benthic fauna biomass in all stations.

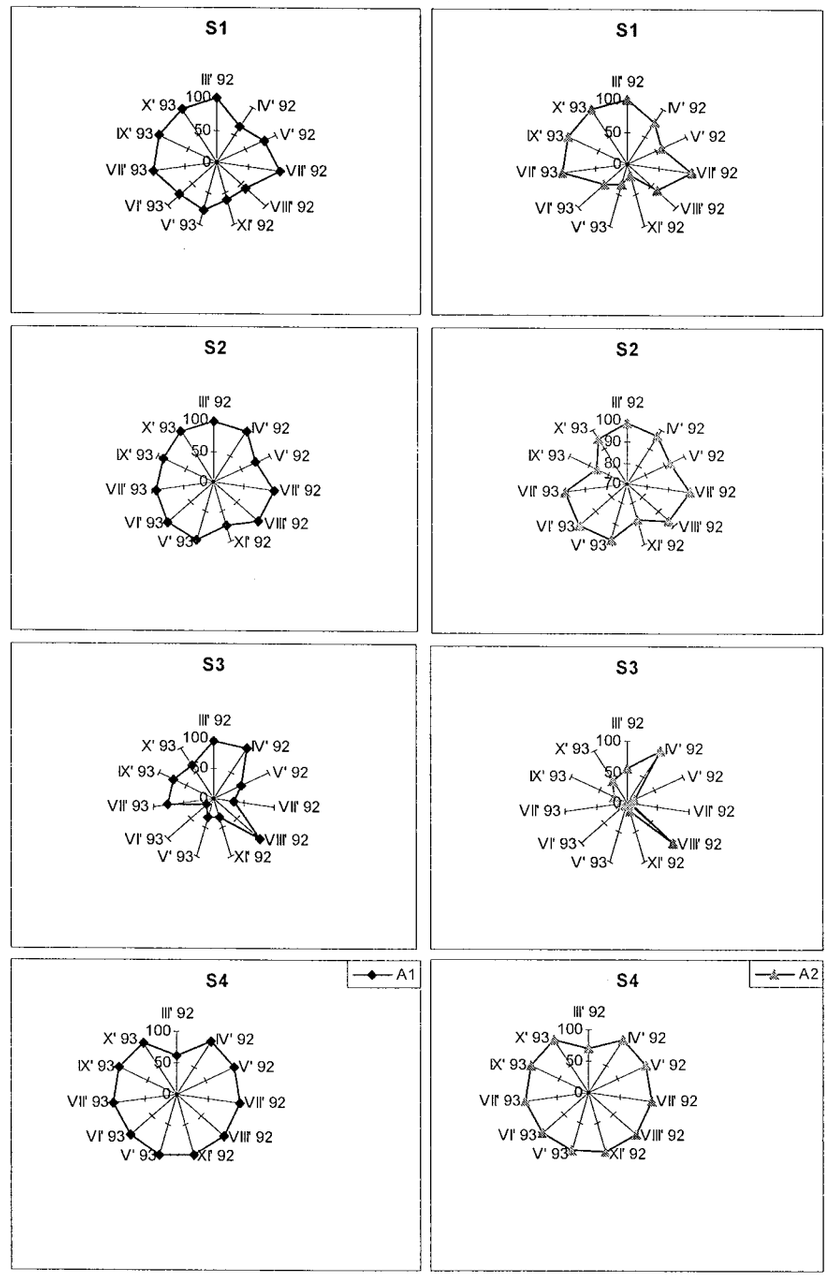

Fig. 1 – Monthly averages of the proportions Oligochaeta comprised of the total number (left) and biomass (right) of the benthic community in the River Danube, during 1992–1993.

The proportion comprised by Oligochaeta of the total benthic community ranged between 13% and 100% as number of individuals and between 3 and 100% as biomass (Fig. 1). In 1992 and 1993, over 97% of the total number of macroinvertebrates was represented by oligochaetes excepting stations S_1 and S_3 were the values were of 84% and 66% respectively. Over 84% of the total benthic biomass belongs to oligochaetes excepting station S_3 where this group comprise only 41% in 1992 and 21% in 1993. The differences recorded among stations are related to physical, chemical, hydrologic, morphometric and geographic peculiarities of each station.

Two species of Naididae (*Pristina sp.* and *Paranais sp.*) and 11 species of Tubificidae: *Potamothrix moldaviensis* (Vejdovsky and Mrazek 1902), *Psammoryctides barbatus* (Grube 1861), *Psammoryctides albicola* (Michaelsen 1901), *Limnodrilus hoffmeisteri* (Claparede 1862), *Limnodrilus claparedeanus* (Ratzel 1868), *Limnodrilus udekemianus* (Claparede 1862), *Limnodrilus profundicola* (Verrill 1871), *Spirosperma ferox* (Eisen 1879), *Isochaeta michaelseni* (Lastockin 1937), *Tubifex newaensis* (Michaelsen 1903) and *Tubifex tubifex* (Muller 1774) were identified for the entire Oligochaeta community. It should be noticed that the sampling technique used was not particularly effective for collecting Naididae. Besides, no clitellate specimens of *T. tubifex* were identified. In this case the species diagnoses was based only on the external morphological characters.

In each station, at each sampling time, the Oligochaeta community consists in one to nine species but frequently only three to four species have high abundances (Fig. 2). Among them: *L claparedeanus* (F = 100%) comprised over 13% as number and over 8% as biomass of the Oligochaeta structure in all stations; *P. moldaviensis* (F = 50 – 100%) presented large fluctuations of its abundance in the range 7 – 68% as number and 6 – 46% as biomass; *T. tubifex* (F = 67 – 100%) comprised up to 37% as number and 26% as biomass of the total Oligochaeta assemblage and *T. newaensis* (F over 50%) accomplished low percentage as number of individuals (less than 25%) but between 9% and 54% of the total biomass of the Oligochaeta community, excepting station S_3 where it was not identified over the study period (Table 1). The other species were sporadic (per station frequencies less than 20%), the size of their populations consistently kept around the critical level of species persistence (frequently less than 50 individuals m^{-2}) (Fig. 2).

Therefore, we consider that in order to assess the role of the Oligochaeta community within the integrating ecosystem the functional assessment of the four species is needed.

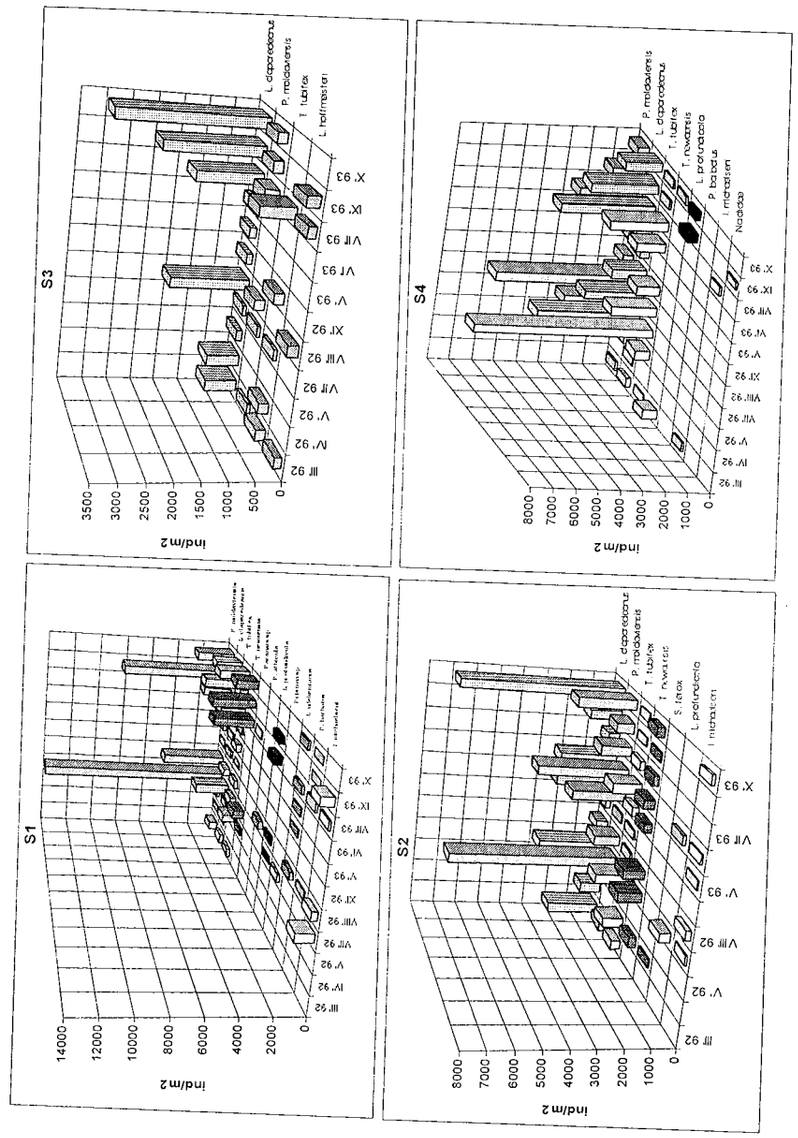

Fig. 2 – The structure of the Oligochaeta communities in the lower River Danube during 1992–1993.

5. CONCLUSIONS

The analyses of the benthic community structure during 1992–1993 interval along the Danube river sector downstream Brăila revealed that:

- the Oligochaeta community represents a constant and dominant component of the benthic fauna;
- three to four species of Oligochaeta had high frequencies of occurrence and dominated the community structure; they are characteristic species with important role in functioning of the integrating ecosystems;
- in stations S_1 and S_2 among six to ten identified Oligochaeta species *L claparedeanus*, *P. moldaviensis*, *T. tubifex* and *T. newaensis* represented over 90% of the total number and biomass of the Oligochaeta community;
- in stations S_3 and S_4 among the four and respectively seven identified species *L claparedeanus*, *P. moldaviensis* and *T. tubifex* represent over 93% as number and over 86% as biomass of the total Oligochaeta assemblage (Table 1).

Table 1

Dynamics of the proportions of each Oligochaeta species to total Oligochaeta community as number (A1) and biomass (A2), in the River Danube during 1992 and 1993

STATION	D1				D2				D3				D4			
	1992		1993		1992		1993		1992		1993		1992		1993	
SPECIES	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
L. claparedeanus	13	8	17	10	32	20	49	29	71	76	78	87	30	22	49	45
P. moldaviensis	68	46	41	27	41	17	38	22	7	8	8	6	30	38	26	34
T. tubifex	7	3	15	5	13	9	6	4	15	15	8	6	37	26	20	11
T. newaensis	3	38	21	53	10	54	5	44					2	13	0	9
I. michaelseni	6	1	3	1	1	0	1	0					0	0	0	0
P. barbatus	0	1	0	1									0	1	0	
L.'udekemianus	1	1	1	2												
L. hoffmeisteri									7	0	5	0				
L. profundicola	1	0	2	1	2	0	0	0					0	0	3	1
P. albicola	1	1	0	0												
Pristina sp.	1		0													

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PHYSICO-CHEMICAL ASPECTS OF LENTIC TERRESTRIAL ECOTONES OF DANUBE DELTA

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Lentic ecotones play an important role as coupling zones between aquatic and terrestrial ecosystems. They have specific physical and chemical characteristics, and also unique plant and animal communities. Ecotone size and configuration depend on shore slope and water level fluctuations, on depositional and erosion processes. Most of the organic matter produced or accumulated in lentic ecotones is utilized "in situ". The vegetation can play an important role in removing nutrients from the substrate and groundwater (2). The paper presents a physico-chemical characterization of two ecotonal zones of Danube Delta, during floods, in the spring of 2000–2002 period. Lentic-terrestrial ecotones were studied in comparison with the adjacent aquatic ecosystems, in order to evidence the differences between them.

Key words: ecotone, nutrients, Danube Delta.

1. INTRODUCTION

The "ecotone" concept was generally defined as the transitional zone between vegetation types and frequently included the notion that ecotones contain abiotic and biotic components found in one or both of the adjacent biological communities (7).

In 1988, Holland will give a more appropriate definition: "an ecotone is a zone of transition between adjacent ecological systems "having a set of characteristics uniquely defined by space and time scales and by the strength of their interactions".

After this definition, the ecotone is no longer regarded as a simple, static zone where two communities join, but rather a dynamic zone changing through time and possessing properties of its own and also he is no longer considered only as entity, isolated from the landscape level processes.

In 1971, the Ramsar Convention of Wetlands established the importance of lentic ecotone between aquatic and terrestrial ecosystems in biodiversity preservation. This ecotone has new characteristics than the adjacent ecosystems (aquatic and terrestrial), with an increased biodiversity, offering also conditions for species dispersion and rapid colonization (3).

This type of ecotone has also a major role in nutrients retention and transformation (4). Retention occurs generally when the nutrients are assimilated and stored in plants or immobilized on sediment level. Authors have shown that nutrient dynamics can differ significantly. Nitrite was the only form that was

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exported from the ecotone to the adjacent aquatic ecosystem. However, in the ecotone was an uptake of ammonia, nitrate, P-ortho, dissolved organic nitrogen, dissolved organic phosphorus, particulate nitrogen, particulate phosphorus from the adjacent aquatic ecosystems. The seasonal dynamic was associated with plants metabolism and with the bacterial activities on the substrate surface.

The nutrient exchanges on the ecotone-aquatic ecosystem boundary are a lot more intense than at the water-soil interface.

Verhoeven *et al.* (1988) studied nutrient relations between wetlands and adjacent open water ecosystems in the Netherlands. Electrical conductivity (a measure of the overall nutrient content) of surface water decreased more rapidly in the ecotone when the wetland surface was flooded from an adjacent ditch. The authors suggested that changes were due to active plant uptake in the ecotone.

The small water bodies partly separated from the main water body have special physico-chemical conditions. Atmospheric precipitation, winds, ice cover and other physical parameters are of greater importance here than in typical land and water habitats. These habitats are under a variable influence of detritus from land and lake origins, which accumulate there. This, in addition to the small volume of water, causes higher concentrations of organic and mineral substances and more frequent oxygen deficits than in deeper parts of the lake (5).

With few exceptions, hydrological conditions and nutrients have a temporal dynamics, and the nutrient retention, storage and transformation capacity of the vegetation vary in an inverse correlation with water velocity through the ecotonal zone. Due to this retention capacity, the ecotone represents a real buffer zone.

All these unique characteristics gave us reasons to study the differences between lentic-terrestrial ecotone and adjacent aquatic ecosystems.

2. MATERIAL AND METHODS

In 2000–2002 period were studied the following lentic-terrestrial ecotonal areas of Danube Delta:

- Roşu zone – it is an ecotonal area with predominance of phragmites, which limits Roşu-Puiu channel;
- Erenciuc zone – near Erenciuc channel – with two sampling stations:

– Erenciuc – Caraorman zone – which limits a channel to Caraorman sandbank, placed in north-western part of Erenciuc Lake;

– Erenciuc – Saint Georg zone – placed at the intersection of Erenciuc channel with Saint Georg branch, in the southern part of Erenciuc Lake.

The samples were collected in spring of 2000–2002 period (at high water level) as it follows:

– in spring of 2000 and 2001 from Roşu ecotonal area and Roşu Lake;

– in spring of 2000 and 2002 from Erenciuc ecotonal areas and Erenciuc lake;

The depth, transparency, temperature, pH, dissolved oxygen were measured in the field. The other analyses were performed in the lab, after samples preservation:

– total and dissolved organic matter content – oxidability with $K_2Cr_2O_7$;

– dissolved organic nitrogen (DIN: $NO_2^-NO_3^-$, NH_4^+) – spectrophotometry;

– total phosphorus (PO_4^{3-}, Porg) – spectrophotometry.

3. RESULTS AND DISCUSSION

Roşu ecotonal area (2000–2001 period). The ecotonal surface increase significantly during floods period, the water depth of these zones being strictly correlated with the amplitude of hydrological regime.

Depth and transparency registered in 2000 year are higher than in 2001, in the ecotonal area and also in lake (Table1). Transparency index T/A reaches higher values (near 1) in ecotone, but in lake this index is rather small (0.24) (Fig. 1).

The temperatures reached values between 17–22 °C, the minimal value being registered in 2000 year in ecotone.

The pH decreases from 2000 to 2001 year, remarkable differences being noticed in ecotonal area (9.38 in 2000 and 6.7 in 2001).

Dissolved oxygen content of water is lower in 2000 than in 2001, and again, significant differences were registered in ecotonal area (6.56 mgO/l in 2000 and 9.35 mg O/l in 2001). Oxygen saturation exceeds 100% (Figs. 1–2), with one exception: the ecotonal area in 2000 year, when the value was only 68.11%.

The organic matter content of the water reaches values between 9.02 and 26.8 mg C/l, with a maximum in the lake in 2001 year (Fig. 2). In 2000 year, the rate of dissolved organic matter (DOM) from total organic matter (TOM) was three times higher in ecotone than in lake, possibly due to vegetation metabolism.

Nutrient dynamics differs significantly in lake towards the ecotone area. In lake, dissolved inorganic nitrogen (DIN) varies around 1 mg N/l, but in ecotone he is lower than 0.8 mg N/l (Fig. 1).

Total phosphorus, instead, reaches higher values in ecotone than in lake (with approximately 50%), due to the higher amount of organic phosphorus (Figs. 1–2) DIN/TRP ratio is comprised between 24 (in ecotonal area) and 32 (in lake), above the critical values of 10, which indicate the phosphorus as a limiting nutrient (Table 1).

The organic matter content of sediment reaches higher values in lake than in ecotone (a mean value of 18% in lake towards 5% in ecotone).

Erenciuc ecotonal area (2000 and 2002 years). Depth and transparency of the lake in 2000 year are higher than in 2002, but for the ecotonal area the values are approximately equal. Transparency index reaches maximum value in ecotone, but he has also high values in lake (Figs. 3–4).

Table 1

Physico-chemical parameters of studied ecosystems

Zone	Roşu Zone				Erenciuc Zone					
Year	2000		2001		2000			2002		
Parameter/St.	Lake	Ecotone	Lake	Ecotone	Lake	E Zone	P Zone	Lake	S Zone	E Zone
Depth(m)	3.30	0.62	3.05	0.17	3.50	0.2	0.2	2.55	0.20	0.25
Transparency (m)	0.77	0.55	0.60	0.17	2.50	0.2	0.2	1.5	0.20	0.25
T(°C)	20.9	17.6	18.9	21.6	20.0	19.5	21.0	15.7	11.6	15.4
PH	8.86	9.38	7.99	6.7	7.33	7.4	7.2	7	6.75	6.69
O_2 (mg/l)	9.54	6.56	14.28	9.35	2.95	2.46	2.3	8.71	3.09	3.90
CCOCr (mg O/l)	24.07	43.35	71.5	51	44.9	30.6	55.1	17	26	22
DOM (mg C/l)	3.06	14.51	–	–	2.3	9.56	13.77	3.31	8.45	7.72
DOM/TOM (%)	33.3	89.3	–	–	13.6	83.3	66.6	51.9	86.7	93.6
NH_4^+ (mg N/l)	0.71	0.215	0.61	0.47	0.04	0.18	0.32	0.83	1.13	0.97
NO_2^- (mg N/l)	0.0035	0.0046	0.058	0.018	0.0054	0.0042	0.0015	0.0061	0.0252	0.0258
NO_3^- (mgN/l)	0.454	0.609	0.299	0.22	1.51	0.045	0.045	0.633	0.249	0.045
TRP (µg P/l)	31.5	21.5	35.5	67.5	131	73	12	44	100	37
Org-P (µg P/l)	40	25.5	16.5	83	8	5	3	30	353	79
DIN/TRP	37	38	27	10.5	11.9	3	30.5	33	14	28

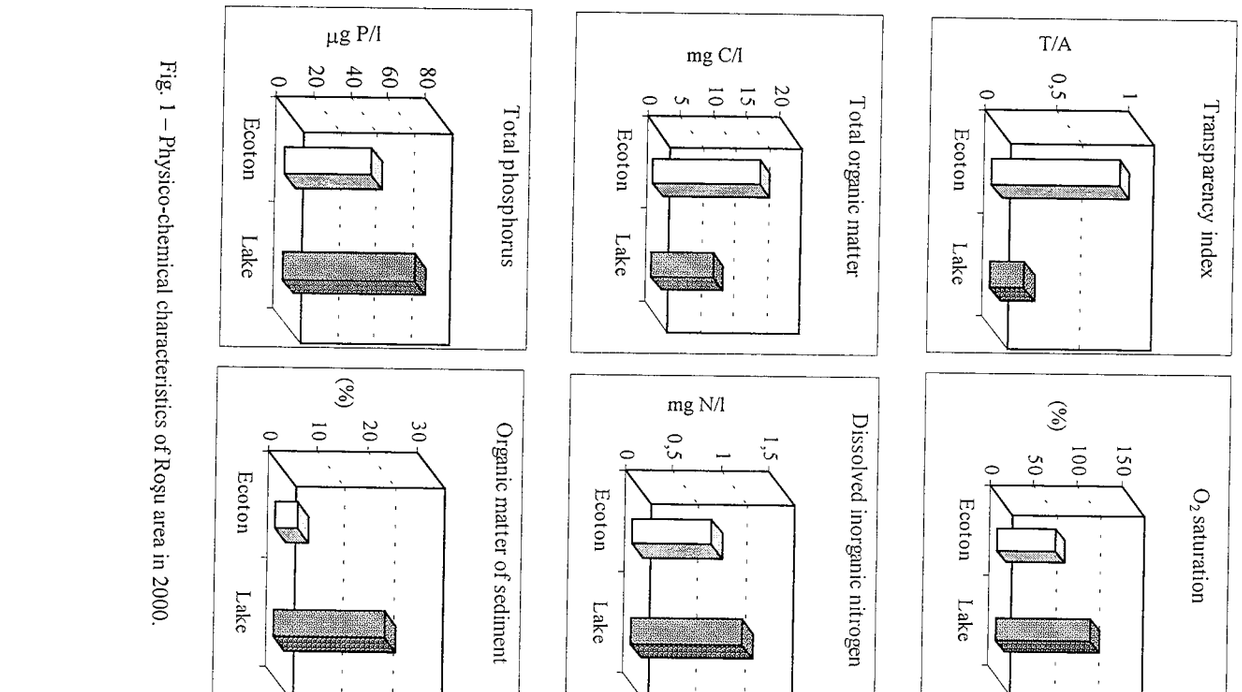

Fig. 1 – Physico-chemical characteristics of Roşu area in 2000.

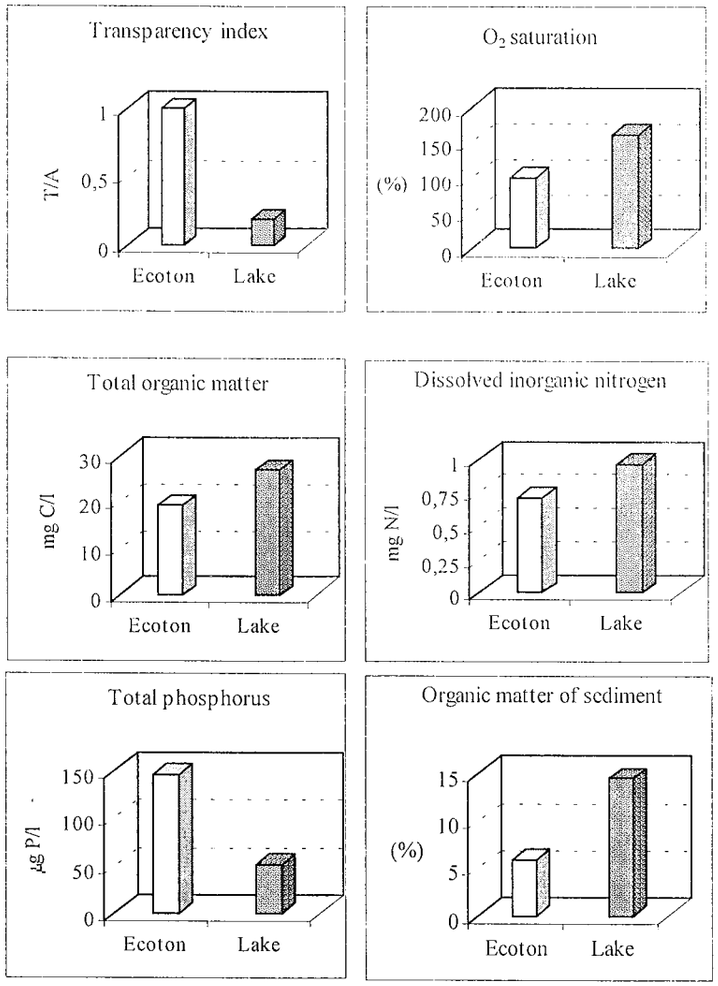

Fig. 2 – Physico-chemical characteristics of Roşu area in 2001.

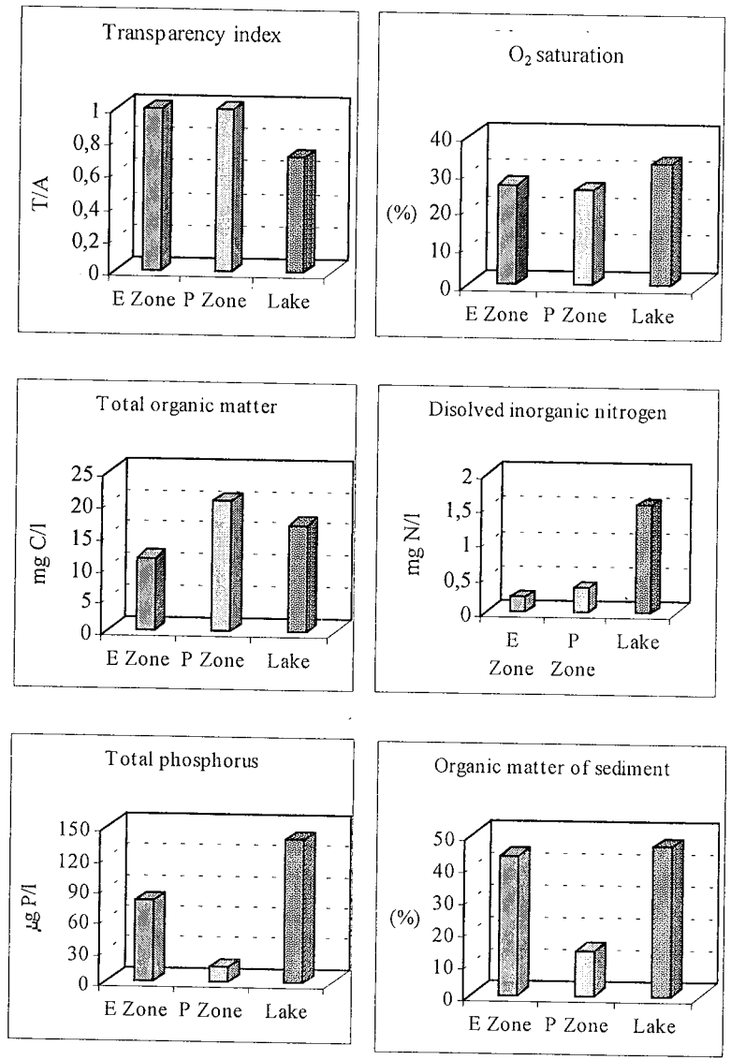

Fig. 3 – Physico-chemical characteristics of Erenciuc area in 2000.

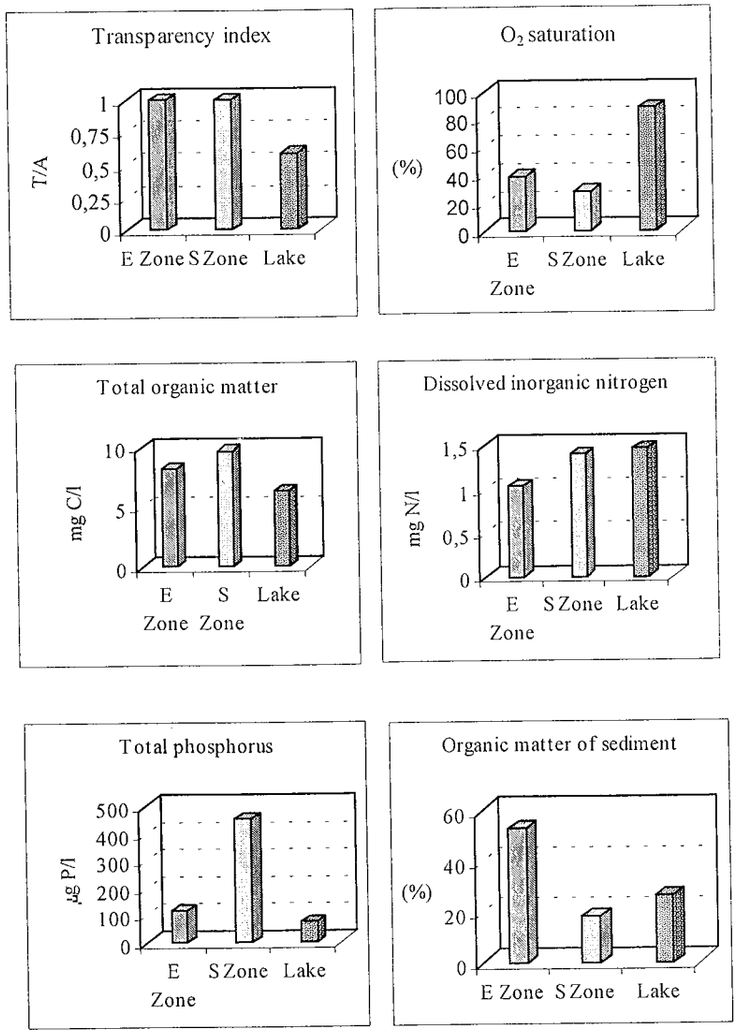

Fig. 4 – Physico-chemical characteristics of Erenciuc area in 2002.

The temperature is higher in 2000 year (20 °C) than in 2002 (14.5 °C).

pH reaches slightly higher values in lake than in ecotone, with a mean value of 7 (Tab.1).

Dissolved oxygen content is lower than in 2002 year, in both zones (E, P). In 2000 year, the values are nearly the same in the ecotone areas and adjacent lake (2.46 mg O/l, 2.3 mg O/l in the ecotone, respectively 2.95 mg O/l), but in 2002 year the differences are significant: mean value 3.50 mg O/l in ecotone towards 8.71 mg O/l in lake (Tab.1).

The oxygen saturation is very low in ecotone as in lake, with a mean value of 30% (with one exception: in lake, in 2002 year, were registered 88%) (Figs. 3–4).

Organic matter content of water reached higher values in 2000, both in lake and in ecotonal areas (16.84 mg C/l in lake, and even 20.6 mg C/l in P zone (Figs. 3–4).

However, in 2002 the values registered in ecotone are approximately with 50% higher than in lake (approx. 9 mg C/l towards 6.38 mg C/l) (Fig. 4).

Considering also the lack of oxygen, it is possible that the higher values reached by the organic matter content of water determined the lower values registered for dissolved oxygen, due to decomposing processes. We may notice that in lake, in 2002 year, was the highest value of oxygen saturation (88%) but the lowest TOM content of water (6.38 mg C/l).

On the sediment level, we may notice also the high content of organic matter, both in lake and in ecotonal area. In 2002 year was reached the highest value in E ecotonal area (52%).

Dissolved inorganic nitrogen (DIN) content of the lake is higher than in ecotonal area, both in 2000 and 2002 year. In 2000, the differences were significant (1.51 mg N/l in lake and only 0.3 mg N/l in ecotone), but in 2002 DIN content of ecotone increased, exceeding 1 mg N/l.

Total phosphorus (TP) has a completely different dynamics. In 2000, the value reached in lake was a lot higher than in the ecotone (139 µg P/l towards 78 µg P/l in E zone or 15 µg P/l in P zone). In 2002, we registered an opposite situation: the values are lower than in both ecotonal areas (Fig. 4), over 75% of total phosphorus being represented by organic phosphorus.

DIN/TRP ratio varies between 12 and 33 in both ecosystems, with one exception: E zone in 2000 year, where was reached the smallest value (3).

4. CONCLUSIONS

Even depth and transparency are a lot higher in adjacent lakes, in ecotonal areas transparency index has always-high values, near 1;

The pH is slightly lower in ecotonal areas, with one exception: R zone in 2000 year, were it was registered a high value (9.38);

The general dynamics of dissolved oxygen content of the water is that in ecotonal areas the values are lower than in the adjacent aquatic ecosystems. These results are in accordance with the results obtained by Pieczynska in 1972.

Organic matter content of the water is generally lower in lakes than in ecotonal areas;

Dissolved inorganic nitrogen generally decreases from the lakes to the ecotonal areas.

Total phosphorus has an irregular dynamics: the high amount of TP found in ecotonal areas is due mainly to organic phosphorus, while in lakes the inorganic phosphorus has the main contribution;

Nutrient dynamics, with higher values in lakes for DIN and TRP was in accordance with the results obtained by other researchers (4). The main differences were registered for org-P: in 2001 in Roşu zone and in 2002 in Erenciuc zone the results show higher values in ecotone, while in other periods the values were higher in lakes (in accordance with Peterjohn & Correll results);

DIN/TRP ratio, generally higher than the critical values 7–10, indicates phosphorus as limiting nutrient.

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NEAR-SHORE *POLYCHAETE* ASSEMBLAGES OF THE SOUTHERN PART OF THE ROMANIAN BLACK SEA COAST

VICTOR SURUGIU

The shallow-water polychaete assemblages of sandy and rocky substrata were analysed at 8 sites situated along the southern part of the Romanian Black Sea in August 1998 and August 1999. During the study 21 species were identified. On sandy bottom *Spio filicornis* had the highest abundance and *Neanthes succinea* possessed the highest biomass value. The most abundant species on hard bottom were *Pseudobrania clavata*, *Neanthes succinea*, *Polydora* cf. *ciliata* and *Fabricia stellaris stellaris*, respectively. Uni- and multivariate analysis suggest that pollution is the main factor affecting the distribution of polychaetes in the Romanian coast.

Key words: Polychaeta, Black Sea, Romanian coast, community structure, anthropogenic impact.

1. INTRODUCTION

The composition and distribution of polychaetes inhabiting shallow-waters of the Romanian coast has been fairly well documented (6, 9, 10, 19, 20, 27, 30). Although polychaetes on sandy bottom have been studied quantitatively by many authors (1, 3, 4, 11, 28, 29), a little has been published on polychaetes on hard substrate (2, 12, 22).

The aims of this study are: (a) to provide new information on the composition and distribution of the near-shore polychaetes along the Romanian coasts and (b) to make a comparison between the structure of polychaete community delineated in the present study, mainly formed by pollution and eutrophication of the Black Sea, and that reported in the previous studies.

The southern part of the Romanian Black Sea coast, with a total length of 85 km, extends between Cape Midia (44°21' N, 28°42' E) and Vama Veche (43°43' N, 28°35' E) (Fig. 1). Due to the predominance of the northern winds, the main current flows in a southward direction.

In the north of Constanţa, the sediment is composed of fine, quartz sands of fluvial origin, with a mean grain-size ranged between 132 and 350 μm (13, 14). In the south of Constanţa, the bottom is predominantly composed of rocks, with sarmatian limestone (5).

Surface water temperature of the Romanian coast range from 26°C in August to 0.5°C in February. Salinity values varies from 15.9 ‰ to 18.3 ‰, according to

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the distance from the outflows of Danube river. Dissolved oxygen values range from 7.15 to 15.73 mg l^{-1} (5), however, after summer algal "blooms", drastic decrease in dissolved oxygen concentration, down to 1.43–2.86 mg l^{-1} or even zero, can occur. Mean values of nutrient concentrations are 262.0 µg l^{-1} for the phosphates and 112.2 µg l^{-1} for the nitrates (23).

The study area is of importance since it has been subjected to many anthropogenic disturbance in the last three decades, such as seaside urbanisation and industrialisation, coastline arrangement works, enlargement of seaport's network, intense marine traffic, regulation of river run-off, etc. All this man-made activities inevitably disturbed near-shore communities, including polychaetes.

2. MATERIAL AND METHODS

Sampling procedure

This study was carried out in August 1998 and August 1999, the period when recruitment of many polychaetes occurs (4, 20). Benthic samples were taken from 8 sites at 0.3–5 m depth, with sand substrate (Năvodari, Mamaia and Constanța) and hard substrate (Agigea, Eforie Sud, 23 August, Mangalia, and Vama Veche) (Figure 1). Sandy bottoms were sampled by a Ekman grab covering an area of 0.02 m^2. Hard bottoms were sampled by SCUBA diving and considering an area of 25×25 cm. At each site three replicates were taken. Polychaetes retained on a sieve with 0.5 mm mesh were fixed in 10% formalin in seawater and preserved in 70% ethyl alcohol.

Data analysis

After their identification to species level, polychaetes were counted and wet formalised weights determined for each species in each sample, by pooling individuals within species. In order to have comparable between-sites data, all the results were referred to square metre.

The structure of polychaetous annelid communities over the sites was analysed in terms of species composition, abundance (A), biomass (B), frequency (F) and various diversity indices.

Species richness (d) is given as Margalef's index (21). Diversity of the communities is calculated using the Shannon-Wiener diversity index (H') on a log base 2 (25). Equitability (J') is expressed as Pielou's evenness index (24). The Bray-Curtis similarity coefficient (7) was calculated between sites on a log-transformed abundance data, in order to down-weight the contribution of common species. Hierarchical agglomerative clustering was performed on similarity matrix using group-average linking. Non-metric Multi-Dimensional Scaling (MDS) was applied to plot the results derived from the cluster analysis (15).

Similarity breakdown, as proposed by Clarke & Warwick (8), was used in order to define discriminating species by breaking down the average dissimilarity

between sample groups into the separate contributions from each species. In the same manner were defined characteristic species by analysing the contributions of each species to the average similarity within group.

Clustering, ordination and similarity breakdown were carried out using PRIMER computer software package developed at the Plymouth Marine Laboratory.

3. RESULTS

A total number of 2086 individuals belonging to 21 polychaete species were collected during this study (Table 1).

For sandy bottoms the leading species in terms of abundance was *Spio filicornis* (400 ind m^{-2} in average), closely followed by *Neanthes succinea* (350 ind m^{-2}) (Table 2). The latest species contributes much to the total biomass, with an average of 4.517 g m^{-2}.

Hard-bottoms were highly dominated by *Pseudobrania clavata*, with an average abundance of 4708 ind m^{-2} (Table 2). The larger-bodied species *Neanthes succinea*, which came in the second place with respect to the mean abundance (2277 ind m^{-2}), prevail in determining the overall biomass, in some places giving up to 46.485 g m^{-2} (Table 1).

From Fig. 2 can be seen that sandy bottoms are characterised by smaller total values of abundance and biomass than those of hard substrata. The highest total abundance as well highest total biomass were recorded in site 23 August, being of 15,501 ind m^{-2} and 56.459 g m^{-2}, respectively (Table 1). Despite the lowest abundance value was found in site Mamaia (with 696 ind m^{-2}), the lowest total biomass was recorded in site Năvodari (1.347 g m^{-2}), due to dominance in this site of small-bodied species *Spio filicornis*.

Number of species and species richness generally increased from Năvodari to Vama Veche (Fig. 3). Number of species varied between 3 in site Năvodari and 11 in site 23 August. In much the same way varied the species richness index (from 0.20 to 0.72, respectively).

Shannon-Wiener diversity (H') varied between 0.7 in site Năvodari and 2.3 in site 23 August, whereas evenness (J') ranged between 0.346 at Constanța to 0.743 at Mamaia (Fig. 4).

The results of the cluster analysis are shown in Fig. 5. Two main clusters can be distinguished at around 35% similarity level. The first group included sites Năvodari, Mamaia and Constanța, which correspond to sandy substrate, and the second group included sites Eforie Sud, Agigea, Mangalia, 23 August and Vama Veche, which correspond to rocky substrate. The lower similarity of site Constanța with Năvodari and Mamaia can be attributed to its somewhat transitional position between sandy and hard substrata.

Table 1

Abundance *A* (ind m^{-2}) and biomass *B* (g m^{-2}) of the polychaete species at different sampling sites

Species	Năvodari		Mamaia		Constanța		Agigea		Eforie Sud		23 August		Mangalia		Vama Veche	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Harmothoe imbricata (Linnaeus, 1767)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	1.980
Harmothoe impar (Johnston, 1839)	0	0	0	0	0	0	0	0	0	0	50	1.577	0	0	115	0.323
Nereiphylla rubiginosa (Saint-Joseph, 1888)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0.244
Syllis gracilis Grube, 1840	0	0	0	0	0	0	0	0	0	0	17	0.110	0	0	615	1.167
Syllis hyalina Grube,1863	0	0	0	0	0	0	0	0	0	0	50	0.045	0	0	0	0
Pseudobrania clavata (Claparède, 1863)	0	0	0	0	30	0.002	8889	0.509	2044	0.245	2950	0.169	1156	0.066	8500	0.487
Nereis zonata Malmgren, 1867	0	0	0	0	0	0	0	0	0	0	17	0.574	0	0	0	0
Neanthes succinea (Frey & Leuckart, 1847)	44	0.097	400	10.092	607	3.363	4089	30.192	444	1.773	5617	46.485	156	0.861	1077	37.219
Hediste diversicolor (O.F. Müller, 1776)	0	0	59	6.425	30	1.278	0	0	0	0	0	0	22	0.947	0	0
Perinereis cultrifera (Grube, 1840)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77	0.600
Platynereis dumerilii (Aud. & M. Edw., 1833)	0	0	0	0	0	0	0	0	0	0	3583	6.335	0	0	0	0

Table 1

(continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Spio filicornis (O.F. Müller, 1766)	1067	1.183	118	0.097	15	0.012	0	0	0	0	0	0	0	0	0	0
Polydora cornuta Bosc, 1802	0	0	0	0	0	0	356	0.168	0	0	0	0	0	0	0	0
Polydora cf. *ciliata* (Johnston, 1838)	0	0	0	0	15	0.008	1022	0.748	0	0	1817	0.924	89	0.065	1231	0.905
Polydora websteri Hartman, 1943	0	0	0	0	0	0	400	0.391	0	0	0	0	0	0	0	0
Capitella capitata (Fabricius, 1780)	0	0	15	0.009	0	0	0	0	44	0.080	0	0	0	0	0	0
Capitella minima Langerhans, 1881	133	0.067	104	0.044	0	0	44	0.023	0	0	233	0.121	67	0.028	731	0.378
Heteromastus filiformis (Claparède, 1864)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0.100
Nerilla antennata O. Schmidt, 1848	0	0	0	0	0	0	0	0	400	0.003	0	0	0	0	0	0
Fabricia stellaris stellaris (Müller, 1774)	0	0	0	0	0	0	44	0.006	0	0	50	0.007	3489	0.465	0	0
Janua pagenstecheri (Quatrefages, 1865)	0	0	0	0	0	0	0	0	0	0	1117	0.112	0	0	0	0
Total	1244	1.347	696	16.667	697	4.663	14844	32.037	2932	2.101	15501	56.459	4979	2.432	12460	43.403

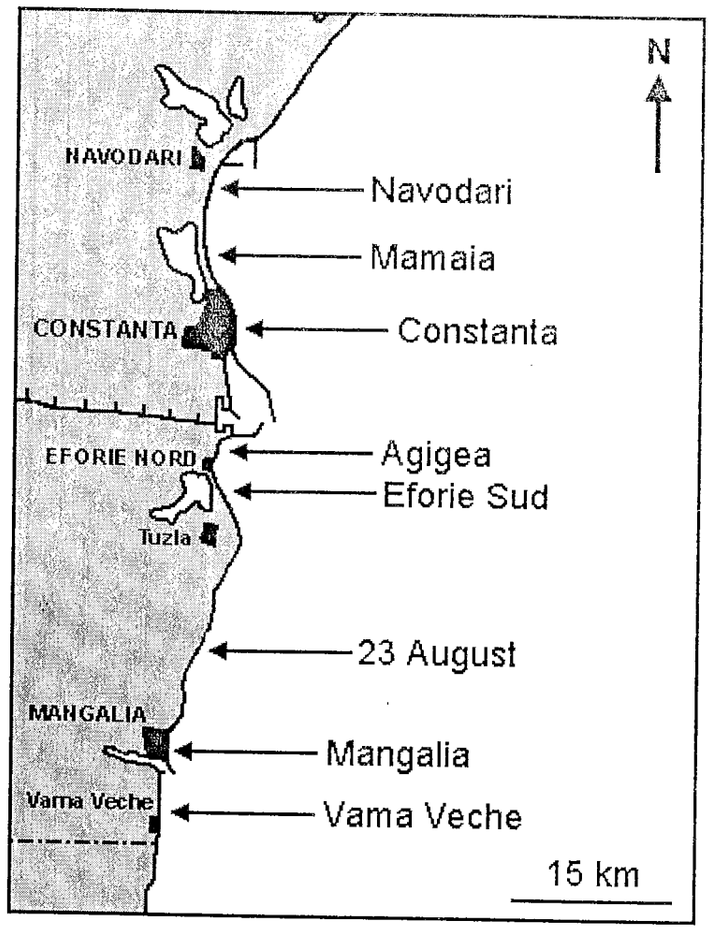


Fig. 1 – Southern sector of the Romanian Black Sea littoral with the location of sampling sites.

The same groupings of sites emerge from MDS ordination plot (Fig. 6). Moreover, MDS reveal some gradual change in community structure along sites in response to other environmental factors, such as range of salinity from north to south and differences in the depth.

Table 2 shows the results of similarity breakdown between and within sandy and hard substrata. It can be seen that 7 species (33% of the total number) are responsible for most of the dissimilarities between sandy and hard bottoms. These species account for up to 65% of the observed dissimilarities. Thus, a good discriminating species between sandy and rocky bottoms can be considered *Pseudobrania clavata*, *Spio filicornis* and *Polydora* cf. *ciliata*.

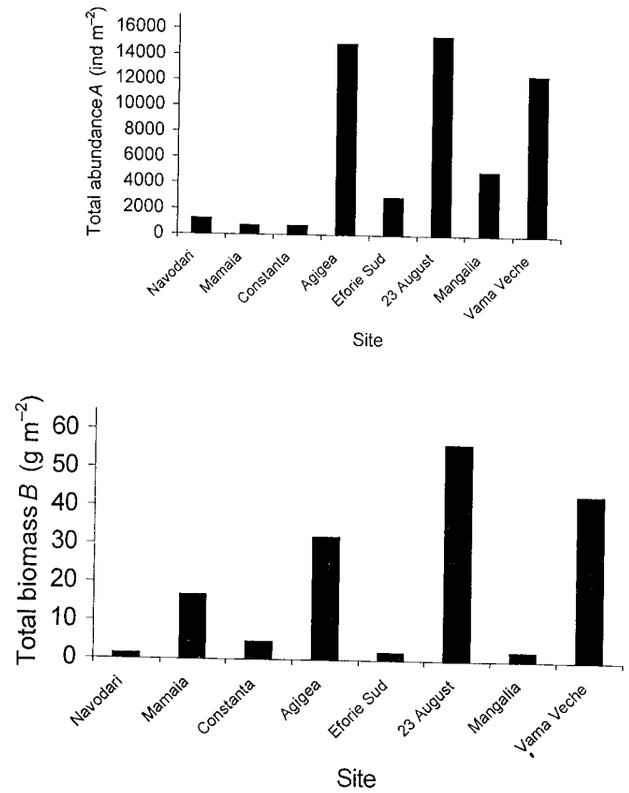


Fig. 2 – Variation in total abundance (ind m^{-2}) and total biomass (g m^{-2}) along sampling sites.

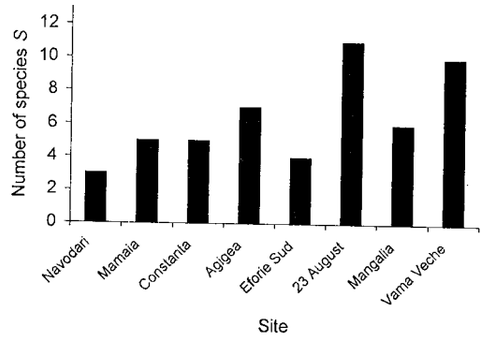


Fig. 3.

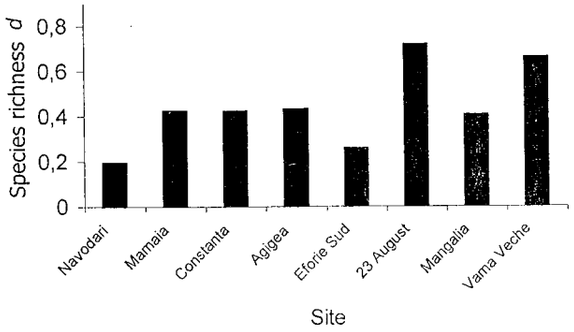
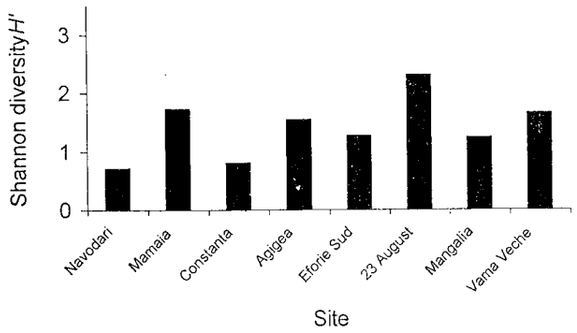


Fig. 3 – Variation in total number of species (*S*) and species richness (*d*) along sampling sites.



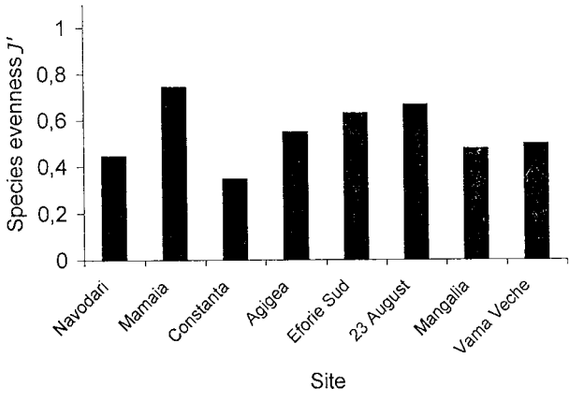


Fig. 4 – Variation in diversity (*H'*) and evenness (*J'*) along sampling sites.

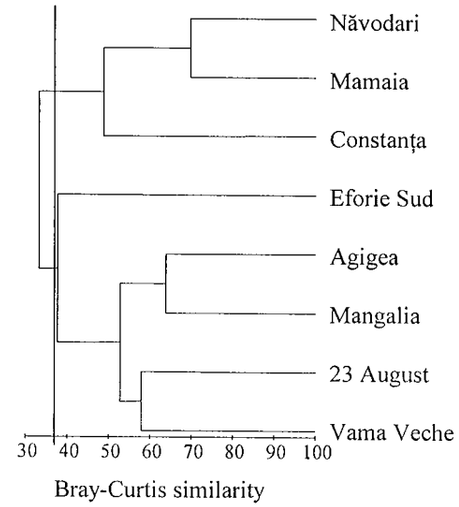


Fig. 5 – Similarity dendrogram of sampling sites. Two groups of sites defined at an arbitrary similarity level of 35% are indicated by line.

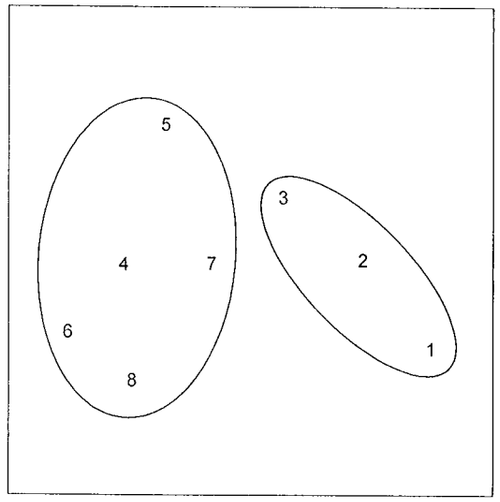


Fig. 6 – Non-metric multi-dimensional scaling (MDS) ordination plot in two dimensions of sampling sites with superimposition of the two groupings of sites resulted from the cluster analysis: 1 – Năvodari, 2 – Mamaia, 3 – Constanța, 4 – Agigea, 5 – Eforie Sud, 6 – 23 August, 7 – Mangalia, 8 – Vama Veche (stress = 0.04).

Table 2

Similarity breakdown between and within sandy and hard substrata: $\bar{A}_s$ – average abundance within sandy substrata, $\bar{A}_r$ – average abundance within rocky substrata, $\bar{\delta}_{sr}$% – dissimilarity percentages between sandy and hard substrata. $\bar{S}_s$% – similarity percentages within sandy substrata. S_r% – similarity percentages within rocky substrata

Species	$\bar{A}_s$	$\bar{A}_r$	$\bar{\delta}_{sr}$%	$\bar{S}_s$%	$\bar{S}_r$%
Pseudobrania clavata	10	4708	17.53	—	37.42
Spio filicornis	400	0	12.41	32.73	—
Polydora cf. *ciliata*	5	832	10.52	—	14.57
Fabricia stellaris stellaris	0	717	8.06	—	5.28
Capitella minima	79	215	6.42	14.66	10.85
Hediste diversicolor	30	4	5.63	—	—
Neanthes succinea	350	2277	4.63	42.62	29.50
Total:	874	8752	65.19	90.01	97.62

In determining the similarity within sandy bottoms a major role play *Neanthes succinea*, *Spio filicornis*, and *Capitella minima*, that account 90% of the contribution. More than 92% of the contribution to the similarity within rocky bottom is given by only 4 species (*Pseudobrania clavata*, *Neanthes succinea*, *Polydora* cf. *ciliata*, and *Capitella minima*), the first two of which were found at very high abundance within the group. These species must be regarded as characteristic to these communities.

4. DISCUSSION AND CONCLUSIONS

Comparing the results obtained in the present study to surveys undertaken some 30 years ago, it can be ascertained substantial changes in the qualitative composition and quantitative distribution of near-shore polychaetes along southern sector of the Romanian littoral.

For instance, if in the past in fine sublittoral sands were identified 27 polychaete species (4), now could be identified only 7. Similarly, it was observed a reduction of number of polychaete species of hard bottoms from 35 species (12) to only 20 in the present study. The same decrease of species richness of polychaetes was reported by Ţigănuş (28, 29, 30).

These changes in the specific composition of polychaetes in front of Romanian seashore can be attributed mainly to increased levels of organic pollution and anthropogenic eutrophication of coastal waters in the north-western part of the Black Sea in the last decades. As a result, coastal waters are

characterised by frequent phytoplankton "blooms" which led to the creation of zones in the bottom layers of water with hypoxia and anoxia and to the subsequent mass mortality of bottom organisms, including polychaetes (16).

In conditions of simplification of coenotic structure of bottom communities an explosive development is showed by euryoecious and opportunistic species. One of these species that now can be found at a high abundance and biomass is *Neanthes succinea*, which represents 23.3% of the total abundance and 81.8% of the total biomass. Losovskaya (17) even speak about setting up in some areas of the northwestern part of the Black Sea of new biocoenosis with *Neanthes succinea*, which replaced mussel biocoenosis. The well-marked euryoecia, the high tolerance to survive to temporary hypoxia and anoxia, and raised capability of propagation of *N. succinea* confer to this species very large ecological valence.

Losovskaya (18) indicated also that the organic enrichment of sandy sediments had as the result the increase in amount of small-bodied infaunal detritivorous polychaetes such as spionids and capitellids.

The increased "corpse fallout" led to the strong deposition on hard substrata of detritus and fine sediments and suffocation of epifauna. The construction of different coastline protection structures intensified the obstruction of macro- and mesoporal spaces by producing behind spur dikes and breakwaters of circular currents and by creating stagnant areas. This can explain the decrease in number of epibenthic polychaetes from rocky substrata such as *Nereiphylla rubiginosa*, *Syllis gracilis*, *Exogone naidina*, *Nereis zonata*, *Perinereis cultrifera*, *Platynereis dumerilii*, *Fabricia stellaris stellaris*, and *Janua pagenstecheri* (16, 17, 26).

Some more stenohaline species, which populated the southern part of the Romanian Black Sea, more stable in respect to the saline regime than the northern one, were also influenced by the construction of the Danube – Black Sea Canal which lowered salinity values and thus limited their areas of propagation southward.

Despite the fact that there is no data to which univariate measures can be correlated, it can be concluded that low values of diversity and evenness indices, i.e. increased dominance, indicates increased levels of environmental stress in shallow-waters of the Romanian littoral.

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ENCEPHALON CHARACTERISTICS OF SOME LEUCISCINAE SPECIES (PISCES, CYPRINIDAE)

CARMEN BĂLESCU

The present paper aims at comparatively rendering the main peculiarities of the encephalon at some species belonging to the Leuciscinae subfamily: *Alburnus alburnus, Hypophthalmichthys molitrix, Pelecus cultratus, Rutilus rutilus, Rhinichthys atratulus, R. cataractae, R. chrysogaster, R. osculus* with two subspecies: *R. o. klamathensis* and *R. o. robustus.* At the level of each vesicle, there can be noticed great variations, both intraspecific and interspecific, regarding their aspect, according to the length of their body, life way and environment. From a morphological point of view, the encephalon of the studied species belongs to 3 main groups: basic (primary) cyprinid, chemosensory and octavolateralis. At the studied species, the variability of the encephalon divisions and subdivisions is low and mean, oscillating between normal parameters.

1. INTRODUCTION

The present paper aims at comparatively rendering the morphology of both intraspecific and interspecific encephalon at the 8 species of Cyprinidae belonging to the Leuciscinae subfamily:

– *Alburnus alburnus* (Linnaeus, 1758) – it live in rivers, lakes, pools, brackish water; pelagic; omnivorous;

– *Hypophthalmichthy molitrix* (Valenciennes, 1844) – its origins are in China's rivers, but it adapted itself in Romania, as well, in midwater; vegetarian nourishment;

– *Pelecus cultratus* (Linnaeus, 1758) – good swimming species; in rivers, fresh water lakes, brackish water lakes, less salty sea areas; pelagic; feeds with plankton (young), various invertebrates and small fish (adults);

– *Rutilus rutilus* (Linnaeus, 1758) – slow rivers, stagnant water and brackish water; they swim in the midwater (neobenthic); omnivorous nourishment;

– *Rhinichthys cataractae* (Valenciennes, 1842);

– *Rhinichthys atratulus* (Hermann, 1804);

– *Rhinichthys chrysogaster* (Girard, 1857);

– *Rhinichthys osculus klamathensis* and *robustus* (Girard, 1857).

The species of *Rhinichthys* genus populate the fast north-American rivers; they are benthic and feed themselves with various invertebrates; the lower mouth presents a pair of short barbels.

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This paper aims at presenting:

– which of the encephalon divisions contribute to the intraspecific and interspecific variability of the brain;

– the correlation among the different divisions and subdivisions with the main sensorial organs, meant to notice to what degree the brain morphology can be ecologically interpreted.

2. MATERIAL AND METHODS

The cyprinids species were supplied by the kindness of Mr. Acad. dr. doc. Bănărescu P. and I want to thank him. I worked on samples preserved in alcohol. After the encephalon was removed from the skull using classical methods, we performed the macroscopic analysis. We analysed the following divisions and subdivisions of the encephalon: olfactory bulbs, olfactory tracts, cerebral hemispheres, hypothalamus lobes, optic lobes, corpus cerebelli, valvula cerebelli, myelencephalon.

These divisions and subdivisions were measured along the sections of maximum dimension. Their length was reported to the total length of the encephalon that includes the olfactory bulbs and olfactory tracts. We also took into account the mathematical statistical methods (Ceapoiu, 1956). As mathematical statistical parameters of the samples, we used: the arithmetical mean (x), variation (S^2), standard deviation (S), variability coefficient (S%) and standard deviation of the arithmetical mean (S x), on the basis of which there can be estimated the variability of the encephalon divisions and subdivisions. At certain species, such as: *Hypophthalmichthys molitrix, Alburnus alburnus* and *Rutilus rutilus,* where we studied many samples, we used as main criterion the length of the body. We worked with dimensions classes, establishing many groups: small sized individuals, which correspond to the first group, great sized individuals, which correspond to the fifth group in the case of *Alburnus alburnus* and *Rutilus rutilus* species and to the third, in the case of *Hypophthalmichthys molitrix* species.

3. RESULTS AND DISCUSSIONS

Characterization of the encephalon

At most species belonging to this subfamily, both the rostral and caudal parts of the two halves of the telencephalon (cerebral hemisphere) are approximately equal. The smallest hemispheres are noticed at *Hypophthalmichthys molitrix* (Fig. 1. f., g.), while the biggest ones are characteristic for *Rhinichthys* genus (Fig. 2). On the surface of the telencephalon, there can be noticed a furrows that delimitate the tubercles. At *Pelecus cultratus* and the species of *Rhinichthys* genus, there can be noticed a new furrows and tubercles. They are clearer and more numerous at the following species *Rhinichthys atratulus, R. cataractae,* and *R.*

osculus robustus (Fig. 2 a., b., c., f.). Their presence could explain the role they play in increasing the surface of the cerebral hemispheres. Smell is well developed in these species. At small sized individuals belonging to *Alburnus alburnus, Rutilus rutilus, Hypophthalmichthys molitrix, Rhinichthys chrysogaster* species, there cannot be noticed any longer furrows and tubercles on the surface of the hemispheres. Thus, the hemispheres surface seems to be even. The length of the olfactory tracts directly depends on the fish's body length. Long tracts are noticed at large sized individuals and short tracts at small sized individuals. The longest tracts olfactory are noticed at *Pelecus cultratus* and *Hypophthalmichthys molitrix* (Fig.1.e., f.). The shortest tracts are characteristic to the species belonging to *Rhinichthys* genus (Fig. 2.) and at small sized individuals corresponding to the first group from *Alburnus alburnus* and *Rutilus rutilus*. At the individuals belonging to the species *Rhinichthys atratulus* and *R. osculus robustus*, the olfactory bulbs present their posterior half placed on the ventral and anterior part of the cerebral hemispheres (Fig. 2.a.,b.).

The form of the hypothalamus lobes is quite varied. The lateral lobes are a little far off at their posterior part at *Hypophthalmichthys molitrix* (Fig. 1.f., g.) and the species belonging to *Rhinichthys* genus (Fig.2). The surface of these lateral lobes is crossed by 3 sulci: medianus, longitudinalis and mammillaris. At *Hypophthalmichthys molitrix,* there can be noticed only the sulcus mammillaris. At *Rhinichthys atratulus* and some *R. chrysogaster* individuals, on the surface of the lateral lobes, there can be noticed tubercles, as well: mammillary, median and longitudinal (Fig. 2.a., b., d.). The smallest hypothalamus appears at *Hypophthalmichthys molitrix*. At this species, the large and rounded hypophysis is placed above the median lobe. Behind the median lobe, there appears a pair of lobes at the species belonging to *Rhinichthys* genus, respectively an elongated formation at *Hypophthalmichthys molitrix, component* that I attribute to the saccus vasculosus (more obvious to the species belonging to *Rhinichthys* genus).

The optic lobes, appear under many forms. At the species belonging to *Rhinichthys* genus (Fig. 2), especially at *Hypophthalmichthys molitrix* (Fig. 1.f., g.), certain individuals of *Pelecus cultratus* (Fig.1.e.), the optic lobes are far off along the entire median line, more caudal than rostral. Thus, there can be clearly noticed the torus longitudinalis and a part of the valvula cerebelli. The most obvious displacement appears at *Rhinichthys cataractae* and *R. chrysogaster* (Fig. 2.c., d.). It is worth mentioning that Evans (1952) presents the optic lobes of *R. cataractae* encephalon, as being rostrally united and caudally outdistanced. The optic lobes are rostrally united and caudally outdistanced; thus, there can be noticed the torus longitudinalis and a part of the valvula cerebelli at certain individuals belonging to *Pelecus cultratus* (Fig. 1.d.) and *Rhinichthys atratulus* species (Fig.2.a.), and only the valvula cerebelli at *Rutilus rutilus and Alburnus alburnus* (Fig.1.a.,c.). The optic lobes are united along the entire median line at small sized individuals belonging to *Rutilus rutilus* (Fig.1.b.) and *Alburnus alburnus* species.

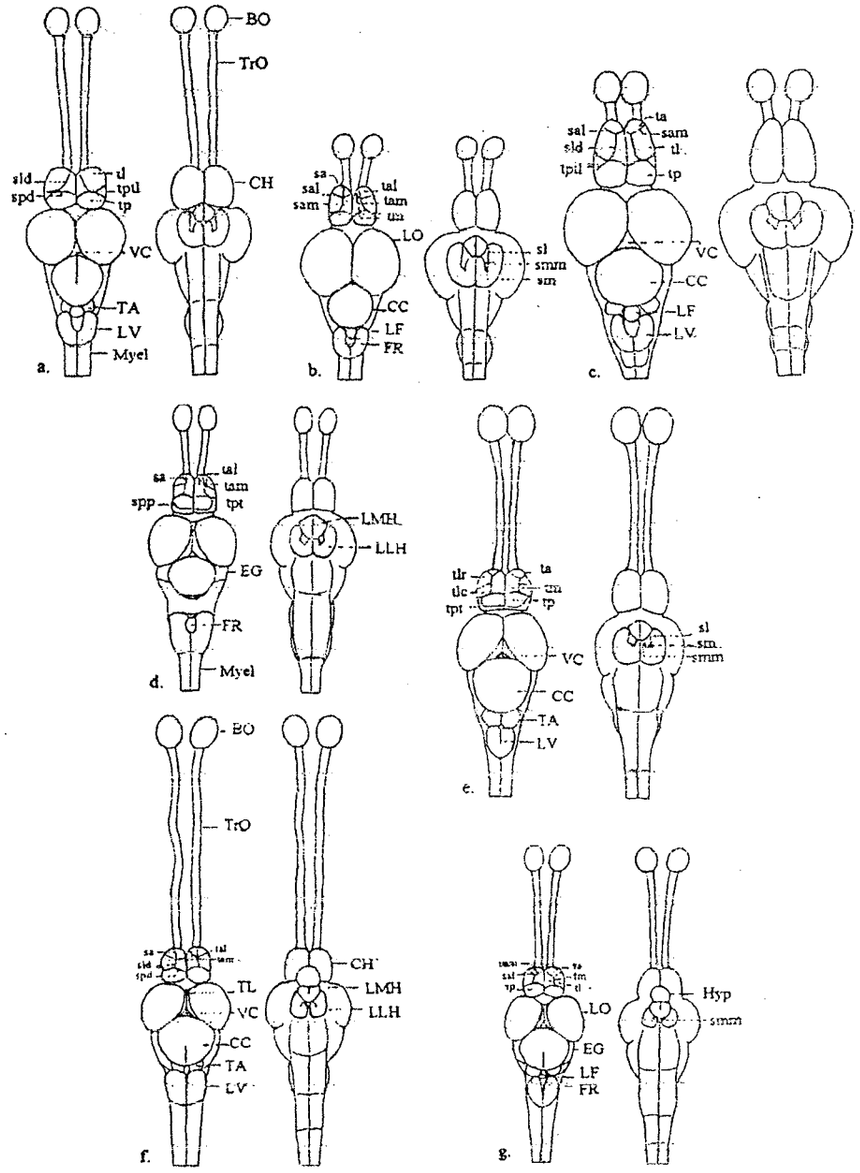


Fig. 1 – Dorsal view (left) and ventral view (right) of the encephalon at 4 species of fish belonging to Leuciscinae subfamily a., b. – *Rutilus rutilus*; c. – *Alburnus alburnus*; d., e. –*Pelecus cultratus*; f. g. – *Hypophthalmichthys molitrix*. Scale – 1,6:1 (a); 2,7:1 (b); 4,1 (c); 2,2:1 (d); 1,5:1 (e.,f.); 2,5:1 (g).

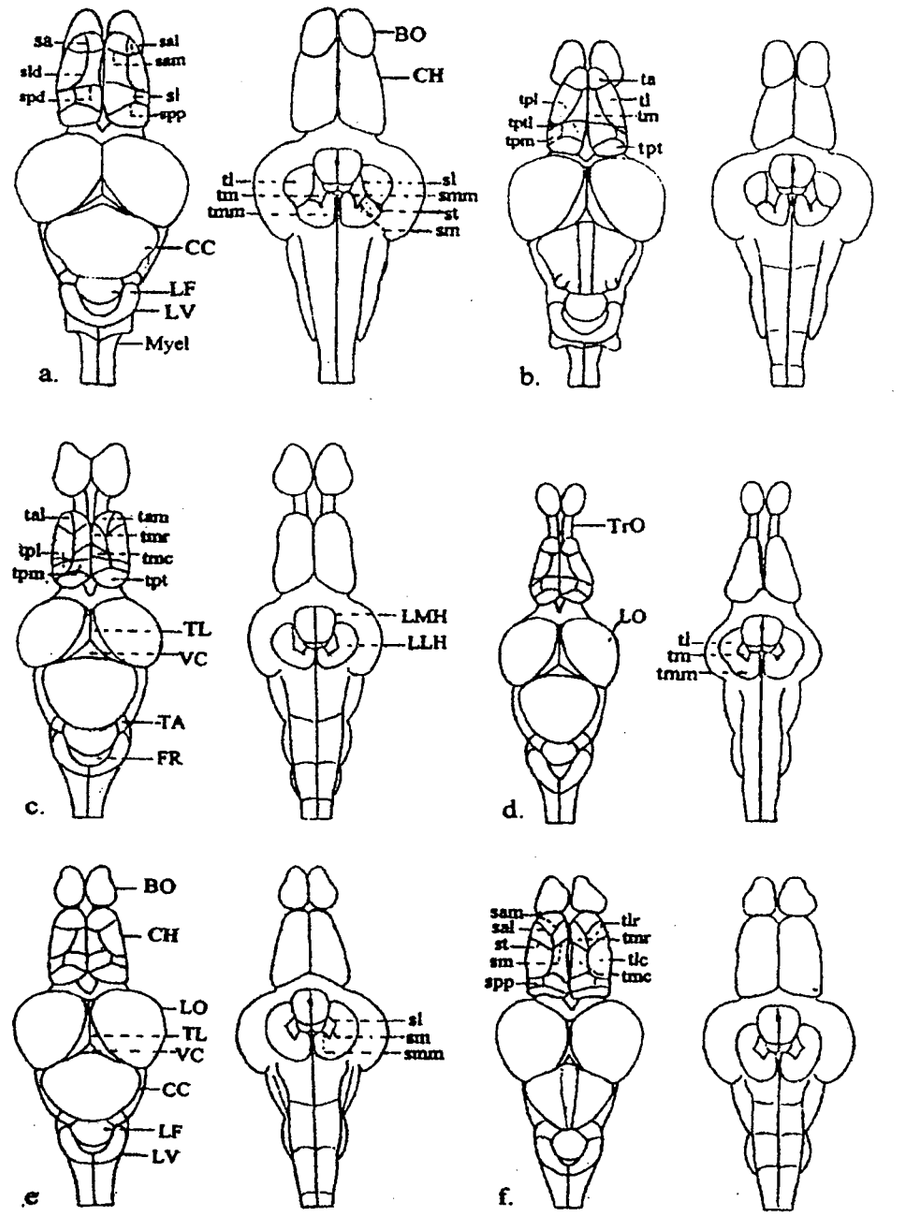
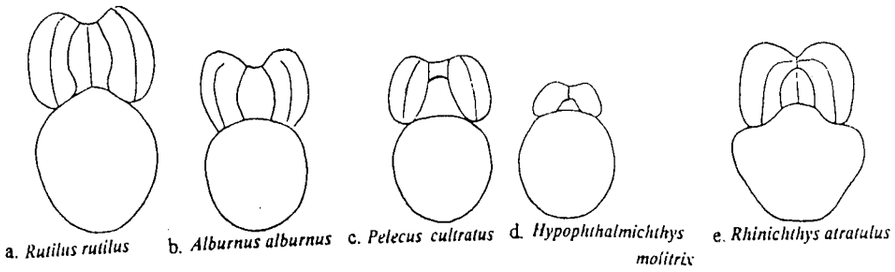


Fig. 2 – Dorsal view (left) and ventral view (right) of the encephalon at 4 species of fish belonging to *Rhinichthys genus*. a., b. – *Rhinichthys atratulus*; c. – *R. cataractae*; d. – *R. chrysogaster*; e. – *R. osculus klamathensis*; f. – *R.osculus robustus*. Scale – 5,4:1 (a., b., d.); 4:1 (c.); 4,4 :1 (e.); 4,7 :1 (f.).

Abbreviations: BO–bulbus olfactorius; TrO–tractus olfactorius; CH–cerebral hemisphere; LH–lobi hypotalami; LMH–lobus medialis hypothalami; LLH–lobus lateralis hypothalami; Hyp–hypophysis; LO–lobus opticus; TL–torus longitudinalis; VC–valvula cerebelli; CC–corpus cerebelli; EG – eminentia granularis; TA–tuberculum acusticum; LF–lobus facialis; LV–lobus vagus; FR–fossa rhomboidea; Myel–myelencephalon; sld–sulcus (s.) laterodorsalis; sa–s. anterior; sal–s.anterolateralis; sam–s.anteromedianus; spd–s.posterodorsalis; spp–s.posteropostremum; st–s.transversalis; sl–s. longitudinalis; sm.–s.medianus; smm–s.mammillaris; ta–tuberculum (t.) anterior; tl–t.laterale; tm–t. medianum; tpl–t.posterolaterale; tpm–t.posteromedianum; tpt–t.postremum; tal–t.anterolaterale; tam–t. anteromedianum; tptl–t.pars ultima tuberculi lateralis; tmr–t.mediorostrale; tmc–t.mediocaudale; tlr–t.laterorostrale; tlc–t.laterocaudale; tmm–t.mammillarum.



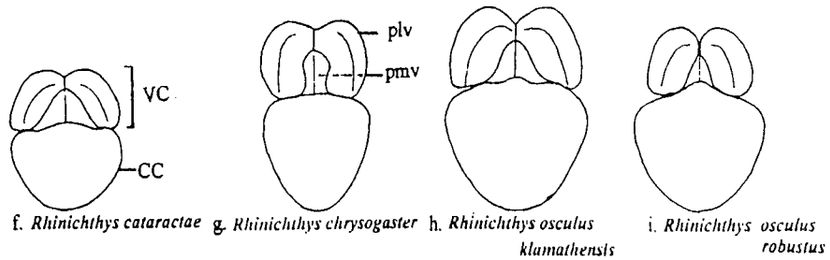


Fig. 3 – Valvula cerebelli at the studied Leuciscinae species. vc – valvula cerebelli; plv – lateral portion of the valvule; pmv – median portion of the valvule. Scale 6,5:1 (a.); 5, 8:1 (b.); 5, 3:1 (c.); 5:1 (d.); 8,4:1 (e.); 6,1:1 (f.); 7,7:1 (g.); 7:1 (h.); 7,3:1 (I.).

With reference to the corpus cerebelli, the species belonging to *Rhinichthys* genus present a quite big cerebellum as they actively seek for food on the rivers' bottom. The form of the cerebellum is different even in the case of the individuals belonging to the same species, being correlated more with the configuration of the skull than with the specific habitats. *Pelecus cultratus* and *Hypophthalmichthys molitrix* species present well-developed eminentia granularis and acoustic tubercles. The acoustic tubercles limit the superior part of the 4th ventricle, being differentiated in 2 rounded anterior lobes and 2 posterior lobes that are quite visible

on the draft. I used the term according to Bănărescu's studies (1949). It has different names in the literature: for example caudal lobe (described by Maller in 1974, quoted by Finger 1984). At most species belonging to this subfamily, the valvula cerebelli is located at the same level with the corpus cerebelli, taking into account its breadth. At *Hypophthalmichthys molitrix*, *Rhinichthys atratulus* and *R. osculus* species (Fig. 3), the valvula cerebelli is a little smaller as breadth than the corpus cerebelli. From a morphological point of view, the valvula cerebelli is poorly developed at *Hypophthalmichthys molitrix*. At *Rutilus rutilus Alburnus alburnus* and *Pelecus cultratus*, the lateral parts are outdistanced at the anterior part. There can be noticed a sulcus longitudinalis on the surface of the lateral portion (except for *Hypophthalmichthys molitrix*). Most species present a median bell shaped portion with a sulcus longitudinalis. The sulcus is transversally placed at *Pelecus cultratus* (Fig. 3).

The presence of the facial lobe is a characteristic of the myelencephalon, which is associated with the external taste and of the vagal lobes, which are associated with the internal taste. According to the development of the vagal lobes and facial lobe, the species of this subfamily belong to three groups (according to the classification proposed by Bănărescu, 1949): the first group – low developed facial lobe and vagal lobes; the third group – well developed facial lobe and moderately developed vagal lobes; the fifth group – inexistent facial lobe at the exterior and reduced vagal lobes. *Alburnus alburnus, Rutilus rutilus* and *Hypophthalmichthys molitrix* belong to the first group; they are species that swim in the midwater. Behind the small and round facial lobe, the fossa rhomboidea is visible, deep and presents the shape of a triangle with the basis directed towards the anterior part. At *Hypophthalmichthys molitrix*, the elongated lobes of the vagal are outlined a little above the myelencephalon; they are slowly anterior outdistanced. At some individuals, the vagal lobes are near each other along the median line, covering the facial lobe, which individualize itself at the exterior through the detachment of the vagal lobes (Fig. 1.f.). The species of *Rhinichthys* genus belong to the third group; they are benthic species, the facial lobe is large, prominent, bulging, and rhomboidal. Except for *Rhinichthys osculus*, all the other species of the genus present a slowly rounded anterior portion due to the pressing of the corpus cerebelli; it is bulging and visible only at its posterior part, but it is not far off from the corpus cerebelli. At *R. cataractae*, the facial lobe is larger (Fig. 2.c.). At *R. osculus robustus,* it can be entirely seen without being necessary the removal of the cerebellum (Fig. 2.f.). The fossa rhomboidea is crescent, larger (*R. atratulus, R. cataractae*) or narrow (*R. osculus*), respectively like a triangle with the basis rostrally placed. At *R. atratulus,* there is a pair of lobes located on the lateral-

posterior parts, behind the vagal lobes. *Pelecus cultratus* belong to the fifth group; it is a surface, pelagic species. In the anterior and median part of the vagal lobes, there can be noticed a small transversal section that reunites them at this level. This group is in a way arbitrary, as we noticed that at *Hypophthalmichthys molitrix* the facial lobe is extremely small, hard to observe, as compared to the other two species from the first group. This is why I consider that this species should be included within an intermediary group between the first and the fifth groups.

On the basis of the morphological aspects correlated with the measurements of the subdivisions and taking into account the data supplied by the specialized literature (Kotrschal and colab., 1988, 1991, 1992, 1998), we noticed that the analysed species of cyprinids belong to 3 groups: the group of basic cyprinid brain, of generalized type; the group of chemosensory brain; the group of octavolateralis brain.

The basic cyprinid brains are characterized by well-developed visual lobes, while the octavolateralis and gustatory lobes are moderately developed. This type of brain is characteristic to the species *Rutilus rutilus* that lives in the midwater and locates the food by means of sight.

Chemosensory brains are characterized by large taste lobes, great valvula cerebelli, while the visual and octavolateralis lobes are less developed. This type of brain can be noticed at the species belonging to *Rhinichthys* genus. The external taste buds predominate at these benthic species; they seek for food on the rivers' bottom by means of their sensorial mouth.

Octavolateralis brains are characterized by large visual and octavolateralis centers, while the chemosensory centers are very small; they can be noticed at *Hypophthalmichthys molitrix, Alburnus alburnus*, *Pelecus cultratus*. At these species the lateral line is well developed, especially at *Pelecus cultratus;* this fact allows a better orientation in the environment and a rapid visual localization.

This setting would correspond to their way of living: the generalized brains are associated with the species from the midwater and omnivorous nourishment regime; the sensorial brains are associated with bottom living and benthic species; the octavolateralis brains are associated with pelagic species, as well as with the ones living in the midwater.

As I had only a few morphological and ecological data and worked on a reduced number of individuals, I cannot draw a conclusion regarding the correlation between the encephalon types of these species and their way of living; thus, considering them hypothesis, I conclude that fish present a great plasticity.

At all the studied species, I have calculated the relative length of the encephalon divisions and subdivisions. At the species with many individuals I have

worked on dimension groups. I have observed that the value of the relative length of the encephalon divisions and subdivisions, decreases from the individuals of the first group of the three species with maximum values, to the individuals of the fifth group (*Alburnus alburnus, Rutilus rutilus*), the third (*Hypophthalmichthys molitrix*) with minimum values (Table 1-3). With regard to the length of the olfactory tract, it increases in direct ratio with the body length: from the individuals of the first group with small length of the body to the individuals of the fifth (*Alburnus alburnus, Rutilus rutilus*) and the third group (*Hypophthalmichthys molitrix*), with small length of the body.

Table 1

The mean of the relative values of the encephalon divisions and subdivisions length and the mean of the body length at the five groups of dimension at *Alburnus alburnus*

Group	Size (mm)	BO/En (%)	TrO/ En	CH/En	LH/En	LO/En	CC/En	VC/ En	Myel/ En
1.	52.38	12.01	2.5	21.37	22.58	27.36	18.20	12.79	28.24
2.	68.24	11.92	8.13	20.07	21.12	24.23	17.23	12.78	29.17
3.	90.62	10.77	12.47	17.89	20.1	22.88	16.92	11.94	27.65
4.	109.5	11.13	14.67	16.31	18.9	21.23	15.72	10.81	27.26
5.	118.6	10.47	16.69	16.16	18.56	19.76	14.37	10.17	28.74

Table 2

The mean of the relative values of the encephalon divisions and sub-divisions length and the mean of the body length at the five groups of dimension at *Rutilus rutilus*

Group	Size (mm)	BO/En (%)	TrO/ En	CH/En	LH/En	LO/En	CC/En	VC/ En	Myel/ En
1.	62.68	10.61	6.79	20.64	21.92	25.91	16.95	11.35	28.44
2.	117.6	9.75	19.58	16.97	18.38	21.75	17.29	10.75	24.61
3.	171	8.88	29.42	14.98	16.18	18.42	15.27	10.34	21.24
4.	240	7.73	38.98	11.93	13.54	13.98	13.09	9.22	19.94
5.	276	7.02	40.27	11.08	12.97	14.32	11.89	8.78	20.27

Table 3

The mean of the relative values of the encephalon divisions and sub-divisions length and the mean of the body length at the three groups of dimension at *Hypophthalmichthys molitrix*

Group	Size (mm)	BO/En (%)	TrO/ En	CH/En	LH/En	LO/En	CC/En	VC/ En	Myel/ En
1.	121.2	7.94	32.40	9.66	10.39	13.62	12.7	5.51	31.75
2.	265	6.52	45.45	6.29	7.22	10.25	10.02	5.12	30.9
3.	349	6.06	46.46	6.26	6.06	9.69	9.69	5.05	30.3

Table 4 that comprises the data regarding the mean of the relative values of the encephalon divisions and subdivisions length for the studied species of cyprinids renders the following aspects: the highest value is registered at the level of myelencephalon at all the species. The maximum value of 37.08% is registered at *Rhinichthys atratulus*, while the minimum value of 25.66 % at *Rutilus rutilus*. According to the presented data, it can be stated that the myelencephalon represents a main component, as there are located important cerebral centers (gustatory, acoustic). The optic lobes come secondly after the myelencephalon, as they present high values, at most of the species, no matter the covered ecological niche. The maximum value is registered at *R. atratulus* – 26.84%, while the minimum value at *Hypophthalmichthys molitrix* – 13.01%. Thus, we can say that the optic lobes play a special role in the life of fish, as they use them to easily seek for food. At a small number of cyprinids the cerebral hemispheres occupy the second place with values of: 26.41% at *Rhinichthys osculus robustus*; 24.79% at *R. o. klamathensis*; 22.32% at *R. cataractae*. The mentioned species are benthic; they mainly use their smelling sense to locate the food. The mean values of the corpus cerebelli oscillate between 12.23% at *Hypophthalmichthys molitrix* (the minimum value) and 23.5% at *Rhinichtys osculus klamathensis* (the maximum value). In the case of the valvula cerebelli, the values oscillate between 5.46% (the minimum value) at *Hypophthalmichthys molitrix* and 13.78% (the maximum value) at *Rhinichthys chrysogaster*. Referring to the hypothalamus lobes, it can be noticed that their maximum value, namely 25.06%, is registered at *Rhinichtys osculus robustus*, while the minimum value 9.76% at *Hypophthalmichthys molitrix*. The minimum value of 2.05% of the olfactory tract is noticed at *Rhinichtys osculus robustus* and *R. atratulus* while the maximum value of 34.66% at *Hypophthalmichthys molitrix*.

Table 4

The values of the statistical parameters for 9 species and subspecies of cyprinids. $\bar{x}$ = the mean of the relative values of the length of the encephalon divisions and subdivisions; S $\bar{x}$ = standard deviation of the arithmetical mean; S% = the variability coefficient; (%).

No.	Species	Index	BO/En	TrO/En	CH/En	LH/En	LO/En	CC/En	VC/En	Myel/En
1.	*Alburnus alburnus*	$\bar{x}$ ±S $\bar{x}$ S%	11.39± 0.21 8.16	9.38 ± 0.43 16.08	19.03 ± 0.48 11.32	20.82 ± 0.40 8.69	23.93± 0.62 11.75	17.18± 0.30 8.03	12.22± 0.18 6.84	28.27± 0.25 3.99
2.	*Hypophthalmichthys molitrix*	$\bar{x}$ ±S $\bar{x}$ S%	7.66 ± 0.2 9.06	34.66 ± 1.72 17.27	9.38 ± 0.43 16.08	9.76± 0.46 16.64	13.01± 0.45 12.04	12.23± 0.38 9.98	5.46 ± 0.16 10.18	31.56± 0.64 7.09
3.	*Pelecus cultratus*	$\bar{x}$ ±S $\bar{x}$ S%	10.13 ± 0.8 13.83	28.37 ± 4.97 30.31	11.99 ± 0.85 12.34	13.36 ± 1.16 15.04	16.56± 0.91 9.60	14.26± 0.70 8.55	9.59 ± 0.01 1.46	31.67± 2.95 16.16
4.	*Rhinichthys atratulus*	$\bar{x}$ ±S $\bar{x}$ S%	14.16 ± 0.44 6.99	2.05 ± 2.28 30.73	25.48 ± 0.74 6.51	24.72 ± 0.67 6.06	26.84± 0.47 3.94	22.40± 0.17 1.69	13.33± 0.53 8.94	37.08± 0.47 2.88
5.	*Rhinichthys cataractae*	$\bar{x}$ ±S $\bar{x}$ S%	13.15± 0.18 3.11	2.88 ± 0.36 29.16	22.32 ± 0.43 4.39	19.52± 0.43 4.96	21.32± 0.38 3.98	21.29± 0.82 8.68	11.74± 0.13 2.53	36.53± 0.53 3.28
6.	*Rhinichthys chrysogaster*	$\bar{x}$ ±S $\bar{x}$ S%	11.89 ± 0.39 8.83	4.49 ± 1.21 59.47	20.47 ± 0.38 4.88	20.97± 0.94 11.85	23.21± 0.82 9.34	21.29± 0.46 5.73	13.78± 0.78 15.13	36.71± 0.33 2.42
7.	*Rhinichthys osculus klamathensis*	$\bar{x}$ ±S $\bar{x}$ S%	12.56 ± 0.23 3.66	2.06± 0.46 45.14	24.79 ± 0.30 2.42	23.03± 0.27 2.34	24.56± 0.34 2.81	23.50± 0.75 6.38	13.66± 0.39 5.71	35.60 ± 0.49 2.75
8.	*Rhinichthys osculus robustus*	$\bar{x}$ ±S $\bar{x}$ S%	12. ± 0.23 3.83	2.05 ± 0.32 31.21	26.41 ± 0.91 6.89	25.06± 0.21 1.76	24.81± 0.28 2.53	22.29± 0.25 2.24	12.41± 0.15 2.57	33.38 ± 1.46 4.33
9.	*Rutilus rutilus*	$\bar{x}$ ±S $\bar{x}$ S%	9.95 ± 0.27 12.6	17.29 ± 2.46 65.27	18.33 ± 0.73 18.29	19.92± 0.66 15.28	23.29± 0.92 18.24	16.27± 0.46 11.30	10.86± 0.19 7.12	25.66 ± 0.78 13.95

The variability of the encephalon divisions and subdivisions (from the point of view of the report between the divisions and subdivisions length and the encephalon length) is low (S% oscillates between 0 and 10%) and mean (S% oscillates between 10 and 20%) at the studied species (Table 4). The low and mean variability coefficient of the divisions and subdivisions indicates a certain approaching among these species, probably induced by the common environment they live in.

The variability of the olfactory tract oscillates between 17.27 % (the minimum value) at *Hypophthalmichthys molitrix* and 65.27 % (the maximum value) at *Rutilus rutilus* (Table 4). The great variability might be explained by the fact that the tract represents a linking component between the cells of the olfactory bulb and the ventral area of the hemispheres and it varies according to the size and aspect of the ethmoidal region of the skull.

4. CONCLUSIONS

We studied the external morphology of 8 species of cyprinids belonging to the Leuciscinae subfamily that live in fresh water covering all the niches: pelagic, midwater and benthic.

At the studied species: *Alburnus alburnus, Hypophthalmichthys molitrix, Pelecus cultratus, Rutilus rutilus, Rhinichthys atratulus, R. cataractae, R. chrysogaster, R. osculus* with 2 subspecies: *R. o. klamathensis* and *R.o. robustus*, there can be noticed a great intraspecific and interspecific variation regarding the form of the encephalon, according to the length of the body, way of living and environment.

At the species belonging to *Rhinichthys* genus, there were noticed many furrows and tubercles on the dorsal surface of the telencephalon. Their great number might be explained by the fact that they can play a certain part in increasing the size of the olfactory surface.

The smallest hypothalamus is present at *Hypophthalmichthys molitrix*, while above the median lobe there can be noticed the large and rounded hypophysis. The lateral hypothalamic lobes present variations. At the species belonging to *Rhinichthys* genus and *Hypophthalmichthys molitrix*, there can be distinguished a diencephalon formation with a different aspect, located behind the median lobe and between the lateral lobes.

The optic lobes appear under diverse aspects, both intraspecific and interspecific, 3 situations being characteristic: – when the optic lobes are more caudally outdistanced along the median line (*Hypophthalmichthys molitrix,* and the species of *Rhinichthys* genus); – when the optic lobes are rostrally united and

caudally outdistanced (at the individuals from the second – the fifth groups at *Alburnus alburnus, Rutilus rutilus*, at *Pelecus cultratus* and some individuals of *Rhinichthys atratulus*); – when the optic lobes are rostrally united along the entire median surface (at the individuals belonging to the first group, namely *Alburnus alburnus, Rutilus rutilus* species).

Taking into account the development of the vagal lobes and of the facial lobes from the level of the myelencephalon, the species belong to 3 main groups. The first group presents less developed facial lobe and vagal lobes (*Alburnus alburnus, Rutilus rutilus).* The third group presents a well-developed facial lobe, while the vagal lobes are moderately developed (at the species of *Rhinichthys* genus). The fifth group presents a facial lobe that does not appear at the exterior and the vagal lobes are reduced (*Pelecus cultratus*). *Hypophthalmichthys molitrix*, with a very small and difficult to observe facial lobe, is the intermediary between the first and the fifth groups.

According to the morphological criterion, it comes out that the encephalon of the analyzed species belongs to three main groups: basic cyprinid (*Rutilus rutilus*), octavolateralis (*Alburnus alburnus, Hypophthalmichthys molitrix, Pelecus cultratus*) and chemosensory (the species belonging to *Rhinichthys* genus).

The value of the relative length of the encephalon divisions, subdivisions, is in indirect ratio with the size increasing from large size individuals to the small size individuals. However, the fish length does not represent, in all the cases, a conclusive parameters, the length of certain divisions and subdivisions should depend on.

The variability of the encephalon divisions and subdivisions is low and mean at the studied species, being influenced by the environment factors and fish dimension.

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EPIDERMAL ULTRASTRUCTURE OF PADDLEFISH, *Polyodon spathula*

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The epidermis ultrastructure of the paddlefish (*Polyodon spathula*) is reported for the first time in the literature.
It has been shown that the epidermis is composed only from epithelial cells associated through desmosomes by many bundles of tonofilaments.
There were not observed differentiated mucous gland cells as in other fish species. Towards the surface, the epithelial cells became flattened, with many vacuoles and desmosomes.
Intrusing cells are of two types: melanophores and phagocytic leucocytes. The leucocytes are present at any level of the epidermis and could be exposed at the surface of damaged skin. The function of eosinophilic leucocytes in fish skin was not clearly elucidated, though in salmonids is associated with ectoparasite species.

1. INTRODUCTION

The North American paddlefish is one of two living species of Polyodontidae, the other being the Chinese paddlefish, *Psephurus gladius* (2, 4).

Polyodon spathula is a fish of economic value, introduced from USA in our country at Nucet Station, in 1992.

The number of studies on the paddlefish integument is surprisingly low, even though this fish has a unique skin. These morphological (3, 5, 6, 7, 8), histochemical and immunohistochemical studies (9) have been carried out only in light microscopy.

In this paper, we report some ultrastructural aspects of the epidermis in paddlefish, *Polyodon spathula*.

2. MATERIALS AND METHODS

Three years old *Polyodon spathula* obtained from the Nucet Station in 1998 were used for this study. Following capture, the fish were sacrificed by medullary transection and skin samples were taken from various sites of the body. The small skin fragments were fixed in 2.5% glutaraldehide, buffered at pH 7.4 in 0.1 M sodium cacodylate solution containing 4% sucrose and 1% DMSO, then post-fixed in 1% OsO_4, in the same cacodylate buffer, dehydrated and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, then studied with a Phillips 206S electron microscope operating at 80 KV.

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3. RESULTS AND DISCUSSION

The skin of *Polyodon spathula* as in most teleosts is composed of two main layers: the epidermis, formed by several rows of interconnected cells and the underlying dermis. Paddlefish is covered by a stratified squamous epithelium that in contrast to other fish species has epithelial cells only. The number of layers varies from eight (rostrum) to 12 (gill membrane).

The basal cuboidal layer has an irregular surface closely approximated by a continuous basement membrane (Fig. 1A). The cytoplasm of the cells contains mitochondria, ribosomes, vesicles and is filled with closely packed tonofilaments, usually arranged in bundles. Columns of tonofilament bundles occupy the lateral cytoplasm (Fig. 1B), associated above, through desmosomes, with the epithelial cells of the next layer and below with hemidesmosomes on the basal plasma membrane.

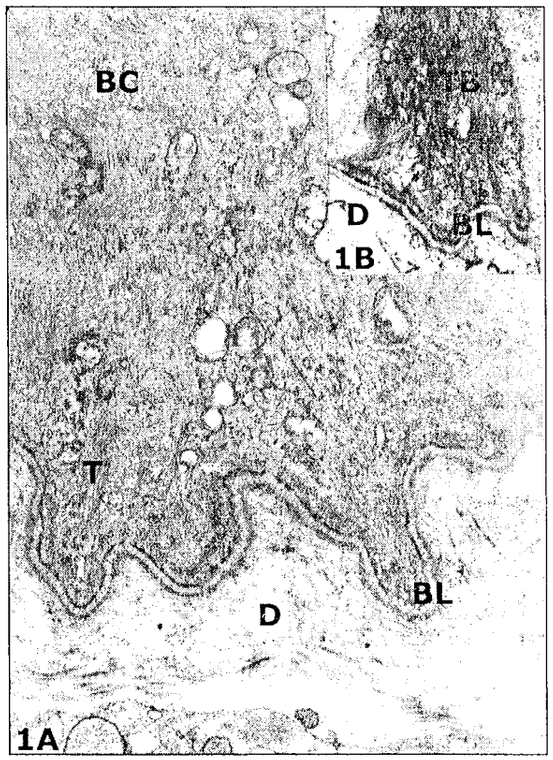

Fig. 1A–B – **A** –Detail of basal cell of epidermis (BC) resting on basal lamina (BL). D-dermis; T-tonofilaments. × 16.000. **B** – TB-tonofilaments bundle. × 20.000.

Previous studies on other species indicated that the great number of tonofilaments seen in the basal cell is characteristic of juvenile stages (9).

The proximal membranes of the basal cells connect through numerous hemidesmosomes to the basal membrane forming the link between the epidermal and dermal parts of the skin. Most organelles are located perinuclear. In the fish the basal layer cells do not constitute a germinal layer because mitotic activity is detectable throughout the epidermis although commonest in the deeper layers.

Cells from middle layer of epidermis are round, oblong or irregular (Fig. 2) and interdigitate deeply with neighboring cells (Fig. 3). Cellular interdigitations are thought to play a role in increasing surface area available for the metabolic exchanges of the cell as well as for other transport activities. Membranes of these cells are rich in desmosomes and the cytoplasm contains tonofilaments, usually arranged in bundles converging toward a desmosome. In some regions of epidermis there are large irregular spaces between epithelial cells. These channels were associated at other species with stress, parasites or virus infections (9). Some cells from midlayer of the epidermis contain few mucus granules in cytoplasm. The epidermis of *Polyodon spathula* in contrast with other fish species including *Acipenser* has epithelial cells only without differentiated mucous gland cells. There is no accumulation of mucus in special cells that migrate to the surface to secrete. A thick compact epidermis and dermis compensate for the small amount of mucus secreted (8).

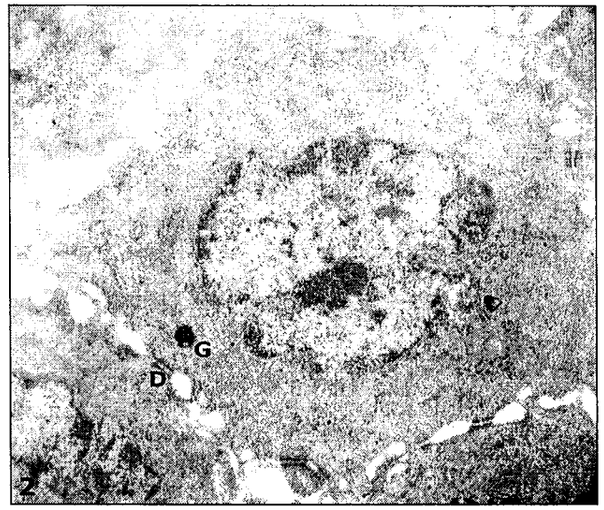

Fig. 2 – Cell from middle layer of the epidermis that contact through desmosomes (D) with neighboring cells; G-secretory granule. × 8.000.

Towards the surface of the epidermis the cells became flattened. The interdigitations appear to be shorter and less interlocking, and the spaces between them are larger (Fig. 4). The cytoplasm is divided into two regions: the perinuclear region that contains mitochondria, dilated Golgi vacuoles and peripheral zone, rich in tonofilaments that partly extend towards desmosomes joining the interdigitating neighboring cells. Higher up in the epidermis, these cells become more flattened and there are fewer dense connections between them. Under plasma membrane are numerous vacuoles.

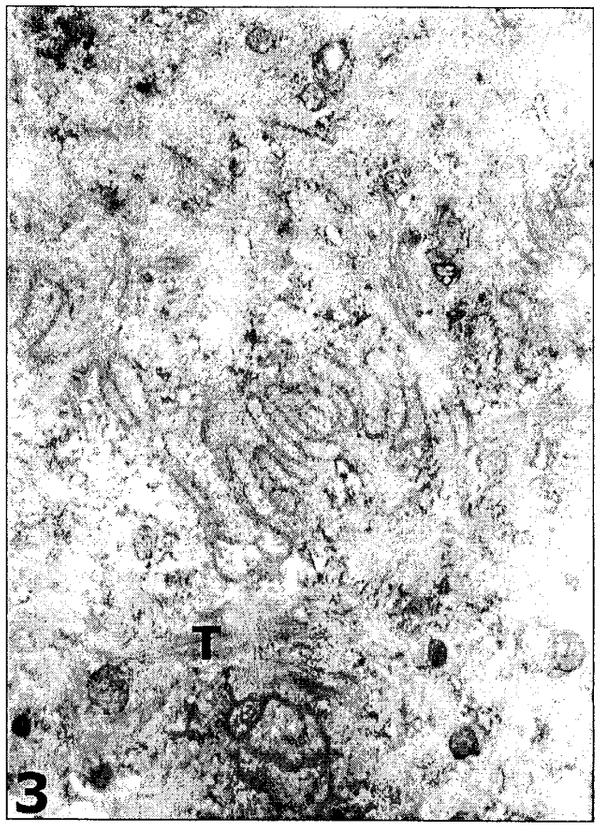

Fig. 3 – Epithelial cells separated by intimately interdigitating processes leading to a complex infolded cell surface.
Note the bundles of tonofilaments (T) converging toward a desmosome.
× 10.000.

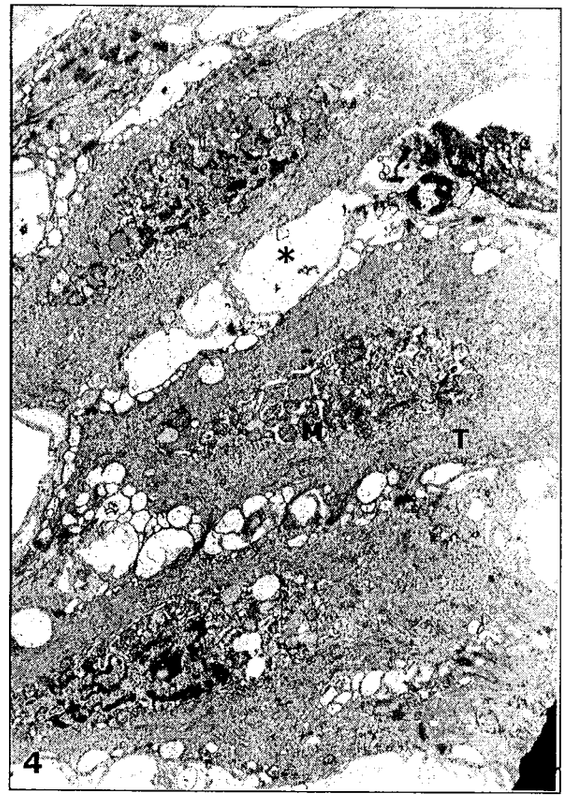

Fig. 4 – Towards the surface of the epidermis the cells are flattened with interdigitations shorter and less interlocking, and spaces between them larger (*). M – mitochondria; T – tonofilaments; × 9450.

The most superficial cells were greatly flattened and with pyknotic nuclei (Fig. 5). Even if the intercellular contacts were disrupted, the desmosomes are present between these cells. These epithelial cells contain remnants of a nucleus, autolyzed organelles and fused tonofilaments.

Intrusive cells within the epidermis that originating elsewhere includes chromatophores and leucocytes.

Melanophores are present at various depths in the epidermis from back, sides, outer surface of operculum and rostrum. The morphology of melanophores was fusiform or irregular, with many cytoplasmic processes that contain melanosomes. The nuclei were centrally located and showed peripheral chromatin and nuclear envelope with slight indentation. Melanin granules were rounded or oval with uniformly electron-dense content (Fig. 6). Other organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and small vesicles could also be observed. These melanophores are primary elements of morphological color change.

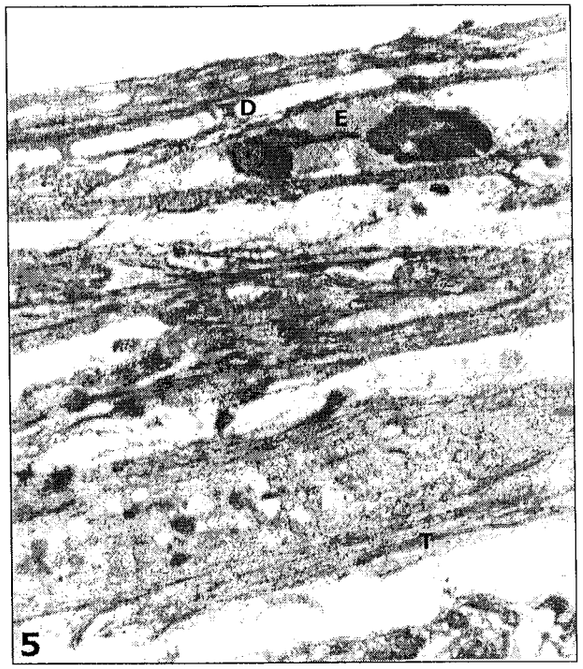

Fig. 5 – The surface cells of epidermis are degenerated but the desmosomes (D) persist between these cells. T-fused tonofilaments. E-eosinophilic leucocytes. × 12.000.

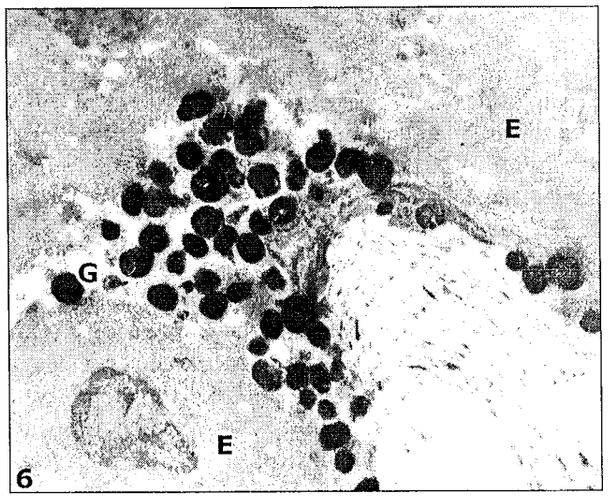

Fig. 6 – Epidermal melanophore located between epithelial cells (E); G-pigment granule. × 10.000.

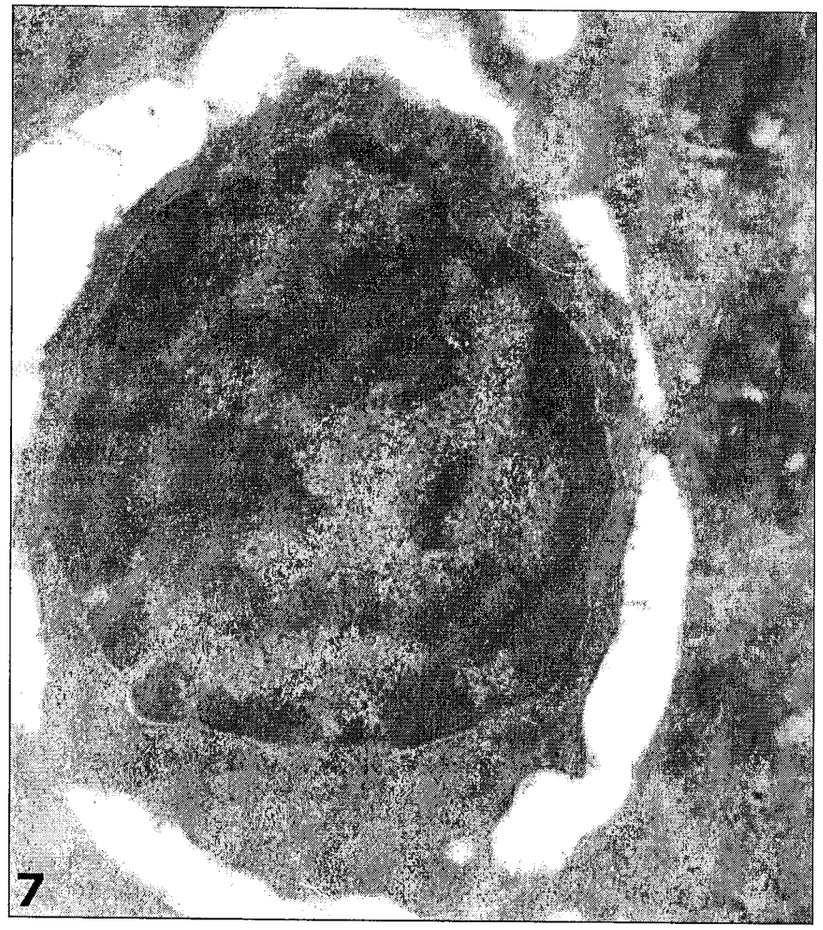

Fig. 7 – Lymphocyte inside channels between epithelial cells. × 20.000.

The leucocytes observed in paddlefish epidermis were lymphocytes and eosinophilic granulocytes. These blood cells are generally phagocytic. Lymphocytes are most numerous in the middle layer of the epidermis (Fig. 7). Eosinophilic granulocytes were more frequent between cells of the superficial layer of the epidermis (Fig. 6). The functions of eosinophilic leucocytes in the fish epidermis have not been satisfactorily elucidated. Large numbers of eosinophilic cells appeared to be associated in salmonid fish with ectoparasite species (1). Leucocytes are most numerous between and above the basal layer cells but may occur at any level of the epidermis and are sometimes exposed at the surface if the skin is damaged (9).

In conclusion, to the best our knowledge this is the first report that describes the epidermis ultrastructure of *Polyodon spathula*.

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STRUCTURAL PECULIARITIES OF THE IMMATURE HUMAN EXTRAEMBRYONIC MEMBRANES

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LUCIA MOLDOVAN*,MARIA CALOIANU*

The aim of this study was to outline the particular aspects of immature human extraembryonic membranes by light and electron microscopy. The space between amniotic and chorionic connective tissue of free fetal membranes, the presence of both syncytio- and cytotrophoblast on the surface of the chorionic villi as well as the occurrence of vitellointestinal duct, resembling with small intestine, are few specific features of human embryonic annexes in early stages of gestation. Histochemical evidence of the collagen and immunohistochemical location of chondroitin sulfate (CS) demonstrated the high synthesis activity of the connective cells from extraembryonic membranes at 8--10 weeks of pregnancy.

1. INTRODUCTION

The extraembryonic membranes are transitional structures of extremely importance for intrauterine foetus accommodation during intrauterine life.

Although the study of free fetal membranes at term represented a very interesting subject since 1960 [1], however, their structure [2] and also of the other immature human embryonic annexes was not a major objective.

Nowadays, a special attention is paid to extraembryonic membranes dynamics in order to elucidate some mechanisms based on cellular and molecular processes during its development, due to the foetus growth as well as its pathological implications.

Collagen is one of the major macromolecular components of the fetal membranes that together with proteoglycans, and elastin provides them strength and elasticity during the pregnancy, and are responsible for the biomechanical properties of the tissue [3]. These matrix components synthesize at the same time with membrane formation.

Our initial studies [4] followed the term structure of these annexes and CS location.

Due to the fact that embryonic membranes are determinative for maintaining the pregnancy under normal conditions until term, we propose to outline some specific aspects of the embryonic annexes in the first gestational quarter, the most critical stage in human placentation.

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2. MATERIALS AND METHODS

We studied human placenta, free fetal membranes and umbilical cord fragments taken at an early stage of pregnancy (8-10 weeks) following voluntary cessation of pregnancy. Tissue fragments were processed both for light and electron microscopy.

For **light microscopy**, tissues were fixed in Bouin solution, dehydrated and clarified in toluene. We performed 4μm (for placenta and fetal membranes) and 7μm (for umbilical cord) sections, and the slides were stained with hematoxylin-eosin and by von Gieson and Azan methods (for collagen).

The CS evidence was made by indirect peroxidase **immunohistochemical method** for light microscopy. The sections were deparaffinated and rehydrated, then immersed in 0.3% hydrogen peroxide for 30 minutes to remove any endogenous peroxidases, thereby reducing background artefacts. Tissue was then washed in 0.1 M phosphate buffer saline (PBS). All incubations were performed in a humid chamber. Then we proceeded for the nonspecific antigens blocking using 2% bovine serum albumin (BSA) in PBS followed by an overnight incubation in primary antibody (antiCS monoclonal antibody; Sigma, C-8035) diluted 1:150 in 2% BSA in PBS. After washing, the samples were maintained for 1 hour in the presence of secondary antibody (peroxidase-conjugated goat anti-rabbit IgG; Sigma) diluted 1:150 in PBS. Substitution of the primary antibody with PBS was used as a control. After that, peroxidase reaction was developed using 3, 3 – diaminobenzidine tetrahydrochloride and H_2O_2 for few minutes. Sections were then dehydrated in alcohols and mounted.

For **electron microscopy** study, placenta fragments were fixed by 2% glutaraldehyde in 0.1M Na-cacodylate, pH 7.4 and postfixed with 1% OsO_4 in the same buffer. The tissue fragments were then rinsed with cacodylate buffer, dehydrated in ethanol and embedded in Epon 812. Subsequently, the sections were picked up on grids and counterstained with uranyl acetate and lead citrate.

3. RESULTS

This study was accomplished with human free fetal membranes, placenta and umbilical cord levels, at cessation of pregnancy (8–10 weeks) and concentrated to outline some particular aspects of embryonic annexes in normal immature pregnancy.

The free fetal membranes consist of a collagen-rich connective tissue (Fig. 1) and cells implicated in biosynthesis of matrix macromolecules. The fetal membranes' connective tissue is covered by a thin epithelium consisting of flattened cells at this early stage of pregnancy. At 10 weeks of intrauterine development, the amniotic connective tissue is not yet attached to the underlying

chorionic connective tissue, the space between them being populated by some macrophages.

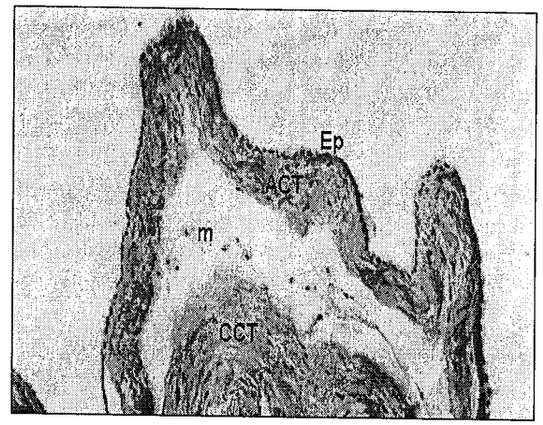

Fig. 1 – Light microscopy view of the immature human free fetal membranes. There is a gap between amniotic (ACT) and chorionic (CCT) connective tissue and few macrophages (m). von Gieson method for collagen, labeled in ACT and CCT (× 16).

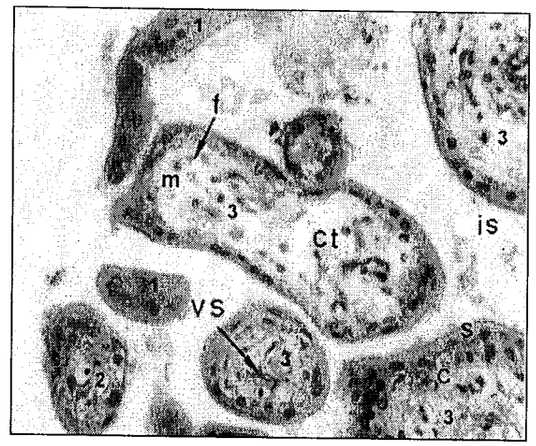

Fig. 2 – Transverse section of immature chorionic villi from human placenta (X 16; hematoxylin-eosin). C – cytotrophoblast; S – syncytiotrophoblast; ct – connective tissue; m – macrophage; f – fibroblast; vs – fetal blood vessel; is – intervillous space; 1 – primary villi; 2 – secondary villi; 3 tertiary villi.

At the placental level, the most important component is represented by chorionic villi. This paper studies the structure of mature intermediate and terminal villi, because of their major role in physiology of pregnancy. These villi exhibit an external trophoblast, which covers a connective tissue and fetal blood capillaries (Fig. 2). In the villous core we noticed a decreased population of fetal macrophages

(Hofbauer cells). These are large, round-shaped cells with a voluminous nucleus and a rich cytoplasm.

At 8 weeks of pregnancy, one may notice all of three types of chorionic villi (Fig. 2): primary, secondary and tertiary villi. The primary villi consist entirely of trophoblast, but the secondary villi are composed additionally of mesoderm content. The tertiary villi are the most developed and are characterized by vascularization. Sometimes, the villi can undergo villous atresia (Fig. 3) and degenerate. Externally, chorionic villi exhibit the cytotrophoblast (Langhans' cells) covered by a single layer of syncytiotrophoblast (Fig. 4). The cytotrophoblast is an almost continuous layer at this age of pregnancy. The syncytial layer shows microvilli like a brush border (Fig. 4) over the entire surface of the villi.

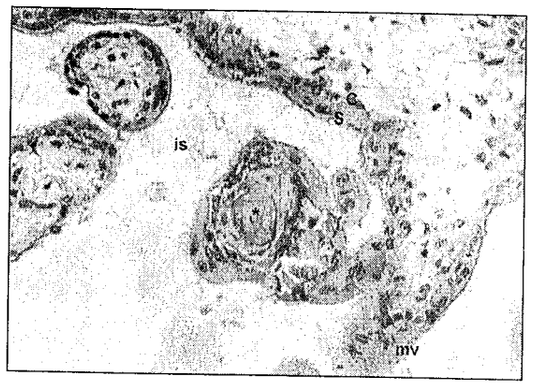

Fig. 3 – Light microscopy view of an immature atretic villus (× 25; hematoxylin-eosin). C –cytotrophoblast; S – syncytiotrophoblast; is – intervillous space; mv – mesenchymal villus.

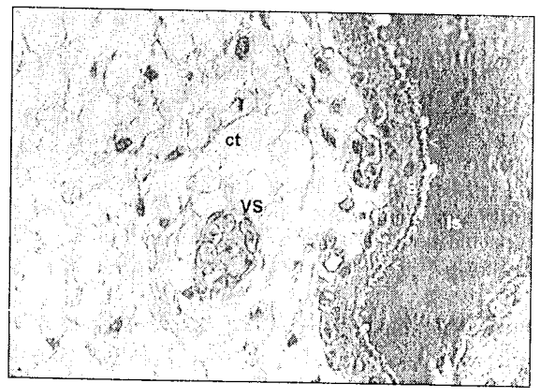

Fig. 4 – Light microscopy view showing the distance between a fetal capillary and trophoblast (× 25; hematoxylin-eosin). C – cytotrophoblast; S – syncytiotrophoblast; is – intervillous space; ct – connective tissue; vs – fetal blood vessel; arrowheads – microvilli.

Ultrastructurally, we found that the syncytiotrophoblast cytoplasm is rich in mitochondria, free ribosomes and rough endoplasmic reticulum (Fig. 5). Under syncytium one may notice the cytoplasm of the cytotrophoblast cell with many mitochondria and profiles of smooth endoplasmic reticulum. The cytotrophoblast is resting on a fibrillar, dense basal membrane beneath which fibroblastic cytoplasmic remnants can be observed. The fibroblasts are fixed connective cells, able to perform macromolecular synthesis.

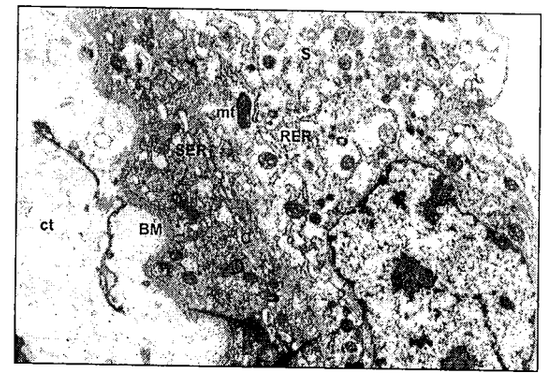

Fig. 5 – Electron microscopy picture of syncytio- and cytotrophoblast from immature chorionic villus (TEM; × 10,500). C – cytotrophoblast; S – syncytiotrophoblast; mt – mitochondria; RER – rough endoplasmic reticulum; SER – smooth endoplasmic reticulum; BM – basal membrane; ct – connective tissue.

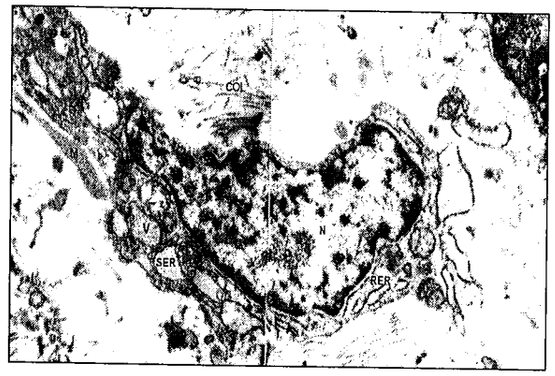

Fig. 6 – Electron microscopy of a fibroblast from an immature chorionic villus (TEM; × 26,000). N – nucleus; V – secretory vesicle; RER – rough endoplasmic reticulum; M – electron-dense material.; asterisk – dilated endoplasmic reticulum.

The fibroblast is a long cell with a voluminous nucleus, little cytoplasm and long cytoplasmic prolongations (Fig. 6). The villous fibroblast is a very active cell that exhibits rough endoplasmic reticulum profiles and dilated reticulum.

Moreover, the presence both of some intracellular vesicles with a fibrillar material and of pericellular collagen fibres and electron-dense material demonstrates the great synthesis ability of this cell.

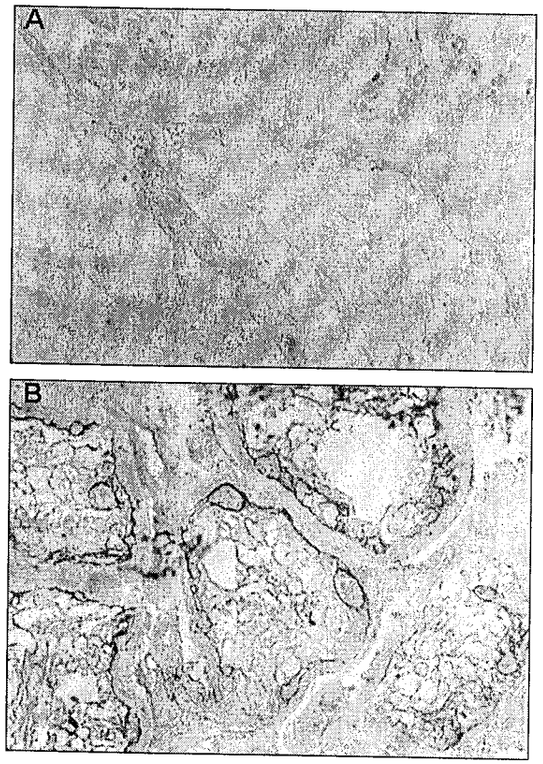

Fig. 7 – CS immunohistochemistry by light microscopy at the level of immature chorionic villus. High immunoreactivity is observed in perivascular area and trophoblastic basal membrane. Chorionic stroma exhibits a weak reaction (× 6.3). No reaction in syncytio- and cytotrophoblast:
A – control (without antiCS primary antibody – no immunohistochemical reaction);
B – sample (incubated with antiCS primary antibody – after immunohistochemical reaction).

The chorionic villi are separated by intervillous space and contact directly the maternal blood. At this time of pregnancy, the villous capillaries can be located at an appreciable distance from a trophoblast (Fig. 4) or come in intimate contact with it forming the vasculo-syncytial membrane (data not shown).

Showing the chorionic villus morphology, the following step was to investigate the distribution of CS. At this level, we noticed a well-defined distribution of this glycosaminoglycan (Fig. 7A, B). CS was immunohistochemical located in the trophoblastic basal membrane, but also in extracellular matrix of the

villus. The strongest immunoreactivity occurs pericapillary in the villus. There is no reaction in syncytio- and cytotrophoblast.

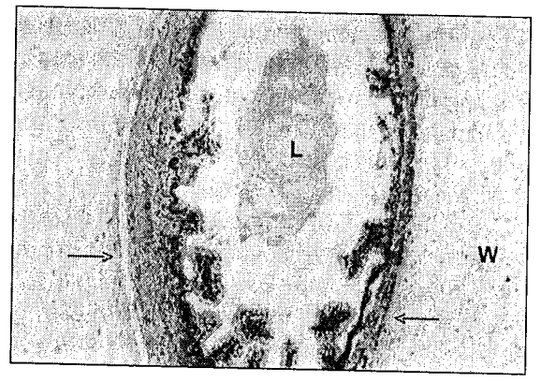

Fig. 8 – Light microscopy view of the vitellointestinal duct from 8–10 weeks-old umbilical cord (× 6.3; Azan method). L – lumen; W – harton jelly; arrows – collagen envelop.

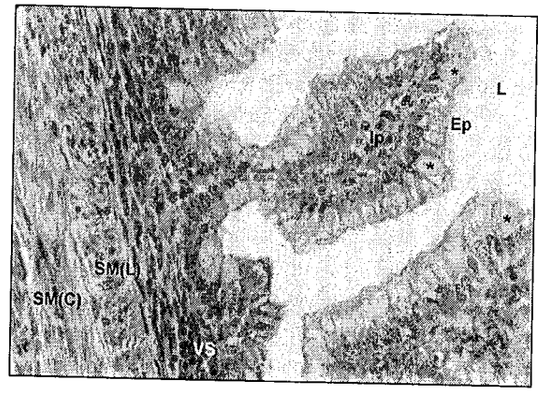

Fig. 9 – Light microscopy view of the vitellointestinal duct from 8–10 weeks-old umbilical cord (× 0; hematoxylin-eosin). Ep – epithelium; lp – lamina propria; L – lumen; VS – blood vessel;
SM(C) – circular layer of the smooth muscle cells;
SM(L) – longitudinal layer of the smooth muscle cells; asterisk – goblet cells.

At 8–10 weeks of pregnancy there is a vitellointestinal duct of tubular shape covered by a collagen-rich envelope in the umbilical cord (Fig. 8). The inner surface of the vitelline duct contains different sizes folds projected in lumen, as simple or budded elevations (Fig. 8).

The transverse section through vitellointestinal duct suggests a primitive intestinal-like structure with three layers: mucosa, submucosa and muscularis. The mucosa consists of epithelial cells, goblet cells and numerous cells in lamina

propria. The columnar epithelial cells (Fig. 9) have a basal voluminous nucleus. The basal membrane is hardly visible on hematoxylin-eosin stained samples.

Goblet cells are found scattered among epithelial cells in the epithelium and have large sizes (Fig. 9). The nucleus is at the base of cell, the apical region is dilated and the submucosa may contain blood vessels. The muscularis is formed of two layers: an inner longitudinal and an outer circular layer (Fig. 9) although in some areas only circular layer may exist or the muscularis may be discontinuous (Fig. 8).

4. DISCUSSION

The free fetal membranes represent an extended fibrous structure, attaching to placenta borders [5] and consists of amnion and chorion, neighboring entities. They contain a different cellular component (epithelial, mesenchymal and trophoblast cells) embedded in a collagenous matrix [6].

Although, we found that the amniotic epithelial cells are flattened in this early stage of pregnancy, our previous study [4] demonstrated their cuboidal shape at term.

The amnion exerts a pressure upon the chorion, although there is no contact between them until the 12th week of intrauterine life [5, 7, 8], an aspect revealed by us in this study. Our previous study [4] showed the presence of a basal pseudomembrane at the junction of amniotic and chorionic tissue in term, but absent in early stages of pregnancy in normal states. The amniotic connective tissue contains a macrophage population that can be activated in pathological conditions (3). They are present in discontinuous and weakened areas, as we noticed in loose regions between the amniotic and chorionic connective tissues.

One of the major macromolecular components of fetal membranes is collagen, that together with another extracellular matrix proteins lends them resistance and elasticity during pregnancy [3]. Our histochemical study concerning collagen location in 8–10 week-old fetal membranes revealed its presence at the level of amniotic and chorionic connective tissue. Extraction and biochemical characterization studies [3, 9, 10] demonstrated collagen occurrence as a major structural element of fetal membranes.

In immature placenta there are three types of chorionic villi: primary, secondary and tertiary ones, while in term only tertiary villi occur.

Cytotrophoblast is unistratified, able to proliferate [7] and delimits the chorionic villus, the functional unit of placenta. Its main activity is to form externally an additional syncytial layer by repeated division without the occurrence of a cytokinesis [7]. Although syncytium nuclei are diploid, they can not undergo cell division. The presence of cytoplasm and of numerous nuclei is provided by trophoblast cells, which have a role to regenerate the syncytium throughout

pregnancy [5], ensuring the major contact with intervillous blood [7]. At the same time with intravillous fetal vascularization extension and villous growth (more than 12 weeks of gestation), the cytotrophoblast becomes discontinuous by Langhans cells cytoplasm fusion with the syncytium on certain portions [5, 11].

During the first 12 weeks of pregnancy, the villus contains connective tissue where the fetal capillaries and macrophages (Hofbauer cells) similar to the amniotic ones occur [5]. Hofbauer cells are present in villous stroma in all the stages of pregnancy [12], although they are rather more abundant in immature placenta comparing to term [7]. During the first month of pregnancy, the villous macrophages are supposed to originate from the mesenchymal cells in the villous stroma, but this supply may be augumented by differentiation from fetal bone marrow-derived macrophages, once the fetal circulation establishes. The role played by macrophages is mainly to control the vasculogenesis and the stromal cell growth [12], but they also represent a population involved in cell response to pathological condition of pregnancy. Nevertheless, fetal macrophage presence in the terminal villi is much reduced compared to stem and intermediate villi, at the same pregnancy stage [13].

This structure is surrounded by cyto- and syncytiotrophoblast [14]. Fetal capillaries represent the finest tips of the blood vessels, ramified at villous level [15]. As our study revealed, even in this early stage of gestation, intravillous fetal capillaries get closer to cytotrophoblast, leading to vasculo-syncytial membrane formation, facilitating the mother-foetus nutritive exchange [5].

Early studies [16] showed that in terminal villi, the fetal capillaries do not exhibit the media, as our studies have showed, in contrast to the stem villi. In this respect, we have followed CS distribution at the villous core level, particularly the perivascular one, in order to see whether terminal villi also have a protection structure at capillary level at this moment.

Histoenzymatic studies [17] demonstrated the CS presence mainly in villous stroma, whereas pericapillarly, heparan sulfate and dermatan sulfate are localized, CS being absent. These data are not consistent with our studies, which revealed a strong immunoreactivity around the fetal capillaries. Moreover, our previous studies [4] localized the perivascular CS in normal umbilical cord at term. Our results are correlated with the CS role in maintaining the hydration and protection against the compression forces [18] due to their ability to attract ions and water molecules.

Vitellointestinal duct is embedded in Wharton gelatinous connective tissue [14, 15], very rich in proteoglycans and connective cells [5], starting with 7 weeks of intrauterine life [19]. At 10 weeks of development it was revealed the presence of some intestinal loops, which retract, by the end of the third month, but remnants of vitelline and allantois ducts may persist until term as a pedicle [14].

Our studies demonstrate the primitive intestinal-like structure of the vitellointestinal duct in human umbilical cord in an early stage of pregnancy (8–10

weeks). These results are supported by data of literature regarding the intestine structure, because we don't know any study about the specific structure of this duct. Thus, intestinal villi are coated by absorptive epithelial cells, goblet cells [20] and rare lymphocytes [21]. The epithelium originates from endoderm [22] and consists of columnar, simple cells [23], as we our data showed as well. The cells produced at intestinal villus base advance upward in the villi and differentiate into four cell types as they migrate [23]: predominating enterocytes, mucus-secreting goblet cells, enteroendocrine cells, hormone secretors and Paneth cells, the last ones being included in intestinal gland structure. The cells differentiate and become functional prior to their removal from villus into the lumen at an interval of 2-6 days [21]. Goblet cells are present among epithelial cells and they are responsible for producing and maintaining protective mucus consisting of high molecular weight glycoproteins [24]. Our studies revealed also their presence in human vitellointestinal duct structure and they are readily visible on the hematoxylin-eosin stained preparations, due to their balloon-like shape at the apical end. Cell nucleus is basal, the cell remainder being occupied by secretory granules filled with mucus. Our studies showed a different distribution of muscular layers in the vitellointestinal duct compared to the primitive intestine. Thus, whereas in intestine there is a circular inner layer and a longitudinal outer one [25], in vitellointestinal duct, the distribution is either opposite, or incomplete.

5. CONCLUSIONS

At early stages of pregnancy, human embryonic annexes exhibit a number of particularities compared to the same structures at term. Among them, it can be mentioned the basal pseudomembrane between the amniotic and chorionic tissues absence, at the free fetal membranes level and a gap between the two of them occurrence. At the placenta level, there is a very strong activity of matrix molecule synthesis carried out by fibroblasts.

The umbilical cord is distinct by the presence of vitellointestinal duct with a similar structure with the primitive intestine.

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ULTRASTRUCTURAL AND FUNCTIONAL CHANGES IN MITOCHONDRIA INDUCED BY HEAVY WATER *IN VITRO*

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VIORICA COROIU *, GH. TITESCU***, I. ŞTEFANESCU***

To study the cell behavior in the presence of heavy water, investigations were carried out concerning changes of mitochondria in CHO, Hep 2 and fibroblast cells cultivated in media with various D_2O concentrations (20%, 65% and 90%) by electron microscopy and MTT assay. Heavy water in the culture medium caused a decrease in the tetrazolium salt conversion into formazan compared with the control, but in a different way than the cell number decreased. At ultrastructural level, the observed changes were dependent on heavy water concentrations in the culture medium and on the exposed cell type. In the case of Hep 2 cells, at 65% D_2O, there were identified swollen mitochondria exhibiting a dense matrix and broken mitochondrial membranes. At 90% D_2O concentration, these changes were much more obvious, mitochondrial cristae being disorganized and dilated, a reduction in mitochondrial matrix being noticed compared to the previous case. At an exposure to 20% D_2O concentration, CHO cells exhibited a morphological polymorphism of mitochondria, with a lower degree of cristae disorganization and the appearance of some intramitochondrial vacuoles. At 90% D_2O, their alteration was more intense, the cells exhibiting mitochondria with different matrix density and cristae disorganization. At 65% D_2O, fibroblast cells show some very long mitochondria, which might arise by fusion of mitochondria of normal size. At 90% D_2O, one may notice the disruption of mitochondrial membranes and the release of mitochondrial content into cytoplasm.

1. INTRODUCTION

The biological effects of deuterium (D), a stable isotope of hydrogen, have been investigated in various biological systems.

In media containing a high amount of deuterium oxide (heavy water) instead of H_2O, growth and division of most cell types and organisms are inhibited (1–4). The effect exerted on mitosis was widely investigated by Lamprecht and Schroeder (5, 6).

In fish (7, 8) and mammals (9), heavy water induces damages in hepatic tissue structure: nuclei pycnosis, vacuolization of the cytoplasm, massive degradation and degeneration.

Heavy water at high concentrations affects a wide range of biological, biochemical and biophysical activities: formation and properties of different blood cells including platelets (10), function of membrane systems (membrane

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depolarization, Ca^{+2} channel activation, interference with ionic exchangers) (11, 12), and lysosome function (13).

Some pharmacological studies suggest that high D_2O concentrations might have antihypertensive effects (14), clinical valuable (15), antitumoral effects by combining with cytostatic drugs and protective effects for pluripotent stem cells during gamma irradiation (16).

Animal and vegetal tissues incubation in heavy water was extensively used as well, for the study of protein synthesis, degradation and turnover in the case of proton density-labeling experiments (17), in order to investigate the role played by water in biosystems structure and function (18) and to suppress solvent signal from the water in proton NMR (19).

The purpose of our study was to examine the effects of heavy water on mitochondrial functions and the relationship between D_2O – induced functional alterations and mitochondrial structural injury.

2. MATERIALS AND METHODS

Cells and incubation media. CHO, Hep 2 and fibroblast cells (between passages 3–8) grown as a monolayer at 37°C in a humidified 5% CO_2 atmosphere were used throughout this study. Stock cultures were maintained in 25 cm^2 polystyrene tissue culture flasks (Nunc) using the media: RPMI 1640 for CHO cell line, MEM for Hep 2 cells and DMEM for fibroblast culture, supplemented with 10% fetal calf serum, 25 mM HEPES, 100 IU/ml penicillin, 100μg/ml streptomycin. Cells were subcultured twice a week. The heavy water (99.96%) was supplied by I.N.T.C.I. Ramnicu Valcea (Romania), on the basis of cooperation agreement. The deuterated culture media were obtained by dissolving the powdered RPMI 1640, MEM and DMEM in water with different deuterium concentrations (25%, 75%, 99.96%) and sterilized by filtration.

MTT assay. The MTT test was carried out on each cell line after 3 days of treatment with D_2O at concentrations ranging from 25% to 99.96%. Cell suspensions were obtained by trypsinization of subconfluent cultures and seeded in 24 well plates at a density of 2×10^4 cells/well for all three-cell lines. The cells were cultured for 24 hours in their respective medium with 10% fetal calf serum, which was replaced with medium containing different D_2O final concentrations (after serum adding 20%, 65%, 90%) in quadruplicate. After 3 days of cell exposure to D_2O, the culture medium was discarded, cells were carefully washed in phosphate buffer and MTT solution (MTT dissolved in culture medium at 5 mg/ml) was added to each well. After 3 hours incubation at 37°C, solubilization of formazan crystals formed in viable cells was achieved by adding 1 ml isopropanol with 0.04 N HCl to each well. After incubation at room temperature for 20 minutes under gentle agitation, the absorbance was measured at 570 nm on a UV-VIS spectrophotometer (Jasco, model V-530). The activity of formazan formation from MTT is expressed as a percentage of the value obtained in control cells.

Determination of cell viability and cell counts. For cell viability and cell count analyses, CHO, Hep 2, and fibroblasts cells were seeded at the same time and the same density and cultured in the same way as for the MTT assay. After 3 days of cell exposure to heavy water, the adherent cells were dispersed with 0.25% trypsin-0.02% EDTA solution and stained with trypan blue 0.4%; unstained viable cells were counted under a bright-field microscope using a hemocytometer.

Transmission electron microscopy. CHO, Hep 2 and fibroblasts cells treated with 20%, 65% and 90% deuterated media for 3 days were fixed for 5 minutes in 2.5% glutaraldehyde in 0.1 m cacodylate buffer, pH 7.4, postfixed for 10 minutes with OsO_4 in the same buffer, serial dehydrated with ethanol and propylene oxide and included in Epon 812. The probes were cut at ultramicrotome and the grids stained with lead citrate and uranyl acetate and visualised at a Philips EM 208 S electron microscope.

3. RESULTS AND DISCUSSION

In the present study, we investigated the effect of heavy water on mitochondrion, which is a highly efficient organelle, essential for the function of cells.

We have chosen the MTT assay and the trypan blue dye exclusion test as indicators of cell viability and proliferation (20). At the same time, the MTT assay could be an indicator of dehydrogenases activities, especially for mitochondrial enzymes. In both methods, the results were expressed as a percentage of control value, in the case of experimental variants treated with heavy water.

The obtained results revealed differences between the two methods of evaluation. In the case of MTT assay, for all the exposed cell types (CHO, Hep 2, fibroblasts), the values obtained for 20% D_2O concentration were higher than the number of viable cells after 3 days of exposure. Also, in the case of media with 65% and 90% heavy water, the values obtained by MTT test were higher (38–45% and 14–19% respectively), compared to the number of cells counted after 3 days (21–27% and 6-8% respectively) (Fig. 1). These results are similar with those obtained by other authors working on digestive cancer cell lines (HepG2, Panc-1, KATO-3, Colo 205 and BALB/c) exposed to D_2O. They observed a higher IC 50 values for MTT assay compared with the trypan exclusion test (21).

The MTT test is based on the reduction of tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to blue insoluble formazan crystals with NADH and NADPH participation. The NADH

dehydrogenases are involved primarily in the energy producing reaction of the respiratory chain: glycolysis, TCA cycle and oxidative phosphorilation. The NADPH dehydrogenases are primarily involved in biosynthetic reductive reactions. These dehydrogenases are located mainly in the mitochondria, and in some cell types a small amount is also present in the endoplasmic reticulum. The sites of the electron transport chain, which are involved in the reduction of MTT dye are proximal to coenzyme Q and cytochrome c (22). The sites and involved enzymes of cellular MTT reduction are yet poorly understood.

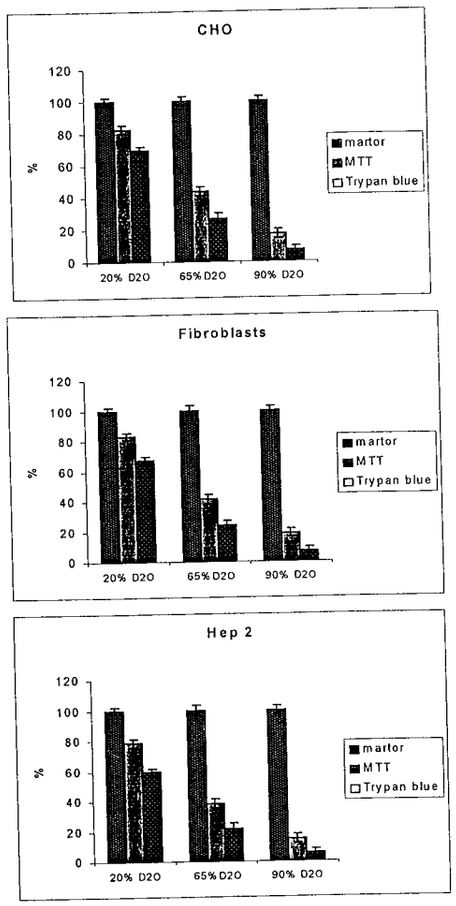

Fig. 1 – Effect of different D_2O concentrations (20%, 65%, 90%) on Hep 2, CHO and fibroblast cells number determined through trypan blue exclusion method and MTT assay.
Data are presented as the percentage of surviving cells relative to the control (150 ppm D).
The values are mean of quadruplicate determination ± SD.

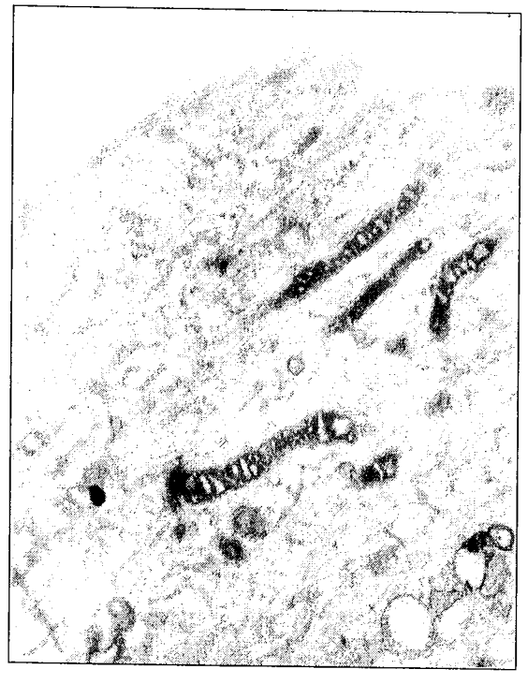

Fig. 2 – Electron microscope image of fibroblast cell mitochondria cultured in medium with 65% D_2O (TEM × 26 500).

MTT assay also was used as an indicator of tetrazolium salt conversion into formazan by mitochondrial dehydrogenases, although some authors questioned the exclusive role of mitochondria in cellular MTT reduction (23). Therefore we considered that the conversion of MTT into formazan generally, is due to dehydrogenases, among them the mitochondrial dehydrogenases play an important role.

In the presence of heavy water in the culture medium, the conversion of MTT into formazan decreased compared with the control, but in a different way than the cell number decreased. It might be considered that heavy water has influenced the MTT reduction acting on dehydrogenases and determining a slight increase of their activities.

At ultrastructural level, the noticed changes depend on heavy water concentrations in the culture medium and the exposed cell type. Thus, at 65% D_2O, fibroblast mitochondria exhibit an increase in mitochondrial matrix density and an ununiform separation of the space between the cristae (Fig. 2). Moreover, some mitochondrial cristae are dilated (Fig. 3).

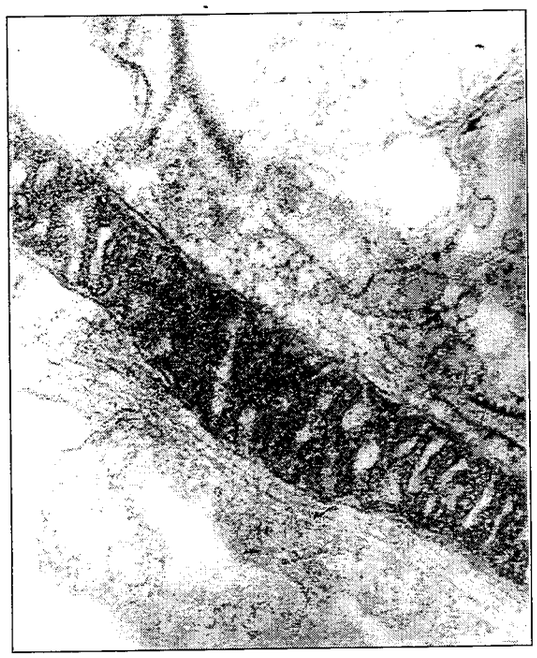

Fig. 3 – Dilated mitochondrial cristae of fibroblast cell exposed to medium with 65% D_2O. (TEM × 78 000).

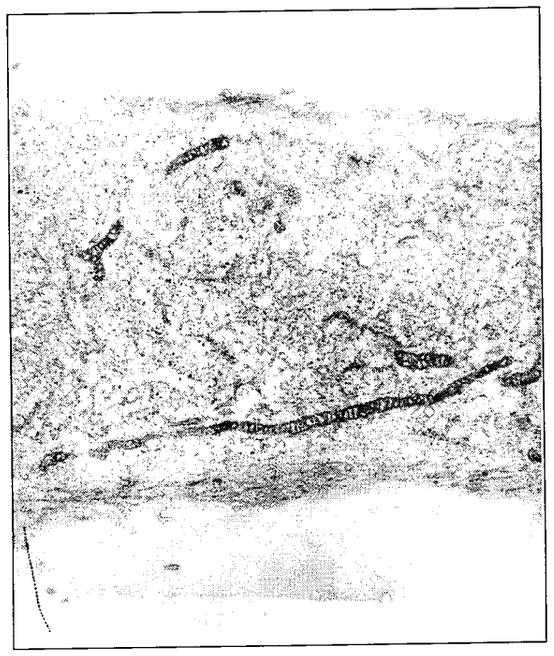

Fig. 4 – Giant mitochondrion in fibroblast cells treated with 65% D_2O (TEM × 10 500).

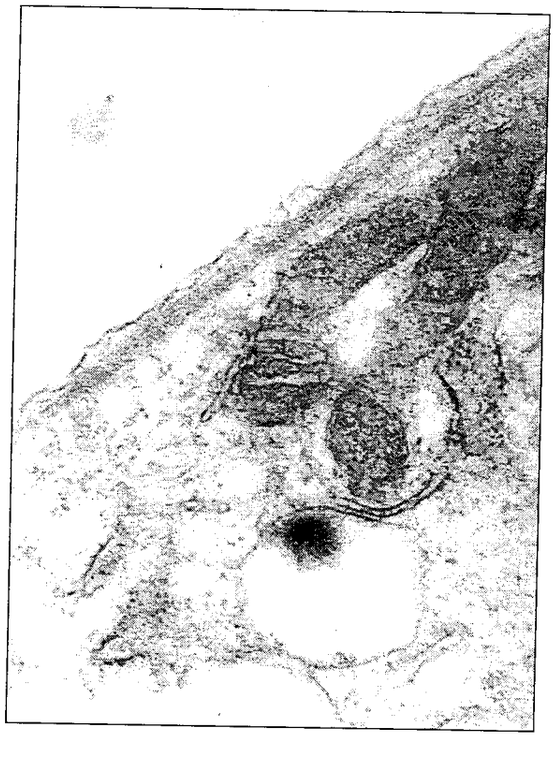

Fig. 5 – Disrupted mitochondrial membranes of fibroblast cells after exposure to medium with 90% D_2O. (TEM × 44 500).

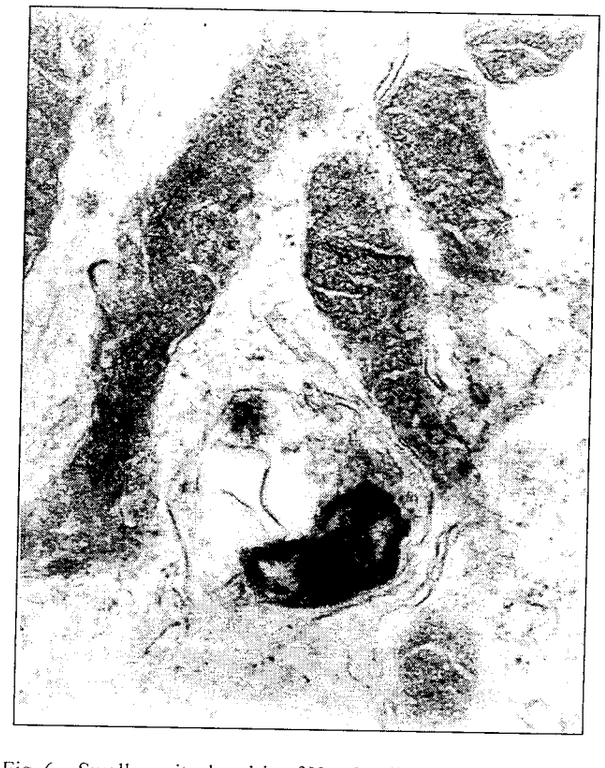

Fig. 6 – Swollen mitochondria of Hep 2 cells exposed to medium with 65% D_2O (TEM × 49 000).

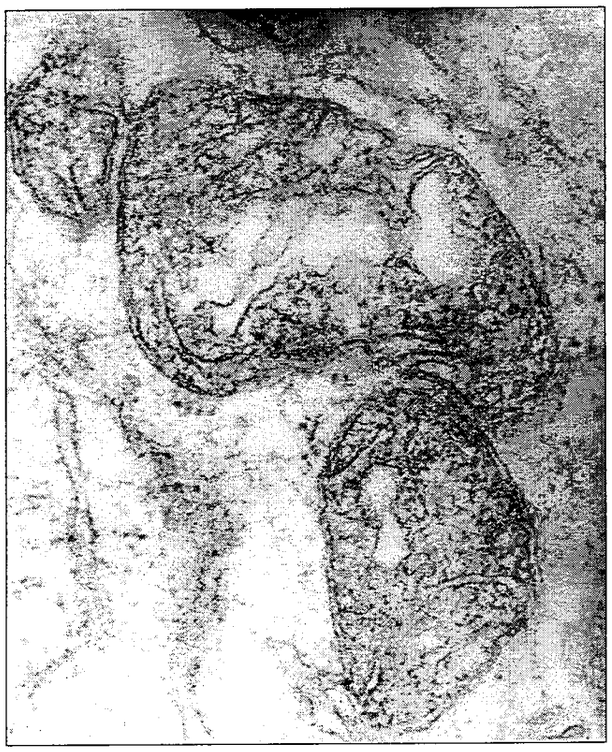

Fig. 7 – Disorganized and dilated cristae of Hep 2 mitochondria after 3 days culture in medium with 90 % D_2O (TEM × 70 500)

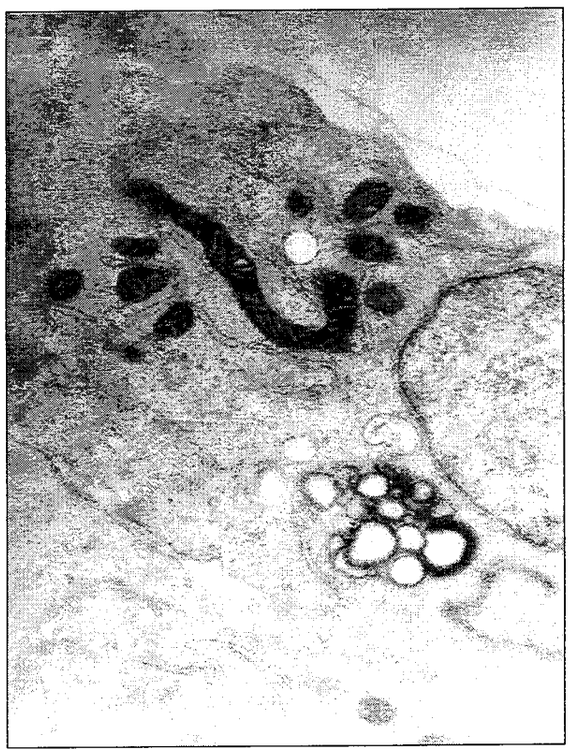

Fig. 8 – Morphological polymorphism of CHO mitochondria. Exposure to 20% D_2O(TEM × 17 600).

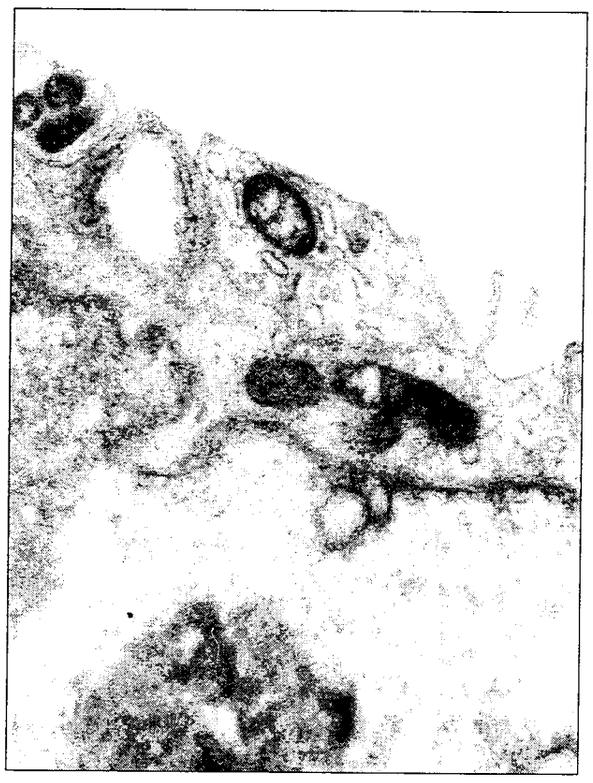

Fig. 9 – Intramitochondrial vacuoles in CHO cells after 3 days culture in medium with 20% D_2O (TEM × 12 000).

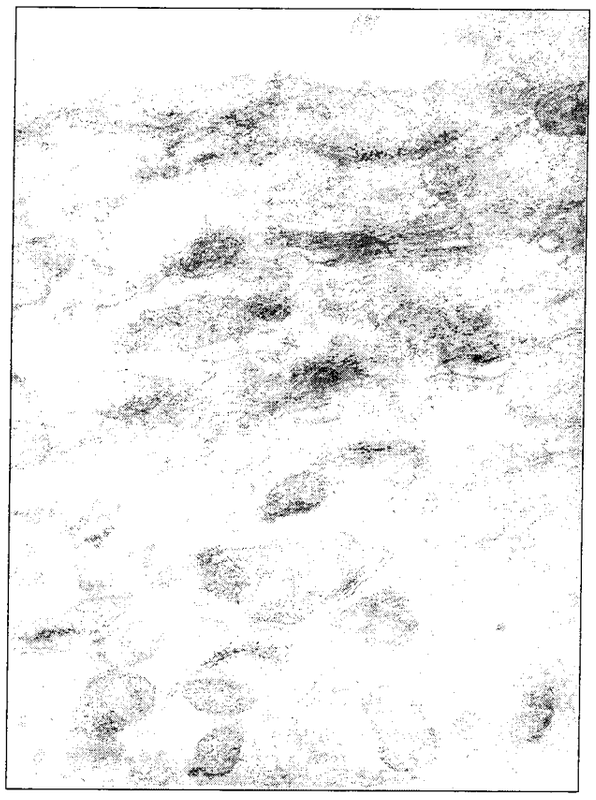

Fig. 10 – Altered mitochondria of CHO cells at 90% D_2O exposure (TEM × 34 000).

A characteristic aspect of these cells response to heavy water is the presence of some very long mitochondria (Fig. 4).

Some of them are "hypertrophic" and these large forms get from fusion of normal size mitochondria. The development of these giant forms was also observed in the case of cell irradiation (24). At 90% D_2O concentration, mitochondrial alterations are more pronounced. One may notice the disruption of mitochondrial membranes and the release of mitochondrial content into cytoplasm (Fig. 5).

In the case of Hep 2 cells exposure to 65% D_2O, mitochondria are swollen and have a bizarre shape (Fig. 6), exhibiting a dense matrix, disorganized mitochondrial cristae and broken mitochondrial membranes. Also in the case of Hep 2 cells, the changes occurred in 90% D_2O medium are more pronounced. Mitochondrial cristae are disorganized and dilated, a reduction in mitochondrial matrix being noticed compared to the previous case (Fig. 7).

CHO cells have a morphological polymorphism of mitochondria (Fig. 8), at 20% concentration exposure, with a lower degree of cristae disorganization and the appearance of some intramitochondrial vacuoles (Fig. 9). At 90% D_2O, their alteration is more pronounced and they exhibit a nonhomogenous matrix (Fig. 10).

The vacuolization and inner and outer mitochondrial membrane disruption were frequently observed after irradiation of different cell types: HeLa, hepatocytes, spleen and adrenal cortex (25, 26). These phenomena are also accompaniated by the translocation of cytochrome c from mitochondria into cytoplasm and cause the caspases activation as an early event that occurs in apoptosis (27). The last data have shown cytotoxic effects exerted by D_2O upon malignant astrocytoma cells (28). The mechanism of D_2O-mediated cytotoxicity involved the induction of apoptosis, which is modulated through the caspase activation pathway (28).

The ultrastructural changes and the cytotoxic effects of heavy water on the cells could be due to a direct action exerted by heavy water on biomolecules through primary and secondary isotopic and solvent effects. The deuteration of biomolecules causes some changes in their metabolic kinetics, particularly in the hydrogen transfer enzymatic reaction due to the double mass ratio of deuterium to hydrogen. The heavy water also has considerably influenced the reaction rate and enzymatic activity in other studied cell systems (hepatocytes) in the case of enzymes involved in glycolysis and gluconeogenesis (29).

In conclusion, the exposure of cells to media with different deuterium concentrations has led to changes in mitochondria structure and activity (the breaking of membranes, vacuolization and swelling, increase in dehydrogenases activity) due to isotopic and solvent effect exerted by heavy water.

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INVESTIGATIONS ON THE ANATOMY AND HISTOLOGY OF SOME INTERNAL ORGANS OF THREE PLATYBUNUS SPECIES (ARACHNIDA, OPILIONIDA)

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MARIA NĂSTĂSESCU**

The paper presents some anatomical and histological aspects of the following organs for three Platybunus species: alimentary canal – pharynx, oesophagus, midgut of *Platybunus pinetorum* (C.L.Koch) 1839, *Platybunus pallidus* Silhavy 1938 and *Platybunus jeporum* Avram 1968; brain of *Platybunus pinetorum* (C.L.Koch) 1839; eye of *Platybunus pinetorum* (C.L.Koch) 1839; internal and external male genitals of *Platybunus pinetorum* (C.L.Koch) 1839 and *Platybunus jeporum* Avram 1968 and excretory system of *Platybunus jeporum* Avram 1968.

Key words: pharynx, oesophagus, midgut, brain, eye, male genitals, excretory system.

1. INTRODUCTION

During time, several anatomical and histological studies in light and electronic microscopy were done for some species of different Opilionid genera. Some of the studies have deep implication in explaining phylogeny (5, 6, 7), others in clearing up Opilionid biology-modalities of feeding, mating, moulting, development (2, 3, 4). We mention the fact that as concerns the Platybunus species, there are few researches regarding their microscopical anatomy.

2. MATERIAL AND METHODS

Adult male specimens of *Platybunus pinetorum, Platybunus pallidus, Platybunus jeporum* were processed by usual histological techniques for light microscopy-they were fixed in Bouin and Carnoy fixators and stained with hemalaun-eosin.

3. RESULTS AND DISCUSSION

The organs studied show the following features:
Alimentary canal
Pharynx

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Platybunus pinetorum – Fig. 1

The pharynx is six corner shaped in transverse section, with a large lumen, lined inside with a thin chitinous cuticule. Two layers of muscular fibres surround the epithelium consisting of one row of cells with clear content and small nuclei:

- circular, contractory musculature;
- radial, dilatatory fibres.

One fat tissue encircles the pharynx.

Oesophagus

Platybunus pinetorum – Fig. 2

The oesophagus penetrates the brain. In transverse section the oesophagus is pharynx like- six cornered shape but with a much narrow lumen. The epithelium has one stratum of cubic, small cells with big nuclei. All over the epithelium there is a thick, fibrous membrane covered with a very thin circular muscular band. The radial, dilatatory muscular fibres lack.

Midgut – median region – ***pars anterior intestinalis***

Platybunus jeporum – Fig. 9

The uniform epithelium of *pars anterior intestinalis* is made up of one layer of columnar cells.

Platybunus pallidus – Fig. 8

The intestinal content covered by a multifilamentous peritrophic membrane can be observed.

The epithelium displays one row of cylindrical cells in exocytosis- producing vacuoles of exocytosis filled with secretion. Most of the vacuoles can be seen in close vicinity of the peritrophic membrane in order to build it up.

Midgut-caeca

Platybunus pallidus

The epithelium is not uniform, but consisting of two types of cells disposed in-groups. In each group, there are small peripherical regenerating cells and tall, cylindrical central cells without striated border. The tall cells are also of two types: some of them, the most numerous, have in their cytoplasm a large number of vacuoles with ferment; the other ones are smaller, their cytoplasm content a less number of vacuoles of phagocytosis- these cells are digestive ones.

Inside the lumen of the caeca, parasite gregarines can be seen – Fig. 7

Brain

Platybunus pinetorum – Figs. 2, 3

The brain displays the features of any arthropod brain: it is covered by one layer of flattened cells with chromatic nuclei. This monostratum gets into the brain, dividing the neurones in several groups, both in supraoesophageal and in suboesophageal nervous masses. Two types of neurones can be distinguished into the supraoesophageal mass:

- very small, granulated neurones placed on the top;

Fig. 1 – *Platybunus pinetorum*
a – the lumen of the pharynx; b – circular, contractory musculature;
c – radial muscular fibres; d – fat tissue.

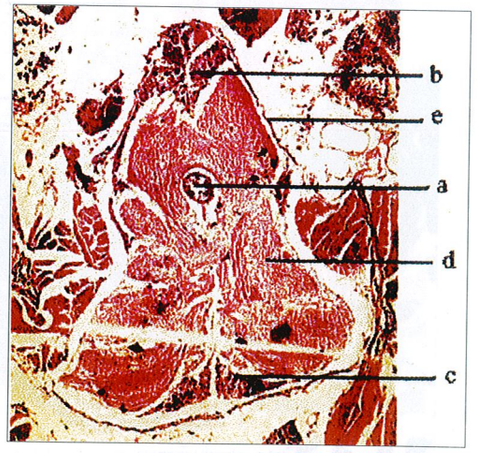

Fig. 2 – *Platybunus pinetorum*
a – oesophagus; b – supraoesophageal nervous mass;
c – suboesophageal nervous mass;
d – neuropil; e – perineurium.

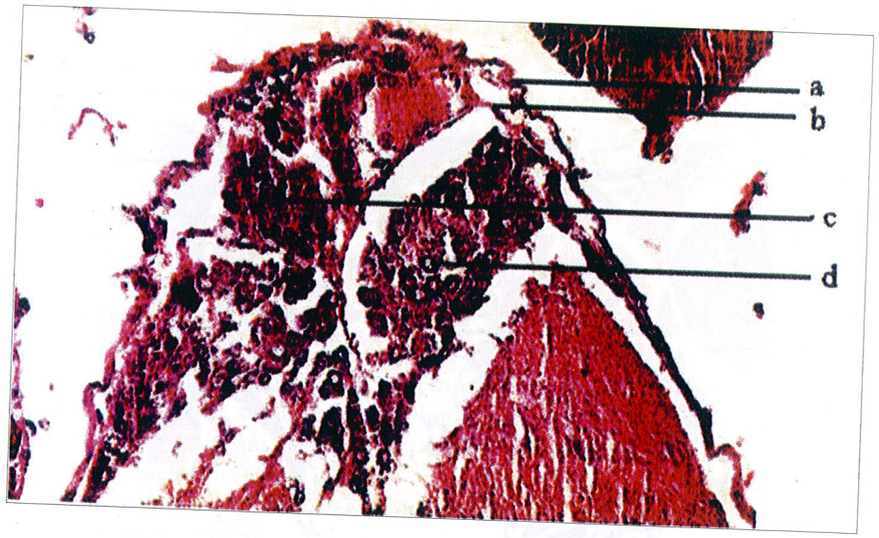

Fig. 3 – *Platybunus pinetorum* supraoesophageal nervous mass
a,b – perineurium; c – granulated, very small neurones; d – sferical neurones of medium size.

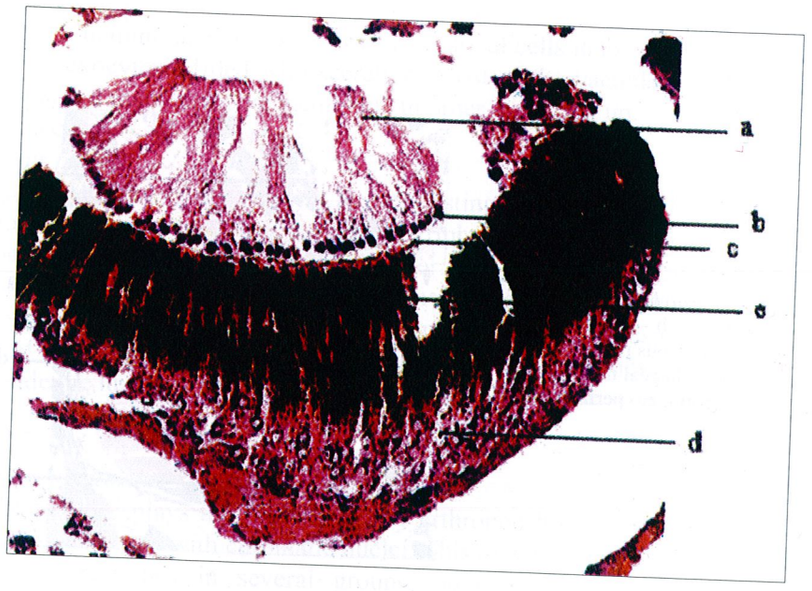

Fig. 4 – *Platybunus pinetorum* eye
a – fibrous lens; b – the vitreous element; c – Graber preretineal membrane;
d – nuclei of the rhabdomeres; e pigment.

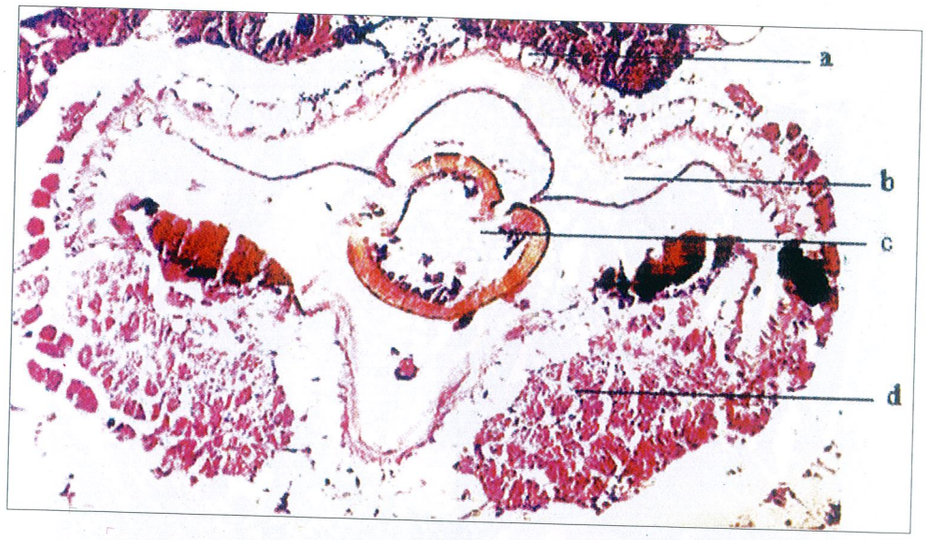

Fig. 5 – *Platybunus pinetorum*
a – the secretory ephitelium of the penis membrane; b – penis membrane;
c – the body of the penis; d – musculature.

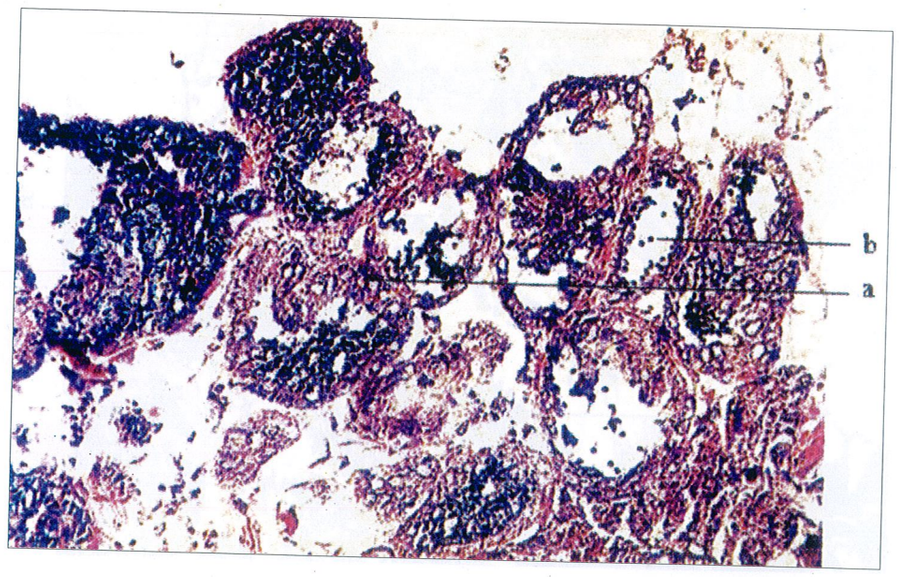

Fig. 6 – *Platybunus pinetorum* spermiducts
a – the epithelium of the spermiducts; b – aflagelated rounded spermatozoa.

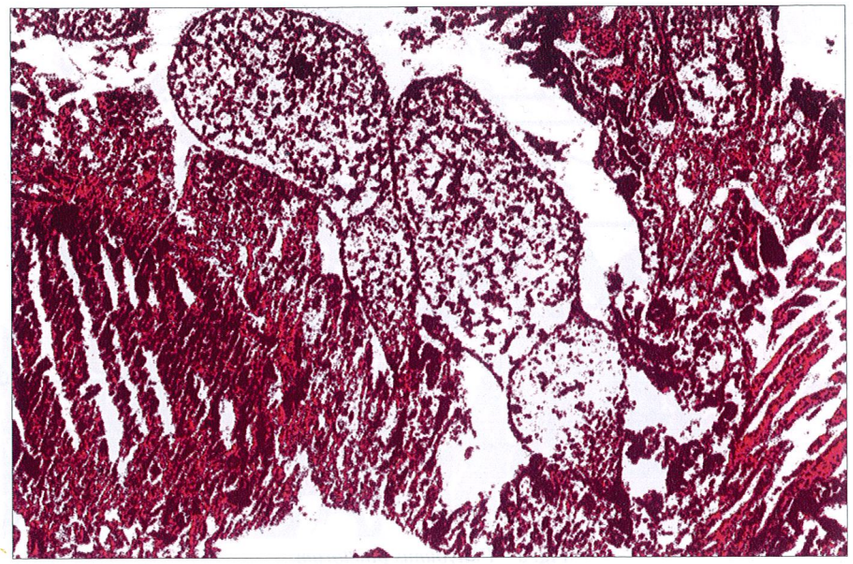

Fig. 7– *Platybunus pallidus* gregarines in the midgut caeca.

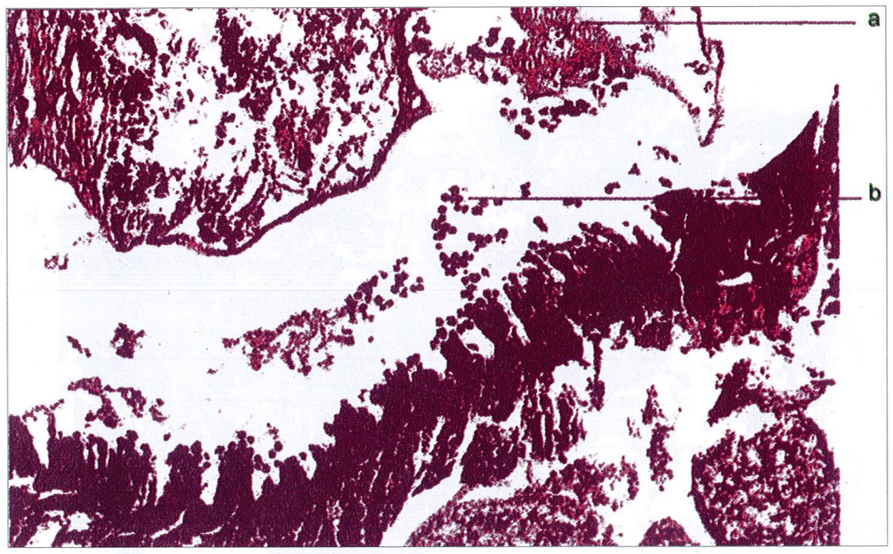

Fig. 8 – *Platybunus pallidus* midgut median region *pars anterior intestinalis*
a – peritrophic membrane; b – vacuoles of exocytosis produced by the uniform epithelium.

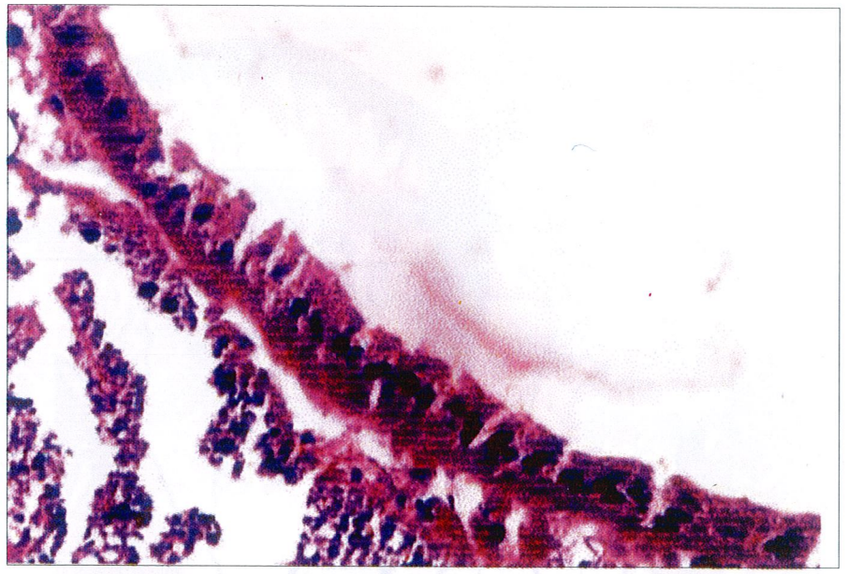

Fig. 9 – *Platybunus jeporum* epithelium of pars anterior intestinalis.

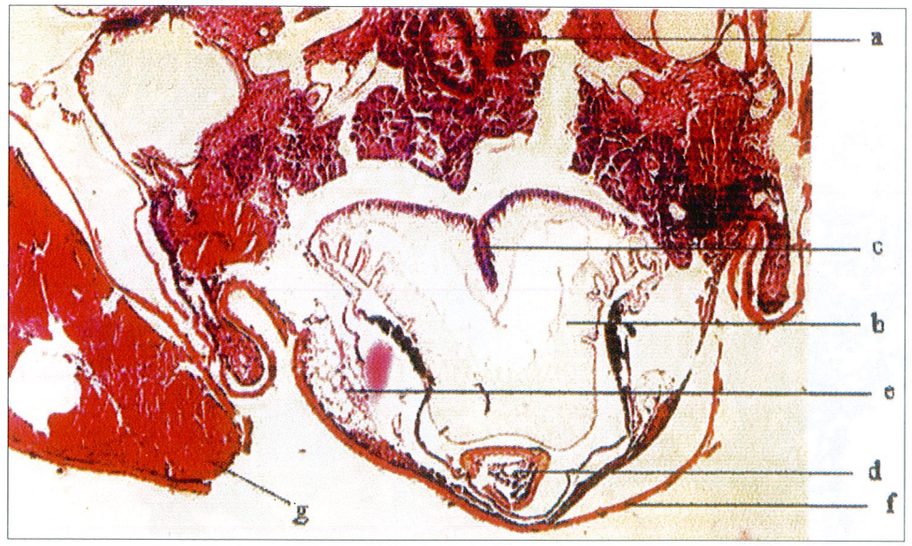

Fig. 10 – *Platybunus jeporum* male reproductive system
a – spermiduct; b – penis membrane; c – generator epithelium of the penis membrane,
d – the body of the penis; e – fat tissue; f – chitinous membrane; g musculature of the coxa.

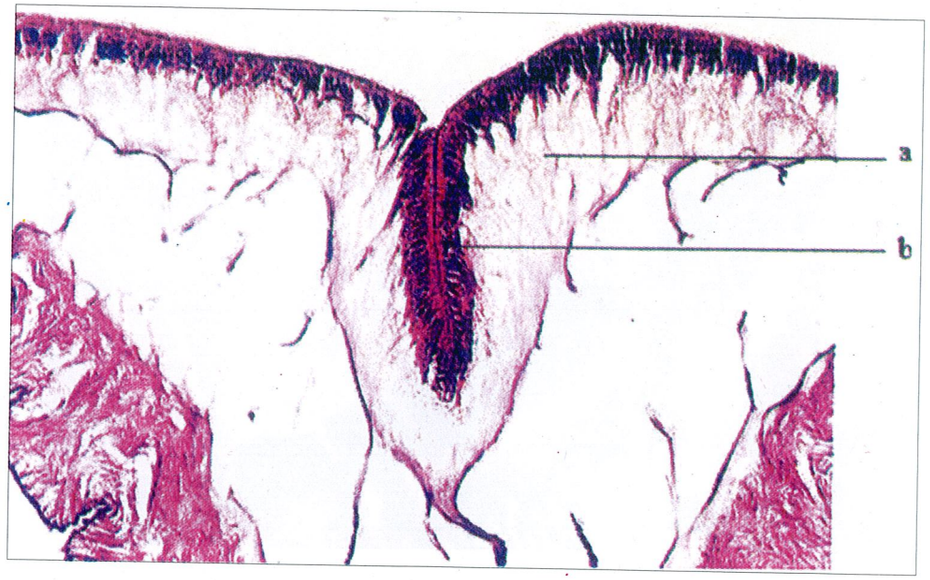

Fig. 11 – *Platybunus jeporum* penis membrane
a – fibrous tissue of penis membrane; b – secretory epithelium of penis membrane.

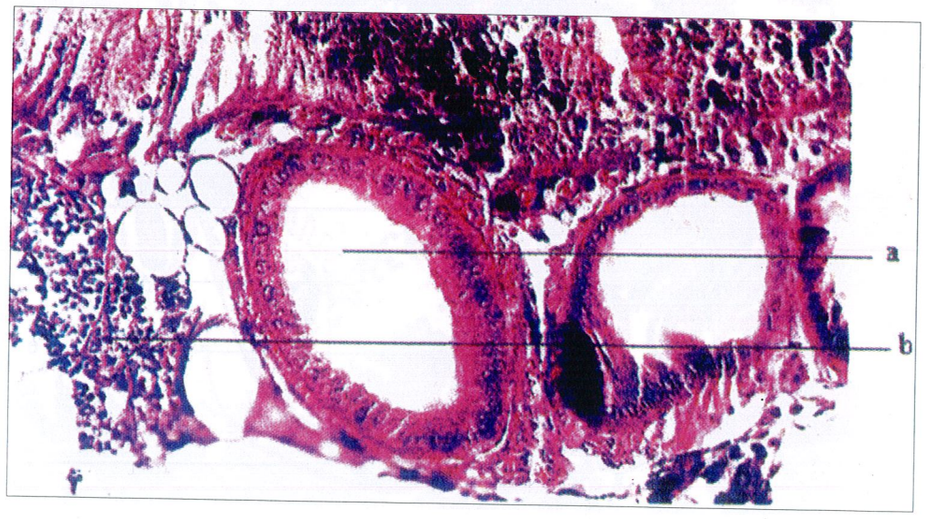

Fig. 12 – *Platybunus jeporum*
a – deferent duct; b – testis–cysts with sex cells.

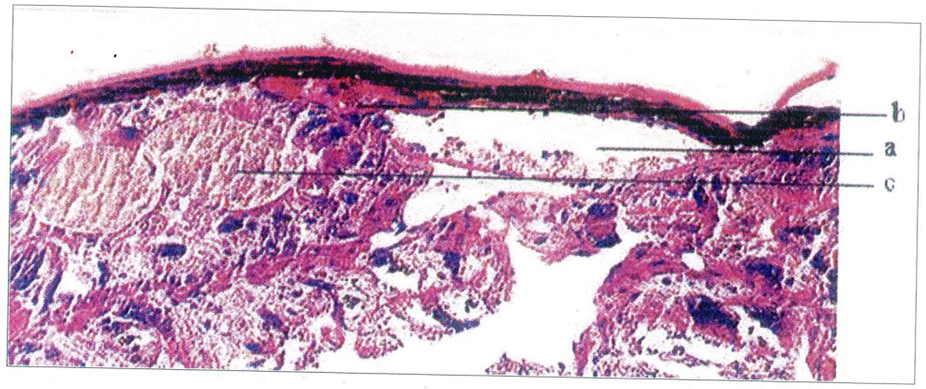

Fig. 13 – *Platybunus jeporum*
a – the cavity of heart; b – alary muscles; c – group of two nephrocytes = pericardial cells.

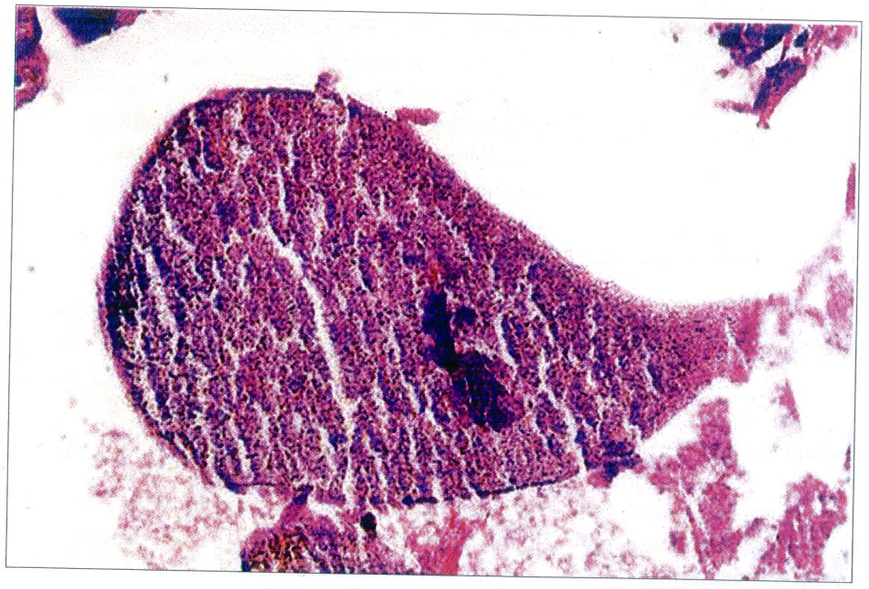

Fig. 14 – *Platybunus jeporum* – large nefrocyte with granular content.

- spherical neurones of a medium size.

Into the suboesophageal nervous mass, stand out groups of large neurones, staining deeply with H-E, with ample GER – suggesting an intens neurosecretory activity.

Eye

Platybunus pinetorum – Fig. 4

The eye is composed of all components of a simple tuber-oculated Opilionid eye:

- a fibrous lens with fibers disposed on the same direction of the rhabdomeres;
- the vitreous element – one row of short cells with deeply stained nuclei;
- a very thin preretineal membrane = the Graber membrane;
- retina consisting of one row of very tall retinal cells – rhabdomeres containing a large amount of pigment lying to near vicinity of the basal nuclei. On the lower part of the rhabdomeres there are groups of rounded small cells with no precise function.

Internal and external male genitals

Platybunus pinetorum – Figs. 5, 6; *Platybunus jeporum* – Figs. 10, 11, 12

There is only a shade of difference between the two species: that is the shape of penis in cross section. Otherwise, all the main structures display a great resemblance:

1. one testis with cysts of sex cells. In every cyst the sex cells are in the same stage of development – spermatogonia, spermatocytes, spermatids; aflagellated rounded spermatozoa can be found into the large genital ducts;
2. different types of spermiducts: narrow efferent ducts, deferent duct with a larger lumen, the seminal reservoir;
3. the penis- enveloped into a membrane with a secretory epithelium;
4. no copulatory muscular organ was put in evidence, but two muscular bands on both sides of the penis;
5. the epithelium of the efferent ducts displays the features of a secretory epithelium-producing the seminal plasm: the large size of nuclei with visible nucleolus, the deep basophily of the cytoplasm.

For *Platybunus jeporum* stand out the penis membrane and its epithelium. The membrane is very thick and the fibers of different thickness intersect one to each other and build up a sort of net. The epithelium that generates the membrane has one row of tall basophilic cells.

Excretory system

Platybunus jeporum – Figs. 13, 14

It is to remark the lack of Malpighian tubes. One pair of twisted tubular coxal glands represents the excretory system. Enormous storage excretory cells-nephrocytes=pericardial cells - with granulated contents are also present. They are symmetrically arranged near the alary muscles in two groups of two cells each group, but they are also scattered in other region of the body, forming two symmetric groups of smaller, numerous and closely packed cells. Probably they come from blood cells, incapable of migrating in blood stream, so occupy fairly constant positions. They are most apt to accumulate waste substances then they may breakdown and be consumed by phagocytes (8). By their granular content they can be easily confused with gregarines.

4. CONCLUSIONS

I. On the basis of bibliographical data as well as on own investigations, we can conclude that the following anatomical and histological features, being similar to a great number of Opilionid species belonging to different genera, can be used by now only for description of Opilionid as a higher taxa-order.

1. the existance of a pharyngeal pump,a suction region of the pharynx; the oesophagus has no dilatatory muscles and penetrates the brain;
2. the uniform epithelium of pars anterior intestinalis, made up of one stratum of cylindrical cells with secretory properties;
3. the epithelium of the midgut caeca consisting of three types of cells: cylindrical cells producing ferment droplets, cylindrical digestive cells and regenerating small cells;
4. the lack of striated border at any level of the intestinal epithelium;
5. the presence in the adult brain of some neurosecretory cells, controlling the cystic spermatogenesis;
6. the lack of pigmentary cells in the eye;
7. the lack from the excretory system of Malpighian tubes, but the presence of some large cells with granular cytoplasm and inclusions serving to a storage excretion;

II. The cystic spermatogenesis, the rounded aflagellated spermatozoa, the lack of a copulatory organ and the presence of two muscles on both sides of the penis.

III. The suction region of the pharynx and a non-dilatatory oesophagus penetrating the brain suggest a way of feeding similar to related Araneae Order, that is: an incomplete extracorporal digestion by spraying on prey some enzymes, liquefaction and sucking up the result. Because the oesophagus has no dilatatory muscles, Opilionid cannot swallow fragments of food larger than oesophagus diameter. The oesophagus can not distend into the brain.

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STRUCTURAL CHANGES CAUSED BY INSECTICIDES ACTION UPON THE FEMALE AND MALE GENITAL SYSTEM OF BOTHYNODERES PUNCTIVENTRIS GERM. (COLEOPTERA CURCULIONIDAE)

VIORICA MANOLACHE

Female and male genital systems of *Bothynoderes punctiventris* were maintained for 4–24 h in the insect haemolymph to which there have been added either Lindan or Furadan 10 insecticides in 0.05% and 0.1% doses.
Structural changes were more strong in ovaries as compared to testis. Alterations were found particularly in follicular cells, but in oocytes as well. Due to its sensitivity, the follicular epithelium represents a sensitive, selective barrier for the oocyte.
In 0.1% dose the insecticides affect the insect reproduction.

1. INTRODUCTION

Insecticides have a strong toxic action on animal and human organisms. Nevertheless, in fighting the pests in agriculture, together with biological, physicomechanical methods, the chemical methods are used as well, thus constituting methods of an integrated fight aimed at the destruction of pests, or at the limitation of their invasion.

A great number of investigations were carried out in insects, revealing the fact that pesticids diminish or abolish the insects prolificity and disturb the eclosion. This fact was also signaled in *Bothynoderes punctiventris* by (5).

Among the researches studying the insecticid action on insects, a particular role is played by those describing histological changes in various tissues. Thus, it may be showed the toxicity degree of the insecticid doses and one may establish propitious time to apply the treatments.

By direct application of Heptaclor and Phosphotox to *Bothynoderes punctiventris* in sublethal and lethal doses at various periods of time of the year, changes were found in female nervous and genital tissues (9).

On the other hand, we consider of particular interest the investigations showing in vitro insecticides influence on animal tissues (1, 4).

In the present work, we aim at revealing the structural changes in *Bothynoderes punctiventris*, a pest of sugar beet cultures, caused by Lindan and Furadan 10 which act on female and male genital system maintained in vitro. In this situation the insecticide acts directly on the hemolymph and thus on the genital tissue.

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2. MATERIALS AND METHODS

Bothynoderes punctiventris Germ. specimens were maintained under normal laboratory conditions for two weeks. Afterwards, microdissections were performed and female and male gonads were collected. They were maintained for 4–24 h in a medium consisting of the insect haemolymph to which there were added either Lindan or Furadan of 0.05 % and 0.1 % doses.

The treated gonads and those from control specimens were fixed in Bouin fixator and processed by histological methods for light microscopy. The sections were stained with hemalaun – eosin.

3. RESULTS

Insecticid action on *Bothynoderes punctiventris* ovary.

Bothynoderes punctiventris as other Coleopteres (2, 3) ovaries consist of meroistic type of ovarioles formed of germarium and vitellarium. Germarium continues to the apical end by a terminal filament and distally by vitellarium. In germinarium, the oogenesis process begins and there oogonia, follicular cells and nutritive cells occur.

In vitellarium, previtellogenous ovarian follicles and then the vitellagenous ones formed. Mature ovocytes are eliminated into oviduct.

Lindan and Furadan 10 insecticides caused the same structural changes no matter the used dose.

We describe the changes in ovary and testis after 24 h in haemolymph with insecticides doses, because after 4 h no significant modifications were noticed.

The influence of 0.05 % dose on ovary at 24 h.

No pronounced alterations are found in germarium. In some areas, some contracted oogonia are noticed. Towards the distal end of germarium, sometimes the process of ovarian follicles formation is blocked. The oocytes have the tendency to agglomerate towards vitellarium, but they do not appear to be degraded (Fig. 1). The alterations appear in previtellogenous follicles. Thus, the follicular cells hypertrophy and their nuclei become pycnotic. Oocyte cytoplasm is sometimes vaccuolised and nuclei have an agglutinated chromatin (Fig. 2). Vitellogenous follicles are less affected. Mature oocytes will be eliminated into oviduct and as in the case of control insects, the corpus luteus forms.

0.1 % dose action on ovary for 24 h.

The observed structural changes are more pronounced than at 0.05 % dose. With this dose, also the ovarioles are of normal aspect, but with obvious alterations. In germarium, the oogonia are smaller than in controls (Fig. 3). Their nuclei are smaller, more chromatic and sometimes they are deformed and even pycnotic. It is suggested that this dose inhibits oogonia growth and therefore the oogenesis is affected.

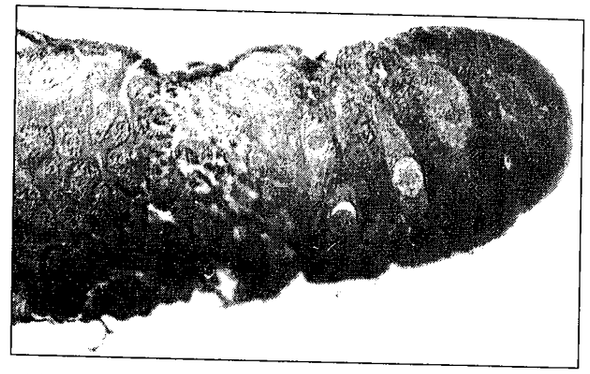

Fig. 1 – The distal end of the germarium at 24 h under Furadan 0.05% action on Bothynoderes (20 ×0.40).

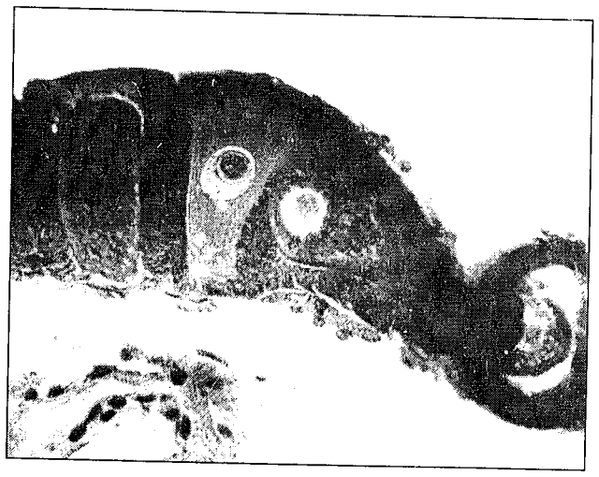

Fig. 2 – Vitellarium with ovarian folicles – 24 h under Lindan 0.05% action (20 × 0.40).

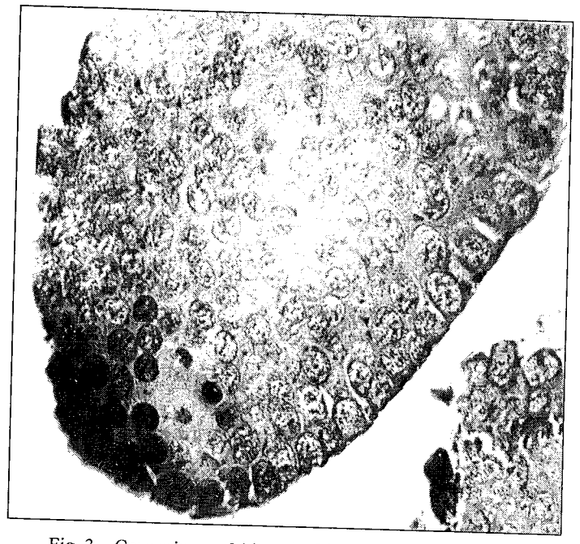

Fig. 3 – Germarium at 24 h under Linadan 0.1% (40 × 0.65).

Fig. 4 – Vitellogenous follicle at 24 h under Furadan 0.1% (40 × 0.65).

Fig. 5 – Seminiferous tubules with isogenic cysts. Action of Furadan 0.05% at 24 h (20 × 0.40).

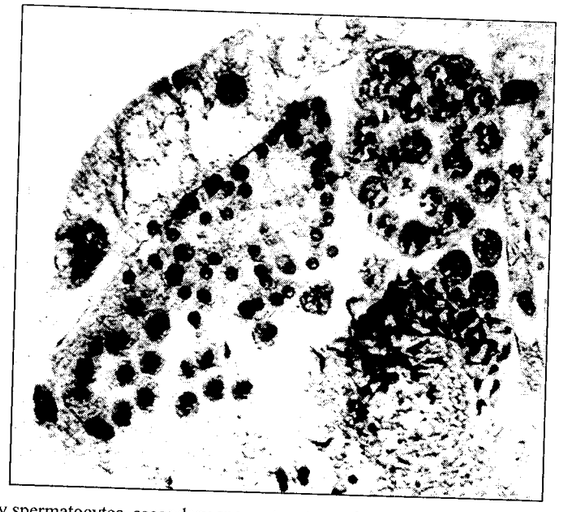

Fig. 6 – Primary spermatocytes, secondary spermatocytes and spermatids were spread by destruction of the cysts walls. The action of Furadan 0.05% at 24 h (40 × 0.65).

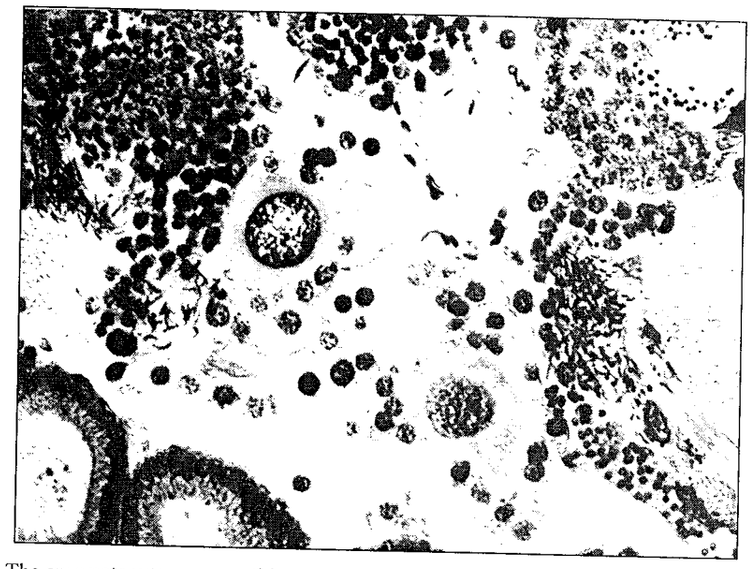

Fig. 7 – The spermatocytes, spermatids were spread. A poliploid cell with phagocytose rol role was observed. Action of 0.1% Lindan after 24 h. (20 × 0.40).

Fig. 8 – The spermatids and spermatozoa were altered (20 × 0.40). The action 0,1% Lindan after (20 × 0,40).

Other oocytes agglomerate abnormally and they are of a smaller size. In cytoplasm, sometimes some vacuoles are noticed and nuclei have chromatin disposed at periphery. In previtellogenous follicles, the follicular epithelium proliferation occurs. The follicular cells are columnar and their nuclei are pycnotic. Oocytes have a vacuolised cytoplasm and their nuclei chromatin is destroyed. The flow of nutritive material from follicular cells towards oocyte being sometimes discontinued, oocytes, deformation occurs as well.

Vitellogenous follicles, as in the case of 0.05% dose, are not too affected. Sometimes, follicular cell hypertrophy as well as nuclei pycnosis are noticed (Fig. 4).

Vitelline granules are not affected. One can find the corpus luteus presence, although the oocyte elimination is less accomplished. We can state that the oogenesis was less affected.

Insecticide action on *Bothynoderes punctiventris* Germ testis.

Bothynoderes punctiventris testis consists of seminiferous tubes. In the seminiferous tubes the sexual cells of spermatogenetic series are disposed in isogenic cysts (Fig. 5). Bothynoderes male sexual cells are less affected by insecticides as compared to the female ones.

With *0.05 % dose of insecticide*, the sexual cells in seminiferous tubes are not affected.

The influence of 0.1% dose of Lindan or Furadan 10.

With this dose little changes begin to be noticed, particularly at the level of cysts with primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 6). The connective walls surrounding the cysts are sometimes destroyed and the sexual cells spread (Fig. 7). Other times, one may notice some confluent germ cells and thus the cell limits disappear. Spermatozoa are degraded as well. The degraded cells are phagocyted by the nutritive cells present in the seminiferous tubes (Fig. 8). Even by these small changes found also in testis, we can say that insecticides destroy a great number of sexual cells.

4. DISCUSSION AND CONCLUSIONS

Treatments with insecticides cause more pronounced structural changes in female genital system compared to the male one. Among the ovariole cells, the follicular cells are particularly affected, having variation of size, shape, cytoplasm and nuclear alterations. The follicular epithelium proliferation was noticed too.

Our results are similar to those obtained by (9, 10) in Bothynoderes by direct application of Lindatox and Fosfotox on insect, as well as in *Nauphoeta cimerea* and *Tanymecus dilaticollis* by Furadan 75 action on female gonads maintained in vitro. Follicular cells react more strongly to insecticides and oocyte reaction represents a secondary phenomenon. The follicular epithelium represents a

selective, sensitive, barrier controlling the passage of substances from haemolymph towards ovocyte.

Following the histological alterations undergone by the follicular epithelium as well as by the perineurium epithelium, as it was found by (8), they represent indexes used to evaluate the toxicity degree.

The structural changes occur throughout the whole vitellarium length, thus diminishing the number of ovarian follicles. The insects prolificity is affected.

Similar, structural aspects were also noticed by Ramade (6) on *Musca domestica* and by Riviere (7) by experiments on Periplaneta. The Lindan influencing not only the insect prolificity decrease, but also the degradation of previtellogenous follicles.

Male genital system is less influenced by Lindan and Furadan 10 doses used by us during the period of time the gonads were exposed.

Nevertheless, by affecting the spermatocytes and spermatids, the number of normal spermatozoa is smaller.

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STRUCTURAL AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY CHANGES INDUCED BY MANGANESE ACUTE INTOXICATION IN *CARASSIUS AURATUS GIBELIO*

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Manganese, an element widely distributed on earth, is a micronutrient, but elevated concentrations could be toxic to aquatic organisms. Mn (II) is a scavenger of superoxide radical and activates a variety of enzymes, but prolonged Mn (II) exposure induces the formation of reactive oxygen species leading to impairment of antioxidant system. The glucose-6-phosphate dehydrogenase catalyses the oxidative branch of the pentose phosphate pathway, generating NADPH, is known as a sensitive target to oxidative stress. After acute exposure to 0.5 mg Mn^{2+}/l Carassius auratus gibelio developed specific adaptative responses for each tissue, neutralizing oxidative stress. Because the epithelial layer of kidney, intestine and gill are in direct contact with the pollutant, the necessity of reduced glutathione and NADPH increases and the histochemical reaction for G6PDH is higher in the epithelial layer than in other tissues The pollutant triggers a response soon after the beginning of exposure. Significant effects were noticed on the main tissues involved in the detoxication processes. In liver, kidney and ovary the main pollutant target were the nuclei. These structural changes consisting in karyomegaly, anisokary, pycnosis prove that the metal ion has a deep effect on the nuclear genetic material. The increase of previtellogenic and vitellogenic follicles atresia after manganese acute exposure affects the sexual cells quality, functions and fish reproduction efficiency.

1. INTRODUCTION

The common products of xenobiotic metabolism are the reactive oxygen species and several pollutants perform a part of their toxicity supplying reactive oxygen species ($^1O_2$, $O_2 \cdot^-$, $\cdot OH$, $RO\cdot$, $ROO\cdot$) (17). Most cellular structures are potential targets of oxidative injuries (12). Thus frequently DNA injury (16, 11), lipid peroxidation (12, 26), protein damages (3) and methemoglobin yield (13), occur. Cell sensitivity to oxidants is diminished by antioxidant enzymes: superoxid dismutase, glutathione peroxydase, catalase, glutathione reductase and glucose-6-phosphate dehydrogenase. Oxidative stress appears as a result of xenobiotic action

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inducing perturbations in antioxidant enzymatic systems. The effects induced by the oxidative stress as well as the antioxidant potential vary depending on species, habitat, alimentary behavior.

Reduced glutathione (GSH), the most abundant cellular thiol, is involved in metabolic and transport processes and in the protection of cells against the toxic effects of reactive oxygen species and heavy metals (24). It is capable of complexing and detoxifying heavy metal cations soon after they enter the cells, thus representing the first line of defense against heavy metal cytotoxicity. Heavy metal accumulation in the cells can therefore result in decreased availability of reduced glutathione, due to both GSH binding and oxidation. At the same time, heavy metals as Cd^{2+}, $Hg^{2+}$and Pb^{2+} have also been demonstrated to increase the concentration of GSH in both mammalian (42, 18) and fish tissues (40), suggesting that *in vivo* metal treatment could interfere with GSH metabolism. Thiols have been shown to enhance DNA oxidation (35) and lipid peroxidation (32), catalyzed by transition metal ions via a thiyl radical (R-S·)-dependent mechanism.

Manganese is an element widely distributed in the earth's crust. It is considered to be the twelfth most abundant element and the fifth most abundant metal. Manganese does not occur naturally in a pure state; oxides, carbonates and silicates are the most important manganese-containing minerals.

Manganese in surface waters is a micronutrient, but elevated concentrations are toxic to fish and impair drinking water quality (15).

Despite the fact that manganese is considered an essential element, due to its participation in several enzymatic reactions (either as a cofactor or as an integral part of the enzymatic protein) (10), it can be proved to be toxic in case of overexposure, the main entry route being the gastrointestinal tract and the respiratory tract (39). The main sources of overexposure to manganese are extraction and crushing of Mn ores, iron, steel industry and metallurgical processes, fabrication of dry batteries, the use of pesticides in agriculture.

Mn (II) is reported to be a scavenger of superoxide radical (2). It reduces ·OH to yield $(OH)^{2+}$ (6) and activates a variety of enzymes (41). Prolonged Mn(II) exposure induces the formation of reactive oxygen species according Fenton reaction, leading to impairment of antioxidant system (8). But it appears that the antioxidant enzymes respond to the effects of Mn(II) differently compared to the results obtained with other metal ions (27).

The glucose-6-phosphate dehydrogenase catalyses the oxidative branch of the pentose phosphate pathway, generating NADPH, an electron donor in reductive biosyntheses, which is used for GSH regeneration. This enzyme is known also as a target sensitive to oxidative stress (22).

Our aim was to measure the effect of 0.5 mg Mn^{2+} /l acute exposure by histopatological, histochemical and biochemical studies on *Carassius auratus gibelio* tissues.

2. MATERIALS AND METHODS

Chemicals. Manganese dichloride (tetrahydrate, $MnCl_2 \cdot 4H_20$) was supplied by Fluka. All the other chemicals were supplied by Merck. Ultrapure water (Milli-Q, Millipore) was used for the preparation of all the solutions.

Animals. Freshwater goldfish *Carassius auratus gibelio* of length 13.5–16.5 cm and weight 20.0–30.0 g, were obtained from Fishery Research Station Nucet and housed in a 60 l glass aquarium at 25°C. Fish of a single lot were used throughout the investigation. Prior to exposure, fish were held for 15 days for acclimatization and evaluation of overall fish health under laboratory conditions.

Experimental protocol. For the intoxication experiments, $MnCl_2$ in dechlorinated tap water was added until a final concentration of 0.5 mg Mn^{2+}/l was obtained. During the experiments, fish were exposed for one, two, three and seven days. Ten individual lots were used for every period of exposure. The control one was kept in a glass aquarium filled with dechlorinated tap water. During the experiment the fish were not fed. After one, two, three and seven days the liver, kidney, intestine, gills and gonads were removed and prepared for histological, histochemical and biochemical analysis.

Histological methods. Small tissue fragments obtained from two years old *Carassius auratus gibelio* were fixed in Bouin three hours long. The prelevated fish pieces were embedded in paraffin and cut in 8μm thick slices which were Hemalaun Meyer-Eosine and PAS stained and examinated by Olympus light microscope.

Glucose-6-phosphate dehydrogenase histochemical localization. The tissue samples were frozen in isopentan and liquid nitrogen, and then 8μ slices were obtained using a SLEE cryotome at 24°C. The slices were incubated for 20 min. at 37 °C in glucose-6phosphate 10 mM, $NADP^+$ 0.8mM, PMS 0.32 mM, $MgCl_2$ 5mM, sodium azide 5mM- NitroBT 20μM-phosphate buffer 100mM pH 7.4 then they were washed, desiccated and mounted in PVP.

Enzymatic assay. Liver and kidney tissues were homogenized in ten volumes of 0.1 M Tris-EDTA buffer, pH = 7.4 and centrifuged at 10 000 × g for 30 min at 0–4 °C. Aliquots of the supernatant were utilized for the spectrophotometric determination of the glucose-6-phosphate dehydrogenase activity. The rate of NADPH formation was a measure of the enzyme activity and it can be followed by means of the increase in extinction at 340 nm (20). The proteic concentration was measured according the method of Lowry (20).

3. RESULTS AND DISCUSSION

The Mn^{2+} -induced histopathological effects in Carassius auratus gibelio tissues. Acute manganese exposure induced significant structural changes in

Carassius auratus gibelio tissues. Hepatic tissue was affected after 48 hours of exposure: some hepatocytes were vacuolized and damaged, frequently karyomegaly (Fig. 1) or less nucleolus nuclei with different affinity for the dyes, were observed. Other hepatocytes presented karyohrhexic or pycnotic nuclei. (Fig. 2). The hipertrophic nuclei contained PAS positive granules. The sinusoids were dilatated containing modified erythrocytes. It seems that manganese pollution induced frequently an eccentric location of the nuclei, hepatocyte vacuolization likely accumulating glycogen or lipids.

The liver is the main organ involved in metabolic and detoxication processes. One of the most important reactions induced by the metal ion is the damage to nucleic acids. DNA is modified by adduct formation with xenobiotic, its metabolites or with products of lipid peroxidation. This may result in misreading during replication at the residue itself and at adjacent positions causing G:T and A:C substitutions (17).

Serious damages in renal tissue after only 24 hours of acute exposure on manganese polluted waters were noticed. Many necrotic urinary tubules having lesions of cubic, or columnar epithelial layer, nuclei modifications (binucleated epithelial cells, pycnotic nuclei, nuclei malformations, anizokaric nuclei, denudated nuclei) can be seen (Fig. 3).

48 hours manganese exposure induced visible gut structural changes. Some epithelial cells were vacuolized and had an increased affinity for the dyes. *Stratum compactum* is folded and has unequal thickness or is broken in some areas (Fig. 4). Seven days manganese exposure induced vilosity cylindrical epithelium disorganization and epithelial cells extended necrosis (Fig. 5). The cytosol affinity for the dye seriously decreased and the intensive vacuolization of epithelial cells were also noticed.

Gills examination revealed rarely the epithelial cells lift, and chloride cell proliferation, frequently hyperplasia of gill epithelium, congested blood cells in lamellae stasis and secondary lamellae fusion occur (Figs. 6, 7). The spaces between the pillar cells connecting the afferent and efferent arterioles are modified. The contractile pillar cells control the lacunas diameter regulating blood flow. The thin lamellar walls allow a straight proximity of the blood with aquatic environment. Due to their hyper osmotic state, the freshwater fishes tend to passively loose ions and to introduce the water by diffusion.

The chlorogen cells, located between secondary lamellae on gills filaments, maintain ionic internal homeostasis. These cells, provided with mitochondria and with a tubular network of smooth endoplasmatic reticulum, actively transport Na^+ and Cl^- against the concentration gradient. They must have a special role in metal ion removal and neutralization.

The gill epithelium lift could be due to the hipoosmotic medium influx through epithelial cells, physiologically affected. (33). This lesion could serve as defensive reaction increasing the distance that the metal have to cross for attaining blood vessels.

Although necrosis of gill epithelium was more often associated with heavy metals pollution (23) we rarely noticed these change on our gills pieces. Our

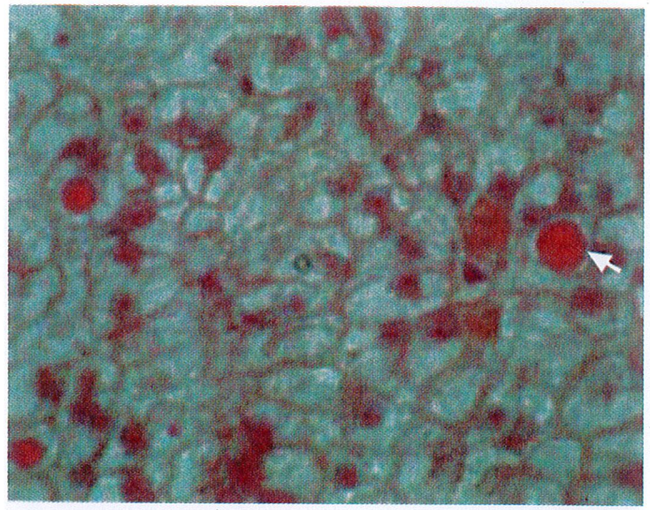

Fig. 1 – *Carassius auratus* liver after 48 hours of manganese acute exposure (H–E). Hepatocyte with karyomegaly or picnotic nuclei (arrow); vacuolized hepatocytes with eccentric nuclei (× 400).

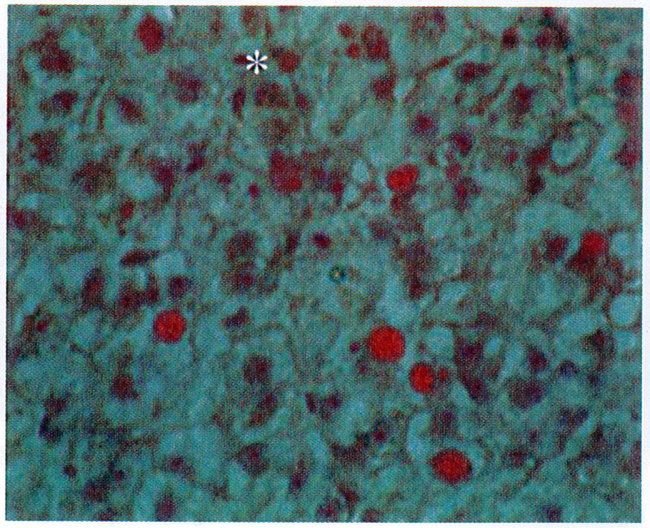

Fig. 2 – Carassius auratus gibelio liver after 48 hours of manganese acute exposure (H–E). Hepatocytes with picnotic nuclei(*)(× 400).

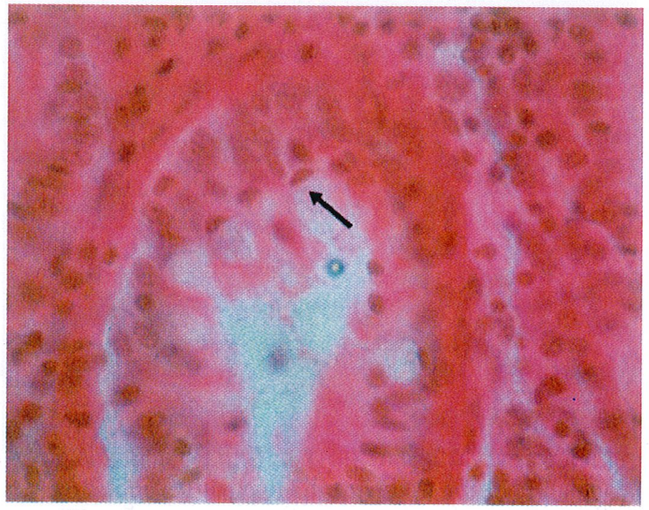

Fig. 3 – *Carassius auratus gibelio* kidney after 24 hours of acute manganese exposure (H–E). Necrotic urinary tubules, with vacuolization (arrow) of cubic or columnar epithelial layer and nuclei changes (× 1000).

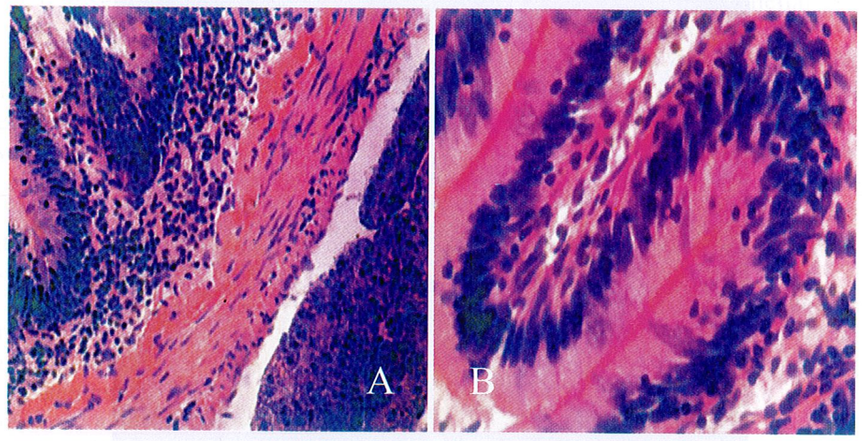

Fig. 4 – Gut structure after 48 hours of acute manganese exposure (H–E)
A – *Stratum compactum* is folded and has unequal thickness (× 200)
B – Epithelial cells vacuolization (× 1000).

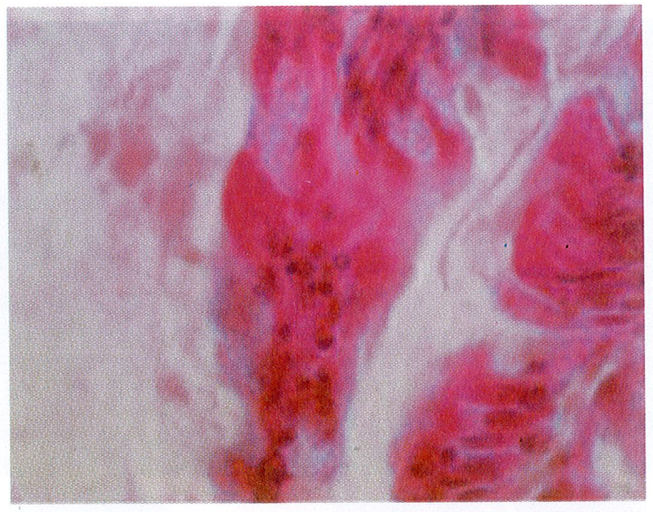

Fig. 5 – Gut structure after seven days of acute manganese exposure (H–E). Disorganisation of cilindric epithelium (× 1000).

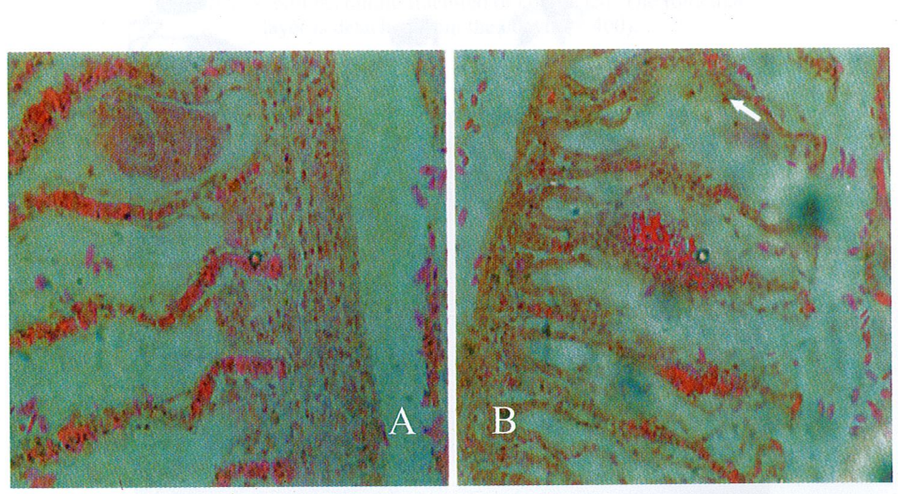

Fig. 6 – Structural gills changes after manganese acute exposure (H–E)
A – Mucous cell proliferation, Mucous secretion (× 1000)
B – Necrosis of gill epithelim (arrow) and lamellar fusion (× 400).

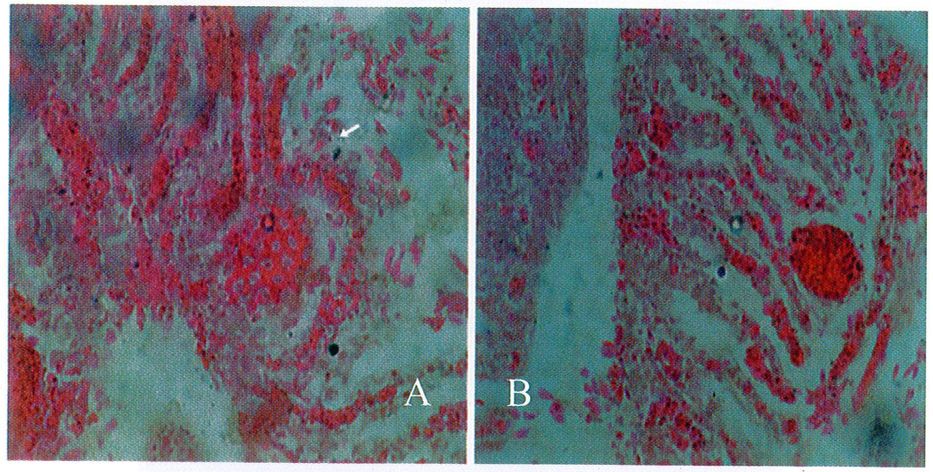

Fig. 7 – Structural gills changes after manganese acute exposure (H–E)
A – Hyperplasia (arrow)(× 400)
B – Lamellar aneurism (× 400).

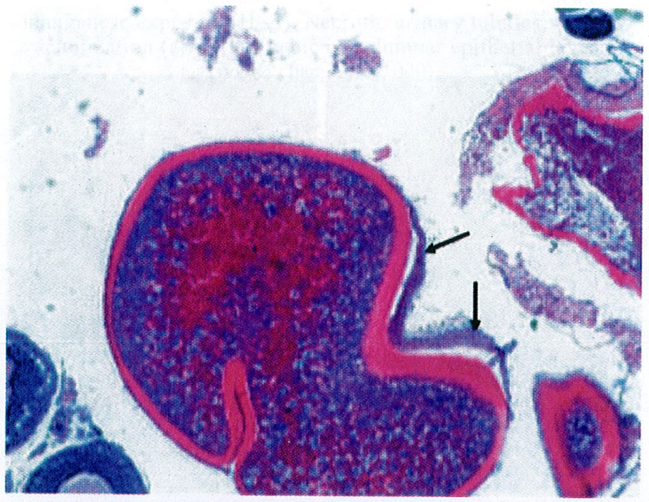

Fig. 8 – Vitellogenic atretic follicle (H–E). *Zona radiata* folding; unequal thickness and disorganization in some areas of *zona radiata*; granulose and thecal layer are detached from *zona radiata* (arrow)(× 200).

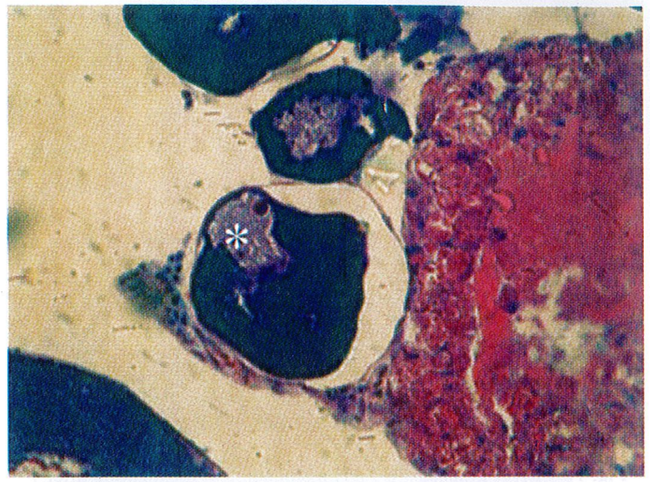

Fig. 9 – Previtellogenic follicle atresia (H–E). The nucleus losses its central location (*) and the nucleols are unequal. The cytosol is intensly basophil becoming fractured or contracted. The follicular layer is detached from the oocyte (× 400).

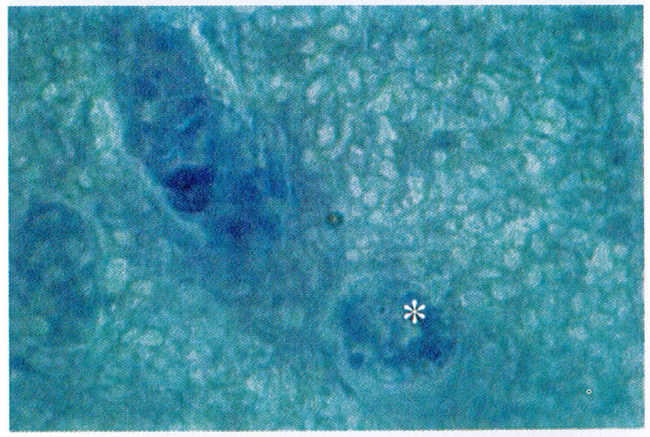

Fig. 10 – Glucose – 6-PDH histochemical localization in control liver (× 1000). Histochemical reaction in macrophages (*).

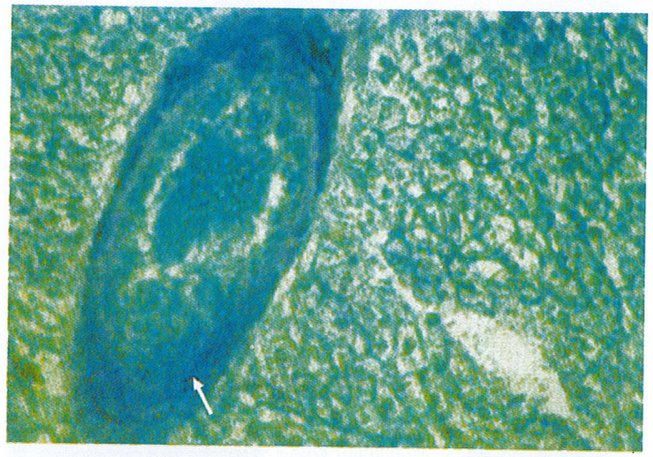

Fig. 11 – Liver glucose – 6-PDH histochemical localization after 7 days of acute manganese exposure. Strong histochemical reaction in hepatocytes citosol in muscular and epithelial layer of the hepatic artery (arrow)(× 400).

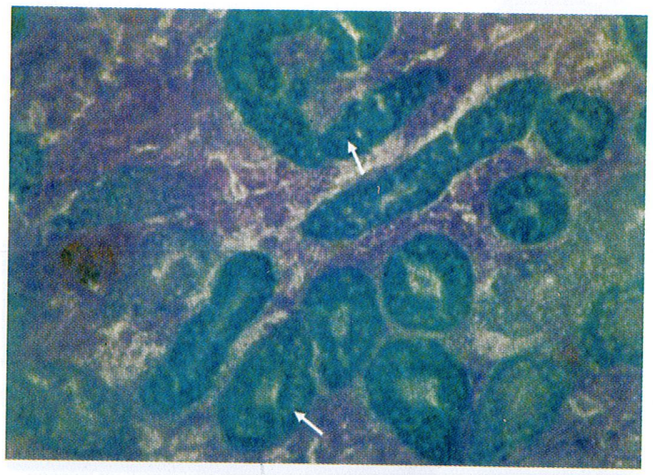

Fig. 12 – Control kidney. The Histochemical reaction is developed in urinary tubules epithelial cells (arrow)(× 200).

Fig. 13 – Seven days kidney intoxication revealed a weak histochemical reaction (× 400).

Fig. 14 – Strong histochemical reaction in control intestinal epithelial layer and in muscular subiacent layer (arrow)(× 200).

Fig. 15 – Stronger histochemical reaction in intestinal epithelial layer after manganese exposure(× 1000).

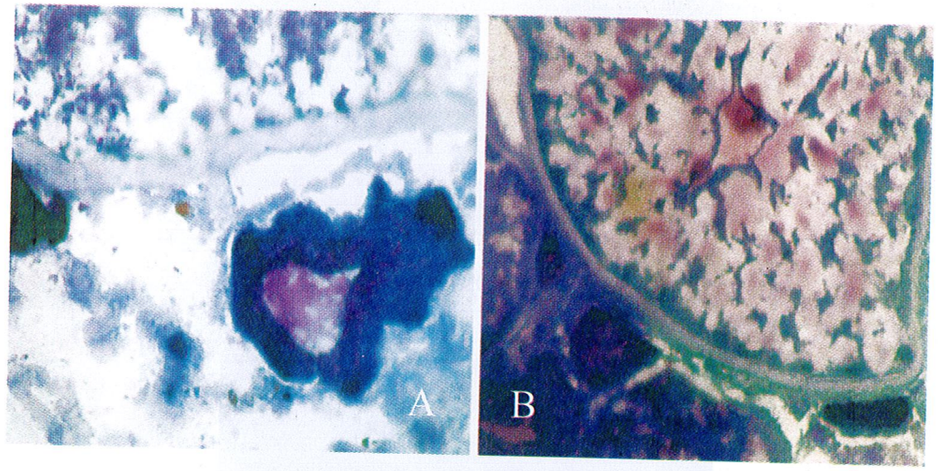

Fig. 16 – Glucose – 6-PDH localization in *Carassius auratus gibelio* ovary.
Strong reaction developed in the oocyte cytosol (× 400)
A – Previtellogenic follicle
B – Vitellogenic follicle.

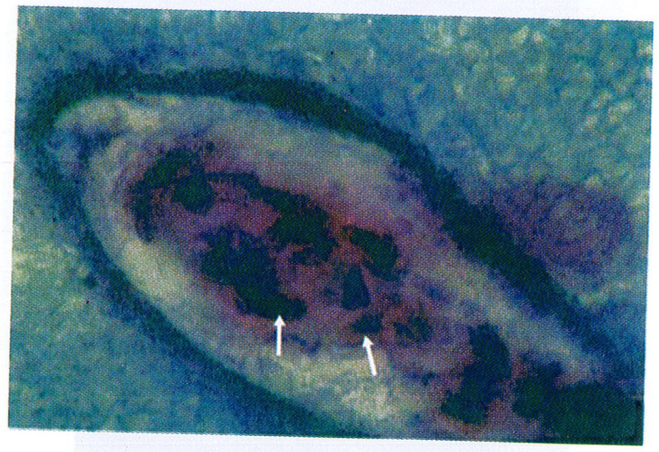

Fig. 17 – Glucose – 6-PDH histochemical localization in *Carassius auratus gibelio* gills (× 1000).

observation are according with Skidmore (35), Lock-van Overbeeke (19), Part-Lock (29) who reported a hyper secretion of branchial mucous branchial cells, alteration frequently associated with heavy metals because mucins, which often are polyanions may be especially effective at trapping metal cations and preventing such toxicants from crossing the gill epithelium. Manganese exposure induced the epithelial rupture and bleeding, lamellar aneurysm, lesions that seem to involve pillar cell disruption (36).

Lamellar fusion could be protective, decreasing the vulnerable gills surface. All these lesions that diminish metal ions intake, induce fish suffocation.

Carassius auratus gibelio ovary contains asyncronous developed follicles. Manganese pollution had induced an increased incidence of atresia in both vitellogenic (Stage V) and previtellogenic follicles (Stage 3) (Figs. 8, 9). Vitellogenic follicle atresia was expressed by *zona radiata* accentuated folding, unequal thickness and disorganization in some areas. *Granulosa* and thecal layer are detached from *zona radiata*. Yolk globules changed their position by invasion of cortical alveoli in the central area. In previtellogenic follicles, oocyte nucleus loses its central location and the nucleoli are unequal. The cytosol is intensely basophil, becoming fractured or contracted. An increase in the number of the atretic follicles was reported after zinc (13), textil-mill effluent (25), acid water (39; 38), lead nitrate (13) copper (13) pollution.

Glucose-6-phosphate dehydrogenase histochemical changes during manganese acute exposure in *Carassius auratus gibelio* tissues

Histochemical localization of glucose 6-phosphate dehydrogenase in control liver revealed no reaction or a very weak reaction in hepatocyte cytosol (Fig. 10). Hepatic tissue presented macrophages with glucose 6-phosphate dehydrogenase intense activity.

Seven day manganese exposure induced a uniform reaction for glucose-6-phosphate dehydrogenase in the hepatocytes cytosol which was stronger in muscular and epithelial layer of the hepatic artery and weaker in subjacent connective tissue (Fig. 11).

The liver G6PD activity increase is a consequence of pollutant toxicity and indicate the operation of hexose monophosphate pathway, since the increased G6PD activity facilitates increased production of NADPH for detoxification processes (28). Several studies revealed an increase of enzymatic activity after pollutant exposure. (31, 32).

In control kidney the histochemical reaction is especially developed, in urinary tubules epithelial cells (Fig. 12). 7 day intoxication revealed a weak reaction in epithelial cells (Fig. 13). We noticed also a strong histochemical reaction in intestinal epithelial layer and in muscular subiacent layer (Fig. 14). Manganese exposure affected the epithelium structure but the intensity of histochemical reaction was higher especially at the apical pole where the contact with the pollutant occurs (Fig. 15).

Our results prove that the enzyme has not a uniform organ distribution but is especially located in the epithelial cells, the first ones contacting the metals ions.

Mn^{2+} induced enzyme activity changes but tissular disorders also appear even after 24 hours of exposure. This ion also stimulated phagocytosis in the liver after 48 hours. This result is according with the Cossarini-Dunier (7) observations. Previous studies sustained that managanese toxicity is due to free radicals release. (4, 9), inducing also mitochondrial energetic metabolism. Mitochondrial disfunction leads to oxidative stress affecting cell defence mechanisms (e.g. gluthathione) and mitocondrial DNA integrity (5).

The histochemical reaction was noticed in the ovary too. It was very intense both in the cortical cytosol of vitellogenic oocytes and previtellogenic oocytes (Fig. 16)

The gills revealed a strong reaction in chondrocytes and a weak reaction in other gill cells types (Fig. 17). The structure of secondary lamellae was seriously damaged.

Effect of Mn^{2+}acute intoxication on glucose-6-phosphate dehydrogenase specific activity in liver and kidney.

Glucose-6-phosphate dehydrogenase is the first enzyme of this pathway. Al-Yousuf et al. (1) noticed that the most important manganese accumulation is in fish liver. About 30% of the glucose oxidation in liver occurs via the pentose phosphate pathway.

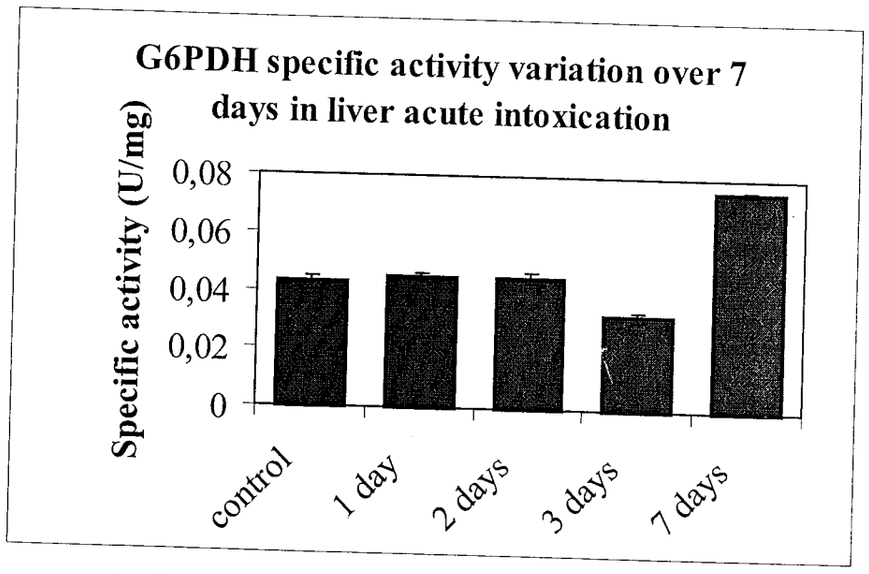

Fig. 18 – Glucose-6-phosphate dehydrogenase specific activity variation over 7 days in manganese acute intoxication of fish liver.

An unsignificant change in liver glucose-6-phosphate dehydrogenase specific activity during the first 2 days, followed by a significant decrease in the third day and a significant increase after 7 days of exposure to the ion, as compared to the

control, occurred (Fig. 18). The significant decrease after the third day of intoxication is probably due to the metal catalysed inactivation of liver glucose-6-phosphate dehydrogenase (22). The ROS stimulated increase of enzyme biosynthesis can be the explanation of the significant specific activity increase of glucose-6-phosphate dehydrogenase after 7 days. The reason could be the necessity for NADPH, used by glutathione reductase to produce GSH, in order to maintain the protein thiols in the reduced state during oxidative stress or to react directly with the metalic ion.

In kidney, a significant increase of the same specific activity after 24 h of exposure, followed by a slight and continuous significant decrease over one week was noticed (Fig. 19). This increase is probably due to the oxidative stress produced by the metallic ion. Mn^{2+} ion can substitute Mg^{2+} in cells. At high concentration Mg^{2+} (and also Mn^{2+}) inhibits the Na^+, K^+-ATPase (30), which modifies the Na^+gradient in the renal epithelial cells. As a consequence, the glucose/Na^+ symport in the apical domain of the epithelial cell can be impaired and a decrease of the intracellular quantity of this monosaccharide could occur. That's why the rate of the first reaction of the pentose-phosphate shunt is probably decreased starting the second day of acute intoxication. Another explanation could be also the attack of the oxygen reactive species on the glucose-6-phosphate dehydrogenase protein.

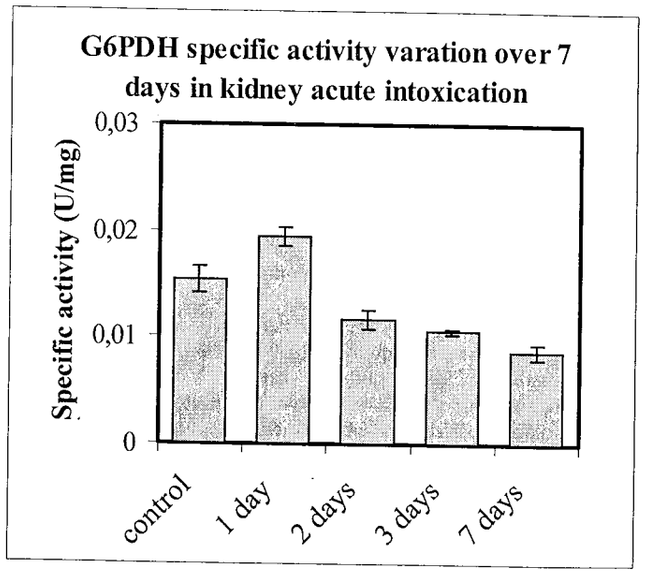

Fig. 19 – Glucose-6-phosphate dehydrogenase specific activity over 7 days in manganese acute intoxication of fish kidney.

4. CONCLUSIONS

Our studies revealed that *Carassius auratus gibelio* acutely exposed to manganese ions developed specific adaptative responses for each tissue neutralizing oxidative stress as it could be noticed from the G6PDH specific activity in liver and kidney. The degree of increase in this specific activity following *in vitro* $MnCl_2$ treatment was higher in liver than in kidney.

Because the epithelial layer of kidney, intestine and gill are in direct contact with the pollutant, the necessity of reduced glutathione and NADPH increases. As a result the histochemical reaction for G6PDH was higher in the epithelial layer than in other tissues.

The pollutant triggers a response soon after the beginning of exposure. Significant effects were noticed on the main tissues involved in the detoxication processes: liver, kidney and gill. In liver, the structural changes appeared sooner than the biochemical ones, which is in according with the functional particularities of this organ.

In liver, kidney and ovary the main pollutant target were the nuclei. These structural changes consisting in karyomegaly, anisokary, pycnosis prove that the metal ion has a deep effect on the nuclear genetic material.

The increase of previtellogenic and vitellogenic follicles atresia after manganese acute exposure affects the sexual cells quality, functions and fish reproduction efficiency.

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H.J. DUMONT & S.V. NEGREA: *Introduction to the Class Branchiopoda. Guides to the identification of the microinvertebrates of the continental waters of the world*: 19. Backhuys Publishers, Leiden, 2002, pp. 1–398.

How far away we are actually from the state of things of scarcely a few decades ago, when these "inferior crustaceans" with biology highly peculiar in many respects were simply classified in "Phyllopods" and "Cladocerans" ! This is shown by the instructive "History of research" (pp. 14–28 of the book), exhaustive and critical account in which "The current phase 1991...."for "Phyllopoda" and "The third phase 1959" for "Cladocera" are given special attention. The present time complexity of the classification of these crustaceans (including some 1000 recent species; should be emphasized that the fossil documents are permanently taken into consideration in this volume) follows from chapter 16 (pp. 240–246) in which Class Branchiopoda is divided in 5 superorders (the 1st one with order Anostraca; the 2nd one with order Notostraca; the 3rd one with two orders corresponding to the group of the conchostracans; the 4th one including only fam. Leptodoridae – formerly belonging to *Cladocera*; and the 5th one, Cladocera, being subdivided in 3 orders). It is possible that the reader did not expect from a volume of "Introduction..." identification keys for superorders, orders, and families (cap. 17) followed by (cap. 18) excellent diagnoses for all of them; supported by a rich illustration (Fig. 125–169 – as well as by the remaining illustration including the numerous habitus drawings) this part of the book will allow not only to specialized carcinologists but also to a wide group of zoologists and naturalists to orientate themselves in the "tangled forest" of this fascinating zoological group.

Chapters 5–15 represent a massive body of updated multilateral information on the group. Are reviewed: morphology, anatomy, reproduction, development and growth, ecology and ethology of feeding, ecology (the title of this section has to be taken in its *sensu lato,* here being also tackled numerous aspects pertaining to ethology and study of populations), biogeography, paleontology, phylogeny (section in which are reviewed the various attempts of phylogenetic reconstruction connected with names like G. Fryer, D. Wallosek, or S. Negrea; one of the author's conclusions being that Branchiopoda is a monophyletic group). For the specialized carcinologist: a mass of accurate information; whereas the attention of a much wider group of naturalists is likely to be attracted by aspects characteristic for the group, like parthenogenesis and gamogenesis, feeding by filtration, cyclomorphosis and other types of polymorphism, vertical diurnal migration of zooplanktonic elements.

Really impressing is the enormous list of references: almost 1500 titles. The considerable effort required for assimilation of this huge amount of information commands admiration.

This book is characterized by the handy and elegant shape of the series *Guides*, and is illustrated with hundreds of well-reproduced drawings from the bibliography. However, the reproduction of the photographs does not reach the high level with which the zoological publications of recent decades have accustomed us.

Introduction to the Class Branchiopoda is an exemplary achievement; and, thanks to the second author (already known through numerous publications on *Cladocera*, including a book on the cladoceran fauna of Romania) it may be considered as a fine achievement of Romanian zoology.

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AVIS AUX COLLABORATEURS

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