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PLANKTONIC COPEPOD PRODUCTIVITY UNDER THE EUTROPHICATION IMPACT (1980–1996), IN THE DANUBE DELTA LACUSTRIAN ECOSYSTEMS

LAURA PÂRPALĂ, V. ZINEVICI

The increase of the ecosystem trophic level determined important changes in structure and function of the biocenosis of the Danube Delta lacustrian ecosystems. The almost whole disappearance of submerged macrophyte stock and the appearance of intense "water blooming" phenomena had influenced the planktonic zoocenosis evolution and, accordingly, the copepod one. The studies were done as a function of the trophic levels: filtrators and predators, over a 16 years period (1980–1996), in 11 Danube Delta lacustrian ecosystems. In addition, the temporal and spatial dynamics of the productivity was considered, on the one hand, and, on the other, the eutrophication impact upon productivity. The eutrophication affected particularly the ecosystems with depths bigger than 1.8 m, in which phytoplanktonic primary producers are dominant, and to a lesser degree colmated ecosystems, with depths smaller than 1.7–1.8 m, characterized by the dominance of macrophyte primary producers. By spatial viewpoint the productivity presents a large variability, the maximum value being registered in the Merhei lake, while the minimum value in the Băclăneşti lake. From the trophic level point of view, the filtrator productivity is 6 times bigger than that of predators. The increase of the Danube Delta lacustrian ecosystem trophic level from meso-eutrophy towards poly-hypertrophy, in 1980–1996 period, induced a 2.2 times increase of the productivity of the algae type primary producer ecosystems, against the macrophytic type primary producer ecosystems. The study carried on some copepod species with dominant biomass, in the Roşu lake, on 1994–1996 period, shows that the highest productivity belongs to the embryons, followed by nauplial forms and copepodids. In the last case the productivity increased from the first stage to V–VI ones.

1. INTRODUCTION

The copepods represent an important zooplankton component (Hutchinson 1967, Teodorescu 1995), which contribute to the main, closely interrelated ecosystem functions such as: the energy and matter circulation and the self-control. This is accomplished by the copepod constant presence and by their high biomass, in spite of a not so wide species diversity (as Ciliata or Rotifera).

The increase of the ecosystem trophic level determined significant changes in the biocenosis structure and function (Zinevici and Teodorescu, 1990_a; 1990_b; 1990_c; 1992; 1996). The almost total decline of the submerged macrophyte stock and the appearance of intense "water blooming" phenomena had influenced the planktonic zoocenosis evolution, including the copepods.

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A double numerical density, a 2.6 times increase of biomass and 2.2 of productivity have been found at the copepod level, as the environmental heterogeneity decreases (Zinevici and Teodorescu 1993).

The estimation of matter and energy quantity existing at a certain moment in the ecosystem requires the determination of the biological productivity, to which planktonic copepods take a part in too.

2. MATERIAL AND METHODS

The studies were carried out over a 16 years period (1980–1996), in the following ecosystems: **Roşuleţ** (1987–1996); **Roşu** (1983–1987, 1989, 1991–1996); **Porcu** (1991); **Puiu** (1983, 1986); **Isac** (1983–1986, 1989, 1994–1995); **Uzlina** (1994–1995); **Merhei** (1980–1983, 1989, 1991–1993); **Matiţa** (1980–1986, 1991–1993); **Babina** (1983–1986); **Bogdaproste** (1983–1986, 1989); **Băclăneşti** (1983–1986). They were done as a function of the trophic levels, namely: filtrators and predators. Also, the temporal and spatial dynamics of the productivity was considered on the one hand, and the eutrophication impact upon productivity, on the other hand. The eutrophication affected predominantly the ecosystems with depths bigger than 1.8 m, in which planktonic primary producers are dominant. The colmated ecosystems, with depths smaller than 1.7–1.8 m, characterized by the dominance of macrophyte primary producers were less influenced.

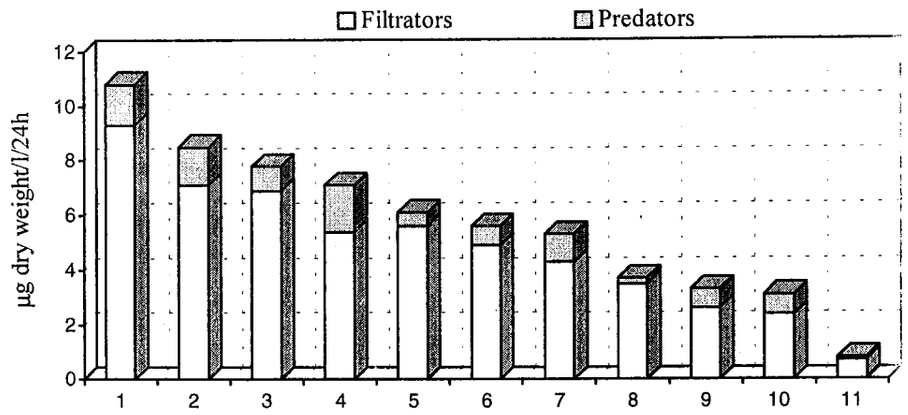
The Winberg, Pechen, Shushkina (1965) method was used for the determination of the productivity (Edmondson and Winberg, 1971), expressed as µg dry weight/l/24h. The development duration was established by means of the development curves, depending on the temperature and ecosystem trophic level (Winberg, 1971).

3. RESULTS AND DISCUSSIONS

Spatial and temporal dynamics. The dynamics of the planktonic copepod productivity from delta biome lakes shows large spatial and temporal variations well correlated with those of the structural dynamics.

The spatial dynamics presents the maximum value in Merhei lake – 10.8 µg dry weight/l/24h (86.11% filtrators + 13.89% predators), and the minimum one in Băclăneşti lake 0.82 µg dry weight/l/24h (87.81% filtrators + 12.19 % predators) (Fig.1).

The planktonic copepod productivity is closely correlated with the structural indices, a fact which evidences the copepod ecologic role in the ecosystem functions. Thus, we can point out that, in an ecosystem descendent ordering of numerical density, biomass and productivity, the Merhei and Isac lakes are situated on the first places, while Roşuleţ, Porcu and Băclăneşti lakes occupy the last ones.



1 = Merhei; 2 = Isac; 3 = Matiţa; 4 = Uzlina; 5 = Puiu; 6 = Babina; 7 = Roşu; 8 = Bogdaproste; 9 = Roşuleţ; 10 = Porcu; 11 = Băclăneşti.

Fig. 1 – The multiannual average dynamics of the planktonic copepod productivity from Danube Delta lacustrian ecosystems.

As regards the biomass, the filtrator value exceeds only 1.5 times over that of the predator one, while in the case of productivity filtrator value is 6 times bigger than that corresponding to predators.

Four stages are clearly distinguished in the temporal dynamics of the productivity evolution (1981–1983, 1984–1987, 1989–1994, 1995–1996).

The strongest eutrophication impact exerted upon copepods, by qualitative viewpoint, was recorded in 1983 year, and upon their numerical and gravimetrical abundance, a year later. The same delay is observed as regards the copepod producing capacity. In 1984 year a sharp productivity decrease took place, lasting whole 1984–1987 interval. A recovery is seen during 1989–1994 years to values higher than those of 1981–1983 period. Another productivity decrease, not below the values of the maximum anthropic impact (1984–1987), was recorded in the last 1995–1996 period (Fig. 2).

Concretely, the average productivity value is of 8.4 µg dry weight/l/24 h in the first mentioned period, followed by a 2.5 times reduction (3.3 µg dry weight/l/24 h). The subsequent recovery equals 9 µg dry weight/l/24 h and the decrease in the last period matches 4.3 µg dry weight/l/24 h.

The seasonal dynamics analysis of the planktonic copepod productivity in 1980–1996 period shows the lowest values in the cold months of the year, respectively in March and December (Fig. 3). It is well known that the temperature and development duration (which, in its turn, is to a great extent upon temperature dependence) are among the determinant factors of the productivity (according to Edmondson and Winberg, 1971).

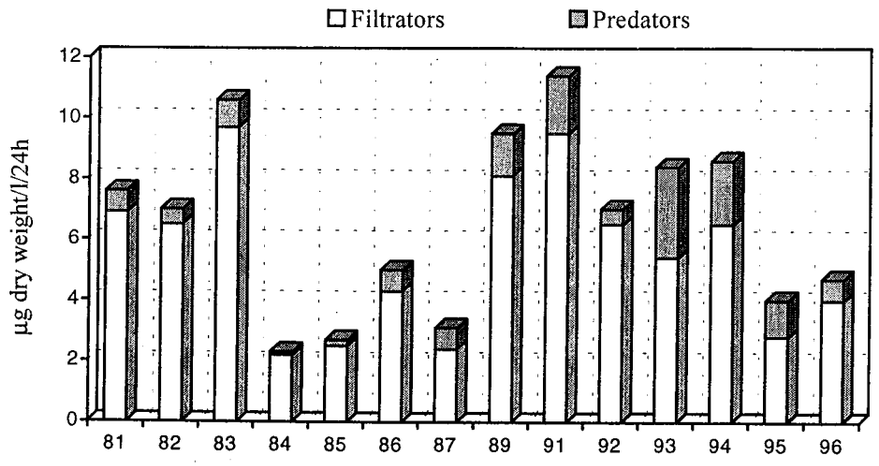


Fig. 2 – The annual dynamics of the planktonic copepod productivity from Danube Delta lacustrian ecosystems.

The seasonal dynamics analysis shows that a 14 times productivity increase against March value, took place at the beginning of spring (April–May), as temperature increases. The maximum values are encountered in summer, respectively in August (10.8 µg dry weight/l/24 h). The productivity begins to decrease, reaching very low values, last in autumn (October) and even zero during the winter.

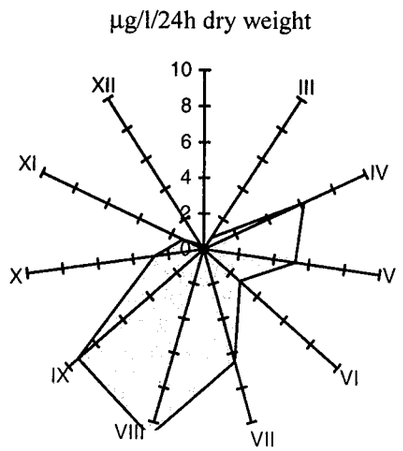


Fig. 3 – The monthly dynamics of the planktonic copepod productivity from Danube Delta lacustrian ecosystems.

As was observed in the case of the structural parameter (number and biomass) analysis, the constituent species have a different ecological role in the frame of the ecosystem. The elements dominant by the productivity viewpoint have a distinct importance (*Eurytemora velox*, *Eudiaptomus gracilis* – filtrators and *Acanthocyclops vernalis*, *Mesocyclops crassus* – predators). The dominant species which are constant forms too have more significance. For exampe, the dominance of *Eurytemora velox*, *Heterocope caspia*, *Acanthocyclops vernalis* and *Mesocyclops crassus* species in the Roşu lake, together with their constant presence, ensure a permanent contribution to biocenosis functions.

The eutrophication impact upon the productivity. The change of the primary producer type in some Danube Delta lacustrian ecosystems induced significant mutations both in the structure and functions, so that the planktonic copepod role grows proportionally with the increase of the ecosystem trophic level.

Consequently, the productivity in the planktonic type primary producer ecosystems is 2.2 times greater than that of the macrophytic type primary producer ecosystems (Fig. 4).

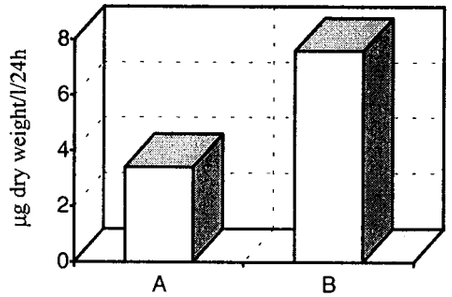
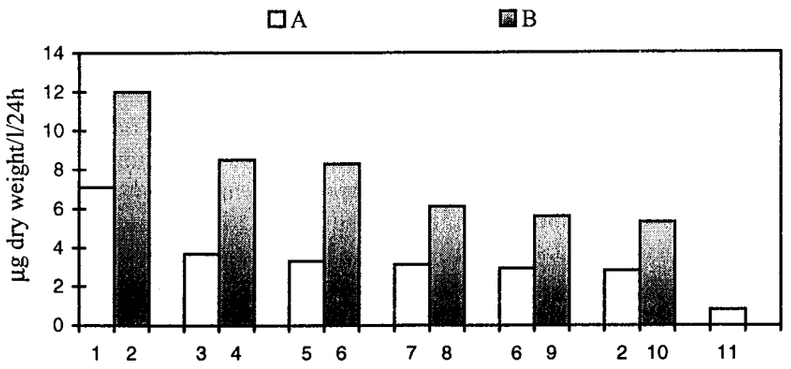


Fig. 4 – The planktonic copepod productivity in Danube Delta ecosystems with:
(A) macrophytic type primary producers;
(B) planktonic type primary producers.

In the macrophyte primary producer ecosystems, the oscillations vary between 0.8 µg dry weight/l/24h (87.50% filtrators + 12.50% predators) in Băclăneşti and 7.1 µg dry weight/l/24h (76.06% filtrators + 23.94% predators) in Uzlina. In the algae type primary producer ecosystems the variations range between 5.3 µg dry weight/l/24h (81.13% filtrators + 18.87% predators) in Roşu and 12 µg dry weight/l/24h (85.83% filtrators + 14.17% predators) in Merhei. (Fig. 5).

The planktonic copepod productivity analysis, as a function of the trophic level, evidences a 2.4 times increase of the filtrator value and 1.4 times that of predators in ecosystems with planktonic type primary producers (Fig. 6) against macrophytic type primary producer ecosystems.



1 = Uzlina; 2 = Merhei; 3 = Bogdaproste; 4 = Isac; 5 = Roşuleţ;
6 = Matiţa; 7 = Porcu; 8 = Puiu; 9 = Babina; 10 = Roşu; 11 = Băclăneşti.

Fig. 5 – The productivity dynamics of the planktonic copepod in Danube Delta ecosystems with:
(A) macrophytic type primary producers;
(B) planktonic type primary producers.

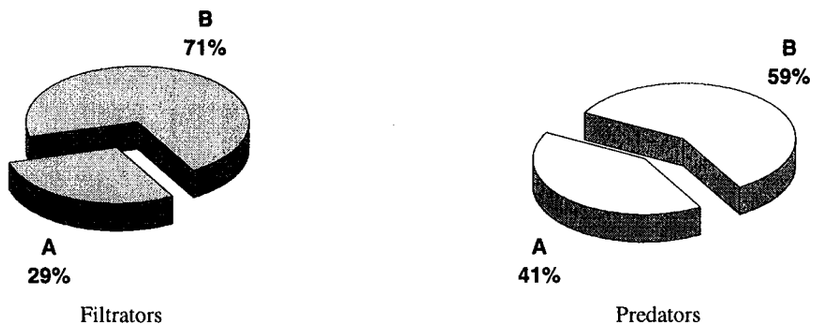


Fig. 6 – The average per trophic levels, of the planktonic copepod productivity abundance in Danube Delta ecosystems with:
(A) macrophytic type primary producers;
(B) planktonic type primary producers.

In view of the limitative role of the trophic base in the ecology of these organisms, the planktonic filtrator copepods are especially favoured by the productivity viewpoint as a result of primary producer type modification. In austerity conditions the predators feed also on detritobacterial aggregates or big size algae resulting from the breakdown of the colonial forms, but this type of feeding is peculiar to filtrators.

The annual dynamics of the copepod productivity, in ecosystems with submerged macrophytes, shows values below those recorded in the other type of ecosystem, (Fig. 7), but with wider variations (the maximum value is 7.4 times against the minimum one while in algae primary producer ecosystems it is 4.6 times over the minimum one).

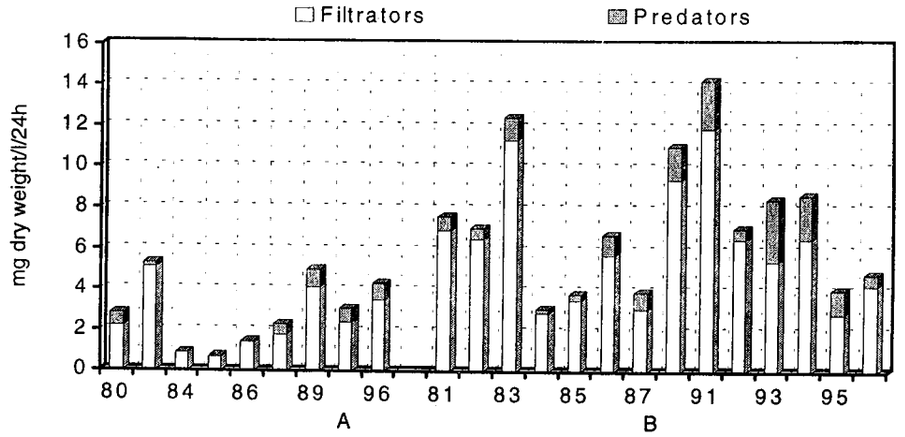


Fig. 7 – The annual average dynamics of the planktonic copepod productivity in Danube Delta ecosystems with:
(A) macrophytic type primary producers;
(B) planktonic type primary producers.

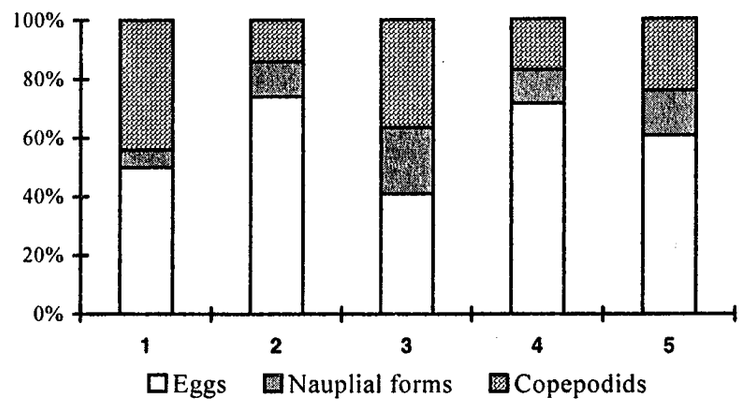
The range of the variations in ecosystems with macrophytes is generated by the high environmental heterogeneity, which enables a wide diversity of the species productivity, while, in the second ecosystem category, the higher values belong to a reduced number of species, best adapted to the new environmental conditions of abiotic (pH, temperature, transparency, O_2) and biotic (nutrients) nature.

Juvenile stages and their contribution to planktonic copepod productivity. A detailed study was accomplished on some copepod species from Roşu lake, in 1994–1996 period to establisi the different juvenile stage contribution to biological productivity of the planktonic copepod. This was based on the principle that the population production represents the sum of the weight increases of each development stage (Winberg, Pechen, Shushkina, 1965). The species selection criteria were the biomass dominance index and the presence of the eggs carrying females. These two criteria were applicable to the species:

among filtrators: *Calanipeda aquae-dulcis,*
among predators: *Acanthocyclops vernalis,*
Cyclops vicinus vicinus,

Mesocyclops crassus,
M. leuckarti.

This analysis evidences that carrying eggs females (embryons) are responsible for most of the total productivity (50% for *Calanipeda aquae-dulcis* and between 40.82% – 73.95% for predator copepods) and nauplial forms or copepodids to a less extent (Fig. 8).



1 = *Calanipeda aquae-dulcis*; 2 = *Acanthocyclops vernalis*;
3 = *Cyclops vicinus vicinus*; 4 = *Mesocyclops crassus*; 5 = *Mesocyclops leuckarti.*

Fig. 8 – The average productivity (%) of the planktonic juvenile copepod in Roşu lake on 1994–1996 period.

As regards the copepodids, the studies confirmed that the highest productivity belongs to the last stage, gradually decreasing towards the first stage (both in the case of calanoids and cyclopids, the last stage productivity is 5 times greater than that of the first stage) (Table 1). This may be due to the intense metabolic processes of the last stage copepodids in order to prepare the adult stage, capable to reproduce.

Table 1

The juvenile stage productivity (%) of the total planktonic copepod productivity in Roşu lake on 1994–1996 period

	E	N	C I	C II	C III	C IV	C V	C VI
Calanoida	50.00	5.83	2.50	4.17	6.67	4.17	12.50	14.16
Cyclopida	61.74	15.22	1.47	3.70	3.99	6.37	7.51	

E = Embryons; N = Nauplial forms; C I–VI = I–VI stage Copepodids

4. CONCLUSIONS

– By spatial viewpoint the productivity presents a large variability, the maximum value being registered in Merhei lake, while the minimum one in Băclăneşti lake;

– At the trophic level, the filtrator productivity is 6 times greater than that of the predators;

– The eutrophication impact exerted upon the annual dynamics of the copepod productivity determined a 2.5 times decrease in 1984 year, comparatively to the 1981–1983 period;

– The seasonal dynamics shows the maximum value of the productivity in August, and the minimum ones in the cold months of the year (March, December);

– The increase of the trophic level of the Danube Delta lacustrian ecosystem, from meso-eutrophy to poly-hypertrophy, in 1980–1996 period, induced a 2.2 increase of the productivity in the algae type primary producer ecosystems, comparatively to the macrophytic type primary producer ones;

– The study realized on some copepod species, with dominant biomass, in the Roşu lake, on 1994–1996 period, shows that the highest productivity belongs to the embryons, followed by nauplial forms and copepodids. In the last case the productivity increased from the first stage towards V–VI ones.

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THE ZOOPLANKTON IN THE DANUBE DELTA LACUSTRIAN ECOSYSTEMS I. MULTIANNUAL MEANS OF THE STRUCTURE, PRODUCTIVITY AND BIOMASS RECYCLATION

VICTOR ZINEVICI, LAURA PARPALĂ

The means (calculated for a 20-year period: 1975–1995) of some structural and functional parameters of the lacustrian zooplankton from the Danube Delta, such as taxonomic diversity, numerical and gravimetric abundance, productivity, biomass recyclation rate and time are analysed. These resulted following studies realised in 12 representative lacustrian ecosystems (Roşu, Porcu, Roşuleţ, Puiu, Uzlina, Isac, Iacub, Merhei, Matiţa, Babina, Bogdaproste and Băclăneşti lakes).

1. INTRODUCTION

A significant part of the Danube Delta surface (9.28% namely 3162 ha) is occupied by the lacustrian system. It comprises 668 aqueous basins (2). Their depths range between 0.7 and 4 m, while surfaces between 1 and 1450 ha. They are delimited by reed and plaur islands. The primary consumer structure and productivity reflect the influence of the submerge macrophytes (under unaffected by eutrophication conditions) or of the phytoplankton (in periods of increased nutrient concentration due to human activity).

The different localisation of aqueous basins throughout the delta hydrographical network, as well as their various stages of succession, generated a high ecosystem heterogeneity of the lacustrian system.

The most of aqueous basins are in an advanced stage of colmatage. As a result their depth is generally below 1.7 m, and they are characterised by reduced surfaces (no more than dozens of ha) and high values of the shoreline development.

Only 7.48% of the lacustrian components are less silted, deeper, with larger surfaces (between dozens of ha to 1450 ha) and low values of the shoreline development. Their large surfaces totalize 67.8% of the whole system surface. The research regarding the zooplankton structure, productivity and biomass recyclation was accomplished in the last mentioned ecosystem category.

2. MATERIALS AND METHODS

The studies were carried out in 12 lakes being in the first stage of ecological succession: Roşu L. (1975–1978, 1983–1987, 1989, 1991–1995), Roşuleţ L. (1987), Porcu L. (1976–1978), Puiu L. (1977–1978, 1983), Uzlina L. (1994–1995), Isac L. (1983–1986, 1989, 1994–1995), Iacub L. (1975), Merhei L. (1980–1983, 1989,

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1991–1993), Matiţa L. (1980–1986, 1991–1993), Babina L. (1983–1986), Bogdaproste L. (1983–1986, 1989) and Băclăneşti L. (1983–1986) in the course of 1975–1995 period.

The sampling was performed monthly (from March to November) with Patalas-Schindler sampler. 50 liters of water were filtered for each sample, through an 60 μm mesh net. The samples were collected in 3–5 stations of each ecosystem, from the entire water column in each station.

The parameter calculations were referred to each species, taxonomic group, trophic level and also to total zooplankton. The literature gravimetric data with regard to species, sexes and sizes were used for the biomass calculation (expressed as μg wet weight/l). The productivity (μg w.w./l/24 h) was assessed by the methods: Ilkowska-Stankzykowska (1969) (in the case of Lamellibranchia planktonic larvae), Galkowskaya (1968) (for Rotatoria), Winberg, Pechen and Sushkina (1965)(for Copepoda and Cladocera)(1). The biomass recyclation was determined using two coefficients: P_{24h}/B (daily turnover rate) and B/P_{24h} (daily recyclation time or biomass recyclation time).

3. RESULTS AND DISCUSSIONS

A global characterisation of the lacustrian zooplankton from the Danube Delta implies the multiannual arithmetic mean analysis, comprising the significant peaks of the temporal and spatial dynamics of the most illustrative structural and functional parameters.

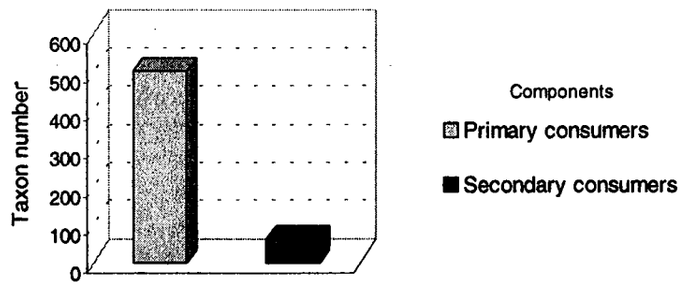

Fig. 1 – The taxonomic diversity (taxon number) of the lacustrian zooplankton depending on the trophic levels (1975–1995 period).

Taxonomic diversity. A large taxonomic diversity characterises the lacustrian zooplankton in the whole 1975-1995 period. The taxonomic spectrum comprises 562 components. 89.15% of them are primary consumers (c_1), the remaining of 10.85% are secondary (c_2) (Fig. 1) (4).

The multiannual mean of the taxonomic diversity reaches 90 taxons ecosystem, referring to lacustrian unit.

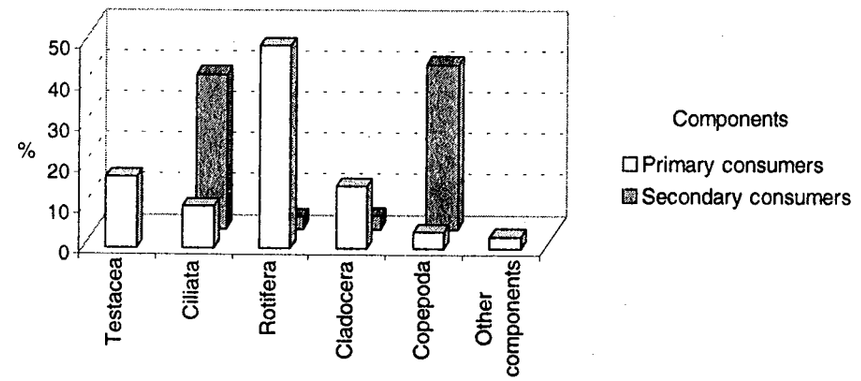

Fig. 2 – The taxonomic structure (%) of the lacustrian zooplankton, depending on the organism group of the two trophic levels (Xa 1975–1995).

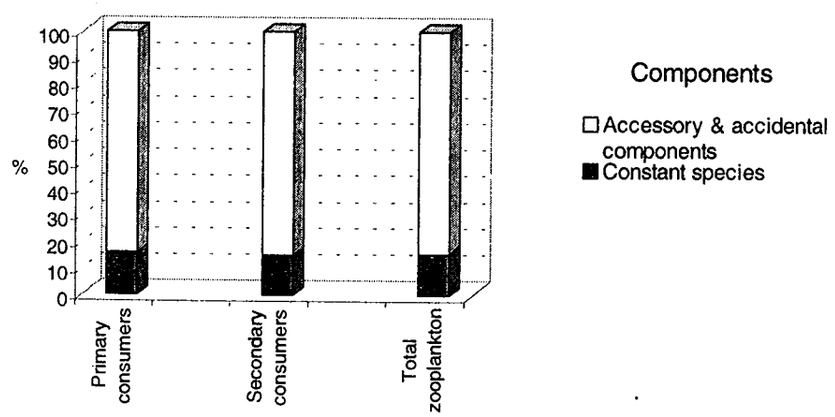

Fig. 3 – The relative abundance (%) of the frequency constant forms in the taxonomic spectrum of the lacustrian zooplankton (Xa 1975–1995).

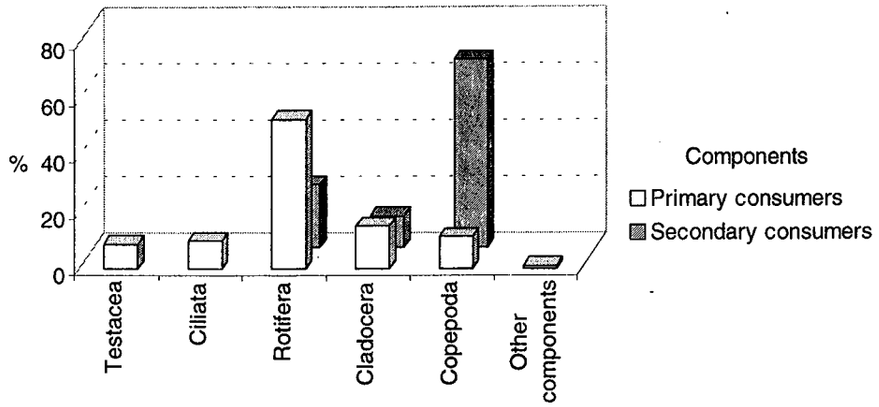

Fig. 4 – The taxonomic structure (%) of the frequency constant forms in the lacustrian zooplankton, depending on the trophic levels (Xa 1975–1995).

Fig. 5 – The numerical density (ex.nr./l) of the lacustrian zooplankton depending on the trophic levels (Xa 1975–1995).

The taxonomic group analysis shows that c_1 rotifers represent about half of the primary consumer taxonomic spectrum, as to the secondary ones the c_2 copepods point out the same proportion. The testacea and respectively c_2 ciliates have a complementary role (Fig. 2).

The frequency constant forms make up 15% of the taxonomic spectrum of both trophic levels (Fig. 3), having an important role in the dynamics of the ecological equilibrium of the community.

The mentioned above determinant role of c_1 rotifers and c_2 copepods becomes more visible in the constant form structure (Fig. 4).

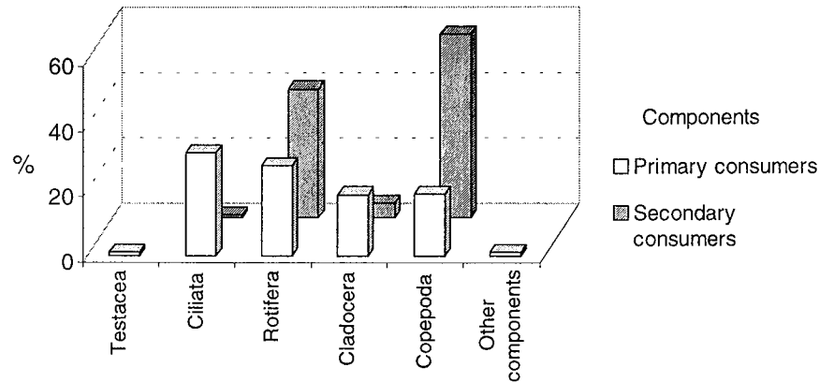

Fig. 6 – The relative numerical abundance (%) of the lacustrian zooplankton, depending on the organism group of the two trophic levels (Xa 1975–1995).

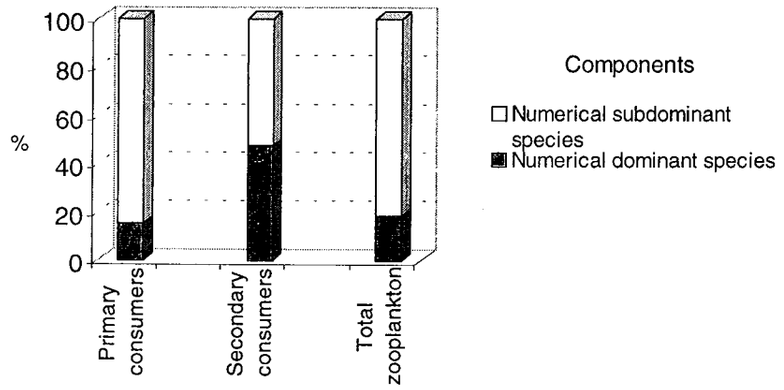

Fig. 7 – The relative abundance (%) of the numerical dominant elements in the taxonomic spectrum of the lacustrian zooplankton (Xa 1975–1995).

Numerical abundance. The mean value is a relatively low (659 ex/l), as for entire study period (Fig. 5) (5). Recent personal data, unpublished yet, prove that smaller than 60 μm components are present in the lacustrian zooplankton, which were removed by 60 μm mesh filtration. Their contribution to real biomass estimation is probably negligible, but seems to be greater regarding the numerical abundance and productivity. This fact will be elucidated in the future. A large dispersion around multiannual mean is observed, as for biomass and productivity. 95.31% of relative numerical abundance is due to primary consumers and only 4.69% to secondary ones (Fig. 5).

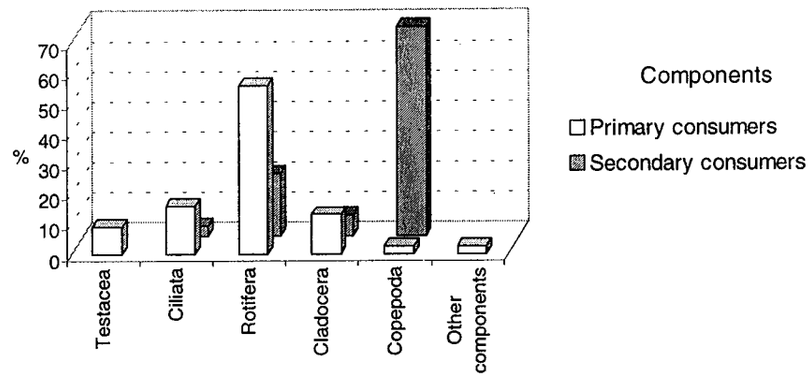

Fig. 8 – The taxonomic structure (%) of the numerical dominant forms in the lacustrian zooplankton, depending on the trophic levels (Xa 1975–1995).

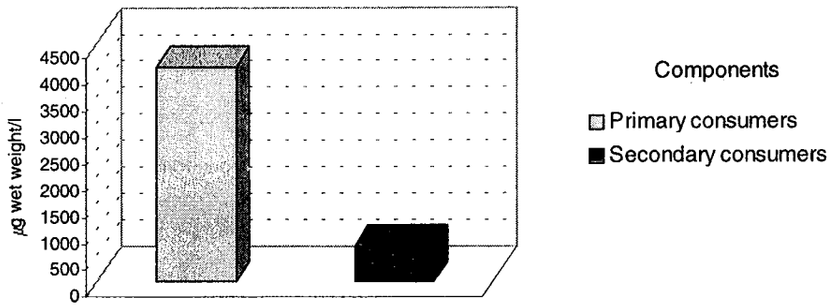

Fig. 9 – The biomass (µg w.w./l) of the lacustrian zooplankton, depending on the trophic levels (Xa 1975–1995).

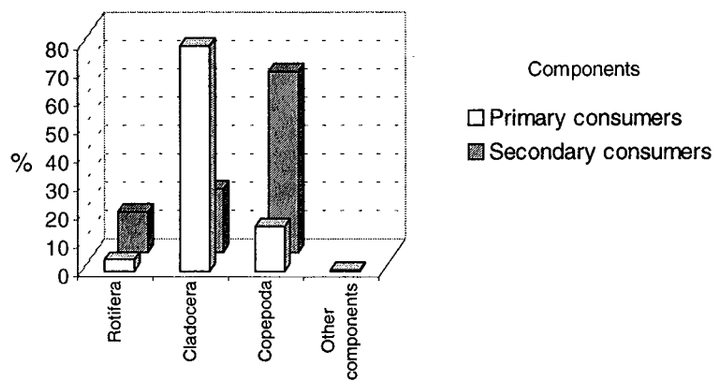

Fig. 10 – The relative abundance (%) of the lacustrian zooplankton biomass, depending on the organism group of the two trophic levels (Xa 1975–1995).

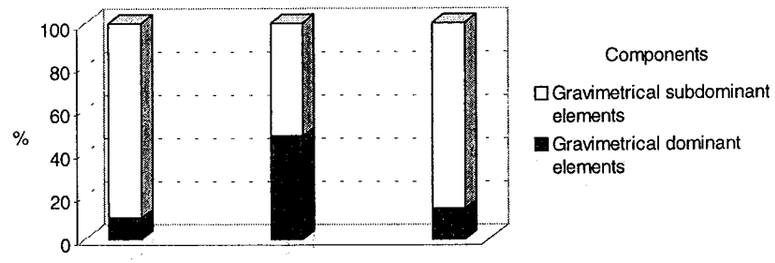

Fig. 11 – The relative abundance (%) of the gravimetric dominant elements in the taxonomic spectrum of the lacustrian zooplankton (Xa 1975–1995).

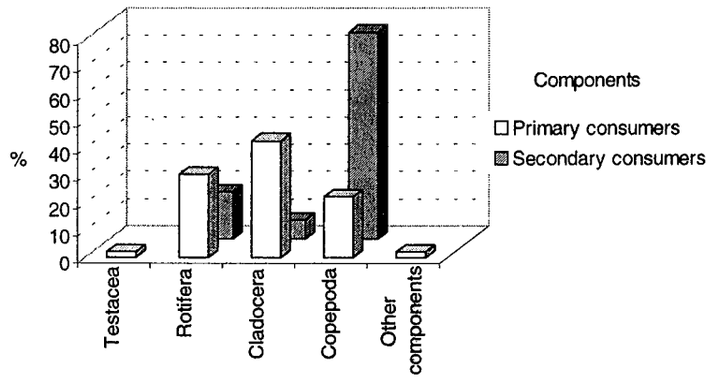

Fig. 12 – The taxonomic structure (%) of the gravimetric dominant elements of the lacustrian zooplankton, depending on the trophic levels (Xa 1975–1995).

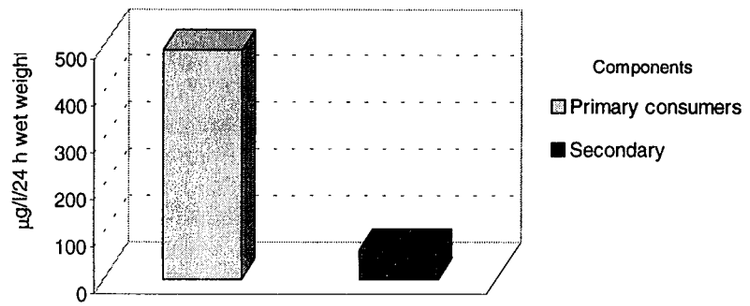

Fig. 13 – The productivity (µg w.w./l/24h) of the lacustrian zooplankton depending on the trophic levels (Xa 1975–1995).

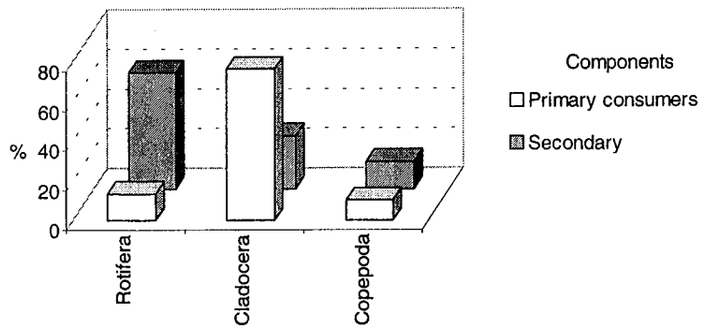

Fig. 14 – The contribution (%) of the organism groups in the zooplankton productivity, depending on the trophic levels (Xa 1975–1995).

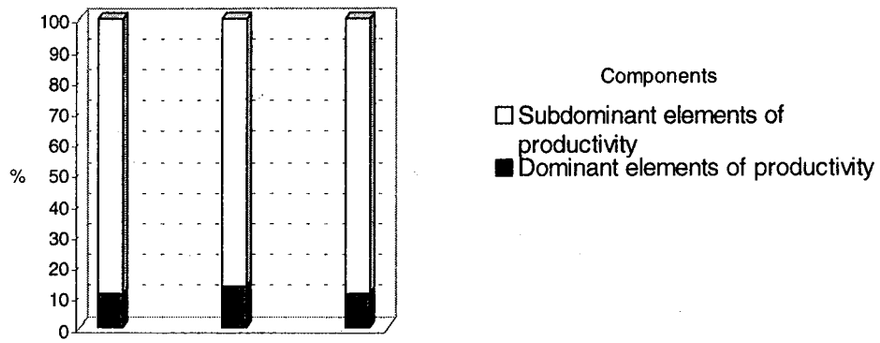

Fig. 15 – The relative abundance (%) of the elements with important role in the lacustrian zooplankton productivity (Xa 1975–1995).

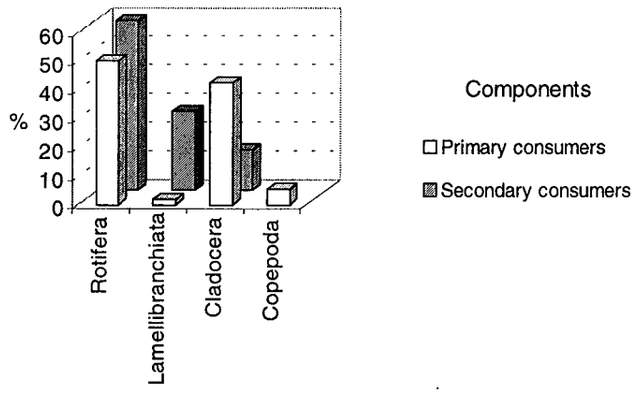

Fig. 16 – The taxonomic structure (%) of the elements with important role in the productivity, depending on the trophic levels (Xa 1975–1995).

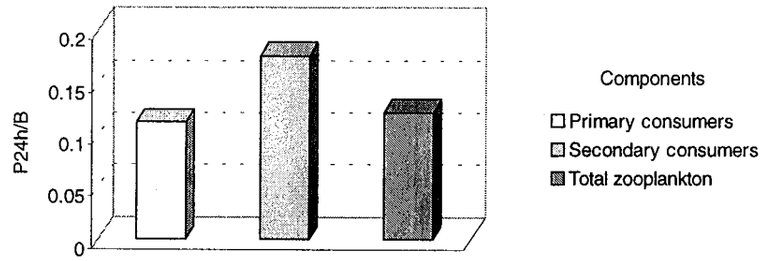

Fig. 17 – The daily turnover rate (P_{24h}/B) of the lacustrian zooplankton, depending on the trophic levels (Xa 1975–1995).

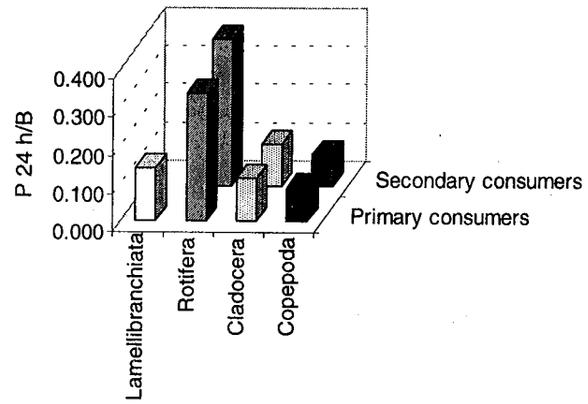

Fig. 18 – The daily turnover rate (P_{24h}/B) of the lacustrian zooplankton, depending on the organism group of the two trophic levels (Xa 1975–1995).

The numerical dominance of the taxonomic groups belongs to ciliats (31.65%) and rotifers (27.73%) as for primary consumers, and to copepods (55.99%) and rotifers (39.16%) as for secondary ones (Fig. 6).

The dominant forms, as a rule, play an important role in the dynamics of the ecological equilibrium. The numerical dominant forms amount to 18.5% of the taxonomic spectrum total (Fig. 7).

The main contribution belongs to rotifers (56%) as for c_1 trophic level and to copepods (68.96%) for c_2 (Fig. 8), like taxonomic structure of this ecological group evidences.

Biomass. The mean value is 4738 g wet weight/l for 1975-1995 period. It resulted from both reduced values in the first period, and significant increased

ones, in the second period. Of the mentioned value, the primary consumers represent 85.89%, the remaining 14.11% being secondary ones (Fig. 9).

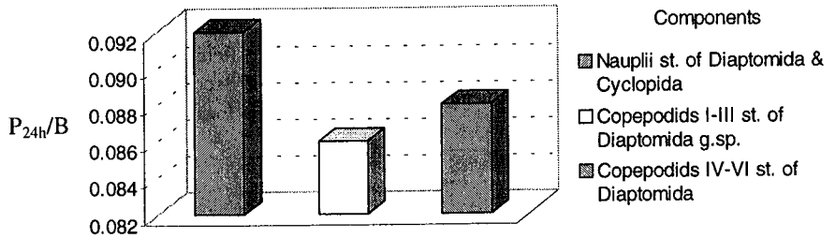

Fig. 19 – The daily turnover rate (P_{24h}/B) of the c1 copepods, depending on the developing stages (Xa 1975–1995).

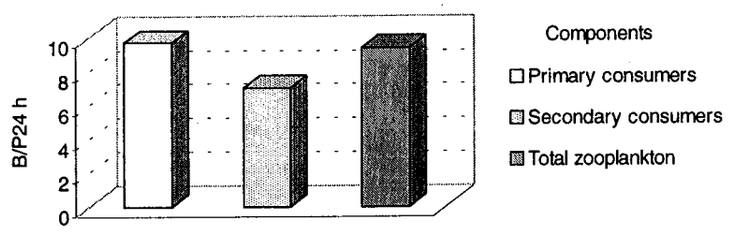

Fig. 20 – The turnover time in days (B/P_{24h}) of the lacustrian zooplankton, depending on the trophic levels (Xa 1975–1995).

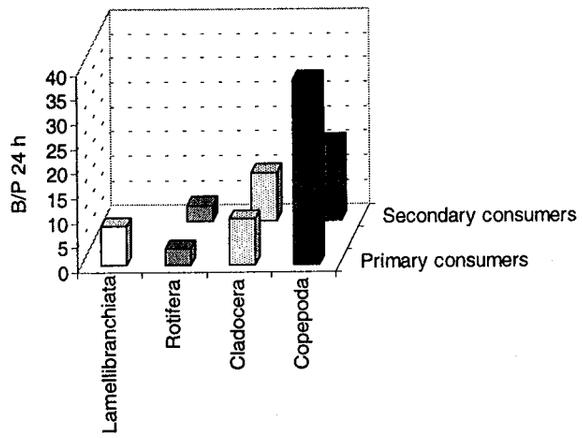

Fig. 21 – The turnover time in days (B/P_{24h}) of the lacustrian zooplankton, depending on the organism group of the two trophic levels (Xa 1975–1995).

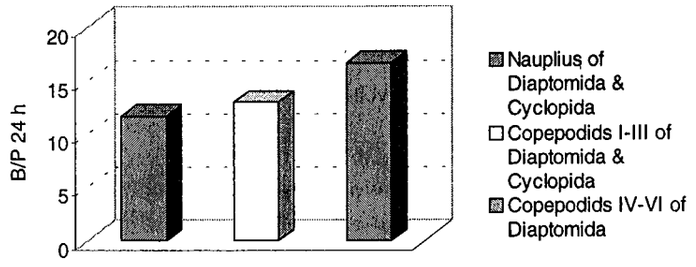

Fig. 22 – The turnover time in days (B/P_{24h}) of the c1 copepods, depending on the developmental stages (Xa 1975–1995).

The taxonomic groups with determinant role in the c_1 zooplankton dynamics are cladocers (79.56%) and copepods (15.66%), as for c_2 are the same groups, but in reverse order: copepods (63.61%), cladocers (22.2%) (Fig. 10).

The gravimetric dominant forms totalize 13.37% of all zooplankton elements (Fig. 11), evident a smaller value than as regards numerical dominant forms.

The cladocers (42.86%) and rotifers (30.61%) are mentioned for their particularly high contribution to primary consumer level, the copepods (75.86%) and, partially, rotifers (17.84%) to the secondary one (Fig. 12), as taxonomic structure of this ecological group shows.

Productivity. The multiannual mean is 547.9 μg w.w./l/24h (Fig. 13), which accounts to 11.56% of the corresponding value regarding the biomass. Of the mentioned value, 89.05% represent primary consumers, the remaining (10.95%) secondary ones.

The main contribution to productivity belongs to cladocers and rotifers (76.54%, respectively 13.38%) in the case of primary consumers, to rotifers and cladocers (59%, respectively 27.17%) as for secondary consumers (Fig. 14).

The dominant forms, having a principal role in the energy dynamics, amount to 11.03% of the taxonomic spectrum total (Fig. 15). A lower proportion against that corresponds to the gravimetric dominant forms and especially to the numerical dominant forms. The main contribution of the rotifers and cladocers, both for primary (50%, respectively 42.6%) and for secondary consumers (62.5%, respectively 25%) (Fig. 16) is evidenced by the relative abundance analysis of the determinant elements of the productivity.

Biomass circulation. The zooplankton of the analysed lacustrian type is characterized by an intense biomass circulation, with significant variations in the function of the trophic level, the community and the development stage.

Daily turnover rate (P_{24h}/B) point out the rate of the biomass recyclation. The multiannual mean is 0.122 for the total zooplankton, in accordance with data obtained by Kajak et al. (3) regarding the zooplankton of some eutrophic lakes in

Poland. The means of the above coefficient calculated function of the trophic level differ significantly (0.113-c_1, 0.176-c_2) (Fig. 17) depending on the various contribution of the communities.

The analysis on communities reveals both for c_1 and c_2, minimum values of copepods (0.088, respectively 0.081), and maximum ones of rotifers (0.335, respectively 0.382) (Fig. 18). In accordance with the copepod food structure in the course of its complete generation, the two trophic level components of the copepod groups differ. Thus, the nauplial forms and I–III and IV–VI stages of diaptomid copepodids, and nauplial forms and I–III stage of cyclopid copepodids comprise the copepods of primary consumers. In the case of c_2 copepods are encountered only IV–V stages of cyclopid copepodids.

In addition, the analysis as function of the development stage shows small differences of the P_{24h}/B in the case of c_1 copepods. The minimum value belongs to I–III copepodid stages (0.086) and the maximum one to nauplial forms (0.092) (Fig. 19).

The **turnover time**, rarely mentioned in the literature, means the average lifetime of species or other component. In the case of lacustrian zooplankton of the Danube Delta the multiannual mean of this coefficient is 9.4 days (Fig. 20). The comparative analysis on trophic level reveals evident differences: 11.71 days as for primary consumers, 6.39 days as for secondary ones, as the daily turnover rate is different. This, in turn, is due to copepod stages, which are included in the two trophic levels. So, as was already mentioned, besides diaptomids, nauplial and I-III stages of copepodids make up c_1 copepodids, while only IV–V stages of cyclopid copepodids form the c_2 copepodids.

The rotifers of the two trophic levels have the shortest development cycles, (3.41, respectively 3.12 days), and copepods the longest (37.61, respectively 16.63 days) (Fig. 21).

In the case of c_1 copepods, if the minimum value belongs to nauplial stages of both diaptomids and cyclopids, the maximum one belongs to IV–V copepodids of diaptomids (16.63 %) (Fig. 22). The turnover time values show small differences (Fig. 21 and Fig. 22), as the analysis of the diaptomid and cyclopid copepodids being in advanced development stages indicates.

4. CONCLUSIONS

– The high ecosystem heterogeneity of the Danube Delta lakes, the large amplitude of the annual hydrological variations and the various trophic levels, influence significantly the multiannual means of the biocenotic parameters.

– The lacustrian zooplankton is characterised by a large taxonomic spectrum (562 taxons) and also by a high multiannual mean of the taxonomic diversity referring to lacustrian unit (90 taxons/ecosystem) in the whole 1975–1995 period.

– The multiannual means of the numerical density (660 ex/l), the biomass (4700 g wet weight/l) and the productivity (550.9 μg w.w./l/24 h) are, conversely to the above parameter, relatively low values.

– The primary consumers/secondary consumers ratio is 6.1 as regards the biomass, 20.3 as for numerical abundance, of 8.1 in the case of productivity, and 8.2 in that of taxonomic diversity.

– The primary consumer review in respect of the taxonomic groups evidences the particular contribution of rotifers to the taxonomic spectrum, of ciliates as for numerical abundance, and especially of cladocers as regards the biomass and productivity; in the secondary consumer case, the cyclopids have a determinant role as to the taxonomic spectrum, the numerical density and the biomass, while the rotifers as to the productivity.

– The constant forms amount to 15% of taxonomic spectrum, and the dominant ones 18.5%, 13.37% and 11.03% for numerical density, biomass and respectively productivity.

– The multiannual mean of the daily turnover rate is 0.122 and that of the turnover time is 9.4 days.

– The rotifers have the highest values of the turnover rate and also the shortest development periods; the copepods, on the contrary, the most reduced and respectively the longest turnover time.

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BIOMASS AND NUMERICAL STRUCTURE OF EDAPHIC FAUNA IN THE UPPER LIMIT OF FORESTRY ECOSYSTEMS FROM BĂTRÂNA MOUNTAIN (BUCEGI MASSIF)

MARIN FALCĂ, LILIANA VASILIU-OROMULU, VIORICA HONCIUC

Nematoda, Enchytraeidae, Lumbricidae, Acari-Oribatida and Collembola were studied in the forestry soil ecosystems of spruce fir upper limit and mixed spruce fir and Larix from Bătrâna Mountain-Bucegi Massif. 78 species of all groups (except Nematoda) were identified in the spruce fir upper limit and 76 species in the mixed spruce and Larix soil ecosystems. It is one of their ecosystemic structure resemblance, some others consisting in floristical composition, climatic and pedological conditions. The dominant animal group, from the numerical point of view, was Acarina-Oribatida (48.3% in the spruce fir upper limit; 61.5% in the mixed spruce-larix forestry ecosystems), followed by Collembola (18.0% respectively 11%). As far as biomass is concerned, mixed spruce-larix soil ecosystem showed 65.4%, comparing with 34.6% of spruce fir upper limit. That is because of earthworm and oribatids species with higher values of numerical densities and so with higher values of biomass density.

1. INTRODUCTION

The structure and function of upper limit forestry ecosystems is important to be known not only for their economic value, but also for their very important ecological protective functions like antierosional, antieolian, climatic protective function and others. These ecosystems represent the first natural barrier against some natural negative processes that frequently occur as torrents, erosion and other degradative processes. A complex ecological study was undertaken on the north slope of Bucegi Massif, on Ialomița Valley, which included spruce fire and mixed spruce fir and Larix. The present paper presents the results of that study on edaphic fauna of Bătrâna Mountain. Objectives of the paper were: 1. establish species composition; 2. establish numerical densities and relative abundance of edaphic animal groups; 3. establish biomass densities.

2. MATERIAL AND METHODS

The study was undertaken in two forest areas located on the down part of Bătrâna Mountain. The first area, *Hieracio rotundati (transilvanico) – Piceetum abietis Br.-Bl et Pawl.39*, is a stand with individuals *Picea abies* reaching 20–25 m high and a herbaceous layer covering 20%–45% from the entire area. Characteristic herbaceous species are: *Hieracium rotundati, Oxalis acetosella, Soldanella hungarica, Homogyne alpina, Deschampsia flexuosa, Luzula sylvatica, Vaccinium*

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mirtillus, Polytrichum alpinum, Dicranum scoparium, Mnium spinosum, Plagiothecium curvifolium. General substrate of calcareous material has favourized the formation of mountain acid brown soil, rich in black calcic moder. The forest is located on an area with 15–17° slope.

The second area, *Piceeto-Laricetum carpaticae* Beldie *67*, is a stand with individuals of *Picea abies* and *Larix decidua ssp. polonica* reaching 18–25 m high, covering 60–75% from the entire area. Herbaceous layer, covering 40–70% from the entire area, is represented mainly through the following important species: *Vaccinium myrtillus* and *Oxalis acetosella*, near which are met weed species like *Veratrum album, Valeriana montana, Calamagrostis arundinacea* and some moss species like *Dicranum scoparium, Caliergonella cuspidata, Timmia austriaca, Hylocomium splendens*. Soil is brown podzolic, formed on loam and reddish clays, residuals on limestone.

Soil fauna was collected using proper technics for each animal group, 10 samples in June, July and August, in each area, subdivided as follows: L=litter; S_1=0–10 cm; S_2=10–20 cm; S_3= 20–30 cm.

Nematoda, Macfadyen borer was used for collecting individuals and O'Connor funnels for extraction individuals from samples.

Enchytraeidae, Macfadyen borer was used for collecting samples and O'Connor funnels for extraction of individuals from samples.

Lumbricidae, a metallic frame with 25/25 cm was used for collecting samples and hand sorting for extraction of individuals from samples,

Acari-Oribatida, Macfadyen borer was used for collecting samples and Berlese-Tullgren, modified by Balogh, for the extraction of individuals.

Collembola, Macfadyen borer was used for collecting samples and Berlese-Tullgren for the extraction of individuals from samples.

Biomass and energetic equivalents were calculated using Petersen H. and Luxton M. indexes (1982). Biomass for Lumbricidae was calculated drying fresh material at 85° C.

3. RESULTS AND DISCUSSIONS

Species composition of edaphic fauna in the spruce fir upper limit is more different as compared with other forestry ecosystem types because of characteristic conditions of soil, vegetation and climatic factors. While, as an example, species composition is richer in the beech forests with species of macrofauna, like earthworms, important in the decomposition processes, in the spruce fir upper limit forests important species are those of microfauna. In the spruce fir upper limit (Table 1), 78 species belonging to Acari-Oribatida, Collembola, Enchytraeidae and Lumbricidae were identified, and 76 species in the mixed spruce fir and Larix. All analysed animal groups showed more species in the spruce fir than in the mixed

forest, except for oribatids with 9 species more in the mixed forest. Overall, the number of species is almost the same in both analysed ecosystems. The same resemblance was noticed between both ecosystems by taking into account other characteristics of them like herbaceous species, climatic and pedologic conditions. However, there are some differences between those soils caused mainly by the less edaphic volume content of the mixed forest, because of its slope higher than that of the pure stand of spruce fir, which determines washing away of the organic material.

Table 1

Species composition of edaphic fauna

Animal group	*Spruce fir upper limit*		*Mixed fir with Larix*	
	species number	%	species number	%
Enchytraeidae	10	12.82	8	10.53
Lumbricidae	8	10.26	6	7.89
Acari-Oribatida	42	53.85	51	67.11
Collembola	18	23.07	11	14.47
Total	78		76	

Among the analysed animal groups, Oribatida are dominant (53.8% in the spruce fire upper limit forests and 67.1 % in the mixed forest), followed by Collembola, far behind (18% in the spruce fire upper limit and 14.5% in the mixed forest). The smallest number of species have Lumbricidae with 10.2% in the spruce fire upper limit and 7.89% in the mixed forest.

Numerical density. As compared with beech forests, where dominant are animal groups of macrofauna, like earthworms, edaphic fauna of spruce fir upper limit and mixed beech and Larix forests is dominated by other animal groups of mesofauna, like oribatids (Table 2, Table 3). Except for Lumbricidae and Enchytraeidae, which are numerically dominant in the spruce fir upper limit forest, all other animal groups are numerically dominant in the mixed beech and Larix forest.

Though the edaphic volume is smaller in the mixed forest than in the pure stand of spruce fir and the slope of the first area is higher than of the second one, numerical densities are higher in the soil of the mixed forest. One of the explanations of that could be a larger area covered with moss in the mixed forest (near 30%), than the pure stand of spruce fir (near 8%). The moss, as pioneer plants, found better conditions for their installation on the parental rock, permanently washed because of its higher slope. Nematoda and especially all Acari groups found better conditions in these habitats which determines the higher numerical density of these populations.

Table 2

Numerical density and relative abundance of soil fauna in the spruce fir upper limit (number of individuals m^{-2})

Animal group	Level	June		July		August	
		Density	%	Density	%	Density	%
Nematoda	L	313 600	73.6	1 224 800	90.6	477 200	78.7
	S_1	221 900	91.5	374 700	90.3	271 200	83.4
	S_2	66 700	80.6	82 700	94.2	106 900	82.7
Enchytraeidae	L-S_2	3 300	0.77	8 200	0.6	11 300	1.8
Lumbricidae	L	19.2	0.004	22.4	0.002	44.4	0.002
	S1	4.8	0.002	9.6	0.002	1.6	0.0005
	S_2	3.2	0.004	–	–	–	–
	S_3	1.6	100	–	–	–	–
Oribatida	L	78 100	18.32	79 100	5.85	76 300	12.58
	S_1	13 700	5.65	31 600	7.62	44 300	13.6
	S_2	13 700	16.56	4 700	5.35	20 100	15.54
Gamasidae	L	–	–	1 500	0.11	2 900	0.48
	S_1	1 200	0.49	400	0.09	1 200	0.37
	S_2	1 200	1.45	–	–	700	0.54
Zerconidae	L	–	–	1 300	0.09	900	0.15
	S_1	1 900	0.78	900	0.22	400	0.12
	S_2	1 100	1.21	400	0.45	1 000	0.77
Uropodidae	L	–	–	–	–	100	0.02
	S_2	100	0.12	–	–	–	–
Trachytidae	L	–	–	400	0.03	900	0.15
	S_1	–	–	–	–	1 100	0.34
	S_2	–	–	–	–	600	0.46
Collembola	L	31 200	7.32	36 400	2.69	8 800	6.40
	S_1	3 800	1.57	7 300	1.76	7 100	2.18
TOTAL		751 428.8		1 854 432		1 063 046	

Biomass density, for all animal groups, shows close values in the analysed ecosystems: 7349.8 mg dry weight m^{-2} in the spruce fir upper limit forest and 7 292.39 mg dry weight m^{-2} in the mixed spruce fir and Larix forest (Table 4 and Table 5). Despite the fact that numerical density has presented higher values in the soil of mixed spruce fir upper limit and Larix forest, for all animal groups, except Enchytraeidae and Lumbricidae, the biomass density showed higher values in the soil of spruce fir upper limit forest. That is because the biomass of earthworms and enchytreids contributes much more than other animal groups to the total amount of biomass in the spruce fir upper limit forest. It is to notice that all Acari groups and Collembola presented higher values of numerical and biomass densities in the mixed spruce fir upper limit forest, though soil volume of this area is smaller than in the spruce fir upper limit forest. But moss, as was underlined before, represents a preferable habitat for these animal groups.

Table 3

Numerical density and relative abundance of edaphic fauna in the mixed spruce fir annd Larix (number of individuals m^{-2})

Animal group	Level	June		July		August	
		Density	%	Density	%	Density	%
Nematoda	L	344 400	56.8	1 126 300	86.1	636 100	79.4
	S_1	233 000	87.3	536 100	91.7	236 200	82.0
	S_2	88 000	93.9	75 800	68.3	79 200	95.4
Enchytraeidae	L-S_2	4 900	0.8	7 700	0.6	6 800	0.8
Lumbricidae	L	6.4	0.001	1.6	0.0001	14.4	0.001
	S1	1.6	0.0006	16	0.003	–	–
	S_2	9.6	0.01	9.6	0.009	–	–
	S_3	–	–	1.6	100	–	–
Oribatida	L	214 900	35.4	134 500	10.3	110 200	13.7
	S_1	25 900	9.5	31 600	5.4	32 700	13.4
	S_2	3 400	3.6	31 500	28.4	2 800	3.4
Gamasidae	L	4 500	0.7	2 100	0.16	2 600	0.32
	S_1	1 500	0.6	1 600	0.3	1 000	0.35
	S_2	1 200	1.3	1 500	1.35	900	1.1
Zerconidae	L	9 900	1.6	1 700	0.13	1 600	0.19
	S_1	1 000	0.37	2 900	0.5	1 100	0.38
	S_2	1 100	1.16	2 100	1.9	–	–
Uropodidae	L	–	–	100	0.02	–	–
	S_2	–	–	100	0.09	100	0.12
Trachytidae	L	300	0.05	1 400	0.11	2.000	0.24
	S_1	–	–	1 000	0.17	200	0.07
Collembola	L	27 400	4.5	33 400	2.55	41 700	5.2
	S_1	6 000	2.3	11 500	1.97	10 700	3.7
TOTAL		967 417.6		2 002 928.8		1 165 914.4	

Table 4

Biomass density of soil animal groups in the spruce fir upper limit (mg dry weight m^{-2})

Animal group	Level	June	July	August
Nematoda	L	15.68	61.24	23.86
	S_1	11.1	18.74	13.58
	S_2	3.34	4.12	5.35
Enchytraeidae	L-S_2	105.6	262.4	361.6
Lumbricidae	L	849.6	435.2	568
	S1	432	739.2	51
	S_2	216	–	–
	S_3	288	–	–
Oribatida	L	1138.97	712.85	584.06
	S_1	134.09	167.48	205.11
	S_2	18.02	166.95	14.84

Table 4 (continued)

Animal group	Level	June	July	August
Gamasidae	L	–	11.55	22.34
	S_1	9.24	3.08	9.24
	S_2	9.24	–	5.39
Zerconidae	L	–	1.3	0.9
	S_1	1.9	0.9	0.4
	S_2	1	0.9	1
Uropodidae	L	–	–	0.1
	S_2	0.1	–	–
Trachytidae	L	–	3.08	6.93
	S_1	–	–	8.47
	S_2	–	–	4.62
Collembola	L	84.24	98.28	104.76
	S_1	10.26	19.71	19.17
TOTAL		3 328.38	2 706.28	2 010.1

Table 5

Biomass density of edaphic animal groups in the mixed spruce fir upper limit and Larix (mg dry weight m^{-2})

Animal group	Level	June	July	August
Nematoda	L	17.22	56.32	31.81
	S_1	11.15	26.81	11.81
	S_2	4.44	3.79	3.96
Enchytraeidae	L-S_2	156.8	246.4	217.6
Lumbricidae	L	992	256	481.6
	S1	224	838.4	–
	S_2	648	520	–
	S_3	–	256	–
Oribatida	L	413.93	419.23	404.39
	S_1	72.61	167.48	234.79
	S_2	72.61	24.91	106.53
Gamasidae	L	0.01	0.016	0.02
	S_1	0.011	0.012	0.07
	S_2	0.009	0.011	0.006
Zerconidae	L	9.9	1.7	1.6
	S_1	1	2.9	1.1
	S_2	1.1	2.1	–
Uropodidae	L	–	0.1	–
	S_2	–	0.1	–
Trachytidae	L	2.31	–	–
	S_1	–	–	0.7
Collembola	L	73.98	90.18	112.59
	S_1	10.26	31.05	28.89
TOTAL		2 711.34	2 943.51	1 637.54

4. CONCLUSIONS

The study of numerical and biomass densities of soil animal groups from a pure stand of spruce fir upper limit forest and mixed spruce fir and Larix could be concluded as follows:

– as far as the number of species is concerned, a clear separation could be made between the soils of the studied ecosystems: all animal groups showed a higher number of species in the mixed spruce fir upper limit and Larix, except for oribatids with 9 species smaller in the pure stand, as compared with the mixed one;

– numerical densities of all animal groups showed higher values in the mixed spruce fir upper limit forest, except for Enchytraeidae and Lumbricidae. One explanation of that consists in the small volume of soil in the mixed forests, with rocks near the surface and moss which covered them and the area with a high slope. Because of that, even small quantities of organic material are washed down, remaining a thin organic layer, which covered the rocks. Enchytraeidae and Lumbricidae species have populated better the beginning forming soil of spruce fir upper limit forest;

– biomass densities presented higher values in the soil of pure stand of spruce fir upper limit than in the mixed forest because of Enchytraeidae and Lumbricidae with higher biomass values in the first ecosystem.

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CONTRIBUTIONS À LA CONNAISSANCE DES SOUS-FAMILLES *CRYPTOCEPHALINAE*, *HISPINAE*, *CASSIDINAE* (FAM. *CHRYSOMELIDAE*, ORD. *COLEOPTERA*) D'OLTÉNIE

AURELIAN LEONARDO ILIE

In this work there are presented 32 species, among which 2 are rare for the Romanian fauna: *Hispella atra* L. and *Cryptocephalus pygmaeus* Fabr. and the first species is mentioned for the first time in the region of Oltenia. There are however 4 species less frequent in the fauna of Romania, but not rare: *Cryptocephalus biguttatus* Scop., *Cryptocephalus virens* Suffr., *Cryptocephalus vittula* Suffr., *Cassida vittata* Vill.

Oltenia's crisomelide fauna is also very interesting from the zoogeographical point of view.

1. INTRODUCTION

Les recherches sur la faune des chrysomélidés d'Olténie sont dûs à quelques travaux sistématiques et écologiques [1, 2, 4, 5, 6, 8, 9, 10] dans lesquels sont examinés Coleoptera et parfois d'autres ordres d'insectes.

Une recherche plus ample sur cette famille, dans la zone d'Olténie n'a pas été realisée jusqu'à présent.

2. MATÉRIEL ET MÉTHODES

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

On a utilisé la nomenclature existente [3] mais en ce qui concerne la dispersion des chrysomélidés dans la région paléarctique on a consulté [7, 13].

3. RÉSULTATS

Les coléoptères étudiés appartiennent à 6 genres, en totalisant 32 espèces. Par la suite on présente ces espèces en mentionnant les départements dans lesquels se trouvent les endroits de collectes, ainsi que les auteurs qui ont publié les espèces et les années des publications.

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Sous-famille *Cryptocephalinae* Gyllenhal, 1813

Pachybrachys sinuatus Mulsant et Rey, 1859

Racoviţă (Vâlcea), Ilie, Chimişliu – 2000; Cărbuneşti (Gorj), Ilie, Chimişliu – 2000; Ilie – 2000. Espèce européenne.

Pachybrachys hieroglyphicus Laicharting, 1781

Cărbuneşti (Gorj), Ilie, Chimişliu – 2000; Bumbeşti-Jiu (Gorj), Ilie – 2000. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) octacosmus Bedel, 1891

Tismana (Gorj), Marcu – 1928; Tâmbureşti (Dolj), Bobârnac – 1974; Craiova (Dolj), Ilie – 2000; Ilie, Chimişliu – 2000; Segarcea (Dolj), Gura Motru (Mehedinţi), Leamna (Dolj), Logreşti (Vâlcea), Ilie, Chimişliu – 2000; Caraula (Dolj), Beloţ (Dolj), Pietrele Albe (Gorj), Roeşti (Vâlcea), Preajba (Gorj), Ilie – 2000. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) vittatus Fabricius, 1775

Piatra Cloşani (Mehedinţi), Marcu – 1928; Rînca (Gorj), Bobârnac – 1974; Cărbuneşti (Gorj), Racoviţă (Vâlcea), Ilie, Chimişliu – 2000; Pietrele Albe (Gorj), Ilie – 2000. Espèce européenne.

Cryptocephalus (Cryptocephalus) violaceus Laicharting, 1781

Tismana (Gorj), Marcu – 1928; Vîrvoru de Jos (Dolj), Ilie – 1999; Cheile Sohodol (Gorj), Ilie, Chimişliu – 2000. Espèce européenne.

Cryptocephalus (Cryptocephalus) moraei Linnaeus, 1758

Călimăneşti (Vâlcea), Ochs – 1921; Turnu Severin (Mehedinţi), Cloşani (Mehedinţi), Tismana (Gorj), Marcu – 1928; Ciuperceni (Dolj), Baia de Aramă (Mehedinţi), Bobârnac – 1966-1971; Vîrvoru de Jos (Dolj), Ilie – 1999, Negoi (Dolj), Cheile Sohodol (Gorj), Leamna (Dolj), Logreşti (Gorj), Ilie, Chimişliu – 2000; Roeşti (Vâlcea), Pietrele Albe (Gorj), Preajba (Gorj), Ilie – 2000. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) sericeus Linnaeus, 1958

Judeţul Mehedinţi, Fleck – 1906; Piatra Cloşani (Mehedinţi), Tismana (Gorj), Marcu – Baia de Aramă (Mehedinţi), Cabana Parîng (Gorj), Bobârnac – 1974; Vîrvoru de Jos (Dolj), Ilie – 1999, Chimişliu – 2000. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) bipunctatus Linnaeus, 1958

Piatra Cloşani (Mehedinţi), Marcu – 1928; Preajba (Gorj), Ciuperceni (Dolj), Bobârnac – 1974; Vîrvoru de Jos (Dolj), Ilie – 1999; Craiova (Dolj), Caraula (Dolj), Roeşti (Vâlcea), Pietrele Albe (Gorj), Ilie – 2000; Bistreţ (Dolj), Bucovăţ (Dolj), Ilie, Chimişliu – 2000. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) virens Suffrian, 1847

Valea Sohodol (Gorj), Bobârnac – 1974. Espèce sud-est européenne et asiatique.

Cryptocephalus (Cryptocephalus) octopunctatus Scopoli, 1763

Tismana (Gorj), Marcu – 1928; Valea Sohodolului (Gorj), Bobârnac – 1974. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) biguttatus Scopoli, 1763

Bunaica (Gorj), Bobârnac – 1966–1974. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) marginatus Fabricius, 1781

Novaci (Gorj), Bobârnac – 1966–1974; Bucovăţ (Dolj), Ilie – 2000. Espèce sudique et central-européenne.

Cryptocephalus (Cryptocephalus) flavipes Fabricius, 1781

Turnu Severin (Mehedinţi), Gura Văii (Mehedinţi), Bahna (Mehedinţi), Marcu – 1928; Novaci (Gorj), Rînca (Gorj), Tismana (Gorj), Bobârnac – 1974. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) hypochaeridis Linnaeus, 1758

Piatra Cloşani (Mehedinţi), Marcu – 1928. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) frenatus Laicharting, 1781

Bahna (Mehedinţi), Marcu – 1928. Espèce eurosibérienne.

Cryptocephalus (Cryptocephalus) aureolus Suffrian, 1847

Piatra Cloşani (Mehedinţi), Tismana (Gorj), Marcu – 1928. Espèce européenne.

Cryptocephalus (Asiopus) apicalis Gebler, 1830

Rînca (Gorj), Bobârnac – 1974; Espèce eurosibérienne.

Cryptocephalus (Burlinius) ocellatus Drapiez, 1819

Gura Văii (Mehedinți), Bahna (Mehedinți), Cloşani (Mehedinți), Cloşani (Mehedinți), Marcu – 1928. Espèce répandue en Europe centrale et Asie mineure.

Cryptocephalus (Burlinius) fulvus Goeze, 1777

Piatra Cloşani (Mehedinți), Marcu – 1928. Espèce eurosibérienne.

Cryptocephalus (Burlinius) pygmaeus Fabricius, 1792

Piatra Cloşani (Mehedinți), Marcu – 1928. Espèce central-européenne.

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Sous-famille *Hispinae* Gyllenhal, 1813

Hispella atra Linnaeus, 1767

Preajba (Gorj), Ilie – 2000. Espèce paléarctique.

Sous-famille *Cassidinae* Gyllenhal, 1813

Pilemostoma fastuosa Schaller, 1783

Tismana (Gorj), Marcu – 1928. Espèce eurosibérienne.

Hypocassida subferruginea Schrank, 1776

Başcov-Calafat (Dolj), Hormuzachi – 1904; Tismana (Gorj), Marcu – 1928; Craiova (Dolj), Ilie – 2000; Ilie, Chimişliu – 2000; Caraula (Dolj), Pietrele Albe (Gorj), Ilie – 2000. Espèce paléarctique.

Cassida (Odontionycha) viridis Linnaeus, 1758

Craiova (Dolj), Caraula (Dolj), Pietrele Albe (Gorj), Ilie – 2000; Bucovăț (Dolj), Poiana Mare (Dolj), Negoi (Dolj), Cheile Sohodol (Gorj), Tîrgu Logreşti (Gorj), Ilie, Chimişliu – 2000. Espèce paléarctique.

Cassida (Pseudocassida) murraea, Linnaeus, 1767

Romula (Olt), Hormuzachi – 1904; Vîrciorova (Mehedinți), Marcu – 1928; Valea Sohodol (Gorj), Bobârnac – 1974; Bucovăț (Dolj), Ilie – 2000; Pădurea Băniei (Dolj), Poiana Mare (Dolj), Plenița (Dolj), Orodel (Dorj), Cărbunești (Gorj), Racoviță (Vâlcea), Ilie, Chimişliu – 2000. Espèce euroasiatique.

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Piscu Sadovei (Dolj), Bobârnac – 1974; Craiova (Dolj), Ilie – 2000; Ilie, Chimişliu – 2000; Caraula (Dolj), Ilie – 2000; Segarcea (Dolj), Negoi (Dolj), Ilie, Chimişliu – 2000. Espèce paléarctique.

Cassida (Cassida) prasina Illiger, 1798

Tismana (Gorj), Bilta (Dolj), Bobârnac – 1974. Espèce euroasiatique.

Cassida (Cassida) nebulosa Linnaeus, 1758

Secui (Dolj), Hormuzachi – 1904; Aluniş (Mehedinți), Marcu – 1928; Mîrşani (Dolj), Halînga (Mehedinți), Preajba (Gorj), Bobârnac – 1974. Espèce paléarctique.

Cassida (Cassidulella) vittata Villers, 1789

Tismana (Gorj), Marcu – 1928. Espèce paléarctique.

Cassida (Cassidulella) nobilis Linnaeus, 1758

Tîmbureşti (Dolj), Cloşani (Mehedinți), Baia de Aramă (Mehedinți), Bobârnac – 1974; Craiova (Dolj), Ilie – 2000; Lunca Jiului (Dolj), Ilie, Chimişliu – 2000. Espèce paléarctique.

4. DISCUSSIONS

Les trois sous-familles analysées comprennent un nombre de 32 espèces. On mentionne une espèce pour la première fois dans la faune d'Olténie: *Hispella atra* L.

Cette espèce a été collectée dans l'année 1999 dans la zone sous-carpatique, dans les environs de la localité Preajba (Gorj) sur une pré constituée des espèces de

graminées: *Poa sp., Agropiron sp.*, en confirmant de cette manière les données de la littérature de spécialité concernant les plantes hôtes de cette espèce. Bien que l'espèce est répandue dans tout le pays, toutefois on peut considérer comme en étant rare, en étant mentionnée avec prédominance dans les travaux taxonomiques du début de notre siècle et seulement par intermittence dans l'intervalle de temps suivant: 1967 – Suceava, un exemplaire (Museé des Sciences Naturelles de Bacău).

En ce qui concerne la sous-famille *Cryptocephalinae*, on remarque la présence d'une autre espèce rare pour la faune roumaine: *Cryptocephalus pygmaeus*. Fabr mentionnée à Piatra Cloşani par O. Marcu (1928); dans les autres provinces du pays l'espèce a été mentionnée par Petri (1921) pour la Transylvanie et quelques exemplaires collectés dans l'année 1968 dans le département de Suceava se trouvent dans la collection du Museé des Sciences Naturelles de Bacău.

Cette sous-famille comprend aussi d'autres espèces plus ou moins fréquentes, mais pas rares dans la faune roumaine: *Cryptocephalus biguttatus* Scop., *Cryptocephalus virens* Suffr., *Cryptocephalus vittula* Suffr. D'autres espèces sont très fréquentes dans toutes les régions du pays: *Cryptocephalus octacosmus* Bed., *Cryptocephalus sericeus* L, *Cryptocephalus moraei* L, *Cryptocephalus vittatus* Fabr., *Cryptocephalus bipunctatus* L.

La sous-famille *Cassidinae* réunit 10 espèces, une étant plus ou moins rencontrée dans la faune roumaine: *Cassida vittata* Vill. Bien que la majorité des espèces de cette famille sont mézophiles il existe aussi des exceptions: les espèces *Cassida nebulosa* L et *Cassida nobilis* L. qui sont neutres par raport à l'humidité et la sécheresse.

Grâce au relief riche et varié d'Olténie, la faune des chrysomélidés est très intéressante du point de vue zoogéographique. Les espèces eurosibériennes sont prédominantes – 13 (40,6%), suivies par les espèces paléarctiques – 7 (21,9%), européennes – 5 (15,6%), central-européennes – 4 (12,5%), euroasiatiques – 3 (9,3%).

En ce qui concerne le régime trophique, on constate la prédominance des espèces polyphagues – 16 (50%), suivies par les espèces oligophagues – 10 (31,2%), monophagues – 4 (12,5%) mais dans le cas de deux espèces (6,2%): *Cryptocephalus virens* Suffr., *Cryptocephalus apicalis* Gebl., la littérature de spécialité ne précise pas les plantes – hôte.

On remarque aussi la présence de trois espèces nuisibles à l'agriculture qui attaquent des plantes appartenant à la famille *Chenopodiaceae*, en particulier la betterave et la follette: *Cassida nobilis* L., *Cassida nebulosa* L., *Cassida vittata* Vill.

5. CONCLUSIONS

Les coléoptères étudiés appartiennent à 6 genres, en totalisant 32 espèces.

On mentionne l'espèce *Hispella atra* L. pour la premiére fois dans la faune d'Olténie et cinq espèces moins fréquentes dans la faune roumaine: *Cryptocephalus pygmaeus, Cr. biguttatus, Cr. virens, Cr. vittula, Cassida vittata.*

On constate la prédominance des espèces polyphagues et aussi la présence de trois espèces nuisibles à l'agriculture.

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THE PRESENCE OF *NAMANEREIS LITTORALIS* (POLYCHAETA, NEREIDIDAE, NAMANEREIDINAE) ON THE ROMANIAN LITTORAL OF THE BLACK SEA

VICTOR A. SURUGIU

Abstract. The finding of *Namanereis littoralis* (Grube 1872), a new species for the Romanian Black Sea coast, is reported. The systematics of the species is briefly reviewed. Some morphological, biological as well as ecological characteristics of this species are given. Some phylogenetic relationships within the genus *Namanereis* are discussed.

Key words: Polychaeta, Nereididae, Namanereidinae, Black Sea, taxonomy.

1. INTRODUCTION

The species *Namanereis littoralis* (Grube, 1872) belongs to the subfamily Namanereidinae, which forms a distinct subgroup within the family Nereididae, characterized by the lack of pharyngeal papillae and paragnaths, by the reduced notopodia and by the presence of three or four pairs of tentacular cirri (9). The Namanereidinae are better known from tropical and subtropical regions for their remarkable capacity to inhabit fresh and brackish waters, but mostly by the adaptation of some members of this subfamily to semi-terrestrial environments (5, 7, 23, 26).

Two species belonging to this subfamily have been reported in the Black and the Mediterranean Seas: *Namanereis pontica* (3, 13,17) and *Namanereis littoralis* (2, 16, 19, 20), although some confusion exists in literature concerning the second, which is usually attributed to *Namanereis pontica*.

In the present paper the occurrence of the species *Namanereis littoralis* on the Romanian seashore of the Black Sea is reported. A description of the species is given together with some ecological and biological observations. Phylogenetic affinities with related species of the genus are also discussed.

Namanereis littoralis (Grube, 1872)

Lycastis littoralis Grube, 1872: 47–48; *Lycastopsis beumeri* Augener, 1922: 42; 1936: 347; Wesenberg-Lund, 1958: 14–17, figs. 9–11; *Lycastopsis augeneri* Okuda, 1937: 307–309, fig. 2a–g; Khlebovich, 1961: 177–178; Khlebovich & B.-L. Wu, 1962: 44, fig. 1A–B; Uschakov & B.-L. Wu, 1965: 196; Uschakov, 1965: 184–185, fig. 62A–E; Buzhinskaja, 1967: 88–89; Imajima, 1972: 39–40, fig. 1a–f; *Lycastoides pontica* La Greca, 1949: 164–165, figs. 13–18; Banse, 1959: 302, figs. 5a–c; *Namanereis littoralis* Hartman, 1959a: 162–163; Glasby, 1999: 89–94, figs. 38a–d, 39; *Lycastopsis pontica* Pettibone, 1963: 150–152, fig. 41a–e; Khlebovich,

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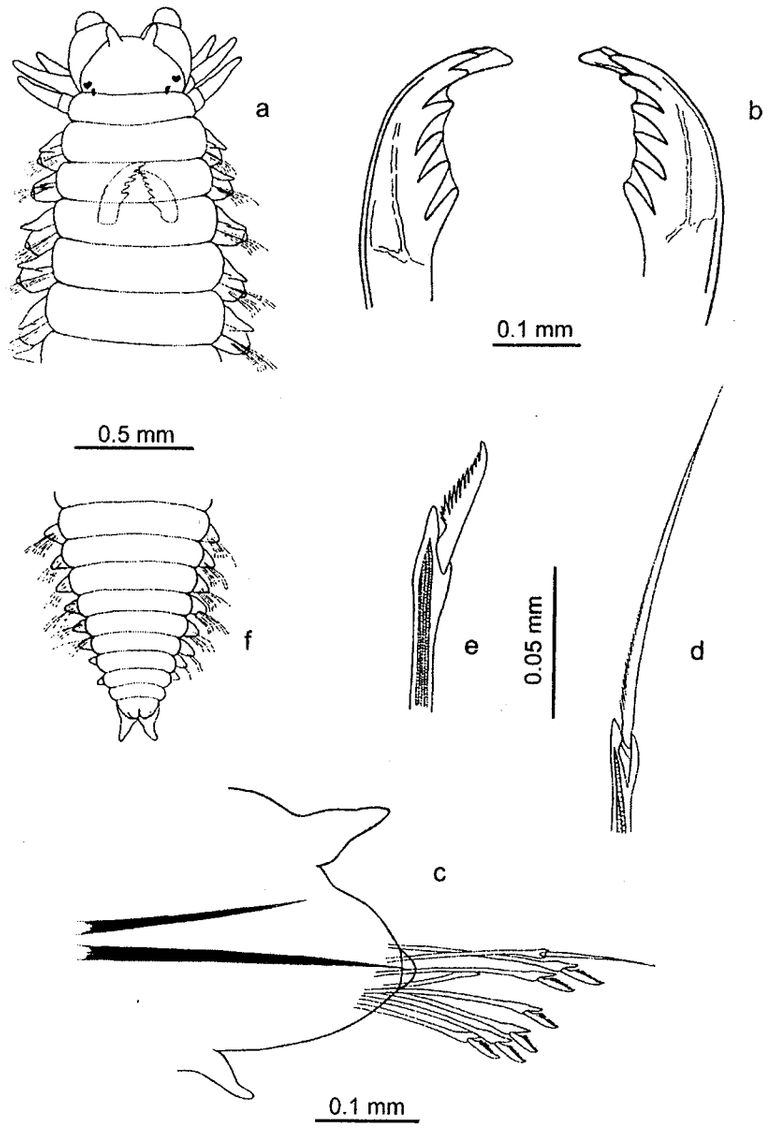

Fig. 1 – *Namanereis littoralis* from Agigea: (*a*) anterior end, dorsal view (jaws visible through the body); (*b*) jaws, dorsal view; (*c*) parapodium from 10th setiger, right side, posterior view; (*d*) supra-neuroacicular sesquigomph spiniger, setiger 10; (*e*) sub-neuroacicular heterogomph falciger, setiger 10; (*f*) posterior end, dorsal view.

1996: 81, pl. 3, figs. 1–8; *Namanereis quadraticeps* Hartman, 1959b: 246; Marinov, 1966: 72–73, figs. 3a–f, 4; *Namanereis pontica* Hartmann-Schröder, 1973: 95–96, figs. 14–17; Marinov, 1977: 116, pl. 15, fig. 1a–e, pl. 37, fig. 2; *Lycastopsis littoralis* Gibbs & Saiz Salinas, 1996: 618–620, figs. 1A–E, 2A, B.

Material examined. The present material consists of thousands of specimens collected from the small beach near Agigea (44°04'53.0" lat. N, 28°38'26.8" long. E) during May-July 1997 and June-October 1998 period and 70 exemplars collected from Cape Tuzla in August 1999. To this can be added the material loaned for comparisons from Zoologisches Institut und Zoologisches Museum der Universität Hamburg: *Namanereis pontica*, 1 ex. from Cuba, coll. Botoşăneanu, det. Hartmann-Schröder (P-13565) and 2 ex. from the Black Sea, Bulgaria, leg. det. Marinov (P-13676).

Description. The maximum length up to 30 mm, with up to 90–100 setigers. Body long, cylindrical, uniform width (1.5 mm), tapering gradually posteriorly. Dorsum convex, venter slightly flattened. Colour of living animals pinkish, resulting from the red color of the dorsal blood vessel on the straw-colour background of the rest of body; preserved in formalin yellowish white, without epidermal pigment. The dorsal longitudinal blood vessel has a slightly sinuous course in the first few segments, with meanders increasing in amplitude considerably toward the posterior end.

Prostomium (fig. 1a) semicircular in shape, twice wider than long, with entire or with slightly cleft anterior margin. Two small, conical antennae on the anterior edge of the prostomium, long as palpophores and wide apart. Pair of biarticulated, thick palps, with swollen palpophores and with distinct, spherical palpostyles. Two pairs of black-brown eyes, devoid of lenses, arranged in a trapezoidal form on postero-lateral border of the prostomium. Anterior pair of eyes oval, irregular, sometimes reniform shape, further apart; the posterior pair slightly smaller, circular and often partially hidden by peristomial fold.

Peristomium, shorter than the following segments, achaetous and apodous. Three pairs of conical, relatively short, almost equal-sized tentacular cirri reaching setiger 2; the hindmost pair of cirri inserted on peristomium and with distinct cirrophores. Eversible pharynx lacking chitinous paragnaths or soft papillae, armed only with two strong, chitinised, brown coloured jaws. These are strongly curved, visible through the body in setigers 2 and 3. Each jaw with single distinct, robust terminal tooth and with 7 lateral teeth, the basal 5 being joined by a membranous sheath (fig. 1b).

Parapodia (fig. 1c) sub-biramous, similar throughout the length of the body, with reduced notopodia, destitute of ligules, with two dark brown or almost black aciculae. Notopodium without podial lobes, but some authors (9, 15) consider

parapodium as having a dorsal parapodial lobe of triangular shape with dorsal cirrus absent. Notoaciculae in the dorsal half of parapodium, completely embedded. Dorsal cirrus triangular, sited half way along length of each parapodium, slightly shorter than podia in the anterior half of the body, becoming slightly longer than podia in the posterior part. Neuropodia with a single acicular neuropodial ligule, conical, obtuse, with a single bundle of setae. Neuroacicula longer and thicker than notoacicula, sited in the middle of parapodium, protruding into retractile tip. Ventral cirrus small, conical, at the base of parapodium. Supra-neuroacicular setae of two types: 1–2 sesquigomph spinigers (fig. 1d) and a single heterogomph falciger (fig. 1e); sub-neuroacicular setae represented only by 3–5 heterogomph falcigers. Spiniger blades long, 2.5 times longer than that of falcigers, slender and finely serrated, tapering gradually to the tip. Falciger blades denticulated, with 8–14 subterminal teeth, longer in supra-acicular falciger than in the sub-acicular ones. Shafts of both falcigers and spinigers with transverse lamellae in the core.

Pygidium (fig. 1f) tripartite, with dorsal anus and with two ventrolateral short, conical, divergent anal cirri.

Biology. About one third of the individuals collected in June and July contained big oocytes, ovoid in shape, measuring 380–460 µm long and 230–310 µm wide, covered by a thin, smooth outer envelope, with a central, spherical, hyaline nucleus and with a parietal, finely granulated yolk. The low number of large eggs (the coelom of the ovigerous individuals contained approximately 40–70 oocytes), as well the semi-terrestrial mode of life suggests that the development may be direct, without free-swimming pelagic larvae. This supposition is strengthened by the fact that the smallest of the juveniles observed, about 2–3 mm long and having 9–10 setigerous segments, were completely developed, resembling adults.

Habitat. The species is found in abundance in the upper littoral zone under isolated rocks overlying coarse and medium grained sand of conchiferous nature. It lives associated with various small turbellarians, nemerteans, nematodes, ostracods, harpacticids, halacarids and amphipods such as *Hyale pontica* and *Gammarus olivii*; very seldom with *Idothea baltica*, *Sphaeroma pulchellum* and Chironomid larvae. After strong storms the species also occurs in the supralittoral mats of decaying seaweed together with the oligochaete *Enchytraeus albidus*. The distribution of the worms is very patchy, in some places reaching 120,100 individuals per square metre, which corresponds to a biomass of 437.6 g/m^2. The Black Sea population had the same habit as that described by Pettibone (22, cf. fig. 41a, p. 151) of coiling up tightly like a compressed spring. Euryhaline species, occurring in seawater (4, 7, 11, 21), brackish water (2, 6) and fresh water (1). Wesenberg-Lund (26) found this species in hyperhyaline lagoons and ponds in

salinity varying from 36 to 130‰. The present material was found in water of salinity ranging from 16.4 to 18.3‰ and at temperature of 15–26 °C.

Distribution. *Namanereis littoralis* has a widespread distribution in the tropical and subtropical regions of the Pacific and Atlantic Oceans, but can spread also in the boreal regions. The following lists the localities from where the species was reported: Black Sea (Sozopol; Agigea; Tuzla); Bosphorus; Adriatic Sea (Rovinj); northern Spain (Ría de Bilbao); New England Region (Massachusetts; Virginia); Caribbean Sea (Havana, Cuba; West Indies; Lesser Antillean Islands); southern coast of Brazil (Desterro, in present Florianópolis); Sea of Japan (Oshoro Bay and Shirikishinai, Hokkaido; Gulf of Peter the Great; Posjet Bay); Yellow Sea (Quingdao); southern Sakhalin (Aniwa Bay), southern Kuril Islands (Iturup, Kunashir, Shikotan, Yurij).

2. DISCUSSION

Due to a great variability of morphological features and to a widespread distribution, *Namanereis littoralis* is regarded by Glasby (7) as a species group. It is possible that the subsequent studies, using criteria other than that of the external morphology, such as DNA sequences, serological methods etc., will show the existence of several sibling species within this taxon, or, vice-versa, will make it possible to synonymise it with other closely-related species.

The status of species *Namanereis pontica* (Bobretzky, 1872), described initially from the Black Sea (Bay of Sebastopol), is confused at the moment. Although the two species are very similar, Dr. C.J. Glasby thinks that *Namanereis pontica* and *Namanereis littoralis* are two distinct and different species, based on the absence of spinigers in the supra-acicular fascicle of the neuropodia in the former and the presence of this type of seta in the second. Also *pontica* has a greater number of teeth on the supra-acicular falcigers (C. Glasby, pers. comm.). In any case, the situation was not completely clarified using cladistical analysis, the both species falling out in the part of the consensus tree that is unresolved (7). Unfortunately, the specimens of *Namanereis pontica* were not available for the present study, but according to the personal communication of Prof. Dr. V. Khlebovich, all specimens from the Black Sea (5 samples) housed in the collection of the Zoological Institute, Academy of Sciences, St. Petersburg present such spinigers in the upper setal bundle of parapodia, so they must be attributed to *Namanereis littoralis*.

The lack of, or strong reduction of, some traditionally used features in nereidid systematics, such as pharyngeal armature and complex structure of parapodia, make it extremely difficult to separate species in the Namanereidinae (7, 9). The most useful features in this sense are the chitinous structures, such as jaws and setae. Another feature used in the separation of Namanereidids was the number

of tentacular cirri. Hartman (9) shows that the species of *Namalycastis* had 4 pairs of peristomial cirri, instead of 3 pairs that are in genus *Namanereis*, with the second ventral pair lacking. The only species of *Namanereis* having 4 pairs of tentacular cirri is *Namanereis quadraticeps*, which according to a cladistic analysis (7) is considered to be the ancestor of both clades *Namanereis* and *Namalycastis*. The loss of the posteroventral pair of cirri during the phylogeny within the genus *Namanereis* can be supported by the existence of some denatured specimens of *Namanereis littoralis*, such as that found in Posjet Bay, which bear a fourth rudimentary tentacular cirrus on the right side of the body (15, cf. Pl. III, fig. 1, p. 154). This can be considered, according to its position, as being the ventral cirrus of the second larval segment, that forms the peristomium by cephalisation of the first setiger. Even Bobretzky (3) indicates for *Lycastis pontica* the presence of one small swelling, hardly visible on peristomium, which in his opinion can be considered as being the primordia of the 4-th tentacular cirus.

In connection with the methods of reproduction and development within genus *Namanereis* there exists relatively few data. Whilst the majority of authors support the possibility of hermaphroditism (5, 9, 18, 22), others consider that the sexes are separate (7), or may involve a parthenogenetic mode of reproduction (6, 8). So further studies must concentrate on the reproductive biology, which may be another aid in species separation within the Namanereidinae.

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SÉLECTION DES ŒUFS DES OISEAUX PARASITES SELON UNE NOUVELLE INTERPRÉTATION

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A new interpretation of the adaptation of some bird species to nest parasitism, e.g. the European Cuckoo, *Cuculus canorus*, is advanced in the present paper. It is almost unanimously accepted that the similarity between the eggs of the parasite bird species and those of the host species has been realized through selection by the host species, which recognize the eggs of the Cuckoo after their colour and accept them in the nest only if they are similar to their own eggs. The author disagrees with this interpretation, because it is a well-established fact that birds do not recognize the eggs after shape, colour pattern or size. They accept all eggs present in their nest. In his opinion, this similarity has gradually been realized by the nest-predatory bird species, which eat especially the eggs whose colour differs from that of the habitat and can easily be observed by the predatory birds. Selection has favoured the eggs of the host species and of the Cuckoo as well, which are most similar to the habitat.

L'une des caractéristiques essentielles de la biologie des êtres vivants est la variabilité, ce qui fut démontré de façon si persuasive par Darwin. La couleur des œufs des oiseaux, ainsi que la forme, la dimension, l'épaisseur de la coque, etc. sont soumises elles aussi à cette implacable loi, et cette tendance à la variabilité a existé et existe chez tous les oiseaux du monde. On constate même aujourd'hui chez les espèces, populations ou même individus différents, à un certain degré de différenciation, surtout en ce qui concerne des œufs. Il y a des cas ou les couleurs des pontes appartenant aux individus de la même espèce diffèrent tellement entre elles, que même un spécialiste est mis dans l'embarras s'il faut établir à quelle espèce appartient une telle ponte ou si elle appartient aux individus de la même espèce.

On va donner par la suite quelques exemples d'oiseaux de l'avifaune roumaine présentant une grande variabilité du coloris des pontes. Ainsi, on rencontre chez la Locustelle luscinioide (*Locustella luscinioides*), Lusciniole à moustaches (*Lusciniola melanopogon*), Rousserole turdoide (*Acrocephalus arundinaceus*), Rousserole effarvatte (*Acrocephalus scirpaceus*), Rousserole verderolle (*Acrocephalus palustris*), Pie-grièche écorcheur (*Lanius collurio*), Pinson des arbres (*Fringilla coelebs*), Bruant jaune (*Emberiza citrinella*), Bruant des roseaux (*Emberiza schoeniclus*), Moineau domestique (*Passer domesticus*), Moineau friquet (*Passer montanus*), Moineau espagnol (*Passer hispaniolensis*), en suite chez la Fauvette à tête noire (*Sylvia atricapilla*), Fauvette à tête grisette (*Sylvia communis*), deux à 18 variations de couleur des pontes et le Pipit des arbres

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(*Anthus trivialis*) champion en ce qui concerne la variabillité du coloris des pontes, qui monte à 32 variantes (W. Makatsch, 1976). Mais, les différences du coloris ne sont pas limitées seulement aux pontes d'une même espèce, mais aux œufs de la même ponte.

Quant au problème du coloris, on constate qu'il n'y a presque aucune couleur, ou nuance simple ou à taches, mouchetée, poitillée, striée, griffée, etc., répartie de la manière la plus diversifiée, qui ne soit retrouvée au cas des coquilles d'œufs des plus de 8680 espèces existant sur la Terre.

Cette grande diversité du coloris est la résultat du processus d'adaptation gouvernant tout le monde ailé et qui a été réalisée au long de l'évolution, conformément au «modèle» qui s'est avéré être le plus favorable à chacune des espèces.

On est unanimement d'accord dans le domaine de l'Ornithologie que cette multitude de couleurs uniformes et de combinaisons du coloris des coquilles d'œufs des oiseaux n'a été autre que le résultat de la permanente activité des oiseaux rapaces des nids sur les œufs des espèces avec lesquelles on vivait en commun. En fonction des conditions géographiques de l'emplacement des nids, de la possibilité d'être dépistés et de l'accès à ces nids, du nombre et de la composante spécifique des oiseaux rapaces des nids, etc., ceux-ci ont «décidé» indirectement le colorit des œufs des espèces qu'ils ravageaient, en éliminant ceux dont le coloris contrastant au milieu trahissait leur présence et dont le sort était ainsi scellé.

En résumant, le coloris des oiseaux a été et est déterminé par les rapaces des nids.

Autant un œuf aura-t-il un coloris semblable au milieu où il a été déposé, d'autant plus ses chances de survivre seront plus grandes, donc la possibilité de continuer sa descendance sera plus grande.

Il est bien connu que les oiseaux, tels ceux nichant aux creux des arbres, dans les tanières, galeries souterraines, etc., ont les œufs claires (blanc, crème), par ex. les oiseaux de proie nocturnes (Chouettes et Hiboux-Strigidae, etc.), Rolliers (Coraciidae), Martins pêcheurs (Alcedinidae), Pics (Picidae), Martinets (Apodidae), Hirondelles rousselines (*Hirundo daurica*), Hirondelles de fenêtre (*Delichon urbica*), Hirondelles de rivage (*Riparia riparia*), Mesange rémiz (*Remiz pendulinus*), Tadornes (Tadornae), Grèbes (Podicipedidae), Canards (Anatinae), Oies (Anserinae), etc., bien que leurs œufs aient un coloris facilement visibile, les rapaces n'ont pas eu accès pour effectuer une sélection. Certaines espèces de Canards ou d'Oies, logées aux creux des arbres ou en tanières, mais à œufs claires sont à l'abri des rapaces dû à leur habitude de couvrir les œufs au moment de leur départ (D. Radu, 1960, 1998).

Il y a encore certaines espèces telles que: les Pigeons ramiers (*Columba palumbus*), Pigeons bisets (*Columnba livia*), les Tourterelles des bois (*Streptopelia turtur*) Tourterelles turques, lesquelles, bien que nichant librement ont cependant les œufs blancs.

Cela s'explique par le fait que ces espèces déposent seulement deux œufs en une ponte, et à partir du premier œuf, la ponte est surveillée à tour de rôle par l'un des parents qui reste debout sur le nid en prévenant l'attaque des oiseaux rapaces des nids (D. Radu, 1960, 1962, 1998).

Il y a aussi des cas inverses, à savoir des espèces qui nichent aux creux des arbres et ont les œufs en couleurs, telles que: l'Etourneau sansonet (*Sturnus vulgaris*), le Rougequeu à front blanc (*Phaenicurus phoenicurus*) le Choucas des tours (*Coloeus monedula*), les Mesanges (Paridae), etc. Cela est dû au fait que l'espèce respective a changé récemment la modalité initiale de ponte, libre et leur abri actuel caché est une adaptation récente.

Le fait que des individus des espèces se trouvent parfois encore aujourd'hui nichant aussi libres (Choucas des tours), ou au demi-creux des arbres (Etourneau sansonet, Rougequeu à front blanc) confirme cette affirmation.

Après cette ample mais nécessaire introduction, on va passer au sujet proposé à traiter dans ce travail.

L'un des plus intéressants phénomènes biologiques du monde des oiseaux c'est le parasitisme. Il a mené à des conséquences étonnantes en ce qui concerne le degré d'adaptabilité dans le monde animal et leur description a intéressé des générations et de chercheurs et de biologistes l'espèce d'oiseau à travers le temps.

Le présent travail se rapporte à l'espèce d'oiseau au plus haut niveau de perfection du comportement relatif à la reproduction parasitaire des oiseaux, notamment celui du Coucou gris (*Cuculus canorus*). C'est un oiseau bien connu aux habitants du pays où il se trouve, tant par la voix caractéristique d'où vient son nom, que par le comportement spécifique, à savoir celui de déposer les œufs dans le nid d'autres oiseaux (environs 300 espèces), qui élèveront sa progéniture dont l'aire de distribution s'étend sur trois continents du Vieux Monde, à savoir l'Europe, l'Asie, l'Afrique.

Certes, ni le coloris des œufs du Coucou n'a échappé au phénomène naturel de la variabilité dont il a été question, fait qui – tel que l'on verra – eut un rôle important au perfectionnement de son mode de reproduction parasitaire.

Le début de l'adaptation du Coucou à la vie parasitaire est apparu à partir d'une mutation du comportement spontané de l'une ou de certaines femelles, à savoir celui de ne plus construire leur propre nid mais de déposer les œufs au hazard, dans les nids de différentes espèces avec lesquelles elles cohabitaient. Les chances que ces œufs ne soient pas observés étaient d'autant plus grandes, qu'ils étaient plus semblables en ce qui concerne la couleur à ceux des nids des oiseaux où ils étaient déposés. Après l'éclosion des oisillons, celui du Coucou, plus vorace et plus fort que ceux des parents d'adoption sera en concurrence avec les autres

«frères» pour la nourriture apportée par les parents de ceux-ci et finalement il restera le seul survivant.

Ainsi, les osillons résultés des parents qui se sont écartés du comportement héréditaire, celui d'élever seuls leur progéniture, héritant la même disposition ont procédé eux-mêmes de manière semblable, en déposant les œufs dans les nids d'autres espèces d'oiseaux.

Un tel comportement s'avérant au cours des ères plus favorable aux oiseaux qui le pratiquaient, a été généralisé à toutes les populations du Coucou, dont le comportement a fixé cette caractéristique avec toutes les «améliorations» gagnées au cours du temps et les exemplaires normaux, donc non parasites, plus désavantagés, obligés à construire des nids, les défendant contre les ennemis, couver les œufs et élever les oisillons, ont diminué progressivement jusqu'à leur complète disparition (D. Radu, 1960).

L'exposé très succinct, en quelques lignes seulement, a constitué des processus de longue durée, couvrant des millions d'années et ce n'est pas le cas de le traiter en détail, vu qu'il ne se réfère pas directement au thème proposé.

On va s'arrêter seulement à une caractéristique du Coucou, à savoir celle des femelles d'avoir des œufs à divers coloris, en arrivant actuellement que chacune pond des œufs de divers couleurs et dessins, complètement uniformes: blancs ou crème, mouchetés, tachés, striés, pointillés, griffés, à dessins variables, etc., semblabes aux œufs des espèces parasitées; Il y a ainsi des œufs de Coucou café marron comme ceux des espèces Phragmite (*Acrocephalus schoenobaenus*), Fauvette mélanocéphale (*Sylvia atricapilla*), Globemouche gris (*Muscicapa striata*), oiseaux dont le coloris des œufs est semblable; ensuite des œufs tachés comme la Rousserolle verderolle (*Acrocephalus palustris*), des œufs bleus déposés chez la Rougequene à front blanc (*Phaenicurus phoenicurus*) œufs bleus; des œufs blancs comme la Rougequene noire (*Phoenicurus ochruros*), etc.

Ces concordances du coloris des œufs des femelles du Coucou avec ceux des hôtes qu'elles parasitent sont bien connues dans la science et sur leur base on a même établi l'existence des races oologiques chez cette espèce, c'est-à-dire aux œufs à couleurs différenciées à divers exemplaires de celle-ci.

La question qui se pose c'est d'expliquer pourquoi les diverses femmelles du Coucou ont des œufs de diverses couleurs, presque semblables au coloris si varié des œufs des oiseaux auxquels elles donneront leurs propres œufs?

Pour être à même de répondre à cette question il faut rappeler en premier lieu l'existence du phénomène connu aux oiseaux, par lequel, les sens des nouveau-nés (oisillons) porteront l'empreinte des traits caractéristiques de l'oiseau qui les a élevés, tels que la voix, le coloris, les mouvements spécifiques, ainsi que le paysage où ils ont vu la lumière du jour, etc. À leur maturité sexuelle, les femelles chercheront à déposer leurs œufs dans les nids des mêmes espèces qui les ont élevés (Radu, 1974, 1976, 1994 a et b).

Au cas spécial de l'oisillon du Coucou, élevé par une autre espèce, le phénomène sera imprégné lui aussi, selon le modèle indiqué, par les parents adoptifs, et au moment de la maturité sexuelle, celui-ci va chercher la même espèce pour mettre à son tour l'œuf dans le nid de celle-ci. Et comme les œufs des parents de ces oisillons sont si semblables aux œufs du même hôte, les œufs même des oisillons hériteront la capacité de faire des œufs du même coloris. Répété mille fois, ce phénomène a conduit à l'existence des races oologiques du Coucou.

Le phénomène de parasitisme des oiseaux a intéressé de nombreux chercheurs. Celui du Coucou et la modalité de sa réalisation figurent dans des centaines de travaux signés par plusieurs ornithologistes du monde entier et les nombreux films documentaires montrent en détail le mode de développement du comportement parasite, à partir de la phase où la femelle du Coucou étudie le nid du futur hôte où elle dépose son propre œuf et jusqu'au moment où l'oisillon du Coucou élevé par les parents étrangers sera indépendant.

Cependant, dans toute la littérature spécialisée concernant le phénomène de parasitisme du Coucou, ainsi que dans les films documentaires réalisés, il ressort de façon erronée que le phénomène qui a déterminé l'homochromie des œufs des espèces parasites à ceux des espèces parasitées a été déterminé par deux facteurs seulement, notamment, les oiseaux parasites et les parasites. On attribue ainsi à l'oiseau parasité en exclusivité le rôle d'observer l'œuf étranger qui a été mis à son nid et qui, saisissant sa présence par le coloris différent de celui de ses propres œufs, soit jettera l'œuf étranger du nid, soit abandonnera le nid en totalité. Ce n'est qu'au cas où le coloris de l'œuf de l'oiseau parasite sera semblable aux propres œufs que l'oiseau parasité ne pourra l'identifier et l'oisillon parasite pourra avoir la chance de voir la lumière du jour et être élevé par l'oiseau trompé.

Conformément aux observations et aux propres expérimentations, ainsi qu'aux données des travaux ornithologiques authentiques on a démontré de façon incontestable que les oiseaux ne reconnaissent pas leurs propres œufs, mais ils acceptent seulement ceux qui se trouvent dans leurs propres nids, quelles que soient la couleur, la dimension ou la forme. De même dans le cas des oisillons. Au cas de l'oisillon du Coucou, celui-ci est élevé au hazard par les oiseaux parasités, seulement parce qu'il est apparu dans leur propre nid, bien qu'il ne leur ressemble aucunement et deviendra même dix fois plus grand qu'eux-mêmes.

1. Il y a plusieurs décennies, l'auteur a montré que l'oiseau-hote accepte l'œuf du Coucou qu'il ne distingue pas, en règle générale, des siens, ainsi que l'élevage de l'oisillon qui ne ressemble pas aux siens, phénomène mis au compte du comportement instinctif des oiseaux (D. Radu, 1960, 1974 a., 1980, 1992 c.).

2. Dans ce cas on a affaire au phénomène que nous avons appelé «imprégnation inverse», c'est-à-dire celui d'imprégnation de l'oiseau parasité par l'oisillon parasite qui est apparu dans son nid (Radu, 1992a., 1994 b.).

Selon nous, le parasitisme chez les oiseaux est réalisé non seulement par les deux acteurs, c'est-à-dire par les oiseaux parasités et parasites, mais par trois, à savoir: oiseaux parasités, oiseaux parasités et oiseaux rapaces des nids qu'on ne mentionne jamais.

On a montré comment le coloris spécifique des œufs des 8680 oiseaux de la Terre, avec les exceptions mentionnées, a été réalisé, dû aux oiseaux rapaces des nids qui ont éliminé les œufs contrastant avec le milieu et qui étaient donc plus visibles et ont «protégé», indirectement, ceux dont le coloris ne trahissait pas leur présence. Même dans le cas des oiseaux parasites, y compris le Coucou, ce ne sont pas les oiseaux parasités qui ont saisi l'œuf étranger mis dans leur nid, mais il est observé toujours par l'omniprésent oiseau rapace des nids qui élimine en permanence les œufs de l'oiseau parasite, donc du Coucou, quand leur coloris est en contraste avec celui des œufs des oiseaux parasités, déjà adaptés au milieu, restant seulement ceux aussi semblables que possible aux œufs des espèces qu'il parasite. La création des races oologiques à l'espèce du Coucou, montrée plus haut, est par conséquent «l'œuvre» des rapaces des nids, mêmes dans le mécanisme de leur réalisation intervenant aussi le comportement d'imprégnation (imprinting) des oisillons parasites par rapport à l'espèce des parents.

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WEIGHT, DIGESTIVE ENZYMATIC ACTIVITY (LACTASE AND AMYLASE) AND GLYCEMIA VARIATION IN PIGLETS ACCORDING TO THE ONTOGENETICAL STAGE AND THE FEEDING REGIMEN*

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Several groups of Landrace piglets were investigated simultaneously to assess their weight gain, the intensity of the pancreatic and intestinal digestive enzymatic activity (lactase and amylase) and the glucose blood level in accordance to the age and the changed feeding conditions.

The enzymatic activity (both lactase and amylase) displayed a clear dependence on age, with a pronounced adapting character. Pancreatic amylase and, to a lower extent, the intestinal amylase, displayed increased values when the enzymatic preparations (A or B) were fed, or when supplemental starch was given to the animals. The lack of supplementary food and the precarious state of development (minus variants from 14 days) caused the reduction of enzymatic activity.

1. INTRODUCTION

The present paper shows the results of a complex investigation on body weight evolution and on the evolution of several physiological indicators pertaining to digestion and carbohydrate metabolism according to the first stages of ontogenetical development. Such investigations are indispensable to know – and find the ways of intervention – the environmental influences on the metabolic maturation of piglet organism and implicitly on the high mortality rates (20–30%) that may occur between farrowing and weaning (Mersmann 1974).

Together with the preoccupation to highlight the variation of these indicators in "normal" conditions (that characterize the technology used in SCCCP Peris complex), we also monitored the influence of some changes on the technology (early weaning) and feeding.

Considering the particular sensitivity of piglets to the stressing action of some environmental factors during the post-farrowing period, we also tried to show the existence of a possible relation of dependence between the enzymatic activity and the state of stress.

* This experiment was conducted with equipment and reagents donated by Alex. von Humboldt Foundation (Germany).

2. MATERIALS AND METHOD

Several experimental variants were developed for the purposes of our investigation, to monitor the following main aspects:

– the influence of age on weight, enzymatic activity and glycemia;
– the influence of feeding some dietary enzymatic preparations;
– the influence of higher starch levels;
– the influence of depriving the piglets of supplementary feeding during the suckling period;
– the influence of early weaning (21 days).

Table 1 shows the experimental diagram.

Table 1

Experimental diagram

Variant	Age (days)	Weight determination	Lactase determination	Amylase determination	Glycemia determination	Feeding regimen	Experimental days
1	2	3	4	5	6	7	8
Control	1	-	yes	yes	-	suckling	-
	7	-	yes	yes	yes	suckling	-
	14	-	yes	yes	yes	suckling	-
	21	yes	yes	yes	yes	suckling	7
	28	-	yes	yes	yes	plus add.	14
	38	yes	-	-	-	feed	24
Feed A	14	-	-	-	-	suckling plus diet 0-1 plus enzymatic preparation A	-
	28	-	-	yes	yes		12
	38	yes	-	-	-		
Feed B	14	-	-	-	-	suckling plus diet 0-1 plus enzymatic preparation B	-
	28	-	-	yes	yes		12
	38	yes	-	-	-		
Starch	14	-	-	-	-	suckling plus diet 0-1 plus 15% corn meal	-
	28	-	-	yes	yes		9-12
	38	yes	-	-	-		
No additional feeding	14	-	-	-	-	suckling, no additional feeding at 14 days	-
	28						
	38						7
No additional feeding	14	-	-	-		diet 0-1, no suckling	-
	28	-	-	yes	yes		17
	38	yes	-	-	-		17

The experiments used Landrace piglets from our own complex. To avoid possible additional stressing influences we always used groups of piglets from the same litter. Each group consisted of 6–10 piglets, 2–4 of which were slaughtered.

Among the digestive enzymes considered to have a determining role for the material and energy metabolism of piglets during the first stages of the ontogenetical development we selected the lactase (β-galactosidase: β-D-galactoside-galactohydrolase, EC 3.2.1.23) and amylase (a-1 4-glucan-4-glucano-hydrolase, EC 3.2.1.1.).

Lactase activity was assessed according to the method of Bergmeyer (1970), modified after our optimization tests as follows:

– potassium phosphate buffer (0.1 M; pH = 7.0)	0.630 ml
– lactose solution (70 mg/l)	0.200 ml
– NAD^+ (10 mg/ml)	0.040 ml
– Mg SO_4 (0.1 M)	0.020 ml
– galactoso-dehydrogenase (5 mg/ml)	0.010 ml (dilution 1:3)

The enzymatic reaction was started with an "enzyme" solution: 100 ml (supernatant obtained after the homogenate was centrifuged).

The determinations were done with an Eppendorf (Germany) spectrophotometer, at 366 nm.

Amylase activity was assessed with the method described by Rick and Stegbauer (1970), based on the determination of the reducing groups.

For both enzymes we used a temperature of determination (ET = AT) of 38 °C.

The activity of the two enzymes was investigated on two types of tissue: pancreas and the small intestine. The sample from the small intestine was taken from the proximal part (jejunum), 8–10 cm from the stomach.

The sample of 0.300–0.600 g tissue were passed through a potassium phosphate buffer solution (0.1 M, pH = 7.0) kept all the time on ice (0–4°C), making a dilution of 1:11 (weight/volume). The sample was thereafter triturated with a Ultra-turrax homogenizer (2 × 30", with 30" breaks). In most cases the samples were processed within the next 1–3 days, being kept meanwhile in the freezer (–8–12 °C), after trituration, the samples were passed into a Janetzky cooler centrifuge for 20' at about 3000 rpm. All used reagents were supplied by Boehringer Mannheim and Merk companies (Germany).

For the variant assessing the influence of age (variant 1), we conducted determinations on piglets aged 1, 7, 14, 21 and 28 days (for the enzymatic activity and glycemia) and 30 and 38 days respectively (for body weight evolution).

Early weaning was performed at 21 days, the piglets remaining in the same pens, the sow being moved away. Control slaughtering was performed after 7–8 days (variant 5).

In variant 4, with no supplementary feeding, we compared the data from the groups fed normally (according to the technologic flow from the complex) starting from the age of 14, with the results of the groups with no additional feeding, in which sow milk was the only feed for 7 days.

For variant 3, with additional starch, we supplemented the usual diet (0–1 for suckling piglets) with some 10–15% more starch (150 g high quality corn meal for 1 kg forage).

The influence of the dietary enzymatic preparations (variant 2) was monitored on two sub-variants: enzymatic preparation A (*Aspergillus orizae*) and B (*Bacillus subtilis*), both produced by the Institute of Biology and Animal Nutrition Baloteşti (eng. M. Vintilă). These preparations were included (5%, 50 g for 1 kg feed) into the normal diet starting from the age of 14 days.

The piglets were always slaughtered in the morning between 9–11, sampling being performed immediately after slaughtering by beheading.

Glycemia was determined immediately after the blood sample was collected using the photocolorimetric method (King, 1951).

Weight assessment was done by individual weighing at slaughtering or at the end of the experiment.

I. Body weight evolution

Table 2 shows the results of all experimental variants.

Table 2

Body weight evolution in the experimental variants

Group	Animal number	Age, days	Feeding regimen	Duration, days	Average weight, kg	Deviation
1	2	3	4	5	6	7
Control I	8	21	Suckling plus additional feeding at 14 days	-	5.36	0.61
II	10	30		-	5.90	0.43
III	9	38		-	7.33	0.55
A	8	38	suckling plus enzyme preparation A	12	7.68	0.81
B	7	38	suckling plus enzyme preparation B	12	8.00	0.45
Starch	9	38	suckling plus 15% corn meal	9-12	8.21	0.58
No additional feeding	7	38	suckling, no additional feeding	7	6.70	0.45
Weaning	6	38	suckling plus diet 0-1 for 14 days	17	7.53	0.51

For a better view of the weight difference, we built diagrams showing the influence of age (Fig. 1) and of the feeding regimen (Fig. 2).

The dependence of weight on both the ontogenetical stage and the feeding regimen is obvious. Thus, the average values for the weight of groups fed supplemental starch (8.21 kg at 38 days) and enzymatic preparation B (8.00 kg at 38 days) are clearly superior to the control group (7.33 kg).

Piglet weaning at 21 days did not reduce the weight gain, which was slightly higher than in the control group (7.33 kg).

Lower weight was observed in the group with no additional feed starting with the age of 14 days (7.00 kg).

II. Enzymatic activity

1. Age influence

Table 3 shows the data on lactase activity.

Table 3

Lactase activity according to age

Tissue type	Piglet number	Enzymatic activity, U/g fresh tissue					Average weight at 21 days, g
		1 day	7 days	14 days	21 days	28 days	
Small intestine	4	10.12	2.80	–	3.18	2.50	3200
Pancreas	4	6.20	2.66	3.18	2.48	2.75	

It may be observed that the activity of this enzyme is much more intense in the intestinal tissue immediately after birth (10.12 U/g fresh tissue), decreasing during 7 days close to the level of the pancreatic tissue: 2.80 compared to 2.66 U/g f.t.

This evolution remained within the same limits after 21–28 days too.

Table 4 shows the results of amylase activity (pancreatic and intestinal) at 1, 7, 14, 21 and 28 days from birth.

The enzyme activity in the small intestine increased by about 50% from 1 to 7 days, remaining thereafter at a constant level.

Pancreatic amylase had a particular affinity, reaching high values from the first day, continuing this trend throughout to the age of 28 days included.

Table 4

Amylase activity according to age

Tissue type	Piglet number	Enzymatic activity, U/g fresh tissue					Average weight at 21 days, g
		1 day	7 days	14 days	21 days	28 days	
Small intestine	4	73.33	125.82	208.97	111.37	116.39	3200
Pancreas	4	588.96	1163.20	1088.72	3918.75	6685.95	

It is interesting the group of 14 days, in which the slaughtered piglets were minus-variants (weighing about 1200 g). In this case the values of intestinal amylase were almost two times higher than the plateau settled on day 7, while the pancreatic amylase did not display a growing trend, characteristic to this type of tissue during the first 28 days post partum.

2. Influence of the enzymatic preparations

Tables 5 and 6 present the results obtained when the enzymatic preparations (A and B) were added to piglet diets for 12 days.

Table 5

Influence of the dietary enzymatic preparation A

Tissue type	Piglet number	Feeding duration, days	Enzymatic activity, U/g fresh tissue			Average weight at slaughtering
			control at 28 days	experimental	deviation	
Pancreas	4	12	6685.95	16225.50	1512.00	3925
Small intestine	4	12	116.39	104.50	33.00	

Table 6

Influence of the dietary enzymatic preparation B

Tissue type	Piglet number	Feeding duration, days	Enzymatic activity, U/g fresh tissue			Average weight at slaughtering
			control at 28 days	experimental	deviation	
Pancreas	4	12	6685.95	18031.25	3280.18	5150
Small intestine	4	12	116.39	215.76	48.11	

Concerning the first preparation (A), based on *Aspergillus oryzae* (amylolytic activity in the preparation: 1000–2000 IU/g), we did not observe any influence on the activity of intestinal amylase. The pancreatic amylase, however, displayed a much higher activity, both compared to the control (28 days) and to the other experimental variants, in which we also observed the increase of activity.

The enzymatic preparation B based on *Bacillus subtilis* (amylolytic activity in the preparation: 600 IU/g) had the most obvious influence. The activity of intestinal amylase increased compared to the control (the highest from all variants), while the activity of the pancreatic amylase also reached the highest values.

3. Influence of additional starch

Table 7 shows the experimental results for the pancreatic and intestinal amylase in the piglets that received a diet for 9–12 days (starting with the age of 14 days) changed from the normal diet (diet 0–1) by the addition of more (15%) starch as high quality corn meal.

The results show an increase in the activity of the pancreatic amylase, while the intestinal enzyme remained at the level of the control group.

Table 7

Influence of the additional dietary starch

Tissue type	Piglet number	Feeding duration, days	Amylase activity, U/g fresh tissue			Average weight at slaughtering
			control at 28 days	experimental	deviation	
Pancreas	4	9–12	6685.95	11240.62	1757.03	5375
Small intestine	4	9–12	116.39	119.61	28.65	

4. Influence of (not administering) additional food

The data for this experimental variant, in which the piglets aged 14 days did not receive additional food, are presented in Table 8.

Table 8

Influence of (not administering) additional food

Tissue type	Piglet number	Feeding duration, days	Amylase activity, U/g fresh tissue			Average weight at slaughtering
			control at 28 days	experimental	deviation	
Pancreas	4	7	3918.75	5843.25	1397.19	4350
Small intestine	4	7	11.37	59.12	22.53	

A decreasing trend can be observed for the activity of the intestinal amylase (compared to the control at 21–28 days), while the activity of the pancreatic amylase increased significantly (by about 70%).

5. Influence of early weaning

Table 9 shows the data for the piglets weaned at 21 days.

It may be noticed that the activity on intestinal amylase decreased compared to the control (not significantly, though). The activity of the same enzyme in the pancreas, however, increased compared to the control and even compared to the group that did not receive additional food (variant 4).

Table 9

Influence of early weaning at 21 days

Tissue type	Piglet number	Feeding duration, days	Amylase activity, U/g fresh tissue			Average weight at slaughtering
			control at 28 days	experimental	deviation	
Pancreas	4	7	6685.95	1501.49	5285	5285
Small intestine	4	7	116.39	75.63	30.43	

III. Blood glucose

Table 10 shows the average blood levels of glucose.

Table 10

Blood level of glucose according to the age and feeding regimen

Variant	Piglet number	Blood glucose, mg%			
		7 days	14 days	21 days	28 days
Control	3	48.67	59.00	66.15	78.50
Enzymatic preparation A	2	–	–	–	56.50
Enzymatic preparation B	2	–	–	–	57.00
Additional starch	2	–	–	–	52.00
Weaning at 21 days	2	–	–	–	69.00

Concerning the relation between glycemia and age, the data seem to show a constant increase up to day 28.

In the groups with changed feeding regimen, the results are less significant. The highest values were recorded in the animals fed supplementary starch, while

the lowest values were recorded in the piglets fed the enzymatic preparation B (*Bacillus subtilis*).

It may be said that our results are in good agreement with the literature (as values) for pigs (Stanton *et al.*, 1973). It is worthy mentioning the slight hypoglycemia trend in some piglets during the first 4 days after farrowing.

3. CONCLUSIONS

1. Several groups of Landrace piglets were investigated simultaneously to assess their weight gain, the intensity of the pancreatic and intestinal digestive enzymatic activity (lactase and amylase) and the glucose blood level in accordance to the age and the changed feeding conditions.

2. Piglet average weight increased compared to the control (7.33 kg at the average age of 33 days) under the conditions of feeding an enzymatic preparation based on *Aspergillus oryzae* (7.68 kg) and *Bacillus subtilis* (8.00 kg), as well as when additional starch was fed (8.21 kg). Piglet weaning at 21 days (the control at 38 days) did not decrease body weight (7.53 kg). The non-administration for 7 days of supplementary food (diet 0–1, starting with the age of 14 days), resulted, however, in lower body weight (6.70 kg).

3. The enzymatic activity (both lactase and amylase) displayed a clear dependence on age, with a pronounced adapting character. Pancreatic amylase and, to a lower extent, the intestinal amylase, displayed increased values when the enzymatic preparations (A or B) were fed, or when supplemental starch was given to the animals. The lack of supplementary food and the precarious state of development (minus variants from 14 days) caused the reduction of enzymatic activity.

4. Glycemia had values within the ranges known from literature for this ontogenetic stage (1–28 days): 48.67 – 78.50 mg%.

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GROWTH AND DEVELOPMENT OF THE ZEBRAFISH (*BRACHYDANIO RERIO*) OOCYTES

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The purpose of this study is to follow ultrastructural dynamics appearing during zebrafish (*Brachydanio rerio)* oogenesis. The paper shows sequential events which lead to cytodifferentiation. One of these refers to the Golgi complex formation. Nucleolar organization varies during the oocyte development. A most striking morphologic aspect is related to the massive extrusion of nucleolar material, essentially ribonucleoproteins into the ooplasm. Related to our ultrastructural observations, some speculations concerning vitellogenesis are presented.

1. INTRODUCTION

There are fundamental differences between somatic and germinal cells. Germinal cells development (gametogenesis) starts early during embryo development. Primordial germinal cells will follow few mitotic divisions to become spermatogonia and oogonia, respectively. Both spermatogonia and oogonia undergo successive divisions and morphostructural changes (cytodifferentiation) to become haploid specialized cells able together to restore the diploid set of chromosomes and to start the development of a new organism.

Special morphostructural changes that took place during gametogenesis were recorded for different species. There is a general known pattern of these changes which leads to the differentiated gamets formation, but particular aspects species-specific must be investigated because of their importance to understand the fundamental process of cytodifferentiation.

The oocyte growth and development revealed very interesting subcellular and molecular events among which we mention: nuclear-cytoplasmic exchanges, cell-cell cooperation (oocyte-follicular cells relationships), vitellogenesis, etc., which contributed to the basic knowledge of cell physiology.

Indeed, until now there is a long list of investigated species for their gametogenesis process including all large groups of vertebrates and amphibians [5], fishes [2], [9], mammals [4], [6], [7].

Zebrafish (*Brachydanio rerio*) became a model system used by many laboratories as a power tool to investigate vertebrate development. Related to this, zebrafish oogenesis study appears as a necessary background. Here we will follow ultrastructural changes which took place during zebrafish oocytes growth.

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2. MATERIAL AND METHODS

Ovaries collected from mature females of zebrafish containing oocytes in all stages of development were fixed in 10% neutral formalin or Formol-calcium and 5–6 μm thick sections were stained with hematoxylin-eosine.

Small fragments of ovarian tissue were prefixed for 2 hours in 2.5% glutaraldehyde buffered to pH 7.2 with 0.2 M sodium cacodylate, then washed and subsequently post-fixed in 2% solution of osmium tetroxide buffered to pH 7.2 with 0.1 M sodium cacodylate. Fixed tissue for electronmicroscopy was dehydrated, infiltrated with propylene oxide and embedded in Epon. Ultrathin sections were stained with uranyl-acetate followed by lead citrate.

3. RESULTS AND DISCUSSIONS

The oogonia are small cells with a large nucleus and a small nucleolus located in the central area. The cytoplasm is relatively scarce in membranous organelles. Endoplasmic reticulum appears as very few small elongated profiles, the mitochondria are round and reduced as number, Golgi apparatus is weakly developed but abundant ribosomes can be detected. No cytoplasmic binding can be observed between adjacent oogonia, which can explain asynchronous development of oogonia inside of ovarian stroma. In order to appreciate the stage of oocytes of the zebrafish, we used the scale published by Malone and Hisaoka (1963). According to this scale the youngest oocytes assigned to stage I, oocytes range from ca. 10 to 20 μm in diameter. These are spherical or ovoidal in shape. To some extent of oocyte periphery elongated folicular cells can be seen in close contact with adjacent oocytes (Fig. 1). Short cell extensions as oolemma microvilli can be seen around oocytes oriented towards follicular cells. At the periphery of the microvilli, an amorphous deposited material can be detected. Later on this material appears as continuous electronodense layer around plasmalemmal oocyte in close contact with follicular cells. The oocytes nucleus is large and is located in central or eccentric position. 1–4 or more nucleoli can be counted. Some nucleoli have a peripherial position inside of nucleus (in close vicinity of the nuclear envelope). Moreover, at this stage the nucleolus exhibited an inner part ("pars fibrosa") and a cortical part "pars granulosa" (Fig. 3). Nuclear envelope exhibits numerous nuclear pores.

Nucleoli are fundamental structures that reflect the state of activity and/or differentiation of each cell. The nucleolar structure, number, size, shape, intranuclear location are characteristics of a specific cellular type, but such a nucleolar organization is not constant and varies during the cell cycle. During oocytes growth, dramatic changes of nucleolar organization are revealed [3], [8].

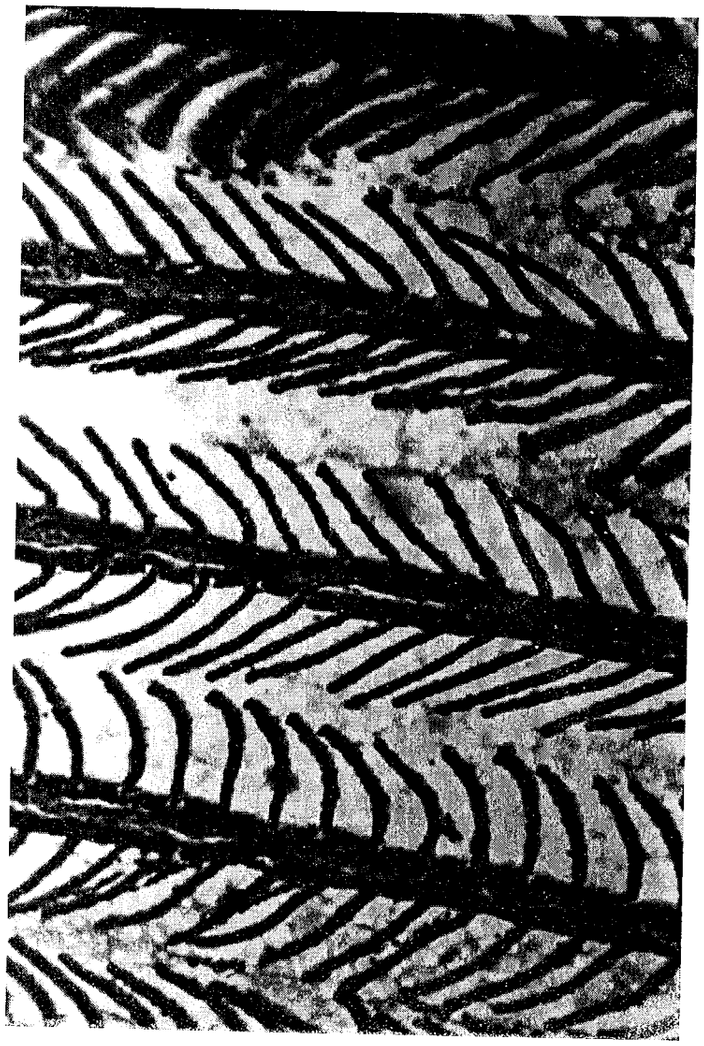

Fig. 1 – Gill structure of *Cyprinus carpio* control (20 × 0.40).

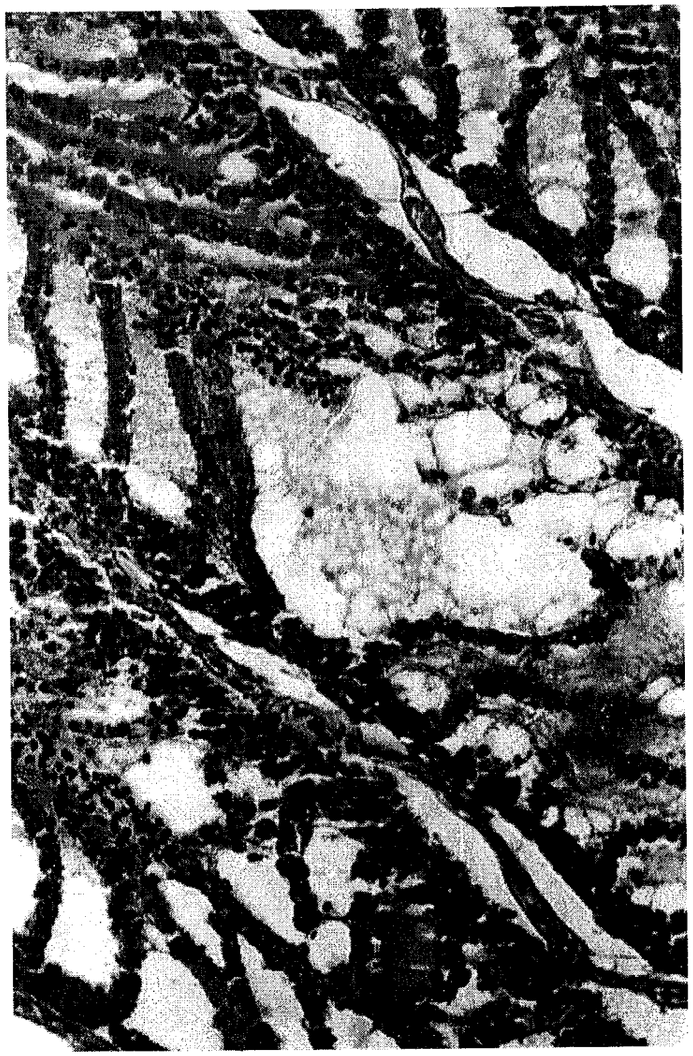

Fig. 2 – Gill structure of *Cyprinus carpio* intoxicated by 0.1 mg/l lead dose, for 24 h (40 × 0.65).

Fig. 3 – Gill structure of *Cyprinus carpio* intoxicated by 0.1 mg/l lead dose, for 96 h (40 × 0.65).

Fig. 4 – Gill structure of *Cyprinus carpio* intoxicated by 0.2 mg/l lead dose, for 24 h (40 × 0.65).

Fig. 5 – Gill structure of *Cyprinus carpio* intoxicated by 0.2 mg/l lead dose, for 96 h (40 × 0.65).

Fig. 6 – Gill structure of *Cyprinus carpio* intoxicated by 0.2 mg/l lead dose, for 7 days (40 × 0.65).

Fig. 7 – Gill structure of *Cyprinus carpio* intoxicated by 0.5 mg/l lead dose, for 24 h (40 × 0.65).

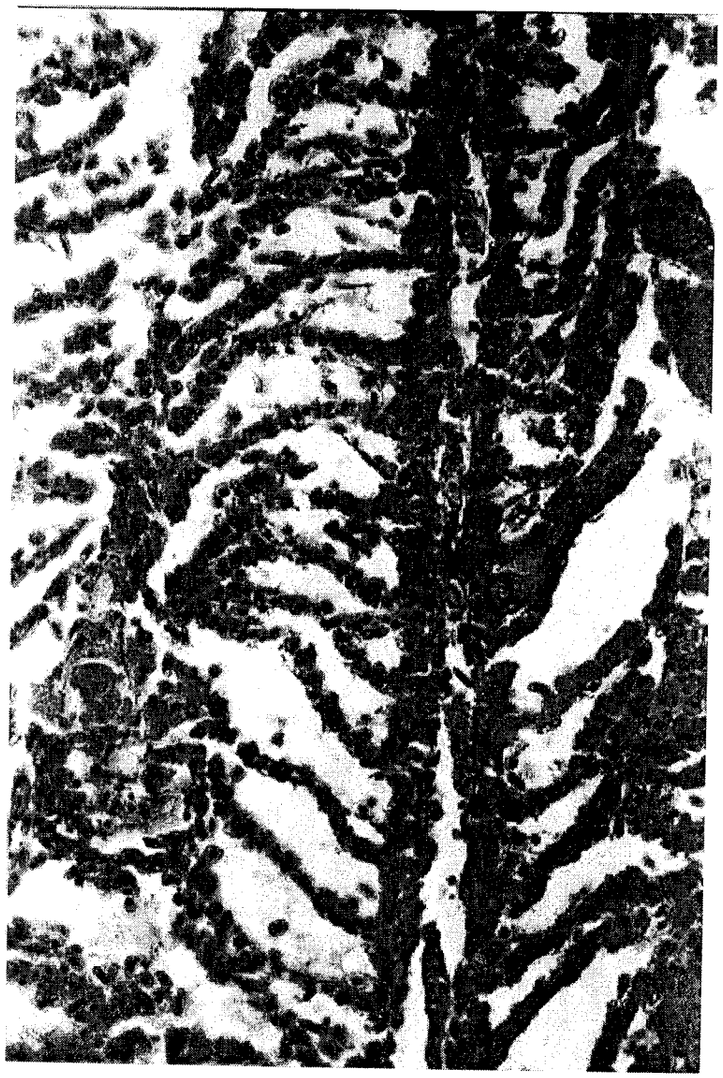

Fig. 8 – Gill structure of *Cyprinus carpio* intoxicated by 0.5 mg/l lead dose, for 96 h (40 × 0.65).

Fig. 9 – Gill structure of *Cyprinus carpio* intoxicated by 1 mg/l lead dose, for 24 h (40 × 0.65).

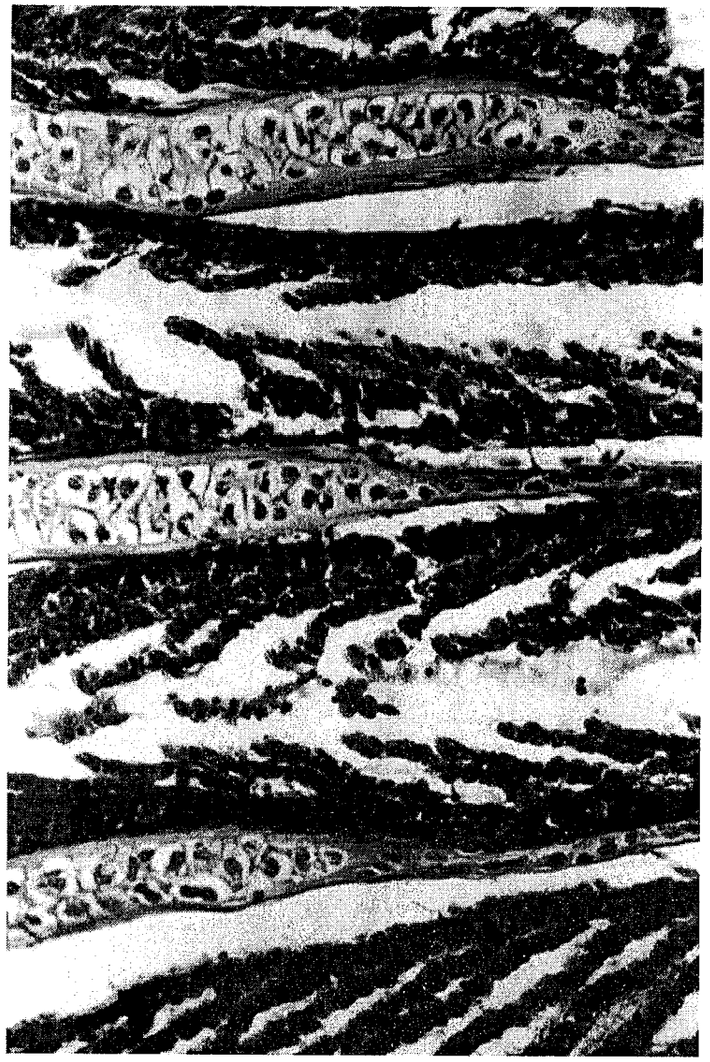

Fig. 10 – Gill structure of *Cyprinus carpio* intoxicated by 1 mg/l lead dose, for 96 h (40 × 0.65).

Fig. 11 – Gill structure of *Cyprinus carpio* intoxicated by 1 mg/l lead dose, for 7 days (40 × 0.65).

During the oocytes growth, sequential morphologic modifications and rearrangements of the various nucleolar components took place. The most striking ultrastructural aspect is related to the massive extrusion of nucleolar material, essentially ribonucleoproteins into the ooplasm.

During previtellogenetic stage the density of nuclear pores is increasing, nuclear envelope becomes folded and these lead to a remarkable increase of the surface exchange between the nucleus and the cytoplasm. There are frequent images showing a massive passage of nucleolar material through nuclear pores inside of the cytoplasm (Fig. 3 and Fig. 4). This ultrastructural aspect sustains the idea that gene amplification (DNA repetitive synthesis) is followed by passage of multiplied informational genetic material from the nucleus to the cytoplasm. Indeed, nucleolus is the morphological expression of a complex machinery where repetitive genes of ribosomal DNA (rDNA) are transcribed into ribosomal RNA (rRNA) the latter being processed and packaged into ribosomal subunits [10].

Recent studies demonstrated that specialized molecules termed karyopherins are able to transport proteins and RNA between the nucleus and the cytoplasm also during oogenesis [1].

The ooplasmic matrix of young oocytes is filled with ribosomes.

During stage II, oocytes range from 20–100 μm and this is the maximum of previtellogenetic growth state. At this stage the space between oocytes and adjacent follicular cells is progressively enlarged. Moreover, follicullar cells grow in size. Oocytic cytoplasm is gradually enriched in organelles. Smooth endoplasmic reticulum is progressively developed, but rough endoplasmic profiles can be also observed. The mitochondria were predominantly round in shape but elongated mitochondria with transverse oriented cristae can be seen as well (Fig. 2). Their number is remarkable increasing by division (Fig. 5). Mitochondrial matrix appeared densely, containing few small electronodense granules.

In some cytoplasmic areas, few short profiles of the endoplasmic reticulum display evaginations (Fig. 5 and Fig. 6). Vesiculated endoplasmic reticulum becomes in intimate contact with mitochondria. Such small shedded vesicles originated from the endoplasmic reticulum with a less electronodense content become coalescent and suggest to participate in dictyosome genesis. Indeed, a forming face (*cis* face) where shedded vesicles from the endoplasmic reticulum fuse to form saccular components of dictyosome as well as a maturing golgian face (*trans* face), where micro- and macrovesicles filled with a relative electronodense material can be observed (Fig. 6). A gradient increase of electronodensity of saccular content from the *cis* to *trans* face is remarkable. Such kind of images are also described by Anderson (1968), in an electron microscopic analysis of the cytological changes during the maturation of oocytes from the pipefish.

Cytoskeleton components are very scarce inside of the ooplasma. Very few actin filaments can be seen and sometimes microtubules can be detected (Fig. 5).

During the development and growth of oocyte, no other cytoplasmic organelle grows in number, as is the case of ribosomes.

An important event which occurs during oocyte growth of the teleost as well as amphibian oocytes is the accumulation of copious quantities of yolk (vitellogenesis). The yolk material which is gradually accumulated has a chemical content and a dynamic location according to oocyte growth stage. This material deposited inside of the ooplasma is the reserve of nutritive substance for the beginning of embryo development [8].

The yolk deposits during zebrafish oocytes differentiation appear polymorphic. Some of them are electronodense, others are less electronodense or electronlucent and fused yolk vesicles can be seen (Figs. 7–9).

Yolk precursors are derived from two sources: (1) endogenous from oocyte and (2) exogenous (an extra-oocyte source) [2], [8].

Yolk precursors formed exogenously are taken from the adjacent follicular cells as well as from the surrounding fluid by the oocyte through the process of micropinocytosis.

During the previtellogenetic stages of zebrafish oocyte differentiation (stage I to stage III) follicular cells increase in size and parallel with microvilli formation, other oolemmal specialization derivatives which can be recorded are pinocytotic vesicles. When the process of invagination of the pits is completed, the result is the formation of a coated ooplasmic vesicle (data not shown) whose interior is thought to contain the precursors of yolk [2], [5].

Subsequently, these coated vesicles lose their coat and fuse one another to form smooth surfaced vesicles with electronodense interior, which represent nutritive reserve.

The endogenous origin of the yolk reserve may have different ways: it seems that mitochondria, endoplasmic reticulum and Golgi apparatus can be implied in this process. Indeed, during previtellogenetic stages of zebrafish oogenesis, the number of mitochondria is increased and these become clustered together with long profiles of the endoplasmic reticulum (Fig. 2). Moreover, a close association of shedded vesicles from endoplasmic reticulum tubules and Golgi complexes whose saccules display a gradual electronodensity of content are related with neighbour micro- and macrovesicles of *trans* face (Fig. 6) which are thought to contain also yolk precursors [2], [8]. The role of the nucleus in yolk formation in zebrafish oocytes is suggested by the fact that there is a sudden decrease in the number of nucleoli and stainable RNA in nuclei of stage IV to stage V oocytes which is precisely recorded at the time when the extravesicular yolk vigorously increases in

the perinuclear zone. At this time, a lot of extruded ribonucleoproteic material into the cytoplasm will contribute to the yolk synthesis [9].

Structures resembling membrane stacks of annulate lamellae can be seen with some frequence in the close vicinity of yolk vesicles (Fig. 9).

With the subsequent growth of oocyte, yolk vesicles increase in number and size. Parallel with microvilli originated from the oocyte plasma membrane development, the amount of an amorphous electronodense material attached to the oolemma is increased (Figs. 10 and 11). Moreover, follicular cell extensions become in a contiguity relation with oocyte microvilli (Fig. 11). Finally, at the periphery of the mature oocyte the so-called zona radiata which displays an external amorphous part and an internal part as a decorative network is formed (Fig. 12). The striated aspect of zona radiata is given by numerous canaliculi which traverse this membrane. Cell extensions originated from follicular cells and oocyte follow these canaliculi. In such a way, the oocyte and follicular cells become in a contiguity relation [8]. To some extent the zona radiata of the teleost remembers zona pellucida of mammals. Recent studies [4], [6] showed that zona pellucida has a complex composition and like zona radiata it undergoes biological changes during oocyte maturation. Moreover, it is supposed that because in both cases of teleost and mammals at the level of periovular membranes, where cell extensions originated from the oocyte and follicular cells become in contact, important cell-cell communications took place.

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A TEST OF TOXIC ACTION TO ANIMAL MODELS OF SOME BACTERIAL CULTURES USED IN BIOTECHNOLOGICAL PROCESSES

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The present dynamic extension of biotechnological applications based on the use of microorganisms imposes an evaluation of the potential effects on the status of health for all those who are handling such microorganisms or their products.

This paper presents the results concerning the estimation of the toxicity degree by determination of 50% lethal doses (LD_{50}) for some bacterial strains and consortia. The following bacterial cultures were used: *Pseudomonas sp.* producer of biopolymer of type pseudozan (inoculum 1, d=$4.9*10^{10}$ cell/ml susp.), bacterial consortium degrading n-alkanes (inoculum 2, d=$7.4*10^{10}$ cell/ml susp.), *Pseudomonas putida* producer of biosurfactants (inoculum 3, d=$1.3*10^{11}$ cell/ml susp.), bacterial consortium degrading residual hydrocarbons contained in oily sludges (inoculum 4, d=$5.3*10^{10}$ cell/ml susp.). Laboratory toxicity tests were carried out on 140 white mice of Naval Medical Research Institute (NMRI) breed. Duration of experiments was 14 days.

Adequate methods were used to establish the 50% lethal doses for each type of inoculum. It was found that the inoculum 1, with minimum lethal dose (mLD) of 36.8 ml/kg mouse, inoculum 2, with LD_{50} of 48.97 ml/kg mouse, inoculum 3, with LD_{50} of 77.62 ml/kg mouse, and inoculum 4, with LD_{50} of 28.84 ml/kg mouse have no toxic effects.

1. INTRODUCTION

Nowadays, the impact of biotechnology on bioindustry, environment and social life can be evaluated through a lot of applications of microbiological methods. A large range of bacterial cultures and bacterial products are used in petroleum industry and in bioremediation processes [4,12,13,16,24]. Once released, interactions with biotic and abiotic factors can disperse microbial agents over considerable distances [18].

The United States Environmental Protection Agency and Federal Register statutes regulated the release of microbiological agents mandate that microorganism used not to be pathogenic. However, because of the increased exposure potential and possibility for the use of identified or unknown opportunistic pathogenic agents, it is imperative to determine if a microorganism has the probability to infect workers or the general public [5,6,17].

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Because people may come into contact with these microorganisms or their products in the industrial setting during production or application, recent researches at national and international level focused on the risks on health status of people who handle such microorganisms or their products [10,15,22]. Therefore, several *in vivo* and *in vitro* systems have been used to determine if biotechnology agents and their products are potentially hazardous. Many of these use rodent models to determine morbidity and establish LD_{50} values through toxicological tests which address acute oral, dermal, pulmonary and subchronic toxicity/pathogenity, oncogenicity studies, primate pathogenicity studies [21].

This paper is a part of a large study concerning the pathogenic and toxic action of some bacterial cultures and bacterial products which are used in biotechnological processes. Isolated from the environment, the tested bacterial cultures were: *Pseudomonas sp.*, a pure culture producer of biopolymer of type *pseudozan*; bacterial consortium degrading n-alkanes (paraffins); *Pseudomonas putida*, producer of biosurfactants, and bacterial consortium degrading residual hydrocarbons contained in oily sludges. This paper presents the results concerning the estimation of the toxicity degree by determination of the 50% lethal doses (LD_{50}) for the above mentioned bacterial strain and consortia and the estimation of various experimental doses, for each type of inoculum.

2. MATERIAL AND METHODS

Bacterial cultures. In this experiment four inoculum types were used, denoted from 1 to 4, corresponding to the four bacterial cultures in suspension, with the following values of density:

1. Bacterial strain of *Pseudomonas sp.* producer of biopolymer of *pseudozan* type, $d = 4.9 * 10^{10}$ cell./ml susp.;
2. Bacterial consortium degrading n-alkanes (paraffins), $d = 7.4 * 10^{10}$ cell./ml susp.;
3. Bacterial strain of *Pseudomonas putida* producer of biosurfactants, $d = 1.3 * 10^{11}$ cell./ml susp.;
4. Bacterial consortium degrading residual hydrocarbons contained in oily sludges, $d = 5.3 * 10^{10}$ cell./ml susp.

The bacterial strains which represent the types of inoculum 1 and 3 were isolated from samples of soil, the inoculum 2 from samples of residual paraffins of hydrocarbons, and inoculum 4 from samples of petroleum sludges. These bacterial cultures were studied by a research team from Microbiological Laboratory of the Institute of Biology of the Romanian Academy, aiming their characterization, to trace the metabolic potentials and to assess the application expectations [2,14,23].

Animal models. For the toxicological test 140 white mice of NMRI breed from Darvari biobase were used. The main criteria for selection were the following: youth, both sex and weight (20g ± 2g). The animals were acclimatized (7 days) and they were not used for any previous experimental procedures; females were nulliparous and nonpregnant. Preparing the experiment, 4 lots (30 mice/lot) were constituted for any type of inoculum and one reference-lot (20 mice). Each lot was divided in 2 sublots: A (n=10) and B (n=20) and, taking into account the inoculum types (1–4), the sublots A were denoted by 1A, 2A, 3A, 4A, and sublots B by 1B, 2B, 3B, 4B ("n" means the number of mice).

Experimental plan. Duration of this experiment was 14 days, denoted from 1 to 14. The sublots A were inoculated on the first day, being included in the probing period (days 1–3). Each individual of sublots A (1A, 2A, 3A, 4A) was inoculated with 0.5 ml of corresponding inoculum type. The modifications of behaviour were estimated through the clinical examinations (they being daily recorded) and the results facilitated the adjustment of administration doses corresponding to the sublots B. Beginning with the 4th day, the sublots 1B, 2B, 3B, 4B were introduced in the experiment. The used doses for each mouse, corresponding to the inoculum types, were as follows: $1 \rightarrow 0.7$ ml; $2 \rightarrow 0.6$ ml; $3 \rightarrow 0.7$ ml; $4 \rightarrow 0.3$ ml.

Inoculation. The suspensions of bacterial cells were inoculated by oral route using a probe, correlated with the calculated doses [9].

Data processing. The experimental data concerning the mortality percentage of each lot facilitated the estimation of LD_{50} for each inoculum by the probit method, through the calculation of the regression line [1,20].

The values of the used doses were noted on the category axis and the frequency effects (percentage) on the values axis. It was obtained a diagram representing a curve like "S" character. Because the biological experiments are more precise when the effect grows more rapidly simultaneously with the dose, it is necessary to change the "S" curve to a rectangle by substituting the percentage with the probit and the experimental values of capacity with logarithm values.

Three regression lines were plotted using the limits of security (min., max.). The three diagrams contain: the minimum values of probit (the lower regression line), the maximum values of probit (the higher regression line), and LD_{50} experimental values (the middle regression line).

3. RESULTS AND DISCUSSION

As a result of the inoculation of the toxicologically tested samples, changes have been established by observation and measuring, determined by the administered doses. The biological response measured by different parameters allowed the

establishment of experimental doses, which have dynamic effects, and of toxic doses which have mortal effects.

Two categories of qualitative effects have been discovered during clinical observations. The mortal effect (mortality), expressed through a percentage of mortality corresponding to the lethal dose (for each type of inoculum), belongs to the first category of qualitative effects. The recorded results concerning the dose and mortality, the subsequent changes (the determination of the logarithm of the dose, of the probit units) and also the minimum and maximum limits of the probit are presented, according to the used protocol, in Table 1.

Table 1

Preliminary results on acute toxicity determination of the four bacterial cultures

Lot	Sublot	No. of mice	DOSE			MORTALITY		PROBIT		
			ml/mouse	ml/kg mouse	Log. of dose	No. of dead mice / total	%	Units of probit	Limits	
									Min.	Max.
I	A	10	0.5	25.0	1.3979	0	0%	–	–	–
	B	20	0.7	36.8	1.5663	1/20	5%	3.36	1.90	4.35
II	A	10	0.5	36.3	1.5599	3/10	30%	4.48	3.50	5.39
	B	20	0.6	30.0	1.4771	4/20	20%	4.16	3.42	4.84
III	A	10	0.5	27.2	1.4346	0	0%	–	–	–
	B	20	0.7	36.8	1.5658	4/20	20%	4.16	3.42	4.84
IV	A	10	0.5	25.0	1.3979	4/10	40%	4.75	3.83	5.64
	B	20	0.3	15.0	1.1761	3/20	15%	3.96	3.14	4.69

Under these given experimental conditions, the maximal tolerated dose (MTD), with a value of 25 ml/kg.mouse, and the minimal lethal dose (mLD), with a value of 36.8 ml/kg.mouse, were determined for the first inoculum. For the three other types of inoculum, the LD_{50} values were graphically estimated as it is shown in Figure 1 (the 2nd inoculum), Figure 2 (the 3rd inoculum) and Figure 3 (the 4th inoculum).

Interpreting the LD_{50} values for the 2nd, the 3rd and the 4th types of inoculum and taking into account the mLD value for the first type of inoculum, it can be appreciated that the four types of tested bacterial cultures within the described experiment do not have toxic effects.

Correlating the values of the determined toxic doses of lots (1–4) and sublots (A,B), it can be established the hierarchy of the inoculum types, taking into account the biological response, as follows: 1, 3, 2, 4 (Table 2).

Data from the laboratory studies on animals, concerning the possible effects upon health determined by biotechnological agents and their products, point out

Inoculum: Bacterial consortium degrading n-alkanes (paraffins);
$d = 7.4 * 10^{10}$ cell./ml suspension

$LD_{50} = 48.97$ ml/kg

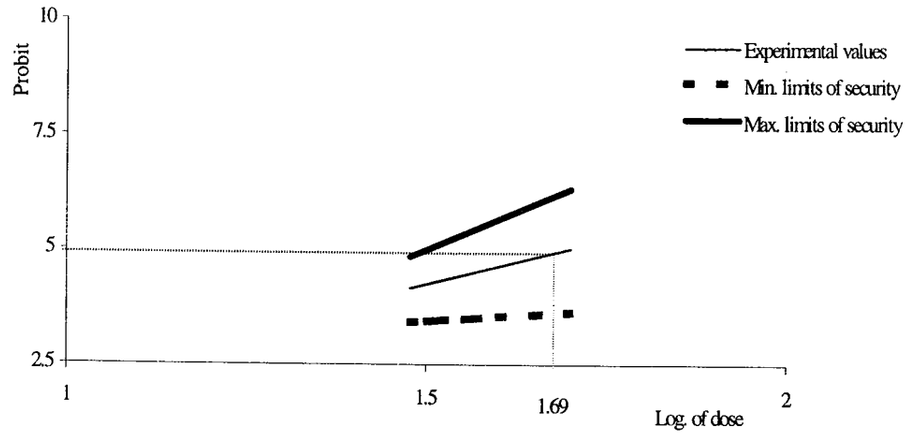

Fig. 1 – Acute toxicity (LD_{50}), lot 2.

Inoculum: *Pseudomonas putida* (producer of biosurfactants);
$d = 1.3 * 10^{11}$ cell./ml suspension

LD_{50}=77.62 ml/kg

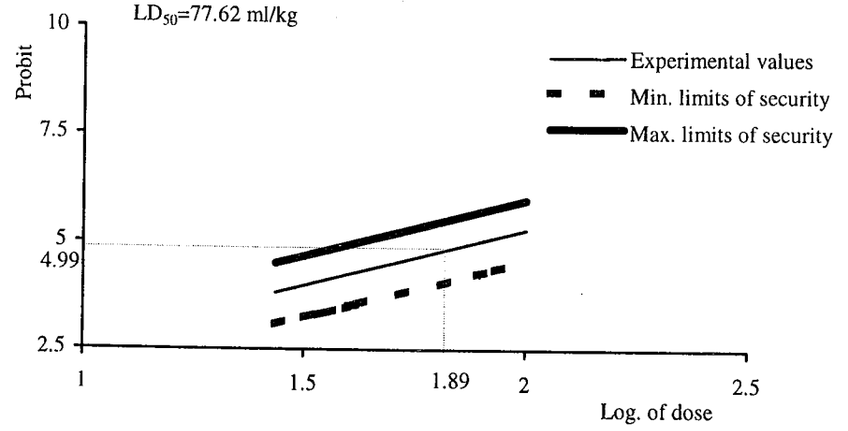

Fig. 2 – Acute toxicity (LD_{50}), lot 3.

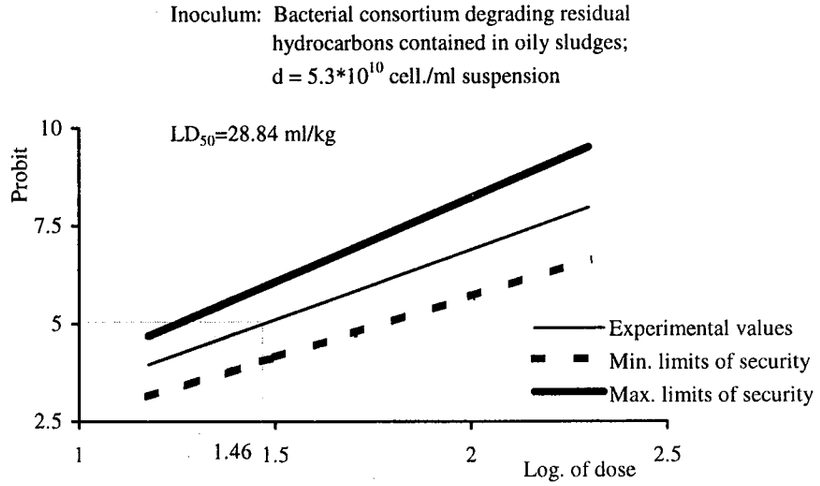

Fig. 3 – Acute toxicity (LD_{50}), lot 4.

Table 2

Types of determined doses

Lot	Sublot	Used dose (UD) (ml/kg mouse)	LD_{50}	Relationships between doses
1	A	25.0	–	UD = MTD
	B	36.8	–	UD = mLD
2	A	36.3	48.97	$mLD < UD < LD_{50}$
	B	30.0	48.97	$mLD < UD < LD_{50}$
3	A	27.2	–	UD = MTD
	B	36.8	77.62	$mLD < UD < LD_{50}$
4	A	25.0	28.84	$mLD < UD < LD_{50}$
	B	15.0	28.84	$mLD < UD < LD_{50}$

similar results [9]. Thus, a research team from North Carolina University studied a lot of species (excerpted from the environment) affiliated of genus *Pseudomonas* (*Pseudomonas putida PPO301-pRO103*) concerning the biochemical and genetic properties and their role in bioremediation processes. Toxicological tests (LD_{50}) of these biotechnological agents to conventional animal models (mouse, rat) were made.

In these experiments, the oral-, subcutaneous-, intraperitoneal- and, mainly, inhalation-way of inoculation were used [7,8].

The final results and the estimation for human related to those types of inoculum pointed out that they do not have toxic effects [3,11,19].

The second category of qualitative effects consists of the various aspects of behaviour of the animals models: normal or some alterations of behaviour. These qualitative effects were expressed by percents of action, depending on the experimental doses. The reference lot did not record any alterations.

4. CONCLUSIONS

1. The characteristics of frequency distribution of action percents concerning the experimental doses were correlated with LD_{50} values of inoculated bacterial suspensions.

2. The type 2 and 4 (bacterial consortium) of inoculum caused more intense effects comparatively with the type 1 and 3 (pure bacterial culture) of inoculum. The results concerning toxicological interpretation are not significant comparing them to the reference lot.

3. All types of inoculum (1–4), represented by:

- A pure bacterial culture containing the strain of *Pseudomonas sp.* produced of pseudozan ($d = 4.9 * 10^{10}$ cell./ml susp.);
- A bacterial consortium degrading n-alkanes ($d = 7.4 * 10^{10}$ cell./ml susp.);
- A pure bacterial culture, containing the strain of *Pseudomonas putida* producer of biosurfactants ($d = 1.3 * 10^{11}$ cell./ml susp.);
- A bacterial consortium degrading residual hydrocarbons contained in oily sludges ($d = 5.3 * 10^{10}$ cell./ml susp.)

are considered to have no toxic effects.

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STRUCTURAL CHANGES CAUSED BY LEAD ACTION UPON THE GILLS OF *CYPRINUS CARPIO* L. (PISCES) YOUNG FISH

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Our investigations on the action of various lead doses (0.1; 0.2; 0.5 and 1 mg/l) during various intoxication times (ranging from 24 h to 7 days) upon *Cyprinus carpio* L. young fish gills revealed a number of important cell and tissue structural changes. Gill disorganization was such that it resulted in gill lamellae destruction by: disappearance of epithelial cell limits and of pilastrum cells: mixing up of different cell types at lamellae level, their disposition in several layers; cell hypertrophy, nuclei deformation and pycnosis; dilatation, even breaking of blood vessels endothelia, white cells' extravasations; affection of connective, cartilaginous, muscular tissues; eventually, the appearance of completely atypical structures. The found changes are, generally, directly related to toxicant dose in water and to intoxication time.

1. INTRODUCTION

The influence of various noxious substances in surface and underground waters (discharges in surface effluents) exerted upon the fish fauna has been much investigated from various points of view. Our attention was caught by the studies on some metals' accumulation in various organs and tissues of fresh water fish [2,4,7,11,15,16].

But in specialty literature we found less data concerning the implications of metals in structural changes at the level of different organs and tissues in fish. Among these we mention Jancovici's [5] work concerning the changes generated by several metals among which the lead at the level of gills of *Barbus meridionalis petenyi* H., *Gobio gobio* L. and *Leuciscus cephalus* L. species. Also, interesting for us is the work by Gill et al. [3], regarding cadmium influence on gills of another fresh water fish, *Puntius conchonius* Ham.

In the present paper we show the results of our investigations on cell and tissue structural changes generated by lead at the level of *Cyprinus carpio* young fish gills. We have chosen to study this species of economic interest, a species occurring both in natural inner waters of our country and in those artificially arranged, as well as in the Danube.

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2. MATERIALS AND METHODS

Static tests were carried out on *Cyprinus carpio* young fish at different times, the fish being subjected to the action of lead doses ranging between 0.1; 0.2; 0.5 and 1 mg/l.

As concerns the environment conditions under which our observations were made, we mention that:

– regarding the pH of the water with lead, at all the analysed concentrations, this showed a significant decrease as soon as the toxicant was introduced, but gradually and concomitantly with a slight increase in temperature, it reverted to the original value;

– the temperature of water with toxic substance varied a little (within the ranges of 19°–20.5° C), *i.e.* slightly increased during the period of 7 days observation, in relation with the environment temperature.

After 24 h, 96 h or 7 days, some specimens of the intoxicated fish were killed, dissected and parts of gills were fixed in Bouin. The material was further processed by classic histological methods and then analysed by light microscopy.

3. RESULTS

All the changes found by us are related to control structure (Fig. 1). We do not describe the normal gill appearance of *Cyprinus carpio*, since that was well illustrated by Dornescu and Mişcalencu [1].

0.1 mg/l lead dose action

Time of exposure to toxicant action: 24 h

Concerning the changes caused by lead, our observations confirm the fact that gill, one of the vital organs in fish, proved to be affected beginning even with the lowest tested dose, namely 0.1 mg/l lead.

At this concentration, within a short time of fish intoxication (only for 24 h), the gill lamellae began to disorganize (Fig. 2). In many stretches they were completely torn off and the cells were detached and dispersed. In some lamellae which maintained their structural integrity, one might dinstinguish elongated and flattened nuclei of cells at the periphery of lamella unistratified respiratory epithelium. Also, the pilastrum cell nuclei were noticed generally as being very chromatic and sometimes they were dislocated from their vertical position. The blood capillaries of gill lamellae were broken and red cells were dispersed, either within or outside the lamella.

Disorganization was also noticed at the level of gill lamella pluristratified epithelium, where cell fusion and nuclei pycnosis occurred.

In the gill axis, the cartilaginous skeleton appeared with cartilaginous cells, sometimes bigger and other times of normal appearance. Nevertheless, the

connective tissue of gill pluristratified lamella axis exhibited hypertrophied cells. The blood vessels were broken and white cells were dispersed. Musculature surrounding the cartilaginous axis did not seem to be affected and the elongated nuclei of these fibres had a normal structure.

Time of exposure to toxicant action: 96 h

During the period of 96 h of intoxication with the same dose of 0.1 mg/l lead, gill degradation continued further (Fig. 3). In this respect, in some stretches there was noticed a dislocation from the normal position of lamellae epithelial cells, as well as an agglomeration of nuclei towards the lamella edge. The pilastrum cells were affected too. The blood vessels were broken and red cells were dispersed alongside the pluristratified lamellae.

It occurred also the deterioration by breaking of blood vessel endothelium in the pluristratified epithelium of gill lamellae. The connective tissue of the gill axis was altered.

We point out that at 0.1 mg/l dose during 96 h it was noticed an alternation of the stretches in which the gill lamellae were more destroyed compared to those subjected to the toxicant action for 24 h only, with stretches in which the lamellae had an apparently normal aspect.

0.2 mg/l lead dose action

Time of exposure to toxicant action: 24 h

At 0.2 mg/l lead dose during the first 24 h of *Cyprinus carpio* young fish intoxication the gill structural changes accentuated compared to those caused by 0.1 mg/l lead dose (Fig. 4). In this respect, a higher disorganization of gill lamellae epithelium was observed. At its level, beside the fact that the cells were higher, hypertrophied, they detached, forming cell clusters pertaining to several lamellae. Also, it occurred a destruction of the pilastrum cells, which became less numerous.

Other changes occurred also at the level of the gill lamella pluristratified epithelium, where nuclei pycnosis was noticed. The blood capillary dilatation took place as well.

The connective tissue in the gil axis also altered.

The musculature adjacent to gill axis cartilage was strongly affected compared to adductive muscles at the gill basis.

Time of exposure to toxicant action: 96 h

At the same dose of 0.2 mg/l lead, but after 96 h of fish intoxication, the gill degradation was much higher (Fig. 5). The most pronounced changes also occurred at the level of gill lamellae, which appeared broken with a disorganized unistratified epithelium, hypertrophied cells, some of them completely destroyed. In other stretches, different cells were mixed, displayed in several layers, hence an atypical structure. The blood vessels, much affected, appeared dilated, fused, ever

destroyed. Disorganization was noticed also at the level of gill lamella pluristratified epithelium.

The gill musculature and the cartilaginous tissue were affected only partially. A more pronounced change consisted of the hypertrophy of a small number of cartilaginous cells.

Time of exposure to toxicant action: 7 days

At the 0.2 mg/l dose for 7 days, a pronounced gill disorganization was found (Fig. 6). Thus, many lamellae were broken. Also, cell settlement towards the free stretch of lamellae was noticed. Gill lamella pluristratified epithelium was also disorganized, with dispersed cells and pycnotic nuclei.

Cartilaginous cell in gill axis did not seem to be affected, in exchange the connective tissue was completely disorganized. Blood vessels were broken and the red cells were dispersed.

The musculature adhering to cartilage had elongated, deformed, pycnotic nuclei.

0.5 mg/l lead dose action

Time of exposure to toxicant action: 24 h

This higher concentration exerted its influence by the fact that gill lamellae unistratified epithelium was strongly affected in many of its stretches (Fig. 7). Thus, the cells were destroyed to such an extent that the cell limits were no more distinguishable. Nuclei of different shapes were pycnotic.

Time of exposure to toxicant action: 96 h

During 96 h of the 0.5 mg/l lead dose action the gill lamellae unistratified epithelium was more strongly affected, i.e. suffered a more pronounced disorganization (Fig. 8). Nuclei of different shapes and sizes appeared dispersed, in some stretches, only cell debris were noticed.

The gill lamellae pluristratified epithelium was destroyed too. The blood vessels in the gill axis were in some stretches very dilated and other times they were completely broken, the blood extravasating.

1 mg/l lead dose action

Time of exposure to toxicant action: 24 h

This dose of 1 mg/l lead, even during 24 h exposure, proved to have a high degree of toxicity for *Cyprinus carpio* young fish gills (Fig. 9). Thus, some gill lamellae had a higher epithelium, in the others, the disorganization consisted in their complete break and dissipation of epithelial cells, particularly of pilastrum cells. Nuclei of various sizes and irregular shapes became pycnotic. Disorganization of gill lamellae structure was more pronounced towards their tips.

Also, there was a strongly disorganized pluristratified epithelium of the gill lamella from which the cells detached and nuclei became pycnotic.

Time of exposure to toxicant action: 96 h

During the 96 h of intoxication, the process of gill degradation strongly accentuated (fig. 10). Thus, the gill lamellae were destroyed. Highly disorganized, the unistratified epithelium had all its nuclei pycnotic. The broken lamellae had dispersed cells. In some stretches, there were noticed cell masses originating from the adjacent gill lamellae. The blood vessels were rarely very dilated, more frequently they were broken.

Also, the gill lamella pluristratified epithelium was completely disorganized and the connective tissue of the gill axis was destroyed.

Time of exposure to toxicant action: 7 days

At the maximum lead dose used by us, 1 mg/l and during the longest time of the (tested) toxicant action, the gill lamellae completely lost their normal arrangement (Fig. 11). Nuclei of different shapes appeared to be either dispersed or displayed in several layers. The blood vessels were more rarely very dilated and usually they were broken and the red cells were extravasated.

In this situation it was observed the strongest lead influence on *Cyprinus carpio* gills, exhibited during our investigations.

4. DISCUSSION

In our investigations we used the lead (from lead acetate) at doses ranging between 0.1 and 1 mg/l, since in the specialty literature the toxico-lethal limits for various fish species vary between 0.1 and 10 mg/l lead [7].

The data obtained as a result of our studies (their results are shown in the present work) reveal a number of cyto-histopathological changes which are preceded by or are concomitant with certain ecophysiological manifestations or biochemical phenomena occurred in the intoxicated fish. In this context, we mention disregulations of the breathing rhythm, the tegument decolorization, bioaccumulation, etc. This means that cytohistopathological changes are closely correlated with physiological condition of the fish subject to inadequate conditions, sometimes even to toxic ones.

The request whether certain structural changes at the level of various organs are or are not specific to one or another substance already preoccupied different scientists. The answer, especially according to Mallatt [6], is the "lack of specificity".

In this respect, we mention that our observations concerning the cyto-histopathological changes occurred under the lead influence are similar to those caused by phenols, at the level of *Cyprinus carpio* gills. Among these changes, in the works by Meşter et al. [8,9] regarding phenols' action, there are mentioned: serious injuries at the level of carp young fish gills expressed by gill lamellae

degeneration, significant dilatation of blood veins and capillaries, accompanied here and there by extravasation of white cells, hypertrophy of the gill cartilaginous cells. The bioaccumulation phenomenon was noticed as well.

Among the pathological structural manifestations, "considered as non-specific reactions", caused by several types of toxic substances, i.e. the lead and ammonium, we outline those related to: gill epithelium blattening, gill lamellae detachment from the skeletal support, dilatation, even the break of blood capillary endothelium with extravasation of white cells, hypertrophy of cartilaginous cells [10,12,14].

Our remarks concerning some structural changes at the level of carp gills, caused by the lead, are similar to those generated by cadmium as mentioned by Gill et al. [3]. In the case of cadmium, in the form of cadmium chloride, sublethal doses of 620 and 840 micrograms/l actioned for 12 week intoxication of *Punitius conchonius* fish gills. In this situation, pathogenic changes consisted in: gill epithelium disorganization, necroses, cell fragment accumulation, capillary congestion, atrophy of the pilastrum cell system. For that reason the gill injuries cause serious breathing disturbances correlated to tissue hypoxia [3].

A much studied phenomenon during the last decades is bioaccumulation. This, shared by several types of living beings, relates to a number of substances among which the metals, implicitly the lead. We insist upon this (bioaccumulation), since the lead quantities bioaccumulated from the Danube water by fish and namely the *Chondrostoma nasus* L. species are not negligible [15]. The respective quantities are: 0.25 mg ions lead/kg of fish wet weight at km 2420 of the Danube, reaching the maximum of 0.79 mg ions lead/kg fish wet weight at km 2410 of the same river. In this last condition, the lead bioaccumulation is much higher than in the liver and the kidney of the same fish [15].

Also of interest are the studies on some heavy metals accumulation in the tissue of fresh water fish *Varicorhinus trutta* revealing the fact that the rate of Cu and Zn accumulation in gills is directly proportional to the respective amounts of metals in water [13]. Other researchers [11], referring to Cu and Cd accumulation in certain organs of *Heteropneustes fossilis* and *Channa punctatus* species, outline the fact that the gill accumulates a higher amount of these metals compared to the liver and the kidney.

More recent studies on the effect of fish contamination in the waters, within a radius of 10 km away from the Chernobyl nuclear powerstation, refer also to the lead accumulation. Thus, lead was detected in higher quantities in the skeleton rather than in the muscle [4]. The respective paper outlines the interesting fact that according to the World Health Organization standards (1972), the fish for human alimentation can contain < 0.3 micrograms lead/g dry substance.

Certainly, it was the bioaccumulation that contributed to the accentuation in time of the changes generated by the original action of lead concentrations upon

the structure of fish gills tested by us. Thus, the irreversible structural changes eventually led to the death of all the fish specimens of the experimental batches.

5. CONCLUSIONS

1. The lead (in the lead acetate) in doses of 0.1;0.2;0.5 and 1 mg/l induced in *Cyprinus carpio* (carp) young fish of one in a summer, cell and tissue structural changes at the gill level.

2. The discovered changes are directly related to the toxicant dose existing in water and to the period of intoxication.

3. The most significant structural changes are those caused by 1 mg/l lead dose during 7 days, namely: disorganization, even destruction of gill lamellae by cell hypertrophy, disappearance of epithelial cell limits and of pilastrum cells, the mixing up of various cell types at lamellar level, cell clusters forming, nuclei pycnosis, their diffusion or their arrangement in several layers, dilatation, even the break of blood vessels, with white cell extravasation, affection of muscular, connective and cartilaginous tissues of gills, eventually, the appearance of atypical structures.

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VAGUS-MEDIATED DECREASE OF PLASMA GLUCOSE LEVEL IN RESPONSE TO INSULIN INJECTED INTO THE LATERAL HYPOTHALAMUS

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The present experiments were performed to see whether and how insulin interacts with the lateral hypothalamus (LH) in the regulation of the peripheral glycemia. Initially, 0.2 µl insulin were injected into the LH and blood glucose level was measured. Because the glycemia was determined to be 23% lower than the base-line value, control experiments were done. Corroborating the hypoglycemic effect with the results of the controls, we reached the conclusion that the decreased glycemia was a specific response to the injected insulin. In order to establish whether the applied insulin acts locally, or it diffuses to some other areas to trigger its effect, the same doses were injected into the same area of LH ablated rats. Since the glycemia remained unaffected, we concluded that LH was strictly responsible for the hypoglycemiant effect. To verify a possible vagal involvement, bilateral vagotomy was further performed, before insulin intrahypothalamic injection. Blocking the hypoglycemic response, that operation showed that the vagus nerves were the pathways for the hypoglycemiant signals. Considering that the endocrine pancreas could be one of the target organs for these signals, we finally measured the plasma glucose levels before and after intra-LH injection of insulin in STZ-diabeted rats. The result was the lack of the hypoglycemic response and the conclusion was that ß-cells were probably involved in the LH-triggered effect of insulin. Thus, we may consider LH as an insulin sensitive glucoregulator, which probably controls the pancreatic insulin secretion by means of parasympathetic signals.

1. INTRODUCTION

The presence of insulin in the central nervous system (CNS) has been clearly established [8,25], but there are still many questions related to its functions and origin.

Although insulin-related molecules seem to be synthesized within the CNS [1, 7, 8, 13], Plisetskaya *et al.* [20] could not detect significant insulin mRNA levels in the salmon brain.

On the other hand, many data indicate the presence of a blood-brain barrier transport system which underlies the delivery of the circulating insulin into the brain [24, 25].

Within the brain, insulin interacts with specific receptors [1, 7, 8, 23], which are mainly distributed in the cerebral cortex, olfactory bulbs and hypothalamus [25].

Whatever the source of the brain insulin would be, there are several lines of evidence indicating that this hormone is involved in crucial processes into the CNS, such as the stimulation of the glucose entry and glycogen synthesis [11], cell and fiber outgrowth and neuron specific enzymes [2, 9, 12, 22].

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Inhibiting norepinephrine release from nerve terminals [30] and its neuronal reuptake [26], as well as affecting the functions of sensory nerves [4], insulin acts as a neuromodulator.

By means of complex interactions with central effectors of feeding, such as serotonin, cholecystokinin, bombesin, norepinephrine and NPY [17, 25, 31, 35], brain insulin might have a key role as a signal for CNS in the regulation of energy balance and body weight [25].

Consistent with this hypothesis, the most suggestive action of insulin is the reduction of food intake and body weight when it is infused into the brain, particularly in the hypothalamic regions [14, 15].

Taking into consideration the link between food intake, energy balance and plasma glucose levels, and the fact that the LH seem to be directly involved in the regulation of the peripheral glycemia [10, 29], we performed the present experiments to see whether insulin and LH interact in the regulation of blood glucose levels, some of our observations being reported previously [21, 27, 28].

2. MATERIALS AND METHODS

Male albino Wistar rats (200–300 g wt.) were used. They had free access to food and water until 18 hours before the experiments.

The animals were anesthetized with Nembutal (30 mg/kg) and then placed into a stereotactic apparatus.

After a small hole was made in their skull, the needle of a microsyringe (0.2 mm diameter size) was implanted into the LH, according to the coordinates established by Bures *et al.* [3]: 2 mm posterior to bregma, 1.3 mm lateral to the midline, and 8 mm ventral to the horizontal zero plane.

Blood samples were taken from the caudal vein before the implantation of the needle for establishing the glucose base-line value. The next probes were taken 5 minutes after the implantation of the needle, and then at 5, 15 and 30 minutes after the injection of 0.2 µl insulin (0.008 U) into the LH.

The plasma glucose concentrations were determined enzymatically, using the method of Werner *et al.* [34].

Control experiments were made to establish the simple mechanical effect of the needle, as well as the effect of similar doses of saline injected into the same LH area.

Sterotactic injections of insulin were also performed into the destroyed area of LH ablated rats.

The lesioning of LH was made 5 days before the injection of insulin, using the coordinates mentioned before. A 2 mA anodal current was administered for 15 seconds by the use of insulated electrodes (except for the tip), with a diameter of 0.2 mm.

The consequences of the bilateral vagotomy on the glycemic changes observed after intrahypothalamic injection of insulin were also studied. The vagus nerves were isolated and sectioned in the vicinity of the carotid arteries. The first blood sample was taken just before the vagotomy, and the second one 10 minutes after this operation. The next four probes were collected at the previously mentioned time intervals.

In order to verify a possible pancreatic reaction in response to the intrahypothalamic injected insulin, the ß-pancreatic islets of another lot of animals were blocked with streptozotocin (STZ). For this, 4 mg/100 g STZ were intraperitoneally administered, three days before insulin injection.

Following the experiments, animals from each experimental series were killed by perfusing their hearts with 40% neutral formalin. The fixed brains were cut for anatomical control, using the coordinates mentioned for LH location.

3. RESULTS

A) EFFECT OF INTRAHYPOTHALAMIC INJECTION OF INSULIN ON PLASMA GLUCOSE LEVEL

As it can be seen in Fig. 1, the simple implantation of the needle (point 2 on abscissae) into the LH induced a small, but significant ($p<0.01$) decrease of plasma glucose level.

Following the application of 0.2 µl of insulin, the glycemia progressively decreased, and 30 minutes after the injection, the plasma glucose level was 23% ($p<0.05$) lower than the base-line value.

B) CONTROL PROBES

To establish whether the detected decrease of glycemia was only an extension of the hypoglycemic effect induced by the needle, or it really was due to the application of insulin, the evolution of plasma glucose levels without insulin injection was determined in a new set of experiments.

The implantation of the needle produced its previously established hypoglycemic effect, but the glycemia remained stable along the determinations if insulin was not applied (see Fig. 1).

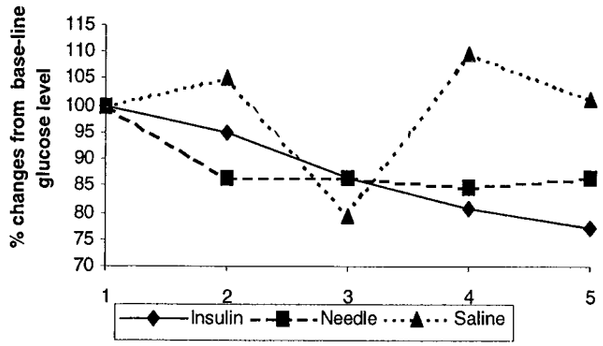

Fig. 1 – Plasma glucose concentrations after: a) the injection of 0.2 µl insulin into the LH (Insulin); b) the simple implantation of the needle into the LH (Needle); c) the injection of 0.2 µl saline into the LH (Saline). 1 – Base-line glucose level. 2 – Sample at 5 minutes after the implantation of the needle. 3, 4, 5 – Samples at 5, 15 and 30 minutes after the injection of insulin.

In comparison to insulin, the same dose of saline injected into LH induced only a transitory hypoglycemic effect. Thus, after a sudden decrease, fifteen minutes following the injection, plasma glucose returns to its initial value (see also Fig. 1), this result showing that the reaction to intrahypothalamic insulin is a specific response.

C) EFFECT OF INSULIN INJECTED INTO THE ELECTROLYTICALLY DESTROYED LH REGION

In order to verify the specific LH contribution to the established hypoglycemic answer, insulin was further injected into the same region of LH-ablated rats.

The LH ablation itself produced a severe and significant increase (42.9 %, $p<0.01$) of peripheral glycemia.

In the rats subjected to LH ablation five days before the experiments, insulin injected in the destroyed area determined only small and insignificant changes of plasma glucose levels (see Fig. 2).

Thus, the conclusions resulting from this experimental series are both that the LH has an important role in controlling peripheral glycemia, and that this structure indeed was responsible for the hypoglycemic response determined in our previous experiments.

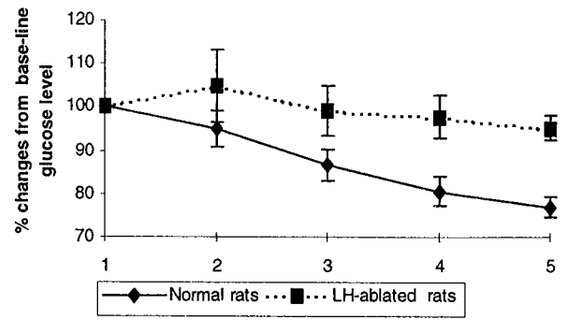

Fig. 2 – Effects of 0.2 µl insulin injected into the LH of LH-ablated rats comparative to those obtained in normal animals. 1 – Base-line glucose level. 2 – Sample at 5 minutes after the implantation of the needle. 3, 4, 5 – Samples at 5, 15 and 30 minutes after the injection of insulin. Results are means ± SE.

D) EFFECT OF BILATERAL VAGOTOMY ON HYPOGLYCEMIC RESPONSE TO INTRA-LH INJECTED INSULIN

In order to establish whether the hypoglycemic signals generated by the LH in response to the injected insulin are transmitted by means of the vagus nerves, we sectioned these pathways ten minutes before insulin application and watched for a possible hypoglycemic effect.

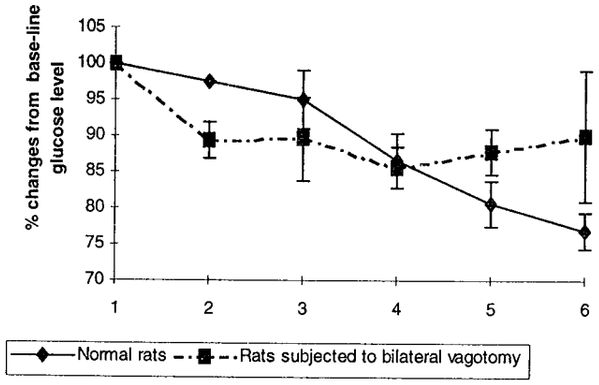

Fig. 3 – Effects of 0.2 µl insulin injected into the LH of the rats subjected to prior bilateral vagotomy comparative to those obtained in normal animals. 1 – Base-line glucose level. 2 – sample at 10 minutes after the bilateral vagotomy. 3 – Sample at 5 minutes after the implantation of the needle. 4, 5, 6 – Samples at 5, 15 and 30 minutes after the injection of insulin. Results are means ± SE.

Bilateral vagotomy itself produced a decrease of plasma glucose concentration.

In contrast to the initial experiments, after this hypoglycemic effect, neither the implantation of the needle, nor the injection of insulin induced further changes in plasma glucose levels (see Fig. 3).

Starting from this observation, one could say that the vagus nerves may be considered the main pathways by means of which the hypoglycemic signals reach certain peripheral targets.

E) EFFECT OF INTRAHYPOTHALAMIC INJECTION OF INSULIN IN STZ-DIABETED RATS

Taking into consideration the results above, we presumed that an increase in blood insulin levels might be a possible reason why the glycemia decreases in response to the injected insulin. If this hypothesis was correct, then the elimination of ß-pancreatic cells prior to insulin injection would result in blocking its hypoglycemic effect.

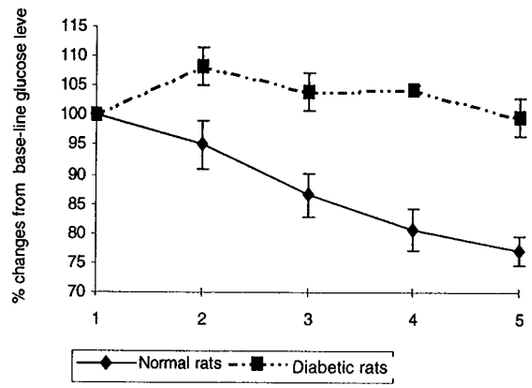

Fig. 4 – Effects of 0.2 µl insulin injected into the LH of STZ-diabeted rats comparative to those obtained in normal animals. 1 – Base-line glucose level. 2 – Sample at 5 minutes after the implantation of the needle. 3, 4, 5 – Samples at 5, 15 and 30 minutes after the injection of insulin. Results are means ± SE.

As can be seen in Fig. 4, in STZ-diabeted rats, after a small hyperglycemic effect produced by the implantation of the needle, plasma glucose level tends to return to its initial value. Since these changes are transient and insignificant, we may say that, in STZ-diabeted rats, intrahypothalamic injection of insulin does not produce its hypoglycemic effect observed in untreated animals.

4. DISCUSSION

As we have shown, 0.2 µl insulin injected in the LH induced an almost linear decrease of plasma glucose level, the lowest value being reached at 30 minutes after the injection.

Our results confirm and extend earlier findings. Thus, studies by Taborsky and Bergman [32] reveal that, after the infusion of insulin into the third cerebral ventricle of conscious dogs, an increase in plasma insulin concentration induces a decrease in plasma glucose levels. Iguchi *et al.* [10] also reported a significant decrease in plasma glucose level after the injection of insulin into the ventromedian hypothalamic nucleus (VMN) of rat, and a smaller one after the administration of insulin into the LH.

In our experiments, even the implantation of the needle was found to have a hypoglycemic effect. When the implantation was not followed by the application of insulin, the plasma glucose level remained stable until the end of the experiment.

Thus, the decrease of glycemia following the application of insulin is not an extension of the hypoglycemic effect induced by the implantation of the needle, but a specific response to the hormone.

The administration of the same amount of saline into the same hypothalamic region resulted in a completely different evolution of plasma glucose levels.

Corroborating the findings mentioned above, one could point out the first main conclusion of this study. Thus, insulin injected into the LH specifically interacts with some hypothalamic structures inducing a decrease of glycemia.

There are indeed several lines of evidence which suggest a hypothalamic control of plasma glycemia.

Studies by Shimazu *et al.* [29] reveal that the stimulation of LH leads to hepatic glycogenesis by activation of glycogen synthetase.

On the other hand, Oomura and Kita [18] and Oomura *et al.* [19] have proven that the application of insulin exerted an excitatory effect on the electrical activity of certain neurons in the LH.

Taking together our present results and the literature data mentioned above, one could say that insulin injected into the LH exerts a hormonal excitatory effect which further leads to the activation of the hepatic glycogenesis. This link of interactions would finally result in a decrease of plasma insulin content, as we actually established by means of the present experiments.

Starting from this point, we may also understand both the hyperglycemic effect occurring after the electrolytic lesioning of the LH, and the lack of the hypoglycemic response to insulin injected into the LH destroyed area. Thus, considering the hypothalamic glucoregulation process as a counteraction between a "hyperglycemic" VMN center and a "hypoglycemic" LH center, as both Iguchi *et*

al. [10] and Shimazu *et al.* [29] suggested, the removal of the LH would suppress the hypoglycemiant loop of this mechanism, and the effect would naturally be the elevation of plasma glucose levels.

Since we have obtained this expected result, we may consider our findings as evidence for the existence of a hypothalamic glucoregulator consisting of a network which includes different hypothalamic structures. Among them, one can mention the LH, the VMN and maybe some other insulin sensitive hypothalamic nuclei, such as the arcuate nucleus, where insulin has been proven to suppress the expression of mRNA encoding NPY, both in normal rats [25], and in STZ-diabeted rats [31].

Besides the interactions between LH and the hepatic glycogen metabolism discussed above, we presumed that the endocrine pancreas might also be involved in the hypoglycemic effect of the intrahypothalamic injected insulin.

As we expected, the injection of insulin into the LH did not induce the lowering of glycemia in STZ-diabeted rats. This result may be evaluated from at least two points of view.

First, it is obvious to consider that, in the absence of functional ß-pancreatic islets, no insulin-based hypoglycemic process can occur. From this point of view, the endocrine pancreas may be proposed as one of the effectors involved in the discussed hypoglycemic effect.

Second, in STZ-diabeted rats, a local hypothalamic suppression of the hypoglycemic response to insulin may occur as a result of some neuronal structural and functional changes.

Consistent with this hypothesis, there are several lines of evidence indicating hypothalamic lesions in STZ-treated animals. Thus, according to Takasawa *et al.* [33], an analogous mechanism of STZ action occurs both on pancreatic islets and on nerve cells, by depleting intracellular NAD^+ and inhibiting cellular functions. Although we did not find any direct evidence that such alterations affect LH neurons, there are data which describe ultrastructural and morphometric changes in the hypothalamic supraoptic and paraventricular nuclei of STZ-induced diabetic rats [5,6].

Summarizing the discussions above, our opinion is that insulin injected into the LH induces a specific hypoglycemic effect, which probably involves an insulin sensitive neuronal group as a receptor/command area, and both the liver and the endocrine pancreas as peripheral effectors.

Taking into consideration that the hypoglycemic effect is characterized by a fast development both in response to needle's implantation and to the injection of insulin, we presumed that such a response must be supported by a nervous process, both in regard to its triggering, and to the transmission of the signals to the target organs. The most susceptible pathways for playing this role were thought to be the

vagus nerves and the most direct method to demonstrate this hypothesis was to cut them before insulin injection, to see whether a hypoglycemic effect still occurs.

Since the bilateral vagotomy abolished the hypoglycemic effect, we can consider that the vagus nerves are indeed involved in its development, and, by extension, in the global process of the hypothalamic control of plasma glucose concentration.

Consistent with these considerations, previous studies revealed that the CNS controls the peripheral glycemia by means of a direct vagus-mediated effect both on the pancreatic production of insulin [32], and on hepatic glucose metabolism [10, 29]. Moreover, Zbigniew *et al.* [36] showed that chemical stimulation of neurons from the dorsal vagal complex evoked increases of insulin secretion, with a maximum at 15 minutes after the stimulation.

Finally, we can resume all the discussions above with a general conclusion of this study. Thus, in our opinion, the lateral hypothalamus may be considered part of an insulin sensitive nervous network, which controls plasma glucose levels by signals that modulate both the hepatic glucose metabolism, and the pancreatic secretion of insulin, and the link between the center and these target organs is achieved by means of the vagus nerves.

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THE MAIN INTESTINAL PARASITE SPECIES (PROTOZOA AND HELMINTHS), IMPORTANT IN HUMAN PARASITOLOGY IN YEMEN

AL SAKKAF GALAL*, TEODORESCU IRINA**

A total of 1204, randomly selected persons, including all age groups and both sexes were investigated during 1998-1999, in the Republic of Yemen, covering two areas, urban (Aden) and rural (Lahj). Registered increasing infestation levels with protozoan species *Giardia intestinalis*, *Entamoeba histolytica* and decreasing with helminths *Enterobius vermicularis, Ascaris lumbricoides, Trichuris trichiura*. Several environmental, demographic and socio-economic factors play an important role in the spread of the intestinal parasites.

1. INTRODUCTION

The Republic of Yemen is a subtropical country in the Arabian Peninsula, characterised by a variety in the landscape and climate: south lowlands and east coastal areas, with humid and hot weather in summer, moderate dry in winter; north highlands, with hot dry summer and cold winter, rainfall often in the summer seasons; desert areas at the north-east. The Republic of Yemen has an estimated area of 555 000 square km and around 16 million inhabitants (in 1994), with an expected increase until 20 million by the year 2002.

The main human parasite species in Yemen are *Giardia intestinalis, Entamoeba histolytica, Plasmodium vivax, Plasmodium falciparum, Trichomonas urogenitalis, Leishmania tropica, Toxoplasma gondii, Schistosoma haematobium, Schistosoma mansoni, Taenia saginata, Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Ancylostoma duodenale.*

The country showed high increases in the infestation level with malaria. During the last few years 6953 cases were reported in 1991; 12863 cases in 1992; 13535 cases in 1993; 2173 cases in 1994 (the exact number is not known because of the civil war in 1994).

Infestation with *Leishmania tropica* showed an increase in the number of cases.

Infestation with *Schistosoma* species also showed an increase in the number of cases and places; despite the efforts done by the program project for schistosomiasis control, these efforts remain insufficient.

In the control of *Dracunculus medinensis* infestation, good results were registered.

Factors influencing health status in Yemen. Several factors, including socio-economic, demographic, environmental, food hygiene, personal hygiene and health education influenced the health status.

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Socio-economic factors. Yemen shares most of the world's least developed countries, acute socio-economic problems behind recent economic distortions, raising the open and disguised unemployment, soaring inflation rates, diminishing rates of agriculture development and food production and unwise use of natural resources, especially of water. The main socio-economic factors influencing the health status, and suspicion to many parasitic infestations are: previous census data recorded illiteracy rates of about 60 % for the population aged about 10 years or more, dominance for females; bad housing conditions, especially in rural and suburban areas, majority of rural houses constructed without planning considering spaces, rooms, bathrooms, the cattle places, etc.; low opportunity for women contribution to socio-economic and health development, due to their high rate of illiteracy and low education; wrong perceptions regarding women's role beyond their traditional duties at home; rapid succession of pregnancies and births; lack of mass and modern health education and medical information; deficiency of medical, and resort to non-medical personnel for advice and treatment; dominance of traditional medicine.

Demographic factors are: population increase (the crude birth rate is about 52 per 1000 population), and its pressure over the limited, financial, medical and food resources; the pattern of population composition and distribution (52 % under 15 years of age, about 82 % in rural areas); heavy rural to urban males migration; return of more than one million of people to the country after the Gulf crisis, in 1990.

During the last two decades there was registered a population increase (due to high fertility and few declining mortality rate), but a little progress in public health measures. The country's efforts to improve the health status of the citizens are not efficient, because the natural and economic resources did not increase simultaneously.

Recent data (Onis et collab., 1993) about the under 5-years old children in Yemen in 1991–1992 showed that the prevalence of under weight children is 30 %, stunting 44.1 % and wasting 12.7%.

Environmental factors which create difficulties to health services development are: environmental degradation with desertification, depletion of water resources, soil and vegetation; increasing rates of physical, chemical, biological, water, air and soil pollution; excessive use of pesticides, fertilisants and growth stimulating substances, diminishing stocks of fuel-wood; low annual rainfall etc. The most important problem, especially in rural areas, is the insufficient supply of fresh water (about 35 % of the population has access to safe drinking water), which increases the possibility of water-borne, vector-borne and faecal related diseases. The inadequate system of sewage disposal of human excreta (nearly about 75 % of all households in urban areas lack sewerage facilities), together with the inadequate use of chemical pesticides by farmers contaminate the water supply.

Food hygiene. Food being a potential source of infection and infestation, food hygiene implies hygiene in its production, processing, handling, distribution and serving. This involves several aspects of personal hygiene, food handlers and consumers: improper washing of vegetables and fruit which are eaten raw; food exposure to synanthropic vector agents (insects, rodents) or air-borne protozoan cysts, helminths eggs; insufficient cleaning of food ustensils, before cooking; ignoring hands washing, especially the children, before meals and after defecation.

Personal hygiene and health education refers to: lack of mass education about health and healthy living, the dangers of unsanitary habits, unhygienic surroundings; overcrowding of family members in small houses, which influences the sanitary conditions favouring the spread and transmission of many parasitic diseases.

2. MATERIAL AND METHODS

Our research was conducted between November 1998–August 1999, in two different areas in the Republic of Yemen, representative for both urban (Aden) and rural (Lahj) areas. A number of 1204 randomly selected patients were studied, including all age groups and both sexes.

Hospitals of Al-Gamhoria and Bin-khaldon collaborated in performing most of the analysis:

- **Ordinary coproparasitologic methods**: the wet mount with saline salt; the wet mount with iodine; the direct method with Lugol's iodine.
- **Culture methods**: Loffler's medium (Simic method) for *Entamoeba* species cultivation.
- **Staining method**: staining with methylene blue; staining with Quensel-Svenson stain and Turdyve's (staining and fixing) fluid, to differentiate *Entamoeba species* (*E. histolytica*, *E. dispar*, *E. coli*) and *Dientamoeba fragilis*.
- **Questionnaire method,** applied by interview.

3. RESULTS AND DISCUSSION

The results revealed the degree of parasitism from 1204 investigated persons (Table 1), the presence of different species of intestinal non-pathogenic (incolinous, commensal) and pathogenic parasites. A high infestation level was registered (above 66 % in both areas, 63.5 % in urban and about 70 % in rural areas) (Table 2). Protozoan species are the most frequently identified as etiologic agents of parasitic diseases. *Giardia intestinalis* and *Entamoeba histolytica*

prevalence level in 1999, in Aden (96.3 %), is higher as compared with a study done in the north districts of Yemen, in 1985 (53 %), in Sana'a (34 %) and Taiz (61.7 %), in 1994 (Ministry of General Health statistical report 1994), and in the south districts, in 1996 (58.4 % in urban, 64 % in rural areas) (Gamal Z., 1996).

Similar prevalence levels were found in some subtropical countries, with relatively comparable socio-economic and climatic conditions: 64 % in Sudan, 62 % in Iraq and 42.1 % in Nigeria (Gamal Z., 1996).

Table 1

Parasitism in urban (ADEN) and rural (LAHJ) areas

PARASITISM	URBAN		RURAL		BOTH AREAS	
	Number of cases	%	Number of cases	%	Number of cases	%
With parasite	382	63.5	416	69.1	798	66.3
No parasite	220	36.4	186	30.9	406	33.7
Total	**602**		**602**		**1204**	

Table 2

Intestinal parasitism in urban (ADEN) and rural (LAHJ) areas

Parasite species	Urban		Rural		Both areas	
	Number of cases	%	Number of cases	%	Number of cases	%
Giardia intestinalis	198	51.80	201	48.30	399	50.01
Entamoeba histolytica	170	44.50	188	45.20	358	44.86
Taenia saginata	1	0.30	–	–	1	0.12
Trichuris trichiura	8	2.10	13	3.10	21	2.63
Hymenolepis nana	3	0.80	9	2.20	12	1.50
Ascaris lumbricoides	2	0.50	5	1.20	7	0.87
Total parasitism	**382**		**416**		**798**	

Table 3

Comparison of the intestinal parasitic infestation between the cities of SANA'A (1994) and ADEN (1999)

Parasite species	Number of positive samples SANA'A 1994	% of positive samples SANA'A 1994	Number of positive samples ADEN 1999	% of positive samples ADEN 1999
Giardia intestinalis	1124	17.20	198	51.80
Entamoeba histolytica	1095	16.80	170	44.50
Ascaris lumbricoides	601	9.20	2	0.50
Schistosoma mansoni	528	8.00	–	–
Trichuris trichiura	224	3.40	8	2.10
Hymenolepis nana	379	5.80	3	0.80
Enterobius vermicularis	2413	37.00	–	–
Ancylostoma duodenale	40	0.60	–	–
Others species	125	2.00	1	0.30
Total	**6529**		**382**	

Table 4

Comparison of the intestinal parasitic infestation between the cities of TAIZ (1994) and ADEN (1999)

Parasite species	Number of positive samples TAIZ 1994	% of positive samples TAIZ 1994	Number of positive samples ADEN 1999	% of positive samples ADEN 1999
Giardia intestinalis	2845	33.00	198	51.80
Entamoeba histolytica	2479	28.70	170	44.50
Ascaris lumbricoides	700	8.10	2	0.50
Schistosoma mansoni	370	4.30	–	–
Trichuris trichiura	223	2.60	8	2.10
Hymenolepis nana	182	2.10	3	0.80
Enterobius vermicularis	845	9.80	–	–
Ancylostoma duodenale	34	0.40	–	–
Other species	948	11.00	1	0.30
Total	**8626**		**382**	

Table 5

Polyparasitism among population of both the urban (ADEN) and rural (LAHJ) areas

Polyparasitism	Number of cases	%
Giardia intestinalis + *Entamoeba histolytica*	48	55.20
Entamoeba histolytica + *Trichuris trichiura*	31	35.60
Giardia intestinalis + *Trichuris trichiura*	6	6.90
Giardia intestinalis + *Hymenolepis nana*	2	2.30
Total	**87**	

In Aden area, from 382 infested persons, more than 96 % are infested with two protozoan parasites, *Giardia intestinalis* and *Entamoeba histolytica*. Other parasite species (*Trichuris trichiura*, *Hymenolepis nana, Ascaris lumbricoides* and *Taenia saginata*) are present, but with a low prevalence level.

In Lahj area, *Giardia intestinalis* and *Entamoeba histolytica* are also the main parasite species (more than 93 % prevalence values). Parasitism with *Trichuris trichiura*, *Hymenolepis nana*, *Ascaris lumbricoides* registered a slight increase, but *Taenia saginata* was not identified.

Comparison of intesinal parasitism between the cities of Aden (1999), Sana'a and Taiz (1994)

Eight important intestinal parasite species (protozoa and helminths) were identified, according to the Statistical Report of the Ministry of General Health (Yemen 1994), from Sana'a and Taiz.

Sana'a district is located in the north part of Yemen, characterised by its cold weather in the winter and a moderate summer weather, hundreds of meters above sea level.

In Sana'a, the helminths parasite species have a wide spread (64 %), the most important species *Enterobius vermicularis* presenting 37 % of the positive investigated samples. *Ascaris lumbricoides* and *Schistosoma mansoni* registered low values of infestation. From protozoan parasites the most important are *Giardia intestinalis* and *Entamoeba histolytica* (Table 3).

Taiz district is located also in the north part of Yemen, characterised by its moderate weather along the year, the rainfall in summer season, having chronic problems in water resources because of the mountainous nature of the land.

In Taiz district, the most cases are those of giardiasis and amoebiasis (61.7 % altogether); giardiasis is nearly equal in value with those of amoebiasis from Sana'a. *Enterobius vermicularis* prevalence level was found in 3.8 times less cases than in Sana'a. Low values of infestation were registered for *Schistosoma mansoni* and *Trichuris trichiura* (Table 4).

The number of analysed samples in Sana'a and Taiz is much more than in Aden.

In Aden district, a spectaculous infestation increase was registered with the two protozoan parasite species (96.3% altogether), from which *Giardia intestinalis* has higher infestation. No registered cases parasited with *Schistosoma mansoni, Enterobius vermicularis* and *Ancylostoma duodenale,* while other parasite species have registered low values of infestation.

Infestation increase with *Giardia intestinalis* and *Entamoeba histolytica* is mainly related to the demographic factors, which are accompanied by insufficient health services, crowding index, poor sanitation, personal and food hygiene, besides the spread of the biological vectors and climatic conditions.

Polyparasitism in Aden and Lahj areas

Polyparasitism represents the simultaneous presence of two or many parasite species, in or on the same host.

Intestinal polyparasitism was identified an overall prevalence 7.2 % from the total number of 1204 examined persons (Table 5).

From the 87 persons that showed polyparasitism, 55 % have association *Giardia intestinalis-Entamoeba histolytica*; 35 % *Entamoeba histolytica-Trichuris trichiura*. Other associations have low incidence. No cases registered with more than two intestinal parasite species.

Recommendations to improve general and especially rural sanitation

The fact that about 82 % of the people live in villages, the following suggested points are necessary: **mass education, rural planning** for houses, considering spaces, rooms, sewage holds, the cattle places, etc., **water supply, correct disposal of human and animal excreta, populations control** of **synanthropic animals** (insects, rodents) and other **diseases vectors**.

Mass education might be achieved by films, pictures, lectures in usual and easy language, etc. Rural people must be given the knowledge of health and healthy life; they should be convinced that most of the diseases are preventable by observing proper precautions should know the danger of unsanitary habits, overcrowding, effects of ill-ventilation and unhygienic surroundings; avoiding polluted water supplies (protection of sources and storage tanks and general inspection measures during transportation and distribution).

4. CONCLUSIONS

- This research was conducted in the Republic of Yemen, between 1998–1999, in two areas, urban (Aden) and rural (Lahj), with differences in the habits and customs, in climatic, socio-demographic and ecological conditions.
- The number of persons that have undergone stool analysis and collaborated with the interview (Questionnaire) was 1204, randomly selected, including all age groups, with both sexes.

- Varieties of methods were used to demonstrate the presence of intestinal parasite species.
- It was found that several environmental, demographic and socio-economic factors play an important role in the spread of the intestinal parasitic infestation.
- The general situation of the parasitic diseases in the country reflects the deterioration in the environmental and socio-economic conditions, that play an important role in the spread of parasites and parasitic infestation.
- For the intestinal parasites with protozoan species, the infestation level was increasing. The two protozoan parasites, *Giardia intestinalis* and *Entamoeba histolytica*, was showed very important infestation levels, in two areas, among all age groups and both sexes.
- Some helminths like *Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura, Ankylostoma duodenale* and *Taenia saginata* showed a moderate to low infestation level, in correlation with the low rainfall in the country and other environmental conditions.
- There is no significant differences in parasitic infestation among the population in the two areas, urban and rural, with a slightly high prevalence level in the rural areas, perhaps due to the little importance given to the rural area in education and health care services.
- Through introducing new technical methods for general surveys to estimate epidemiological levels of parasitic infestations, the progress in general health, in socio-economic and the environmental conditions, can be realised by both people and government, with financial and technical support.

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