ȘTEFAN NEGREA PhD
(November 09, 1930 – February 20, 2019)

A life dedicated to the study on the creatures of the dark
and damp underground caves
A life dedicated to speleology – Light from the dark

The scientific community of Romanian biologists, as well as many lovers of nature or hiking, have been deeply saddened by the news of the famous biospeleologist Dr. Ștefan Negrea’s disappearance, witness to the renaissance of the Emil Racoviță Institute of Speleology in Bucharest, later becoming one of its most renowned scholars, known everywhere at home and abroad for his papers of speleology, ecology and zoology, especially for those on cladoceran crustaceans and chilopods.

Dr. Ștefan Negrea left us discreetly, lonely after the loss of his dear wife and collaborator on the road of science – Dr. Alexandrina Negrea – Didina, as he usually called her.

Dr. Ștefan Negrea left behind a radiant light of his speleological and hydrobiological research in Romania and abroad.

Dr. Ștefan Negrea highlighted the life and work of some great personalities of Romanian biology.
Dr. Ştefan Negrea spoke to scientists, and to the media, about the caves in our country and elsewhere, showing their treasures, the creatures of the world without sun, with their amazing adaptations.

Dr. Ştefan Negrea worked at the Institute of Speleology of the Romanian Academy, an institution reborn after 1956, under the direction of Emeritus Professor Constantin Motâş (1891–1980). We can say that Ştefan Negrea was linked by speleology, for life, mostly due to Professor Constantin Motâş, who later, prefacing a book, characterized him as follows: “Ştefan Negrea is a naturalist by vocation, and in his veins is flowing a restless blood of an explorer...”.

In these lines, we want to honor our colleague Ştefan Negrea’s memory, but we think it is absolutely necessary to interpose in our note a few words about Prof. C. Motâş, who was his model in life, mentor and colleague, true friend, without whom, with all his talent and hereditary heritage, they could not had been activated for the completion of the young biospeleologist.

Certainly, the passion for knowing life in the dark and humid caves of the earth was transmitted to Ştefan Negrea and other speleologists as a relay started from Emil Racoviţă through Constantin Motâş and from here through the generation living in a troubled world, with many shortcomings, but strengthened and forced to survival, Stephen's generation, to the young generation of the new Millennium. But let the facts speak.

Constantin Motâş was an eminent young man recommended by Emil Racoviţă for a scholarship in France, which he concluded with a doctorate for which he obtains the mark “très honourable avec felicitations du jury”.

Returned to the country, the young biologist with a brilliant training, as we were told, occupied a number of important academic dignities and, after 11 April 1949, Acad. C. Motâş became responsible for the series of volumes “Fauna Republicii Populare Române” [“Fauna of the Romanian People's Republic”], he is arrested (after a political trial and convicted at 25 years); after seven years in communist prisons, under the pressure of colleagues from abroad, he was released and asked to choose one of the institutions in which he worked. He chose the Institute of Speleology, which was being reorganized at national level, almost 10 years after Emil Racoviţă’s death, its founder in Cluj. He was director of the institute from 1956 till 1969, but continued to work at the institute until 1980, sharing the same room with Ştefan Negrea for a long time. Emeritus Professor Constantin Motâş was followed by Prof. Dr. Traian Orghidan, Prof. Costin Rădulescu and Dr. Ioan Povară. Together with them, Ştefan Negrea contributed fundamentally to the “invention” and the development of stigobiology – the study of the creatures of all kinds of underground waters. Without the contribution of stigobiology, speleology would remain a field of geology – carstology, without the knowledge of biodiversity and the evolution of life in underground environments.

Ştefan Negrea was born on the 9th of November 1930 in a family with intellectual roots, that of Priest Vasile and Maria Negrea, in Gura Văii village, Neamț county. He was proud of his family, whom he described in warm,
melancholic tones, when he was at conferences in his native lands, in Piatra Neamț or in Bacău, for example. Important ancestors, as they were mentioned in Ștefan Negrea’s CV, were: Ion Negrea (great-grandfather’s brother, born in Gura Văii), a professor of science, who founded, along with the writer “literature professor” Calistrat Hogasă, Petru Rareș Theoretical High School from Piatra Neamț (now National College) in 1869; Vasile Conța (his grandfather’s primary cousin, Ioan Trifan, born in Dachia) – philosopher, propagator of darwinism and author of „Teoria ondulaţiunii universale” [“Universal Wave Theory”]; Constantin Virgil Gheorghiu (his mother’s primary cousin, born in Petricani in 1916, m. 1992) – priest from 1968 at the Orthodox Church in Paris, prolific writer (he wrote over 50 books, among which “Ard malurile Nistrului” [“Banks of Dniester River are burning”] as reporter of war and the novel “Ora 25” [“Hour 25”], in 1962, which brought him a world fame, and after which the French–Italian–Yugoslav film “The 25th Hour” was made.

ȘTEFAN NEGREA’S SCHOOLS

He spent his childhood and primary school (1936–1940) in his native village, then he attended the gymnasium at Petru Rareș High School in Piatra Neamț (1941–1949).

As a high school student was enchanted by the lessons of zoology which guided him to observe and to discover nature; sometimes he told us how he was waiting for the butterflies to fly, the hairy caterpillars rattling the leaves, bush-cricket singing in the grass, the “black as pitch” crickets taken out with the straw from holes, the ants carrying their colossal burdens.

In high school he was also noted for his literary skills and participated in the student competitions, obtaining for his papers on Soviet literature, compulsory at that time, the First Prize in 1947 and 1948, and in 1949 he became a member of the Literary Cenacle “The New Word” and founding member of the Romanian Writers’ Union Branch in Piatra-Neamț. His skills in writing and literature would reach high levels all Ștefan’s life. In 1949 Ștefan Negrea took the baccalaureate exams receiving the highest mark, “very well”.

A year later, he married (on August 16, 1950) with his former high school colleague – Alexandrina Budăi, the One, Didina as he used to tell her, remaining together all their life. Young Ștefan Negrea felt strongly attracted both to literature and to biology-zoology. Finally, discussing with Didina, he decided and had chosen the second option, the study of nature. Thus, together with his wife, he decides to study biology together and in 1951–1954 the young couple Ștefan and Alexandrina attended the courses of the Faculty of Biology of the University of Bucharest.

As a student he is remarked for his scientific interest and becomes president of a student scientific circle of hydrobiology, being awarded in Bucharest and country
In memoriam Ștefan Negrea PhD

In 1953 he wrote the paper “Hydrobiological and Fishery Research in Snagov Lake”, being supported by Prof. Traian Orghidan (1917–1985) – a remarkable naturalist and scientific personality who, after 1956, was to become one of the dynamic organizers of the Institute of Speleology, and which helped Ștefan Negrea to develop interest in the study of hydrobiology. The paper was awarded with an honorary diploma.

Also in 1953, as a student, Ștefan Negrea and Didina had the great chance to visit the Cave of Vadul Crișului, a cave with resurgent reliefs, with abundant water, very suited to speleological and bio-speleological research, the very first cave visited by them. Being fascinated by its beauties, Negrea spouses were glimpsed by an idea, which became a dream of their future activity; was the desire to explore the cave creatures as zoologists and hydrobiologists at the Speleology Institute of Emil Racoviță and as researchers, together with their masters, Professor Margareta Dumitrescu and associate professor Traian Orghidan, biologists “definitively converted to speleology”.

Next year, in 1954, Ștefan Negrea wrote the paper “Fauna de cladoceri a Lacului Snagov” [“Cladoceran Fauna of Snagov Lake”], which was awarded at the second Student Scientific Conference. For the Bachelor's degree exam, he continued the study of the tiny aquatic crustaceans and prepares the thesis “Contribuții la studiul faunei de cladoceri din lacurile glaciare ale R. P. Române” [“Contributions to the Study of Cladocerans of the Glacial Waters of the Romanian People's Republic”] and passes the exam with the grade “Very well”.

Under the guidance of Prof. Nicolae Botnariuc – the promoter of systemic conception in ecology, Ștefan Negrea prepares the PhD at Bucharest University, studying the small freshwater crustaceans from his group studied during studentship, Cladocera Order, included in Branchiopoda Class, a group he studied minutely his whole life. In 1971 he obtained a PhD in Biology with a doctoral thesis titled “Ecologia cladocerilor din lacurile Jijila, zona inundabilă a Dunării” [Cladoceran Ecology of Jijila lakes, flooded area of the Danube”].

In 1975 he attended a training and scientific study period in France (Paris, Moulis) in the Underground Laboratory of the National Center for Scientific Research, considered the most important biospeleological center in the world at that time.

**SCIENTIFIC ACTIVITY**

The period of the 1950s, when Ștefan Negrea made his university studies and began to work, was a difficult time, with many shortcomings and destructions, as after the war, but also as the setting up of a new political order in the country, the Soviet order brought with the tanks. Then, as a result of a political pressure to organize the Romanian Academy according to the Soviet model, the Committee of Hydrology, Hydrobiology and Ichthyology of Romanian Academy is founded. This
committee gathers around it some distinguished biologists with the initiative and desire to develop biology in Romania, such as Teodor Bușniță (1900–1997), Mihai Băcescu (1908–1999), Ludovic Iosif Urban Rudescu (Rodewald) (1908–1992), Nicolae Botnariuc (1915–2011), A.C. Banu (geographer), Radu Codreanu (1904–1987) and others, who organized a series of researches on the Danube and the Black Sea, employing university graduates.

After graduating his studies, Ștefan Negrea's career takes the lead of scientific research. In brief, after a brief period in Constanța and Brăila, Dr. Ștefan Negrea was a scientific researcher at the “Emil Racoviță” Institute of Speleology of the Romanian Academy, Bucharest, since 1956. In 1966, he was promoted to the position of scientific researcher gr. III, in 1990 he became scientific researcher gr. II, and in 1995 – scientific researcher gr. I. In 2005 he retired, and since 2008 he was associate researcher at “Emil Racoviță” Institute of Speleology.

However, we must mention that at the end of 1954, he was appointed as a research assistant at the Committee of Hydrology, Hydrobiology and Ichthyology of the Romanian Academy, with a job at the Constanța Marine Research Station, the former Bio-Oceanographic Institute, founded by Grigore Antipa. Here he stayed for a short time, but he gave us the “Observations sur la répartition du zooplancton sur le profil Est – Constanța”, published together with Alexandrina Negrea and Lucreția Elian in 1959.

In the period 1955–1956, Ștefan Negrea was transferred to the Brăila Hydrobiological Station of the University of Bucharest, thanks to Prof. Nicolae Botnariuc, who coordinated an important project on the “Study of the Crapina-Jijila Pool in the Danube Flooding Area”. He was among the young researchers; the results of this collaboration are included in the paper “Observații asupra biologiei speciei Fagotia esperi (Fér.) din complexul de bâlți Crapina-Jijila”. [“Observations on the biology of the species Fagotia esperi (Fér.) in the Crapina-Jijila Pond Complex”], published in 1963 together with Nicolae Botnariuc and Alexandrina Negrea.

At the beginning of his career, Ștefan Negrea collaborated with Prof. Constantin Motaș and Lazăr Botășăneanu, making important researches on the biology of the springs and phreatic waters in the central part of the Romanian Plain; the results of the extensive studies were published in a volume of over 330 pages in the Romanian Academy Publishing House (1956).

Among the reference works of Dr. Ștefan Negrea, we mention:

- Cercetări asupra biologiei izvoarelor și apelor freatice din partea centrală a Câmpiei Române (C. Motaș, L. Botășăneanu, Șt. Negrea, 1962, 366 pp.); [Researches on the biology of springs and phreatic waters in the central part of the Romanian Plain];
- Bibliographia Biospeologica Romanica (1937–1963) (V. Decu, Șt. Negrea, 1965);
- Recherches sur les grottes du Banat et d’Oltenie (L. Botoșăneanu, A. Negrea, Șt. Negrea, A. Decou, V. Decou, M. Bleahu, 1967, 397 pp.) [Studies on the caves of Banat and Oltenia];
- The three papers dedicated to parietal, guano and flooring biocenoses of the caves of the Banat Mountains (Șt. Negrea, A. Negrea, 1971–1977);
- Ecologia populățiilor de cladoceri și gasteropode din zona inundația a Dunării (Șt. Negrea, A. Negrea, 1975, 232 pp.) [Ecology of the cladoceran and gastropod populations of the Danube flooding area];
- The volume “Speleology” in the monograph series “The Iron Gates” (editors T. Orghidan, Șt. Negrea, 1979);
- Expediționari români la tropice (Șt. Negrea, 1980, 208 pp.) [Romanian Explorers in Tropics];
- “Cladocera” in the academic series “Romanian Fauna” (Șt. Negrea, 1983, 399 pp.);
- Pe urmele lui Grigore Antipa (Șt. Negrea, 1990) [On Grigore Antipa’s Footsteps];

Dr. Ștefan Negrea was a member of a number of scientific organizations and associations:
- The International Myriapodology Center in Paris;
- The Senate of “Alexandru Ioan Cuza” University of Iași;
- Limnology Society of Romania, affiliated to the International Society of Limnology (SIL);
- Division of the History of Science of the Romanian Committee for the History and Philosophy of Science and Technology (CRIFST) of the Romanian Academy;
- The Leading Board of the Association of Journalists and Writers of Tourism in Romania.

Also, Ștefan Negrea was part of the Editorial Staff of scientific journals of Romania and abroad:
- Academic series “Fauna României” (deputy editor-in-chief since 1983);
- Noema; Studii și Cercetări – Divizia de Istoria Științei a CRIFST;
- Travaux de l’Institut de Speologie “Emile Racovitza”;
- Oltenia – Studii și Comunicări, Științele Naturii (the journal of “Oltenia” Museum in Craiova).
IMPORTANT ACHIEVEMENTS

Over the years, the results of the research undertaken by Dr. Ştefan Negrea materialized in the elaboration of an impressive number of works, with particular reference to the cladocerans (Cruciacea: Branchiopoda), the chilopods (Myriapoda) and the underground environments. He has also carried out numerous expeditions for the scientific exploration of caves in Romania.

- The researches from 1956–1959 were carried out during scientific expeditions aimed at exploring the caves of the Southern Carpathians, Banat and Dobrogea.
- Between 1960–1986, Dr. Ştefan Negrea explored, together with his wife and Dr. Lazăr Botoșâneanu, hundreds of caves and fauna of the Banat Mountains, the Cerna Valley and the Poiana Ruscă, which became very well known in Romania, from biospeleological point of view.
- As a result of these studies, information on more than 240 caves and ravines from Romania were published, and about 30 km of cave galleries were mapped, mostly in the southwestern part of the country.

Since 1961, Dr. Ştefan Negrea has carried out a series of field trips together with members of the “Exploratorii” Speleological Club of Reşiţa, and since 1987 he did field applications at the National School of Biospeleology. Negrea spouses, in collaboration with amateur biospeleologists from this center, studied dozens of caves from the hydrographic basins of Caraş and Bârzava (1980–1984).

Between 1969 and 1973, Dr. Ştefan Negrea participated in 40-day Romanian–Cuban Biospeleological Campaigns in the Cuban Tropical Karst Area, the results contributing to the formation of an overview of the cave fauna within the area. Obtained data were published in four volumes at the Romanian Academy Publishing House (1973, 1977, 1981 and 1983).

In 1990, at Prof. Univ. Dr. Francis Dov Por’s request (University of Jerusalem), in 1990, Ştefan Negrea was included in a team for a 40-day expedition in Israel for the collection of zoological material from the cave and terrestrial environment. The results of the researches were used in a series of papers published under the aegis of the Romanian Academy (1995).

During his long scientific career, Dr. Ştefan Negrea collaborated with numerous colleagues, both from the country (Motaş C., Orghidan T., Botnariuc N., Botoșâneanu L., Decu V., Sencu V.L., Karban G., Elian Lucretia, etc.), especially from “Emil Racoviţă” Institute of Speleology and from abroad: Pospisil P. from Austria, Dumont H.J. from Belgium, Por F.D., Dimentman Ch. from Israel and other famous specialists from France, Spain, Germany, Hungary, Bulgaria, Macedonia, Poland, Czech Republic, Italy, Russia, Cuba, Mexico, USA, etc.

In his 55 years of scientific activity (1954–2009), Dr. Ştefan Negrea has published, single or as collaborator, 406 articles and books with subjects that dealt
with the various fields of biological sciences: classical taxonomy (Cladocera, Chilopoda) aquatic, terrestrial and underground ecology, zoogeography, phylogeny, nature conservation, history of biological sciences and some of its representatives.

- Among these, a valuable scientific contribution is the original, complex study of the crustaceans of the Branchiopoda Class, with reference to ecology, ethology, phylogeny and their classification. Dumont and Negrea made a cladistic analysis of all branchiopod groups, using a total of 42 morphological characters and establish that this class is composed of five superordinates and 11 orders (nine recent, two fossils); the results of these researches materialized in the publication of the monograph “Introduction to the Class Branchiopoda” (H.J. Dumont, Şt. Negrea, 2002, 398 pp.), published in the series “Guides to the identification of the invertebrates of the continental waters of the world”. The paper was awarded the “Emil Racoviță” Prize of the Romanian Academy and now it is considered one of the most valuable carcinology works of the world;

- Dumont and Negrea (1996), reviewing of the cladocerans of the cave waters of the world, present, also, a synthesis of the current knowledge on cladocerans by providing a historical study on concept development, category identification among groundwater species and discussing the existing adaptations and lines of evolution; in addition, they consider that out of the total of 450 non-marine Cladocera, estimated in the world, only \( \approx 20\% \) can occur in underground aquatic habitats, their number (stigobiontes or stigobites) being relatively low (Dumont H. J., Negrea Şt., 1996 – “A conspectus of the Cladocera of the underground waters of the world”, Hydrobiologia 325 (1): 1–30 DOI: 10.1007 / BF00023664);

- In Israel, Negrea and his collaborators studied the morphology, zoogeography and ecology of scutigeromorph centipeds and presented an identification key for species.

Dr. Ştefan Negrea spoke about the caves in our country and abroad, showing us their treasures, the creatures of the world without the sun, with their amazing adaptations. The description of over 40 new species for science, of myriapods (chilopods and diplopodes) and cladoceran crustaceans represents a significant contribution to the knowledge of the cave environment biodiversity.

This way, the specialist Ştefan Negrea evolved, the one who studied taxonomically, ontogenetically, phylogenetically, and biogeographically mostly the Branchiopod crustaceans from the freshwater, brackish, marine, hypersaline and underground regions of the palearctic, neotropical and ethiopian regions, for more than 50 years from embryonic development point of view, but also other invertebrate groups, in terms of ethology, ecology, and biogeography (including palaeobiogeography). Dr. Ştefan Negrea’s books guided young people towards science adventure and decided the orientation to the biological knowledge and training of many people.
He left behind, the result of an agitated life, the search for science, numerous and valuable biological contributions, which, at this early hour after his departure into eternity, we mention by chance and partly as follows:

- Studiul zoologic al peșterilor din România: [Heleobia (Semisalsa) dobrogica (Grossu & Negrea, 1989). (Gastropoda: Rissoidea: Cochliopidae), Biodiversitatea în mediile subterane din România (Șt. Negrea, A. Negrea, A. Ardelean, 2004, 248 pp.). [Zoological study of the caves in Romania: [Heleobia (Semisalsa) dobrogica (Grossu & Negrea, 1989). (Gastropoda: Rissoidea: Cochliopidae), Biodiversity in the underground environments in Romania];
- Studierea biocenozelor parietală, de guano și de planșeu a peșterilor din Munții Banatului (Șt. Negrea, A. Negrea, 1971–1977) [Study of the parietal, guano and flooring biocenoses of the caves in the Banat Mountains];
- Ecologia populațiilor de cladoceri și gasteropode din zona inundabilă a Dunării (Șt. Negrea, A. Negrea, 1975, 232 pp.) [Ecology of the cladoceran and gastropod population in the floodplain area of the Danube];
- Cunoașterea biologiei izvoarelor și apelor freatic din partea centrală a Câmpiei Române (C. Motaș, L. Botoșâneanu, Șt. Negrea, 1962, 366 pp.) [Knowledge of the biology of the springs and phreatic waters in the central part of the Romanian Plain];
- Cercetări asupra peșterilor din Banat și Oltenia (L. Botoșâneanu, A. Negrea, Șt. Negrea, A. Decou, V. Decou, M. Bleahu, 1967, 397 pp.) [Research on the caves in Banat and Oltenia];
- Contribuții speologice la realizarea Seriei monografice Portile de Fier (editori T. Orghidan, Șt. Negrea, 1979) [Speleological contributions to the Iron Gates monographic series];
- Filogenia, evoluția și clasificarea Clasei Branchiopoda (Crustacea) (Ștefan Negrea, Nicolae Botnariuc, Henri J. Dumont) [Phylogeny, Evolution and Classification of Class Branchiopoda (Crustacea)];
- Conспектul Cladocerelor din fauna apelor subterane ale lumii (Ștefan Negrea, Henri J. Dumont) [Cladocerans Groundwater Fauna of the World];
- Evaluarea critică a speciilor de Chilopode din România și Israel – istoricul studiilor, sinonimele, actualizarea nomenclaturii a două înregistrări vechi: Eupolyorthus [Critical evaluation of Chilopod species in Romania and Israel - history of the studies, synonyms, updating the nomenclature of two old records: Eupolyorthus];
- Istoria biologiei românești și biografiile marilor săi slujitori: Pe urmele lui Grigore Antipa (St. Negrea, 1990); Nicolae Botnariuc la 95 de ani [The history of Romanian biology and of its great servants’ biographies: In Grigore Antipa’s footsteps of Grigore Antipa; Nicolae Botnariuc at his 95th anniversary];
- Expediționari români la tropice (Șt. Negrea, 1980, 208 pp.) [Romanian explorers in the Tropics].

Being contemporary with Dr. Ștefan Negrea, we can appreciate him as one of the most important specialists from Romania in the second half of the 20th century in the knowledge of organisms of the underground waters. The recognition of Dr. Ștefan Negrea’s scientific contributions can be exemplified by the many new species and genera to science, which were dedicated to him by a number of Romanian and foreign specialists.

But as many aspects of life and work as Dr. Ștefan Negrea could add, nothing is more relevant than the impressive list of 343 scientific papers, plus 28 books (author and/or editor), 42 chapters of books or independent scientific volumes, approachably edited for the distribution of science all levels of education.

Looking at the list of the distinguished colleague's papers, we will have a better image of the passionate, pedantic naturalist, biospeleologist-zoologist Ștefan Negrea, the slightly proud-spirited one, but with literary talent and profound analyst of the researched subjects, that he presented clearly, by explicit texts, and where it was necessary, by beautiful scientific drawings. We will understand its ability to explain the evolution of organisms from the complex environments, revealing the apomorphic characters, as compared to the plesiomorphic ones, in order to establish cladograms, verified and confirmed later and by methods of molecular biology techniques.

During his career, Dr. Ștefan Negrea wrote valuable pages of the history of Romanian biology, about “people and facts”, witnessing events that marked the development of biological sciences in our country.

In more than 50 years of scientific activity, Dr. Ștefan Negrea worked with passion and tenacity at the service of Biology in Romania, to whom he was devoted to his colleagues, collaborators, by all those who had the privilege to be in around.

Today, when he is in a better world, he left us a light behind, gathered along a life time the darkness of caves. Also, he left us a model of marriage rarely occurred, when spouses share the same concern, and together they go in field trips, share the lab together, consult together on scientific issues, write and publish together, participate in conferences and symposiums. This explains why, after Didina’s eternal departure, Stephen did not find his peace of mind until February 20, 2019, when he also left for eternity.

Farwell beloved friend and colleague in your last underground trip!
Rest in peace, anxious soul in search of light in the darkness of the caves!

Prof. Dr. Marian-Traian Gomoiu, Dr. Dumitru Murariu,
Member of the Romanian Academy Corresponding Member of the
Romanian Academy

Dr. Sanda Maican,
Institute of Biology Bucharest of the Romanian Academy
In memoriam Ștefan Negrea PhD

DR. ȘTEFAN NEGREA – SELECTED PAPERS


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NEGREA ŞT., 2009, Dr. Ştefan Negrea. Curriculum Vitae et Opera Omnia. Studii și Comunicări, II: 81–86.
DIVERSITY AND SPATIAL DISTRIBUTION OF ZOOPLANKTON COMMUNITIES OF DANUBE DELTA LAKES

LARISA FLORESCU*, LAURA PARPALĂ*, IOANA ENACHE**, ALINA DUMITRACHE***, MIRELA MOLDOVEANU*

The high diversity of aquatic communities offers stability and resilience to the ecosystems. The zooplankton has a particularly important role in aquatic ecosystems, by up-taking the organic matter synthetized by phytoplankton and transferring it to higher consumer orders, by grazing and facilitating bacterial decomposition processes, by contributing to the enrichment with nutrients of the water and benthic layers, etc. Our study was focused on assessing zooplankton diversity, spatial distribution and particularities in 21 selected lakes located in three main lake complexes of the Danube Delta. The structural parameters with key role for zooplankton diversity were assessed by linear regressions. The most representative taxonomic groups were Rotifera and Copepoda, the highest number of species and abundance being recorded in Matiţa-Merhei lake complex (164 species, 343 ind. L\(^{-1}\)). In the same complex, the Shannon index was positively correlated with species richness and evenness. In the Roșu-Puțu complex, a negative correlation between evenness and abundance was found as a result of the high number of accidental species; also, a positive correlation between evenness and Shannon index was recorded. The highest diversity was recorded in Isac-Gorgova lake complex, characterized by a positive relationship between abundance and the number of species and between Shannon index and evenness. The correlations with phytoplankton groups indicated the importance of Chlorophyceae and Cyanobacteria for the development of rotifers and copepods. The influence of environmental variables on zooplankton diversity was further tested to explain the dissimilarities between the lake complexes.

Keywords: Copepoda, diversity indices, population dynamics, Rotifera, shallow lakes.

INTRODUCTION

The zooplankton communities play a pivotal role in aquatic food webs as they represent a significant food for planktivorous fish and invertebrate predators. They graze on algae, bacteria, protozoa, and other small invertebrates.

Diversity is a very important trait of any community in a given ecosystem. The variation of diversity may strongly influence the functionality, stability and productivity of the ecosystem (Zhang et al., 2012). Many authors use species richness as a measure of diversity (Stirling & Wilsey, 2001; Bock et al., 2007; Gotteli & Chao, 2013). But community diversity includes two main components: number of species and evenness. Hence some authors recommend to treat these two components separately in order to investigate the determinants of diversity.
Examine along an increasing gradient of trophy, the zooplankton community is represented by species like large cladocerans, diaptomids and cyclopids and small percentages of rotifers, in mesotrophic lakes, while in more eutrophic lakes, the structure is based on small species: small cladocerans, more copepod instars, and dominating rotifers (Gliwicz, 2005).

During early spring, the zooplankton community is dominated by small species, with high reproductive rates, like protozoans and rotifers, while large species, such as cladocerans and copepods, occur later; this dynamics is highly dependent on phytoplankton development and the predators pressure. Moreover, the particular conditions created by wind-induced mixing (Yoshida et al., 2001; Gliwicz, 2005), raise additional difficulties to anticipate the distribution of zooplankton in shallow lakes.

With the beginning of summer, the zooplankton community is restructured as a consequence of temperature increase, dynamics of phytoplankton community and fish predation pressure; small zooplankton genera, such as Thermocyclops, Mesocyclops, Bosmina, Chtydorus, Diaphanosoma, Ceriodaphnia, Moina, and rotifers prevail. As autumn progresses, large genera, like Daphnia and Eudiaptomus, start developing again. This is an effect of both the changes occurred in the phytoplankton community, when edible species have a distinct peak of development, and of the decreased predation pressure. During late autumn and winter, many zooplankton species enter a diapause phase (Sommer et al., 1986).

Generally, the zooplankton diversity in Danube Delta lakes is very high, being influenced however by the natural and anthropogenic drivers in the area. For instance, between 1975–1995, 562 species were recorded (Zinevici & Parpală, 2006), but their number fluctuated widely during this interval: e.g. due to a high impact of eutrophication, a drastic reduction of zooplankton diversity was recorded between 1975–1987 (53.08%) (Zinevici & Teodorescu, 1996; Zinevici & Parpală, 2000). Hence, investigations of freshwater zooplankton community structure could represent a valuable indicator for assessing aquatic ecosystem health (Rocha et al., 1997; Pedrozo & Rocha, 2005; Ţundri, 2015).

The objectives of this study were to assess the diversity of zooplankton and its spatial distribution in selected lakes of Danube Delta, as well as the state of these communities in 2013. Such information plays a major role for the conservation efforts of Danube Delta lakes.

**MATERIAL AND METHODS**

**Study area**

The Danube Delta Biosphere Reserve, located at 45°0’N latitude, 29°0’E longitude, in the Eastern part of Romania encompasses a complex of aquatic and terrestrial ecosystems unique in Europe.

The study was conducted during 2013, in 21 lakes belonging to three lake complexes of the Danube Delta: Roşu-Puiu, Matiţa-Merhei, Gorgova-Uzlina (Fig. 1).
Field sampling and laboratory analysis

Three sampling campaigns were carried out seasonally: in May, July and September. The sampling point was located in the centre of the lake, samples being collected on water column. The redox potential, pH, conductivity, were measured in the field with a multiparameter WTW 340i. The turbidity was measured with a Hanna Instruments turbidimeter and the water velocity was estimated using a flowmeter. The phytoplankton samples (500 ml) were taken on the water column,
without filtration and conserved with formaldehyde 4% in order to estimate the abundance (cells L$^{-1}$). The total phytoplankton biomass and the biomass of different algal groups (expressed as chlorophyll $a$ content, µg L$^{-1}$) were assessed in situ with a submersible fluorometer (Fluoroprobe III, bbe Moldaenke).

The zooplankton was sampled by filtering 50 L of water taken from the water column with a Patalas-Schindler plankton trap (5 L) and plankton nets (65 µm mesh size), concentrated in 10 ml and conserved with formaldehyde solution 4%.

Samples for chemical analyses were filtered through Whatman GF/F glass fibre filters and frozen for further analyses in the lab. Nutrients were determined spectrophotometrically following a modified Berthelot method for N-NH$_4$ (Krom, 1980), Griess-Ilosvay modified method for N-NO$_2$ (Keeney & Nelson, 1982), Tartari & Mosello (1997) for N-NO$_3$, P-PO$_4$ and TP.

The phytoplankton samples were counted in the lab using Utermöhl method (1958), a Zeiss inverted microscope, and specific keys. Zooplankton species were identified using a Zeiss inverted microscope and the following keys: for Ciliata (Grospietsch, 1972; Foissner et al., 1991–1995), Testacea (Bartoš, 1954), Lamellibranchia (Marsden, 1992), Rotifera (Voight, 1956; Rudescu, 1960), Cladocera (Brooks, 1959; Negrea, 1983), Copepoda (Damian-Georgescu, 1963, 1966–1970). At the same time with species identification, the individuals were counted to assess abundance (ind. L$^{-1}$) (Edmonson, 1971).

Data Analysis

For statistical processing, PAST (Hammer et al., 2001) and XLSTAT software were used. The zooplankton diversity was evaluated by species richness, Shannon index and Evenness. A log transformation of zooplankton abundances, phytoplankton and physical-chemical data was applied for multivariate statistical analysis.

RESULTS AND DISCUSSION

The diversity assessment is based on species richness, abundance and also by the distribution of individuals in populations (Magurran, 1988; Legendre & Legendre, 1998; Vădineanu, 2004).

In our study, the highest value of species richness (164 species) was found in Matița-Merhei Lake complex and the lowest (117) in Gorgova-Isac complex (Table 2). The highest zooplankton abundance (343 ind. L$^{-1}$) was recorded also in Matița-Merhei complex, while the lowest abundance (216 ind. L$^{-1}$) was noticed in Gorgova-Isac.

Most of the zooplankton species belong to rotifers, ciliates and cladocerans (Table 2). In freshwater ecosystems, rotifers are known to be the dominant group, both, as species number and abundance (Berzins & Pejler, 1987; Barrabin, 2000; Saler, 2004).
Table 2
Species richness recorded in the three lake complexes of the Danube Delta in 2013

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Roșu-Puiu</th>
<th>Gorgova-Isac</th>
<th>Matița-Merhei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliata</td>
<td>23</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Testacea (Testate amoebae)</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Lamellibranchia</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gastrotricha</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rotifera</td>
<td>78</td>
<td>80</td>
<td>103</td>
</tr>
<tr>
<td>Cladocera</td>
<td>22</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Copepoda</td>
<td>3</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total zooplankton</td>
<td>133</td>
<td>117</td>
<td>164</td>
</tr>
</tbody>
</table>

Also, in our study rotifers were the most abundant group, while cladocerans were not so well represented in abundance. The abundance of copepods (Crustacea) was very high, even if the number of species was low, due to the high number of juvenile stages (Table 3). The copepod communities are frequently dominated by juvenile instars (Pourriot et al., 1997).

Table 3
Annual abundance (ind. L⁻¹) of the zooplankton communities
(1 – Ciliata; 2 – Testacea; 3 – Lamellibranchia; 4 – Gastrotricha; 5 – Rotifera; 6 – Cladocera; 7 – Copepoda; 8 – Ostracoda)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roșuleț</td>
<td>4.35</td>
<td>10.69</td>
<td>7.14</td>
<td>0.00</td>
<td>33.16</td>
<td>28.05</td>
<td>52.43</td>
<td>0.00</td>
</tr>
<tr>
<td>Roșu</td>
<td>16.95</td>
<td>35.76</td>
<td>6.91</td>
<td>0.00</td>
<td>94.55</td>
<td>8.56</td>
<td>39.69</td>
<td>0.00</td>
</tr>
<tr>
<td>Mândra</td>
<td>7.19</td>
<td>67.77</td>
<td>6.00</td>
<td>0.41</td>
<td>126.95</td>
<td>13.16</td>
<td>104.40</td>
<td>0.00</td>
</tr>
<tr>
<td>Erençiuc</td>
<td>4.67</td>
<td>57.00</td>
<td>0.00</td>
<td>0.21</td>
<td>45.79</td>
<td>1.49</td>
<td>73.17</td>
<td>0.00</td>
</tr>
<tr>
<td>Puiu</td>
<td>10.22</td>
<td>70.64</td>
<td>0.09</td>
<td>0.37</td>
<td>173.42</td>
<td>4.85</td>
<td>66.86</td>
<td>0.00</td>
</tr>
<tr>
<td>Tătaru</td>
<td>3.46</td>
<td>13.24</td>
<td>0.00</td>
<td>1.12</td>
<td>10.35</td>
<td>122.32</td>
<td>237.16</td>
<td>0.00</td>
</tr>
<tr>
<td>Gorgova-Isac complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuibul cu Lebede</td>
<td>1.51</td>
<td>7.46</td>
<td>0.00</td>
<td>0.26</td>
<td>26.17</td>
<td>0.36</td>
<td>14.14</td>
<td>0.00</td>
</tr>
<tr>
<td>Isac</td>
<td>2.40</td>
<td>2.42</td>
<td>0.10</td>
<td>0.00</td>
<td>29.24</td>
<td>1.66</td>
<td>236.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Uzlina</td>
<td>3.16</td>
<td>5.87</td>
<td>1.27</td>
<td>0.00</td>
<td>129.12</td>
<td>0.99</td>
<td>102.15</td>
<td>0.28</td>
</tr>
<tr>
<td>Gorgostel</td>
<td>10.00</td>
<td>20.41</td>
<td>0.00</td>
<td>0.84</td>
<td>157.42</td>
<td>41.43</td>
<td>70.32</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Although the highest values of species richness and abundance were recorded in the lakes of Matița-Merhei complex, the Shannon-Wiener and evenness indices were lower than in other complexes. The highest diversity was recorded in Gorgova-Isac complex (Fig. 2).

In order to establish which structural parameter (species richness or abundance) had a decisive role in defining diversity, linear regressions were performed.

In Matița-Merhei complex, the Shannon-Wiener index was significantly influenced by the number of species in ecosystems (Table 4) while between abundance and evenness a negative relationship was found. Also, between Shannon-Wiener
and evenness indices a positive correlation was noticed. In addition, the evenness presented the lowest value in comparison with the other complexes.

Table 4
The relationship among diversity indices and abundance in Matița-Merhei complex

<table>
<thead>
<tr>
<th>R</th>
<th>p-values:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species richness</td>
</tr>
<tr>
<td>Species richness</td>
<td>0.004</td>
</tr>
<tr>
<td>Abundance</td>
<td>0.5</td>
</tr>
<tr>
<td>Shannon</td>
<td>0.73</td>
</tr>
<tr>
<td>Evenness</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

All this explains that, even if the number of species and abundance was higher in Matița-Merhei comparing with Roșu-Puiu and Gorgova-Isac complexes, the individuals were not evenly distributed in the lakes. Some species were dominant or were concentrated in few lakes of the complex, which reduced the evenness and diversity. This was confirmed using Simpson’s Index of dominance (D) that showed higher values, ranging between 0.59 and 0.72 in almost half of the lakes of the complex (Babina, Bogdaproste, Dracului, Matița, Lung, Merhei lakes).

In Gorgova-Isac, the complex with the highest diversity, a positive relationship was recorded between species richness and abundance (Table 5). As new species occurred, the abundance increased significantly, accompanied by evenness, in benefit of the diversity.

Table 5
The relationship among diversity indices and abundance in Gorgova-Isac complex

<table>
<thead>
<tr>
<th>R</th>
<th>p-values:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species richness</td>
</tr>
<tr>
<td>Species richness</td>
<td>0.03</td>
</tr>
<tr>
<td>Abundance</td>
<td>0.62</td>
</tr>
<tr>
<td>Shannon</td>
<td>0.71</td>
</tr>
<tr>
<td>Evenness</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The Shannon-Wiener diversity index from Roșu-Puiu complex was significantly influenced only by the evenness (Table 6). The abundance presented a positive correlation with species richness, while a negative relation was found with evenness. The output of these correlations supports the fact that abundance was the main parameter influencing the diversity, but this was reflected only for few species and in few lakes. As an example, 13% of the total number of species were present only once during the survey.
The relationship among diversity indices and abundance in Roșu-Puiu complex

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>p-values:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species richness</td>
<td>Abundance</td>
</tr>
<tr>
<td>Species richness</td>
<td>0.0008</td>
<td>0.11</td>
</tr>
<tr>
<td>Abundance</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Shannon</td>
<td>0.40</td>
<td>-0.03</td>
</tr>
<tr>
<td>Evenness</td>
<td>-0.44</td>
<td>-0.60</td>
</tr>
</tbody>
</table>

The diversity assessed in the 3 complexes reflects the environmental conditions existing in the ecosystems and also the functionality of the organisms forming the zooplankton communities. In our study, zooplankton was represented by eight taxonomic groups who contributed to diversity in various degrees, both through species richness and abundance. To assess the importance of different groups to the overall diversity of zooplankton community, Simper Analysis (similarity percentage analysis) was used. The results show that, in all the three studied complexes, Rotifera and Copepoda had the largest contribution (77.04 %) (Table 7).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Averages</th>
<th>Av. dissim</th>
<th>Contrib. %</th>
<th>Roșu-Puiu</th>
<th>Gorgova-Isac</th>
<th>Matița-Merhei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotifera</td>
<td>23.57</td>
<td>48.79</td>
<td>80.7</td>
<td>85.5</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Copepoda</td>
<td>13.64</td>
<td>28.25</td>
<td>95.6</td>
<td>106</td>
<td>91.1</td>
<td></td>
</tr>
<tr>
<td>Testacea</td>
<td>4.94</td>
<td>10.22</td>
<td>42.5</td>
<td>9.04</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>Cladocera</td>
<td>4.46</td>
<td>9.23</td>
<td>29.7</td>
<td>11.1</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Ciliata</td>
<td>1.07</td>
<td>2.21</td>
<td>7.81</td>
<td>4.27</td>
<td>6.09</td>
<td></td>
</tr>
<tr>
<td>Lamellibranchia</td>
<td>0.53</td>
<td>1.1</td>
<td>3.36</td>
<td>0.34</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Gastrotricha</td>
<td>0.08</td>
<td>0.16</td>
<td>0.35</td>
<td>0.28</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>0.02</td>
<td>0.03</td>
<td>0</td>
<td>0.07</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

The development of these groups was primarily based on the existing physical-chemical conditions and on the availability of the food resources. For zooplankton, there are two main food sources: phytoplankton communities and secondary detrito-bacterial particles/aggregates (Kim et al., 2000; Freese & Martin-Creuzburg, 2013; Zinevici et al., 2015).

During our investigation, a common trait of the three lake complexes was the fact that Rotifera consumed total phytoplankton, while copepods seem to prefer...
cryptophyte algae, confirming the results of other studies (Antajan & Gasparini, 2004) (Tables 8–10). There is a big resemblance between the food sources used by zooplankton in Roșu-Puiu and Matița-Merhei complex. Although the rotifers and copepods groups prefer green algae and diatoms, in agreement with results for similar ecosystems (Work & Havens, 2003), in the Danube Delta they seem also well adapted to cyanobacterial consumption (Tables 8–9) even if these organisms could be inedible or even become toxic in certain conditions, especially in Roșu-Puiu complex, affected by a long-term eutrophication (Postolache, 2006). Also, the diaptomids are able to use their long antenna and break the cyanobacterial filaments to consume them (Moriarty et al., 1973). The third lake complex, Gorgova-Uzlina, shows a different pattern of biodiversity, due to different environmental factors (Table 10).

### Table 8

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ciliata</th>
<th>Testacea</th>
<th>Lamellibranchia</th>
<th>Gastrotrichia</th>
<th>Rotifera</th>
<th>Cladocera</th>
<th>Copepoda</th>
<th>Ostracoda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyceae (µg chl a L⁻¹)</td>
<td></td>
<td>0.384</td>
<td>0.498</td>
<td>0.493</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyceae (µg chl a L⁻¹)</td>
<td></td>
<td>0.477</td>
<td>0.427</td>
<td>-0.363</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptophyceae (µg chl a L⁻¹)</td>
<td>0.440</td>
<td></td>
<td>0.364</td>
<td>0.703</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total chl. a (µg chl a L⁻¹)</td>
<td></td>
<td>0.583</td>
<td>0.678</td>
<td>0.493</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria (cells L⁻¹)</td>
<td>0.471</td>
<td>0.600</td>
<td>0.483</td>
<td>0.734</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrophyceae (cells L⁻¹)</td>
<td>0.492</td>
<td>0.375</td>
<td></td>
<td>0.480</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysophyceae (cells L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>0.354</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillariophyceae (cells L⁻¹)</td>
<td></td>
<td></td>
<td>-0.522</td>
<td>0.457</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyceae (cells L⁻¹)</td>
<td>0.398</td>
<td>0.437</td>
<td>0.686</td>
<td>0.526</td>
<td>0.780</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (°C)</td>
<td></td>
<td></td>
<td></td>
<td>0.361</td>
<td>0.548</td>
<td></td>
<td></td>
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<tr>
<td>T (°C) sediment</td>
<td>0.693</td>
<td>0.415</td>
<td>0.608</td>
<td>0.732</td>
<td>0.694</td>
<td></td>
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</tr>
<tr>
<td>pH</td>
<td>0.390</td>
<td></td>
<td>0.505</td>
<td></td>
<td>0.670</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>pH sediment</td>
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<td></td>
<td>0.633</td>
<td>0.748</td>
<td>0.703</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cond. (µs/cm)</td>
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<td></td>
<td>0.395</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Turbidity (NFU)</td>
<td>0.596</td>
<td></td>
<td>0.485</td>
<td>0.620</td>
<td>0.561</td>
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</tr>
<tr>
<td>Light intensity (lx)</td>
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<td></td>
<td>0.345</td>
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<td></td>
</tr>
<tr>
<td>Water flow (counts/min)</td>
<td>0.669</td>
<td>0.436</td>
<td>0.648</td>
<td>0.643</td>
<td>0.614</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Water velocity (m/s)</td>
<td>0.462</td>
<td>0.394</td>
<td>0.526</td>
<td>0.664</td>
<td>0.544</td>
<td></td>
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</tr>
<tr>
<td>NH₄⁺ (µg N L⁻¹)</td>
<td>0.535</td>
<td>0.549</td>
<td>0.559</td>
<td>0.779</td>
<td></td>
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<td></td>
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<tr>
<td>NO₃⁻ (µg N L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>0.492</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻ (µg P L⁻¹)</td>
<td>0.517</td>
<td>0.481</td>
<td>0.406</td>
<td>0.537</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP (µg P L⁻¹)</td>
<td>0.384</td>
<td></td>
<td>0.425</td>
<td></td>
<td>0.585</td>
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<td></td>
</tr>
</tbody>
</table>
Table 9
Significant relationships of zooplankton, phytoplankton and environmental variables in Roșu-Puiu (p<0.05)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ciliata</th>
<th>Testacea</th>
<th>Lamellibranchia</th>
<th>Gastrotrichia</th>
<th>Rotifera</th>
<th>Cladocera</th>
<th>Copepoda</th>
<th>Ostracoda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanobacteria (µg chl a L⁻¹)</td>
<td>0.553</td>
<td>0.471</td>
<td></td>
<td>0.633</td>
<td>0.514</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyceae (µg chl a L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>0.567</td>
<td></td>
<td>0.509</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chryrophyceae (µg chl a L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>0.511</td>
<td></td>
<td></td>
<td>0.511</td>
<td></td>
</tr>
<tr>
<td>Total chl. a (µg chl a L⁻¹)</td>
<td>0.609</td>
<td>0.812</td>
<td></td>
<td>0.504</td>
<td>0.644</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria (cells L⁻¹)</td>
<td>0.576</td>
<td>0.550</td>
<td></td>
<td>0.722</td>
<td>0.744</td>
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<tr>
<td>Euglenophyceae (cells L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.528</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrophyceae (cells L⁻¹)</td>
<td>0.538</td>
<td></td>
<td>0.548</td>
<td>0.566</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bacillariophyceae (cells L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>0.504</td>
<td>0.671</td>
<td>0.737</td>
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<tr>
<td>Chlorophyceae (cells L⁻¹)</td>
<td>0.513</td>
<td></td>
<td></td>
<td>0.750</td>
<td>0.796</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (m)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.490</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (m)</td>
<td>-0.501</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (C)</td>
<td></td>
<td></td>
<td></td>
<td>0.491</td>
<td>0.473</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>T (C) sediment</td>
<td>0.626</td>
<td>0.471</td>
<td>0.494</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>pH</td>
<td></td>
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<td></td>
<td></td>
<td>0.640</td>
<td>0.617</td>
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<tr>
<td>pH sediment</td>
<td>0.480</td>
<td>0.655</td>
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<td></td>
<td>0.541</td>
<td>0.576</td>
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</tr>
<tr>
<td>Cond (µs/cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.544</td>
<td>0.535</td>
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<td></td>
</tr>
<tr>
<td>Light intensity (lx)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.541</td>
<td>0.575</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water flow (counts/min)</td>
<td>0.652</td>
<td></td>
<td>0.469</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Velocity (m/s)</td>
<td>0.614</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺ (µg N L⁻¹)</td>
<td>0.493</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻ (µg P L⁻¹)</td>
<td></td>
<td>0.579</td>
<td></td>
<td>0.486</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TP (µg P L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>0.585</td>
<td>0.649</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The environmental parameters are key drivers for the dynamics of biological communities, influencing both phyto- and zooplankton populations (Basu & Pick, 1997; Heneash et al., 2015). Although there is no general agreement regarding all the factors regulating phytoplankton and zooplankton communities in different
aquatic ecosystems (Reynolds, 1988), evidence shows that light, water velocity, temperature, nutrients, and xenobiotics modulate the development of plankton communities and interspecific competitions.

**Table 10**

Significant relationships of zooplankton, phytoplankton and environmental variables in Gorgova-Isac (p<0.05)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ciliata</th>
<th>Testacea</th>
<th>Lamellibranchia</th>
<th>Gastrotrichia</th>
<th>Rotifera</th>
<th>Cladocera</th>
<th>Copepoda</th>
<th>Ostracoda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chryrophyceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.591</td>
</tr>
<tr>
<td>(µg chl a L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total chl. a</td>
<td>0.643</td>
<td>0.694</td>
<td>0.688</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(µg chl a L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0.561</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(cells L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euglenophyceae</td>
<td>0.716</td>
<td>0.678</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cells L⁻¹)</td>
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<tr>
<td>Pryrophyceae</td>
<td></td>
<td></td>
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<td></td>
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<td>(cells L⁻¹)</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Chrysophyceae</td>
<td>0.605</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cells L⁻¹)</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bacillariophyceae</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>(cells L⁻¹)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.596</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻ (µg N L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.772</td>
</tr>
</tbody>
</table>

The investigations carried out in the three lake complexes emphasized that pH was a common factor influencing the development of copepods (Tables 8–10), but the other physical and chemical parameters modulating zooplankton communities had a different influence in each lake complex: while in Gorgova-Isac only nitrates influenced copepods development, in the other two complexes TP and temperature influenced both rotifers and copepods (Tables 8–10). In Matița-Merhei complex, rotifers, cladocerans, copepods and also testaceans are influenced by turbidity, water velocity and NH₄⁺ content (Table 8).

The hydrogeomorphological features of the ecosystems have also key role in defining the development of plankton communities, habitat heterogeneity influencing the distribution of zooplankton individuals. The distribution of zooplankton communities in the three investigated complexes was analyzed based on Detrended Correspondence Analysis (DCA). The results show that although rotifers did not seem affected by the hydrogeomorphological characteristics, being widely spread in all the lake complexes, crustaceans (Cladocera and Copepoda) were found especially in Roșu-Puiu and Gorgova-Isac complexes (Fig. 3).
CONCLUSIONS

Diversity is an important tool to assess the complexity of a community and its stability. In our study, the zooplankton diversity was assessed based on species richness and evenness. In lake complexes exhibiting a negative correlation between evenness and abundance, a higher presence of accidental species was found, determining a decrease of diversity indexes.

Taxonomic groups with significant role in defining traits of diversity were rotifers and copepods. Their role depended on both, community structure and spatial distribution. Water velocity, pH, temperature, turbidity, and nutrients modulated the development of zooplankton communities during the investigated period.

Acknowledgements. This study was funded by the Swiss Enlargement Contribution; project IZERZ0 – 142165, “CyanoArchive”, in the framework of the Romanian-Swiss Research Programme and Institute of Biology Bucharest of Romanian Academy; project no. RO1567-IBB02/2019. The authors thank to Marian Constantin for providing the Danube Delta map and Stela Sofa for technical support, as well as to the team from the Ecological Station Sulina for assistance during the sampling trips.
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  e-mail: ioana.enache_n@yahoo.ro
The paper reports the first occurrence of Branchiostoma lanceolatum (Pallas, 1774) larvae in the Romanian waters in samples collected in July and August 2018 in four stations located in coastal and offshore waters of the Romanian shelf.

**Keywords:** Branchiostoma lanceolatum, Black Sea, Romania.

**INTRODUCTION**

After almost fifty years since the first observation of an adult of Branchiostoma lanceolatum (Pallas, 1774) in the benthic samples collected in shallow waters of the Romanian littoral (Gomoiu, pers. comm.), here we report the first occurrence of pelagic larvae of the species in the national waters.

Branchiostoma lanceolatum is known as a relatively rare species in the Black Sea. Grigore Antipa, during the expedition performed in 1893 all around the Black Sea, documented the presence of amphioxus from Sinope, on the northern Anatolian coast, and from Sevastopol, where it had been found by Ostroumov on coarse sands in association with Ophelia taurica, Polygordius ponticus, Glycera sp., and two species of Synapta (Antipa, 1941).

Only few recent records in literature were found reporting, for example, abundances of 1,109 indv. m\(^{-2}\) (Luth, 2004) in 2004. In the period 1999–2014, B. lanceolatum was found in 38% of the samples collected on the Crimean soft bottoms (Shalovenkov, 2017). Shiganova et al. (2010) signals the appearance of specimens of the lancelet larvae (1.5–5 mm in length) in the layer of 0–160 m above 500 m (sampling point: 44°513’ N, 37°933’ E) at a temperature of 26°C, inferring its potential intrusion in the Black Sea from the Mediterranean Sea. At the Bulgarian coast, it forms in shallow waters (10–30 m) on shelly, medium and coarse sand an association with Protodorvillea kefersteini and Gouldia minima (Todorova et al., 2015; Todorova, 2017). Lately (2011–2017), B. lanceolatum larvae have been constantly observed in the summer-autumn season mainly at depths of
15–40 m in coastal and inner shelf area and rarely, on the outer shelf and open sea. Its average abundance varies insignificantly around 2 ind. m\(^{-3}\) (SD\(\pm\) 5), an exception being recorded in front of Ropotamo river where a peak density of 32 ind. m\(^{-3}\) was found in 2016 (Stefanova K. & Stefanova E., pers. data). To our knowledge, no data is available for the Black Sea, from the Turkish and Georgian coast.

This species has been included in the Black Sea Red Data Book and in the Vulnerable category according to the IUCN criteria, in 1996, due to its continuous decreasing population since the 60’s as result of anthropogenic pressure (Konsulova, in Dumont, 1999). Most data in the literature suggest that its demise is generally related to the change in texture and eutrophication of the coastal sediments.

**MATERIAL AND METHOD**

The larvae were found in the zooplankton samples collected by a Juday net with 150 µ mesh size, deployed at certain depths in the water column in July and August 2018. Out of the eight collected larvae, five specimens were found in the samples collected in front of Mamaia Bay at depths between 23–25 m, and the other three in the samples taken along Sf. Gheorghe and Constanța transects in offshore waters (Fig. 1).

![Fig. 1. The sampling stations (green points) where *B. lanceolatum* larvae occurred in July and August 2018 at the Romanian coast and its occurrence (red points) (according to Konsulova, 1999).](image)
RESULTS

Description of larvae

Along the body three regions are seen: the head with the pharyngeal region, the midgut with the anus opening and the spear-like tail. The notochord and the nerve chord with pigment spots span along entire body length. In the anterior head region the main observable morphological features were: the preoral organ, the club-shaped gland and the anterior and posterior endostyle ridges (Fig. 2b). Within the pharyngeal part, 10–11 primary gill slits were accounted, while a canal-like atrial chamber formed from the level of the third posterior gill slit to the anterior midgut region (atriopore) was noticed (Fig. 2c). In the hindgut, the cells forming the wall corresponding to the future ileocolonic ring (Urata et al., 2007) of brownish colour and the anal opening could be seen (Fig. 2d). The larvae were 5–5.5 mm long, flattened in transverse plan. After all appearances, the larvae were in their late stage of development (premetamorphic larvae). *B. lanceolatum* larvae metamorphose with at least 14 gill slits, 45 to 50 days after fertilization at 23°C (Fuentes et al., 2007).

Fig. 2. a. Larva before metamorphosis into juvenile; b. Left sided head region of larva, cv. – cerebral vesicle, ncp.– nerve cord with pigment spots; c. – club-shaped gland; m. – mouth margin; n. – notochord; po. – preoral organ; c. Branchial region of larva with 11 primary gill slits (I–XI); ac – the canal-like atrial chamber formed from the level of the third gill slit to the anterior midgut region; d. Hindgut with brownish wall cells and the ao – anal opening on the left body side, and the spear shaped tail region (Photo: Teacă A.).

Distribution of specimens found at the Romanian littoral

The 4 specimens collected in July in the station CM 16 were found in the upper surface layer of 0–5 m depth as well as within the thermocline layer at 5–15 m, respectively. Likewise, in the station CM18 the fifth specimens was collected also within the thermocline layer at 10–18 m depth. The distribution of all three specimens...
found in August was confined to the under thermocline layers. The larvae is believed to execute diurnal vertical migration due to their light-guided behaviour (Pergner & Kozmik, 2017). Their diet include algae such as diatoms, dinoflagellates, e.g., *Ceratium* (Kehayias, 2015), the latter being present in great abundances during sampling period. However, the larvae ingest also calanoid copepods or other organic material and small particles of a size similar to that of the larval mouth (Webb, 1969).

The salinity and temperature at the sampling site varied between 17.19 (surface layer) and 18.91 PSU (under thermocline layer) and 23.97°C (surface) and 8.41°C (bottom layer) (Table 1). In the Black Sea, the species has a reproductive peak during the summer season (Konsulova, 1992).

**Table 1**

<table>
<thead>
<tr>
<th>Station</th>
<th>CM16</th>
<th>CM16</th>
<th>CM18</th>
<th>SG07</th>
<th>CT06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude [deg. N]</td>
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<td>44.16838</td>
<td>44.26112</td>
<td>44.27365</td>
<td>43.79941</td>
</tr>
<tr>
<td>Bot Depth [m]</td>
<td>27.4</td>
<td>27.4</td>
<td>27</td>
<td>107</td>
<td>89.7</td>
</tr>
<tr>
<td>Depth [m]</td>
<td>0–5</td>
<td>5–15</td>
<td>10–18</td>
<td>25–60</td>
<td>35–85</td>
</tr>
<tr>
<td>Chla [ug/L]</td>
<td>0.41</td>
<td>9.89</td>
<td>9.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T°C</td>
<td>23.97</td>
<td>12.73</td>
<td>13.65</td>
<td>10.11</td>
<td>8.41</td>
</tr>
<tr>
<td>PSU</td>
<td>17.19</td>
<td>18.49</td>
<td>18.57</td>
<td>18.46</td>
<td>18.91</td>
</tr>
<tr>
<td>O₂ mg.L⁻¹</td>
<td>7.24</td>
<td>7.06</td>
<td>7.95</td>
<td>6.99</td>
<td></td>
</tr>
<tr>
<td>Substrate type</td>
<td>coarse (shell debris)</td>
<td>coarse (shell debris)</td>
<td>sand</td>
<td>shelly mud</td>
<td>shelly mud</td>
</tr>
<tr>
<td>No. Ind./sample</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

**DISCUSSION**

At this moment there is no evidence for the species presence in the adult stage on the Romanian shelf. In our opinion, there are three hypothetic explanations that could be drawn concerning the larvae appearance.

Firstly, it is likely that adult populations to be actually present in the Romanian waters, but with distribution and abundances limited to few habitats, such as the sandy bottoms from the coast and/or circalittoral. For example, the habitats investigated in July correspond to the circalittoral sand and circalittoral coarse (shell debris) sediments (Teacă et al., 2018) that are likely suitable for adults dwelling and spawning.
Secondly, the Romanian sector is on the north-south cyclonic current trajectory, thus the larvae could have been conveyed in our research area simultaneously with the water masses from the Crimean coast.

Third, the favourable hydrometeorological conditions in the collecting period and especially in the previous days, characterised by predominantly W-NW winds that favoured upwelling and increased surface and subsurface cyclonic and anticyclonic eddies in the region of the Romanian shelf.

CONCLUSIONS

After more than fifty years since first observation of an adult of Branchiostoma lanceolatum at the Romanian littoral (Gomoiu, pers. comm.), here we report the first occurrence of pelagic larvae of the species, eight specimens being found in the coastal and offshore Romanian waters within the surface and under thermocline layers.

Three hypothesis regarding their occurrence were assumed: one, the species actually lives within the Romanian habitats but its presence in adult stage has not been observed due to its limited distribution and abundance, the second, the larvae were drifted from the Crimean coast to the Romanian waters with the north-south cyclonic current, and the third, as result of favourable hydromorphological conditions prior and during the sampling period.

Acknowledging that the species has already been declared in the Vulnerable category at the Black Sea level, according to the IUCN criteria and has been enlisted in the 1999 Black Sea Red Data Book, we also propose re-evaluation of its status at the Romanian littoral if its presence will be further confirmed.

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EFFECT OF TEMPERATURE ON THE VIRULENCE OF ENTOMOPATHOGENIC NEMATODES

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Nowadays, the use of entomopathogenic nematodes as a biological control agent is a key component in IPM system. Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) are extraordinarily lethal to many important insect pests, yet are safe for plants and animals. They are the only insect-parasitic nematodes possessing an optimal balance of biological control attributes. The effect of temperature on the virulence of three species of entomopathogenic nematodes, *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* was investigated. Last instar of *Tenebrio molitor* Linnaeus, 1758 larvae were choosing for experiment. In the laboratory, all three nematode species successfully reproduced inside *T. molitor* larvae, *H. bacteriophora* produced the highest number of infective juveniles per larva at 30°C than *S. feltiae* and *S. thesami*. *S. feltiae* caused the highest mortality of larvae at 20°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 10–33°C. Based on the present study, we indicate that entomopathogenic nematodes have well-defined thermal breadths for their development and reproduction.

**Keywords**: entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, *Tenebrio molitor*, biocontrol.

INTRODUCTION

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) are soil-inhabiting insect parasites that possess potential as biological control agents (Gaugler & Kaya, 1990; Kaya & Gaugler, 1993). These nematodes have a symbiotic association with bacteria of the genus *Xenorhabdus* (Akhurst & Boemare, 1990). The bacteria convert the insects into a suitable environment for development and reproduction of the nematodes’ parasitic stages (Poinar, 1990). The only function of the infective juveniles is to locate and parasitize new host (Grewal et al., 1994). Variation among species of entomopathogenic nematodes for temperature tolerance has been reported (Grewal et al., 1993). The aim of this work was determined thermal factor for infection and reproductions of three species of entomopathogenic nematode: *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* (Gorgadze et al., 2016) in laboratory conduction. The species *Neoaplectana*
Manana Lortkipanidze et al. 2

(= Steinernema) thesami was isolated in Mtskheta-Mtianeti Region of Georgia, from infected pupa of a winter moth, Operophtera brumata Linnaeus, 1758 (Lepidoptera: Geometridae). The isolate of S. thesami was maintained in the laboratory of entomopathogenic nematodes of the Institute of Zoology of Ilia State University, Tbilisi, Georgia.

MATERIAL AND METHODS

Nematodes were reared at 25°C in last instar larvae of the wax moth, Galleria mellonella, according to procedures described by (Woodring & Kaya, 1998). The infective juveniles (IJs) that emerged from cadavers were recovered using modified White traps (White, 1927), and stored at 7°C for 7–14 days before use (Kaya & Stock, 1997).

Infectivity of nematodes to last instar of Tenebrio molitor at 8–35°C was tested in a sand-based assay (Grewal et al., 1994). Fifty infective juveniles of a nematode species in 200 μl of distilled water were inoculated into a 5 cm diameter Petri dish containing 3 g dry sand. One last instar larva of T. molitor was placed on dish. The dishes were wrapped with parafilm to reduce desiccation. Insect mortality was recorded during 20 days. Dead larvae were removed from sand, washed in distilled water, dissected and the number of nematodes established recorded. Three Petri dishes were prepared for each species and for each temperature.

Nematode reproductive potential was evaluated in T. molitor larvae. Five last instars larvae were exposed to 500 IJs of each species on a filter paper in 10 cm diameter Petri dish. Dead larvae were transferred to White traps for the recovery of a new generation of IJs. After the start of emergence (from 5 to 14 days), IJs were collected and counted. Total number of IJs produced per host was then determined.

Both treatment was replicated four times, included untreated control dishes, which received only distilled water. Mortality percentage was recorded and corrected with Abbott formula (Abbott, 1925).

RESULTS AND DISCUSSION

Thermal effect for infection and reproduction differed among nematode species.

Infection. S. thesami infected T. molitor larvae at the widest temperature range and killed insects at 10–33°C, H. bacteriophora infected host at between 10–32°C. The two species of nematodes S. thesami and H. bacteriophora wide killed insects between 20–32°C and 10–30°C, whereas S. feltiae infected host at the narrow temperature range (10–25°C) and caused the highest mortality to larvae at 20°C (Fig. 1).
Effect of temperature on the virulence of entomopathogenic nematodes

Reproduction. Thermal breadth for reproduction was for *S. thesami* (20–32°C), for *H. bacteriophora* from 15–30°C, whereas *S. feltiae* reproduced at cooler temperatures (12–25°C) (Fig. 2).

After 5 days’ exposure, each larva was transferred to a separate White trap containing filter paper with distilled water and the total number of emerging IJs, were counted every two days until there was no further recovery. *H. bacteriophora* produced the highest number of infective juveniles – 200,000 per cadaver at 30°C, compared with *S. thesami* and *S. feltiae*. The number of infective juveniles produced by *S. thesami* was 90,000, whereas for *S. feltiae* – 85,000 per insects (Fig. 3).
Fig. 3. Reproductive potential of entomopathogenic nematode species.

*H. bacteriophora* produced the highest number of infective juveniles per larva at 30°C than *S. feltiae* and *S. thesami*. *S. feltiae* caused the highest mortality of larvae at 25°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 12–32°C. *S. feltiae* was the only species that killed the larvae at 10°C, *S. thesami* and *H. bacteriophora* were effective at 15–30°C. The temperature significantly affected the host searching ability of all tested species.

**CONCLUSIONS**

Thermal effect for infection differed among nematode species. Both species *S. thesami* and *H. bacteriophora* were more adapted to warm temperature reproduction, whereas *S. feltiae*, to cooler temperatures.

In the laboratory, all three nematode species successfully reproduced inside *T. molitor* larvae, *H. bacteriophora* produced the highest number of infective juveniles per larva, followed *S. thesami* and *S. feltiae*. *H. bacteriophora* treatment generally caused the highest mortality levels to *T. molitor* at high temperature (30°C), whereas *S. feltiae* caused the highest mortality to *T. molitor* larvae at low temperature (25°C).

Temperature influences the nematodes’ survival, infection, and reproduction, is one of the most important factors limiting the practical uses of the nematodes as biocontrol agents (Jagdale & Gordon, 1998). It has been established that the nematodes are able to adapt physiologically to environmental temperatures. In future research, for field tests will be used the most suitable nematode species for biological control of different pest insects.
Effect of temperature on the virulence of entomopathogenic nematodes

**Recommendations.** All nematodes were significantly different from each other in effectiveness against last instars of *T. molitor* larvae. Temperature is the most influential environmental factor, which has great biological significance. Mortality of larvae and production of IJs in *T. molitor* increased with increasing exposure time and temperature in both experiment.

As an environmental factor, temperature is variable both in space and time (Prosser, 1973). Temperature influences nematode mobility, reproduction and development (Mason & Hominik, 1995).

Farmer through the activities should be acquire knowledge and choose the time and appropriate conditions for the application of entomopathogens in the field, i.e. season of the year, a-biotic factors such as temperature, humidity of soils etc. and biotic factors including living organisms. Environmental conditions – cool weather, dry conditions, UV radiation during application, climatic variation, pests, disease and price risks as well as natural disasters such as droughts and floods, free information about the weather and temperature.

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DATA ON THE COLEOPTERA (STAPHYLINIDAE, CERAMBYCIDAE AND CHRYSOMELIDAE) IN THE FĂGĂRAȘ MOUNTAINS AREA (SOUTHERN CARPATHIANS, ROMANIA)

SANDA MAICAN*, RODICA SERAFIM**, MELANIA STAN**

The paper presents data on the diversity of Coleoptera (Staphylinidae, Cerambycidae and Chrysomelidae families) in the Făgăraș Mountains and its surroundings, based on the study of material preserved in the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest) and the Institute of Biology Bucharest of the Romanian Academy. A total of 148 species belonging to 89 genera and 20 subfamilies are reported. Among the valuable species recorded in this area, we mention: *Cornumutila lineata* (Letzner, 1844) – a highly rare representative of the fauna of Palaearctic coniferous forests and *Ocypus kuntzeni* (G. Müller, 1926) – a mountainous species, possibly endemic in the Romanian Carpathians. Also, *Rosalia alpina alpina* (Linnaeus, 1758) and *Morimus asper funereus* Mulsant, 1863 are species of community interest, included in the Annex II of the Habitats Directive.

*Keywords*: faunistic diversity, Staphylinidae, Cerambycidae, Chrysomelidae, Făgăraș Mountains, Romania.

INTRODUCTION

The first data on the distribution of the coleopterans in the Făgăraș Mountains area were published beginning with the end of the 19th century by several authors, such as: Kuthy (1896), Bielz (1887), Petri (1912; 1925–1926), followed later by Panin (1944), Eliescu et al. (1949), Panin & Sâvulescu (1961), Roșca (1973, 1974), Gruev et al. (1993), Szél et al. (1995), Rözner (2007), Rözner & Rözner (2007), Tăușan & Bucșa (2010), Dascălu et al. (2012), Cuzapan & Tăușan (2016), etc. Data on the Natura 2000 Cerambycidae species reported in this area, respectively *Morimus asper funereus* and *Rosalia alpina alpina*, were published by Tatole et al. (2009).

Recently, Linnell et al. (2016) had published a report that aims to summarise the existing knowledge concerning the biodiversity of the Făgăraș Mountains, giving a list with 125 beetle species based on data published and unpublished sources.
MATERIAL AND METHODS

This paper is based on published and unpublished data on the distribution of staphylinids, cerambycids and chrysomelids in the Făgăraș Mountains and adjacent areas.

The studied material is preserved in the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest) and the Institute of Biology Bucharest of the Romanian Academy. The specimens from the “Grigore Antipa” museum were collected during several trips between 1949 and 2006, that were made mainly in the Avrig River Valley, in the Vâlșan River Valley and Vidraru Lake surroundings.

Most rove beetle species were collected in August 2004 on the occasion of a project regarding the diversity of Făgăraș Mountains fauna, other specimens/species were donated by the Hungarian Natural History Museum (collected in 2003, 2006 and 2008) and only a few specimens/species were collected before the 2000s.

A part of Cerambycidae material belongs to Dr. Nicolae Săvulescu Collection from the “Grigore Antipa” National Museum, which includes material collected between 1952 and 1974 especially in the Capra Peak, the Oticu Peak, the Buda Peak, Pleașa Mts, “Sâmbăta” Chalet. The other part of material was collected in 1985, 1994 and 2004 on the occasion of a Museum’s Research Projects of the Făgăraș Mountains Biodiversity especially Poiana Neamțului Chalet (Valley of Avrig River), Bâlea Waterfall, Vama Cucului Chalet (Transfăgărășan mountain road), Vidraru Lake area (the valleys of Buda, Cumpăna, Oticu Rivers, and so on), Brăduleț (Vâlșan Valley). This material was included in the Museum's Cerambycidae Palaearctic Collection.

The Chrysomelidae specimens that were collected between 2000 and 2002 are recorded mainly from the “Vâlșan Valley” Site of Community Importance ROSCI0268. The collecting sites in the Făgăraș Mountains and its surroundings:

Argeș County: Arefu, Argeș Valley, Brădulet (Brădetu and Galesu, Vâlșan Valley), Buda Peak, Buda Valley, Capra Chalet, Capra Peak, Clăbucet Peak, Clearings of Vâlșan Valley (Brădetu), Cumpâna Valley, Cumpâna Chalet, Cumpâna Peak, Curtea de Argeș, Făgăraș Mountains, Iezer Păpușa Mountains (Lerești), Lunca Gârtii (Stoenești), Moliviș, Mușătești, Oticu Valley, Oticu Peak, Pleașa Mountains, Stan Valley, “Valea cu Pești” Chalet, Vidraru Lake area, Valley of Oticu River.

Sibiu County: Avrig, Bâlea Lake, Bâlea Waterfall, Bârcaciu Chalet, Cârțișoara, Laița River, Negoiu Peak, Negru Peak (Piscul Negru), Poiana Neamțului Chalet (Valley of Avrig River), Porumbacu de Sus, Sebeșu de Sus, Suru Chalet, Turnu-Roșu, Vama Cucului Chalet (Transfăgărășan mountain road).

Brașov County: Sâmbăta de Sus, Sâmbăta Chalet.
Abbreviations for collector’s name:

Other abbreviations:
a.s.l. – above sea level; ID – identification; leg. – legit; Mts – Mountains; spec./specs – specimen/specimens.

RESULTS

The paper reports 148 species belonging to 89 genera and 20 subfamilies from Făgăraș Mountains and surroundings, based on the study of the material preserved in the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest) and the Institute of Biology Bucharest of the Romanian Academy.

The nomenclature and the systematical order follow the Catalogue of Palaearctic Coleoptera (Löbl & Smetana, 2004, 2010).


Among the valuable species recorded in this area, we mention the species Cornumutila lineata Letzner, 1844, a very rare representative of the fauna of Palaearctic coniferous forests (Picea, Larix, Pinus, Abies). C. lineata is a boreo-mountainous species, distributed in Central and Eastern Europe and Siberia. In the Romanian fauna, it was less mentioned: Făgăraș Mountains, Bârcaciu Chalet (Panin & Săvulescu, 1961); Făgăraș Mts, Negoiu Peak (Serafim, 2006), Făgăraș Mts (Lazarev, 2009); Rarău Mt., Valea Putnei (Eastern Carpathians) (Serafim & Maican, 2011).

Ocypus kuntzeni (G. Müller, 1926) is a mountainous species, distributed in the southern and eastern Carpathians and the Transylvanian Plateau and it is possibly an endemic species of the Romanian Carpathians.

Rosalia alpina alpina (Linnaeus, 1758) and Morimus asper funereus Mulsant, 1863 are species of community importance, included in the Annex II of the Habitat Directive.
Family Staphylinidae Latreille, 1802
Subfamily Omaliinae MacLeay, 1825

*Anthophagus alpestris* Heer, 1839

*Material:* 1 spec., surroundings of Capra Chalet, N: 45°34'58", E: 24°37'40", 1535 m a.s.l., 05.08.2004, M.S.

*Geodromicus plagiatue* (Fabricius, 1798)

*Material:* 20 specs, bank of stream Laiţa, 2 km S Cărţișoara, near bridge, 1 km W stream Cărţișoara, N: 45°42'17", E: 24°34'11", 520 m a.s.l., 16.05.2003, Gy.M., ID Adriano Zanetti.

Subfamily Tachyporinae Macleay, 1825

*Lordithon lunulatus* (Linnaeus, 1760)

*Material:* 1 spec., the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 7 specs, the bank of Vidraru Lake (close to “Valea cu Pești” Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

*Lordithon trinotatus* (Erichson, 1839)

*Material:* 2 specs, the bank of Vidraru Lake (near the “Valea cu Pești” Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

*Tachinus laticollis* Gravenhorst, 1802

*Material:* 5 specs, Buda Valley, in cow dung on forest road, 04.08.2004, M.S.; 2 specs, 1 km upstream “Valea cu Pești” Chalet, horse dung, 6.08.2004, M.S.; 1 spec., the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 6 specs, Stan Valley, in cow dung on the forest road, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., 07.08.2004, M.S.

*Tachyporus chrysomelinus* (Linnaeus, 1758)


*Tachyporus dispar* (Paykull, 1789)


*Tachyporus hypnorum* (Fabricius, 1775)

*Material:* 8 specs, Galeșu, Vâlsan Valley, 7–21.07.2001, C.P.

Subfamily Aleocharinae Fleming, 1821

*Aleochara bilineata* Gyllenhal, 1810

*Material:* 1 spec., surroundings of Capra Chalet, N: 45°34'58", E: 24°37'40", 1535 m a.s.l., 05.08.2004, R.S.
**Atheta aeneicollis** (Sharp, 1869)

*Material:* 1 spec., the bank of Vidraru Lake close to forest road (N: 45°27′14″, E: 24°36′13″, 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.

**Atheta castanoptera** (Mannerheim, 1830)

*Material:* 5 specs, the bank of Vidraru Lake close to forest road (N: 45°27′14″, E: 24°36′13″, 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 28 specs, bank of Vidraru Lake (near the “Valea cu Peşti” Chalet), N: 45°23′37″, E: 24°37′50″, 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

**Atheta crassicornis** (Fabricius, 1792)

*Material:* 22 specs, the bank of Vidraru Lake (near the “Valea cu Peşti” Chalet), N: 45°23′37″, E: 24°37′50″, 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

**Atheta leonhardi** Bernhauer, 1911

*Material:* 1 spec., surroundings of Bâlea Lake, 2075 m a.s.l., 26.07.2006, A.Ba., ID Ádám Laszlo.

**Atheta liturata** (Stephens, 1832)

*Material:* 18 specs, the bank of Vidraru Lake close to forest road (N: 45°27′14″, E: 24°36′13″, 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 3 specs, bank of Vidraru Lake (near the “Valea cu Peşti” Chalet), N: 45°23′37″, E: 24°37′50″, 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

**Atheta nigritula** (Gravenhorst, 1802)

*Material:* 3 specs, the bank of Vidraru Lake (near the “Valea cu Peşti” Chalet), N: 45°23′37″, E: 24°37′50″, 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

**Atheta pallidicornis** (Thomson, 1856)

*Material:* 1 spec., bank of Vidraru Lake (near the “Valea cu Peşti” Chalet), N: 45°23′37″, E: 24°37′50″, 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

**Gyrophaena minima** Erichson, 1837

*Material:* 28 specs, the indicator area Valea Cumpâna Mare-Clăbucet 9.2 km, in mushrooms (mixed forest), 08.08.2004, M.S.

**Gyrophaena strictula** Erichson, 1839

*Material:* 2 specs, the indicator area Valea Cumpâna Mare-Clăbucet 9.2 km, in mushrooms (mixed forest), 08.08.2004, M.S.

**Liogluta alpestris** (Heer, 1839)

*Material:* 5 specs, surroundings of Bâlea Lake, N: 45°36′17″, E: 24°36′10″, 2046 m a.s.l., 26.07.2006, A.Ba., ID Ádám Laszlo.

**Myllaena intermedia** Erichson, 1837

*Material:* 6 specs, bank of stream Laiţa, 2 km S Cârţişoara, near bridge, 1 km W stream Cârţişoara, N: 45°42′17″, E: 24°34′11″, 520 m a.s.l., 16.05.2003, Gy.M., ID Ádám Laszlo.
Oxypoda deubeli Bernhauer, 1899

*Material:* 1 spec., surroundings of Bălea Lake, 25.02.2008, 2075 m, A.Ba., ID Ádám Laszlo.

Subfamily Oxytelinae Fleming, 1821

*Anotylus mutator* (Lohse, 1963)

*Material:* 1 spec., Buda Valley, N: 45°28´, E: 24°38´, 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.

*Ochtheophilus praepositus* Mulsant & Rey, 1878


Oxytelus laqueatus (Marsham, 1802)

*Material:* 4 specs, Buda Valley, N: 45°28´, E: 24°38´, 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.; 3 specs, the bank of Vidraru Lake (near the “Valea cu Peşti” Chalet), N: 45°23´37", E: 24°36´56", 960 m a.s.l., cow dung, 7.08.2004, M.S.

Platystethus arenarius (Geoffroy, 1785)

*Material:* 3 specs, Buda Valley, N: 45°28´, E: 24°38´, 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.; 1 spec., Stan Valley, N: 45°22´45", E: 24°36´56", 960 m a.s.l., cow dung, 7.08.2004, M.S.

Thinobius angusticeps Fauvel, 1889

*Material:* 1 spec., Porumbacu de Sus, Şerbota stream, 18.05.2013, leg. & ID György Makraczy.

Subfamily Steninae MacLeay, 1825.

*Stenus comma* LeConte, 1863

*Material:* 1 spec., Curtea de Argeș, the bank of Argeș river, 3.09.1979, leg. A.P.G.; 3 specs, Buda Valley, the bank of Buda stream, N: 45°28, E: 24°38´, 930 m a.s.l., M.S.

Subfamily Paederinae Fleming 1821

*Lathrobium laevipenne* Heer, 1839

*Material:* 1 ♂, Arefu, the bank of Oticu valley, in detritus, N: 45°27´20", E: 24°38´12", 925 m a.s.l., 04.08.2004, M.S.

*Paederus littoralis* Gravenhorst, 1802

Subfamily Staphylininae Latreille, 1802

*Bisnius fimetarius* (Gravenhorst, 1802)

*Material:* 3 specs, Buda Valley, N: 45°28´, E: 24°38´, 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.; 1 spec., the bank of Vidraru Lake (near the “Valea cu Pești” Chalet), N: 45°23’37”, E: 24°37’50”, 830 m a.s.l., horse dung, 06.08.2004, M.S.

*Philonthus cruentatus* (Gmelin, 1790)


*Philonthus decorus* (Gravenhorst, 1802)


*Philonthus marginatus* (Müller, 1764)


*Philonthus spinipes* Sharp, 1874


*Philonthus succicola* Thomson, 1860


*Philonthus varians* (Paykull, 1789)


*Quedius cincticollis* Kraatz, 1857

*Material:* 1 spec., Cumpăna Valley (sourroundings Cumpăna Chalet), N: 45°26’09.24”, E: 24°36’18.22”, 853 m a.s.l. in moos, 03.08.2004, M.S.; 1 spec., Bâlea Lake, 2075 m a.s.l., 26.07.2006, A.B., ID Adám Laszlo.

*Ocypus aeneocephalus* (De Geer, 1774)

*Material:* 1 ♂, 1 ♀, Galeșu (Vâlșan Valley), meadow in plum orchad, between the streams, 600 m a.s.l., 7-21.07.2001, C.P. (Stan, 2010).

*Ocypus fulvipennis* Erichson, 1840

*Material:* 1 ♂, Galeșu (Vâlșan Valley), meadow in plum orchad, between the streams, 600 m a.s.l., 7–21.07.2001, C.P. (Stan, 2010).

*Ocypus kuntzeni* (G. Müller, 1926)

*Material:* 4 ♂, Galeșu (Vâlșan Valley), meadow in plum orchad, between the streams, 600 m a.s.l., 7-21.07.2001, C.P. (Stan, 2010).
Ocypus picipennis picipennis (Fabricius, 1793)
*Material:* 1 ♂, Galeșu (Vâlsan Valley), meadow in plum orchard, between the streams, 600 m a.s.l., 7–21.07.2001, C.P. (Stan, 2010).

Platydacus stercorarius (Olivier, 1795)
*Material:* 10 ♂, 4 ♀, Galeșu (Vâlsan Valley), meadow in plum orchard, between the streams, 600 m a.s.l., 7–21.07.2001, C.P.

Quedius alpestris (Heer, 1839)
*Material:* 7 specs., surroundings of Bâlea Lake, 2075 m alt, 26.07.2006, A.Ba., ID Ádám Laszlo.

Quedius punctatellus (Heer, 1839)

Family Cerambycidae Latreille, 1802
Subfamily Prioninae Latreille, 1802
Prionus coriarius (Linnaeus, 1758)

Subfamily Lepturinae Latreille, 1802
Alosterna tabacicolor Degeer, 1775
*Material:* 5 specs, Buda Peak, 12–21.08.1959; 7 specs, Oticu Peak, 15.08.1959 N.S.; 9 specs, Capra Peak, 17.08.1959, N.S. (Serafim, 2006); 1 spec., Valley of Buda River, 4.08.2004, R.S.; 1 spec., “Valea cu Pești” Chalet surroundings, 6.08.2004, M.S.; 1 spec., Moliviș, 7.08.2004, M.S.

Anastrangalia dubia (Scopoli, 1763)

Anastrangalia sanguinolenta (Linnaeus, 1761)
*Material:* Capra Peak, 2.08.1957 N.S. (9 specs), 17–20.08.1959, N.S. (10 specs), 28.VII.1974 (63 specs); Buda Peak, 12–18.08.1959, N.S. (15 specs), 30.VII.1974 N.S. (21 specs); Oticu Peak, 15–16.08.1959, N.S. (9 specs), 29.07.1974 N.S.
Data on the Staphylinidae, Cerambycidae and Chrysomelidae in the Făgăraș Mountains area


**Cornumutila lineata** Letzner, 1844

**Material:** 5 specs, Negoiu Peak, 11.08.1957, N.S. (Serafim, 2006).

**Judolia sexmaculata** (Linnaeus, 1758)

**Material:** 1 spec., Capra Peak, 2.08.1957, N.S.; 2 specs, Suru Chalet, 28.07.1974, N.S. (Serafim, 2006).

**Leptura annularis** Fabricius, 1801 (syn. arcuata Panzer, 1793)

**Material:** Turnu-Roșu, whithout other data (Serafim, 2006).

**Leptura quadrifasciata quadrifasciata** Linnaeus, 1758


**Lepturobosca virens** (Linnaeus, 1758)


**Pachytodes cerambyciformis** (Schrank, 1781)


**Pachytodes erraticus erraticus** (Dalman, 1817)


**Pseudovadonia livida livida** (Fabricius, 1776)

**Material:** 1 spec., Pleașa Mts, 12.08.1959, N.S.; 2 specs, Buda Peak, 12–21.08.1959, N.S.; 3 specs, Oticu Peak, 15–16.08.1959, N.S.; 3 specs, Capra Peak, 17.08.1959,
N.S.; 10 specs, Poiana Neamțului Chalet surroundings, 19.07.1985, R.S., I.M. (Serafim, 2006); 1 spec., “Valea cu Pești” Chalet surroundings, 850 m, 6.08.2004, R.S.; 1 spec., Stan Valley, 7.08.2004, R.S.; 3 specs, Moliviș, 7.08.2004, M.S.

*Rutpela maculata maculata* (Poda, 1761)


*Stenurella bifasciata bifasciata* (Müller, 1776)

**Material:** 1 spec., Pleașa Mts, 12.08.1959, N.S. (Serafim, 2006); 1 spec., Galeșu, (Vâlsoa Valley), 7.08.1997, C.P.

*Stenurella melanura* (Linnaeus, 1758)


*Stenurella nigra* (Linnaeus, 1758)

**Material:** 1 spec., Sebeșu de Sus (Racovița), 12.V.1986, I.M.

*Stenurella septempunctata septempunctata* (Fabricius, 1792)

**Material:** Turnu-Roșu, whithout other data (Serafim, 2006).

*Stictoleptura rubra rubra* (Linnaeus, 1758)


*Stictoleptura maculicornis maculicornis* (Degeer, 1775)

**Material:** Capra Peak, 2.08.1957, N.S. (1 spec.), 17–20.08.1959, N.S. (19 specs); Oricu Peak, 15.08.1959, N.S. (1 spec.), 29.07.1974, N.S. (3 specs); 11 specs, Buda Peak, 12–18.08.1959, N.S.; 5 specs, valley of Oticu River, 4.08.2004, R.S. (Serafim, 2006); 1 spec., valley of Buda River, 4.08.2004, M.S.
Stictoleptura scutellata scutellata (Fabricius, 1781)


Strangalia attenuata (Linnaeus, 1758)


Oxymirus cursor (Linnaeus, 1758)

Material: 1 spec., Turnu-Roșu, 13.05.1986, I.D. (Serafim, 2005).

Evodinus clathratus (Fabricius, 1792)


Gaurotes virginea virginea (Linnaeus, 1758)


Pachyta lamed lamed (Linnaeus, 1758)

Material: 1 spec., Făgăraș Mts, the road to Clăbucet Peak, 2.08.1954. N.S. (Serafim, 2005).

Pachyta quadrimaculata (Linnaeus, 1758)


Pidonia lurida (Fabricius, 1792)


Rhagium (Megarhagium) mordax (Degeer, 1775)

Material: Capra Peak, 2.08.1957, N.S. (1 spec.), 27.07.1974, N.S. (2 specs); 1 spec., Negru Peak (Piscu Negru), 27.08.1957, N.S. (Serafim, 2005).
Subfamily Spondylidinae

*Tetropium castaneum* (Linnaeus, 1758)

*Material:* Capra Peak, 28.VI.1957, N.S. (5 specs), 17.08.1959, N.S. (1 spec.); 2 specs, Buda Peak, 12.08.1959, N.S; 1 spec., Oticu Peak, 15.08.1959, N.S.; 1 spec., Poiana Neamțului Chalet surroundings, 15.05.1986 (Serafim, 2007).

*Tetropium fuscum* (Fabricius, 1787)


*Saphanus piceus piceus* (Laicharting, 1784)

*Material:* 1 spec., Bârcaciu Chalet surroundings, 10.08.1958, N.S. (Serafim, 2007).

*Spondylis buprestoides* (Linnaeus, 1758)

*Material:* 2 specs, Cumpăna Chalet surroundings, 17.08.1957, N.S. (Serafim, 2007).

Subfamily Cerambycinae Latreille, 1802

*Aromia moschata moschata* (Linnaeus, 1758)


*Callidium coriaceum* Paykull, 1800


*Callidium violaceum* (Linnaeus, 1758)


*Ropalopus clavipes* (Fabricius, 1775)

*Material:* 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2010); 1 spec., Buda Peak, 18.08.1959, N.S.

*Chlorophorus sartor* (Müller, 1766)

*Material:* 2 specs, Galeșu (Vâlsan Valley), 20.08.1998, C.P.

*Chlorophorus varius* (Müller, 1766)


*Clytus lama* Mulsant, 1847

*Material:* 1 spec., Cumpăna Peak, 2.08.1958, N.S. (Serafim, 2009).

*Clytus rhamni* Germar, 1817


*Plagionotus floralis* (Pallas, 1773)

**Molorchus minor minor** (Linnaeus, 1767)

*Material:* 1 spec., Poiana Neamțului Chalet surroundings, 15.05.1986, I.D.

**Rosalia alpina alpina** (Linnaeus, 1758)

*Material:* 6 specs, Pleașa Mts., 12.08.1959, N.S. (Serafim, 2009); 3 specs, Făgăraș Mts, 18.07.1979, N.S.; 1 spec., Brădetu (Vâlcan Valley) (beech forest), N: 45.32555°, E: 24.74644°, 721 m a.s.l., 29.07.2015, observed by M.S., C.C., G.C. (Fig. 1).

![Fig. 1 – Rosalia alpina alpina (Photo: Melania Stan).](image)

**Stenopterus flavicornis** Küster, 1846


**Stenopterus rufus rufus** Linnaeus, 1767


**Subfamily Lamiiinae Latreille, 1825**

**Leiopus nebulosus** (Linnaeus, 1758)

*Material:* 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2010).

**Aegomorphus clavipes** (Schrank, 1781)

*Material:* 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2010); 1 spec., Buda Peak, 18.08.1959, N.S.
Agapanthia (Epoptes) dahli (Richter, 1821)
Material: 1 spec., Turnu-Roșu, 13.05.1986, I.D. (Serafim, 2010).

Agapanthia (Epoptes) villosoviridescens (Degeer, 1755)

Monochamus sartor (Fabricius, 1787)

Monochamus sutor sutor (Linnaeus, 1758)

Morimus asper funereus Mulsant, 1863
Material: 1 spec., Brădetu (Vâlșan Valley) (beech forest), N: 45°19'16.968", E: 24° 45'2.916", 682 m a.s.l., 29.07.2015, observed by M.S., C.C., G.C. (Fig. 2).

Fig. 2 – Morimus asper funereus (Photo: Melania Stan).

Phytoecia (Phytoecia) nigricornis (Fabricius, 1781)
Family Chrysomelidae Latreille, 1802

Subfamily Donaciinae Kirby, 1837

*Plateumaris consimilis* (Schrank, 1781)


Subfamily Criocerinae Latreille, 1807

*Lilioceris lili* (Scopoli, 1763)


*Lilioceris merdigera* (Linnaeus, 1758)


*Oulema erichsonii* (Suffrian, 1841)


*Oulema gallaeciana* (Heyden, 1870)


*Oulema melanopus* (Linnaeus, 1758)


Subfamily Cassidinae Gyllenhal, 1813

*Cassida rubiginosa rubiginosa* O.F. Müller, 1776


*Cassida vibex* Linnaeus, 1767


*Cassida viridis* Linnaeus, 1758

Subfamily Chrysomelinae Latreille, 1802

*Chrysomela vigintipunctata vigintipunctata* (Scopoli, 1763)


*Plagiodera versicolora* (Laicharting, 1781)

**Material:** 1 spec. ♀, Brădetu, upper end of Vâlsan dam lake, 25.07.2000, S.M. (Maican, 2003); valley of Buda River, Făgăraş Mts (Maican & Serafim, 2018 a).

*Plagiosterna aenea aenea* (Linnaeus, 1758)


*Gastrophysa polygoni polygoni* (Linnaeus, 1758)

**Material:** 1 spec., Brădetu (Vâlsan Valley), 16.05.2002, S.S. (Maican, 2003).

*Gastrophysa viridula viridula* (DeGeer, 1775)


*Phaedon armoraciae* (Linnaeus, 1758)


*Phaedon cochleariae cochleariae* (Fabricius, 1792)


*Chrysolina haemoptera haemoptera* (Linnaeus, 1758)


*Chrysolina marcasitica marcasitica* (Germar, 1824)


*Chrysolina polita polita* (Linnaeus, 1758)

Chrysolina fastuosa fastuosa (Scopoli, 1763)


Chrysolina olivieri olivieri (Bedel, 1892)

**Material:** 5 specs, Brădetu (Vâlșan Valley), 24.07.2000, S. M. (Maican, 2003).

Chrysolina varians (Schaller, 1783)


Chrysolina herbacea herbacea (Duftschmid, 1825)


Oreina cacaliae senecionis Schummel, 1844


Oreina intricata intricata (Germar, 1824)


Oreina virgulata virgulata (Germar, 1824)


Leptinotarsa decemlineata (Say, 1824)


Gonioctena pallida (Linnaeus, 1758)

**Material:** Brădetu, Vâlșan dam (Maican, 2003).

Subfamily Galerucinae Latreille, 1802

Galeruca pomonae pomonae (Scopoli, 1763)


Galeruca tanaceti tanaceti (Linnaeus, 1758)


Galerucella lineola lineola (Fabricius, 1781)

Lochmaea caprea (Linnaeus, 1758)

Pyrrhalta viburni (Paykull, 1799)

Agelastica alni alni (Linnaeus, 1758)

Subfamily Alticinae Newman, 1834

Crepidodera aurata (Marsham, 1802)

Neocrepidodera femorata (Gyllenhall, 1813)

Neocrepidodera transversa (Marsham, 1802)

Phyllotreta ochripes (Curtis, 1837)

Phyllotreta tetrastigma (Comolli, 1837)

Sphaeroderma testaceum (Fabricius, 1775)

Subfamily Cryptocephalinae Gyllenhal, 1813

Labidostomis longimana (Linnaeus, 1761)

Smaragdina flavicollis (Charpentier, 1825)

Smaragdina xanthaspis (Germar, 1824)
**Cryptocephalus ocellatus ocellatus** Drapiez, 1819  

**Cryptocephalus octacosmus** Bedel, 1891  

**Cryptocephalus bipunctatus bipunctatus** (Linnaeus, 1758)  

**Cryptocephalus frenatus** Laicharting, 1781  

**Cryptocephalus hypochoeridis** (Linnaeus, 1758)  

**Cryptocephalus moraei** (Linnaeus, 1758)  

**Pachybrachis sinuatus** (Mulsant & Rey, 1859)  

**CONCLUSIONS**

This study completes the data regarding the occurrence of Staphylinidae, Cerambycidae and Chrysomelidae species in the Făgăraș Mountains area, based on the study of the material preserved in the collection of the “Grigore Antipa” National Museum of Natural History and the Institute of Biology Bucharest of the Romanian Academy, and also on literature sources (published by authors between 1998 and 2018).

A total of 148 species (including 44 Staphylinidae species, 53 species of Cerambycidae and 51 Chrysomelidae species) belonging to 20 subfamilies and 89 genera are listed.

According to IUCN Red List of Threatened Species, *Morimus asper funereus* and *Rosalia alpina alpina* are listed as Vulnerable species.
Coleoptera fauna of the Făgăraș Mountains is still less known, and the potential of the area is very large. Therefore, based on the data known so far, we consider that it is necessary to continue the research in order to have an inventory as complete as possible.

Acknowledgements. A part of this study was funded by project no. RO1567-IBB01/2019 from the Institute of Biology Bucharest of the Romanian Academy.

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The relationships between standard length (SL) and otolith length (OL), and width (OW) were examined for kais kingfish (*Cyprinion kais* Heckel, 1843) from Güclükonak location of Tigris River. Sampling was carried out every month using nets from January to December 2015. Otolith samples for analysis were obtained from 135 specimens (101 females and 34 males). Standard length and otolith length measured for all fish and sex was identified. Samples consisted of fish varying in standard length from 6.0 to 14.8 cm, weight from 2.8 to 75.55 g and otolith length from 1.51 to 3.07 mm. The relationship between fish length (SL) and otolith length (OL) was as $y=0.1596x +0.4445$ ($R^2=0.7606$), between fish length (SL) and otolith width (OW) was as $y=0.1201x +0.5561$ ($R^2=0.8155$), between fish length (SL) and otolith weight (OWe) was as $y=0.0003x –0.0017$ ($R^2=0.7632$), between otolith length (OL) and otolith width (OW) was as $y=0.6655x +0.4192$ ($R^2=0.8385$). Analysis for the relation between right and left otolith did not reveal any significant differences (t-test, $p>0.05$).

**Keywords:** *Cyprinion kais*, otolith, kais kingfish, Tigris River, Turkey.

**INTRODUCTION**

*Cyprinion kais* Heckel, 1843 is an endemic freshwater fish seen in the Tigris-Euphrates river basin (Nasri et al., 2010). *Cyprinion* species spread in a region extending from the river Indus to Orontes, Tigris and Euphrates rivers. The description of the two types of *Cyprinion* was made by Heckel in Tigris and Euphrates river systems. One of these species, *Cyprinion macrostomum* Heckel, 1843, has been mentioned in subsequent studies. The other *Cyprinion kais* has been reported in studies in Iraq and Syria. *Cyprinion kais* species have not been mentioned in studies for determination of freshwater fish in our country. However, specimens collected from the Tigris River and the Batman Creek were identified by Bănărescu & Herzig-Straschil (1995) as *Cyprinion kais*. And shows the distribution in freshwater of Turkey, Syrian Arab Republic, Iran and Iraq. In the case of wide distribution, it is under the danger of extinction in Turkey. *Cyprinion kais* is a valuable species for edible and angling. Despite its wide distribution and endangered species, it has not been studied extensively. Compared with *C. macrostomum*, there
is much doubt about its validity and sometimes it is considered synonymous with *C. macrostomum* (Nasri et al., 2010).

Bănărescu & Herzing-Sraschil (1995) and Geldiay & Balık (2007), reported in their studies the number of branched rays in dorsal fin of *C. kais* is around 13–15 and this number is 9–11 in *C. macrostomum*. *C. kais* is flattened from side and its head length is less that the body height. The ratio between the standard length and body depth is ranged from 2.9 to 3.4. The mouth of fish is small and on the ventral and there is a lobe on it. There are a couple of barbels around the mouth. The last branched ray of the dorsal fin is boned and there are denticles on the ray (Bilici et al., 2016).

Although kais kingfish is endemic Tigris and Euphrates basin, biology and ecology have not been well studied (Alkan-Uçkun & Gökçê, 2015). For this reason, in this study, morphometric and meridional properties of *Cyprinion kais* species were tried to be given comparatively in the samples obtained from Dicle River at Şırnak province, Güçlükonak.

**MATERIAL AND METHODS**

A total of 135 specimens of kais kingfish was caught monthly using nets from Tigris River, Şırnak from January to December 2015. Each fish samples was measured to the nearest 1 mm for standard length (SL). Sagital otoliths were removed, cleaned and preserved dry in labeled boxes. Otolith length and otolith width were measured to the nearest 1 μm using Olympus SZ61TR+Olympus LC20. The rigth and left otoliths were dealt with separately. Broken and damaged otoliths were excluded from the calculations.

Differences between the lengths of the right and left otoliths for each species were tested using paired t-test. The relationships between otolith length and fish length were calculated using least squares regression equations to predict the standard length and weight of the original fish from otolith length. The otolith dimensions-standard length relationships were examined by using the following equation: \( y = a + bx \), where \( y \) is otolith length, \( x \) is fish standard length, \( a \) is intercept value, \( b \) is coefficient value (Başusta et al., 2013).

**RESULTS AND DISCUSSION**

The sagittal otoliths of 135 kais kingfish specimens were examined. The standard length of all individuals ranged from 6.0 to 14.8 cm SL (mean 11.37±0.15 cm) and weight from 2.8 to 75.55 g (mean 29.77±1.19 g). The otolith length ranged between
1.51 and 3.07 mm (mean 2.27±0.029 mm), otolith width from 1.28 to 2.48 mm (mean 1.93±0.021 mm) and otolith weight from 0.0004 to 0.0034 g (mean 0.0018±0.00005 g). The age of kais kingfish ranged from one to six years.

The relationship between fish length (SL) and otolith length (OL) was found as $y=0.1596x +0.4445$ ($R^2=0.7606$) (Fig. 1), between fish length (SL) and otolith width (OW) was $y=0.1201x +0.5561$ ($R^2=0.8155$) (Fig. 2), between fish length (SL) and otolith weight (OWe) was $y=0.0003x –0.0017$ ($R^2=0.7632$) (Fig. 3), between otolith length (OL) and otolith width (OW) was calculated as $y=0.6655x +0.4192$ ($R^2=0.8385$) (Fig. 4), between fish total weight (W) and otolith weight (OWe) was determined as $y=0.00003x–0.0008$ ($R^2=0.6367$) (Fig. 5). Analysis for the relation between right and left otolith did not reveal any significant differences (t-test, p>0.05).
Fig. 3. Standard length-otolith weight relationship of *Cyprinion kais*.

\[ y = 0.0003x - 0.0017 \]
\[ R^2 = 0.7632 \]

Fig 4. Otolith length-otolith width relationship of *Cyprinion kais*.

\[ y = 0.6655x + 0.4192 \]
\[ R^2 = 0.8385 \]

Fig. 5. Total weight-otolith weight relationship of *Cyprinion kais*.

\[ y = 3E-05x + 0.0008 \]
\[ R^2 = 0.6367 \]
Despite endemic kais kingfish in Tigris and Euphrates basin, otolith biometry have not been studied. Therefore, this is the first information on otolith biometry of C. kais from Şırnak, Turkey. Otolith length and otolith width were found to highly reliable measurement for determining the fish length.

The maximum estimated age was 6 years for kais kingfish in Şırnak. Alkan-Uçkun & Gökçe (2015) reported a maximum age of four years for C. kais and four years for C. macrostomum from Karakaya Dam Lake. Five years of age was reported for C. macrostomum from Gamasiab River in the Tigris River drainage in Iran (Faghani-Langroudi & Mousavi-Sabet, 2018).

There are a strong linear relationship between fish length and otolith length, and between fish length and otolith width ($R^2>0.76$). Başusta et al. (2013) reported that strong correlation between fish length-otolith length have been found in Salmo trutta macrostigma in Munzur River. Likewise, a strong positive correlation between fish length and otolith length have been found in some other fish species (Şen et al., 2001; Ross et al., 2005; İlyaz et al., 2011; Jawad et al., 2011a, b; İsmen et al., 2013; Yılmaz et al., 2014; Dörtbudak & Özcen, 2015; Bostancı et al., 2017; Mat-Piah et al., 2017).

Also, a relationship between otolith weight and fish length has been used by some authors (Jawad et al., 2011b; İlyaz et al., 2011; Yılmaz et al., 2014; Bostancı et al., 2017, Mat-Piah et al., 2017). These all studies found that there was a positive linear relationship between fish length and otolith weight. In this study, a strong correlations was found between fish length and otolith weight.

**CONCLUSIONS**

Consequently, studies on the otolith biometry of kais kingfish are generally unavailable. Hence, relationship between fish length and otolith size of kais kingfish in Tigris River given in this study provides some tools for the study of food habits of piscivores and size of fish in archaeological samples.

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REPORT ON THE ACCUMULATION OF HEAVY METALS IN THE FEATHERS OF SOME WETLAND BIRDS IN THE DANUBE DELTA (ROMANIA)

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An assessment was carried out in the Danube Delta Biosphere Reserve (Romania, 2015) regarding heavy metal residues in the feathers of native wild bird species that depend on wetlands for all or part of their life cycle. The concentrations of trace elements (As, Cr, Mn, Ni, Pb and Zn) were determined in the feathers of Ferruginous Duck, White Stork, Common Little Bittern, Little Egret, Great White Pelican, Pygmy Cormorant, Common Tern and Western Marsh-harrier. Arsenic concentrations varied between 1.610 mg/kg (Ferruginous Duck) and 3.437 mg/kg (Great White Pelican). The highest chromium concentrations were found in Pygmy Cormorant (11.063 mg/kg) and Great White Pelican (12.368 mg/kg); the highest zinc concentration was found in Ferruginous Duck (163.237 mg/kg). The highest concentrations of manganese and nickel were found in the White Stork (66.541 mg/kg – Mn; 6.276 mg/kg – Ni), Western Marsh-harrier (59.037 mg/kg; 4.075 mg/kg) and Little Egret (64.347 mg/kg; 5.900 mg/kg). The concentration of lead showed values between 3.474 mg/kg (Western Marsh-harrier) and 0.426 mg/kg (Ferruginous Duck), while the other studied species had concentrations up to 0.9 mg/kg. Laboratory analyses revealed important interspecific differences and a high capacity of waterbirds for storing heavy metals in plumage.

Keywords: heavy metals, feathers, birds, Danube Delta, Romania.

INTRODUCTION

At the European level, a series of natural and anthropic limiting factors affect wild bird species, most of which are protected by a number of European and international directives and conventions. Heavy metals are among these limiting factors. There have been several studies regarding heavy metal bird poisoning in Europe (Lebedeva et al., 1997; Hernandez et al., 1999; Berglund et al., 2011; Kitowski et al., 2014).

In Romania, there have been several studies regarding heavy metal concentrations in abiotic elements, plants and fish (Dinescu et al., 2004; Tudor et al., 2006; Vignati et al., 2013; Ilie et al., 2014; Ionescu et al., 2015; Burada et al., 2014; 2015; 2016; 2017; Ștefănuț et al., 2018). However, studies focusing on bird species
are lacking (Fasolă-Mătăsăru et al., 2017). In order to evaluate the degree of heavy metal accumulation in bird species of Community interest from the Danube Delta Biosphere Reserve, a non-invasive protocol was applied. According to literature, this type of monitoring can show annual variations in heavy metal concentrations due to the fact that these substances are integrated into new plumage during the moulting period (Frantz et al., 2012; Gushit et al., 2016).

The present study was carried out during a colonial waterbird breeding census and the monitoring of bird species of Community interest from the Danube Delta Biosphere Reserve on the Romanian territory.

MATERIAL AND METHODS

All the samples were collected (May to August, 2015) in the Danube Delta Biosphere Reserve [DDBR, Găștescu & Știucă (2008)] and its immediate vicinity, in the eastern part of Romania. Sampling locations included several reas: Murighiol, Uzlna Lake, Nufără, Maliu fishpond, Sâlcioara fishpond, Nebunu Lake, Fortuna Lake and Draghilea channel (Fig. 1).

Fig. 1. Locations of sample collection in the Danube Delta Biosphere Reserve.
We sampled moulted flight feathers in feeding and roosting areas, as well as feathers collected from dead individuals (approximately 5 g of feathers per individual). All the samples were primary flight feathers (remiges), as it has been shown that different feathers accumulate trace elements in different concentrations (Guo et al., 2001). The concentration of trace elements (As, Cr, Mn, Ni, Pb and Zn) was determined for Ferruginous Duck – *Aythya nyroca* (1 individual), White Stork – *Ciconia ciconia* (5 individuals), Common Little Bittern – *Ixobrychus minutus* (1 individual), Little Egret – *Egretta garzetta* (2 individuals), Great White Pelican – *Pelecanus onocrotalus* (1 individual), Pygmy Cormorant – *Phalacrocorax pygmeus* (1 individual), Common Tern – *Sterna hirundo* (3 individuals) and Western Marsh-harrier – *Circus aeruginosus* (1 individual).

Sample analysis was conducted by acid mineralization, necessary for trace elements determination, with the help of an Anton Paar Multiwave 3000 microwave oven. The trace element content was analysed using the ICP-MS Elan DRC-e, which is applicable for the determination of small concentrations of a large number of elements. Approximately 1–2 g of each sample was weighed and 5 ml HNO₃ and 2 ml of H₂O₂ were added. After a short pre-reaction time (15 minutes), the recipients were closed with special caps and placed in the protection layer with secured cap, after which it was introduced into the rotor. The power of the device was gradually raised to 600 W over a period of 5.5 minutes and maintained at this level for 4.5 minutes. In the last stage, the power was raised to 1000 W and maintained for 10 minutes. The total mineralization time was 20 minutes, while the total cooling time was 20–25 minutes.

Following the completing of the mineralization program and the cooling time, the quartz recipients were opened, and the contents transferred into 50-ml volumetric flasks that were then filled to the mark with acidified water.

The content of heavy metals was analysed with the help of *Elan DRC-e ICP-MS* applicable in the determination of small concentrations of a large number of elements. Trace elements are nebulized and transformed into aerosols which are then carried by argon into the plasma torch. The resulting ions are trained in plasma and introduced via an interface into a mass spectrometer according to SR EN ISO 17294-2, 2005 (**2005**).

**RESULTS AND DISCUSSION**

We analysed a total of 15 individual samples from eight locations, belonging to eight species (Table 1). The highest concentrations were recorded for Zn, with values between 12,307 to 158,237 mg/kg.
Table 1
Trace element concentrations for sampled feathers

<table>
<thead>
<tr>
<th>Prelevation place</th>
<th>Species</th>
<th>Values (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As</td>
<td>Cr</td>
</tr>
<tr>
<td>Murighiol</td>
<td>Ciconia ciconia</td>
<td>2.856</td>
</tr>
<tr>
<td>Nufără</td>
<td>Ciconia ciconia</td>
<td>2.534</td>
</tr>
<tr>
<td>Maliuc fishpond</td>
<td>Ciconia ciconia</td>
<td>1.625</td>
</tr>
<tr>
<td>Murighiol</td>
<td>Ciconia ciconia</td>
<td>3.12</td>
</tr>
<tr>
<td>Murighiol</td>
<td>Ciconia ciconia</td>
<td>2.659</td>
</tr>
<tr>
<td>Dragilea Channel</td>
<td>Phalacrocorax pygmeus</td>
<td>1.589</td>
</tr>
<tr>
<td>Murighiol</td>
<td>Sterna hirundo</td>
<td>2.776</td>
</tr>
<tr>
<td>Murighiol</td>
<td>Sterna hirundo</td>
<td>1.796</td>
</tr>
<tr>
<td>Uzлина Lake</td>
<td>Circus aeruginosus</td>
<td>2.924</td>
</tr>
<tr>
<td>Nebunu Lake</td>
<td>Egretta garzetta</td>
<td>3.584</td>
</tr>
<tr>
<td>Nebunu Lake</td>
<td>Egretta garzetta</td>
<td>1.709</td>
</tr>
<tr>
<td>Maliuc fishpond</td>
<td>Ixobrychus minutus</td>
<td>2.239</td>
</tr>
<tr>
<td>Salcioara fishpond</td>
<td>Aythya nyroca</td>
<td>1.61</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>2.5122</td>
</tr>
<tr>
<td>Max</td>
<td></td>
<td>3.584</td>
</tr>
<tr>
<td>Min</td>
<td></td>
<td>1.589</td>
</tr>
</tbody>
</table>

All of the six studied elements (As, Cr, Mn, Ni, Pb and Zn) have bioaccumulative and toxic effects on the organism (Hill, 2010). It should be noted that the accumulation of heavy metals (internally, from food and water) occurred during the period of growth of feathers, for about 1–3 months during the previous year, very likely in the Danube Delta (all birds were adults).

The studied birds either moult after the breeding period, as in the case of Aythya nyroca (Cramp & Simmons, 1977), or during the breeding period until the start of migration, for the other species. However, in the case of large species, the moultling of flight feathers can occur over longer time periods (Ramirez & Panuccio, 2019), and the accumulation of these elements could have occurred outside the DDBR.
Also, the question of the origins of these trace elements should be treated with care, as it is very likely that the samples suffered from external contamination, either by atmospheric deposition or by contact with water, as has been shown in the case of Ni, Pb and Zn (Dauwe et al., 2003). In this situation the origin of the contaminants can also be from outside of the DDBR, as all the sampled species belong to species that have migratory populations.

Moreover, some species (the Great White Pelican at least) can frequently feed at great distances from the colony during the nesting period and, again, the accumulation (from food, water and atmospheric deposition) of these elements have occurred outside the DDBR. A comparison of our results with previous studies regarding background concentrations of DDBR ecosystems (water and sediments, Burada et al., 2014; Ibram et al., 2019) showed our values to be lower.

There are also differences (p <0.05) between trace element concentrations found in bird feathers and those found in aquatic macroinvertebrates (Ibram et al., 2019) and fish (Burada et al., 2017). However, these comparisons must be regarded with caution, since the sampling periods were different and samples were collected from different places in the DDBR. Thus, we cannot make definitive conclusions of this kind regarding bioaccumulation phenomena for the studied elements.

The concentration values for arsenic varied between 1.610 mg/kg for the Ferrouiginous Duck and 3.437 mg/kg for the Great White Pelican. In general, the arsenic concentrations show little variation ($S^2 = 0.496$) for the studied species (Fig. 2). The differences between values may be caused by the trophic factor or phenological characteristics of each species. However, when concentrations have low variation, we can consider that these are caused by the natural level of arsenic in the environment.

Comparing the levels of arsenic found in birds with the ones found in fish in the DDBR (Burada et al., 2017), we found higher concentrations in our samples, which can be an indicator of a bioaccumulation process via the trophic pyramid.

![Fig. 2. Arsenic concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.](image-url)
The graphical representation of chromium concentrations (Fig. 3) shows a significant difference of accumulation capacities of this heavy metal between the Pygmy Cormorant – 11.063 mg/kg and the Great White Pelican – 12.368 mg/kg, and other species. These differences are most likely linked to the size of fish that are consumed by different species of birds (including benthic fish that are in close contact with the substrate). The differences between these concentrations can also be linked to the feeding areas, as well as with the different migration periods, different stopover sites and especially with wintering areas.

The minimum values, up to 2.472 mg/kg, were identified for White Storks, a species that frequently consumes its prey from dry habitats, especially amphibians, snakes and lizards, and much less fish (unlike fish, many amphibians and reptiles spend less time or none at all in water).

For some species, the variation of chromium shows no clear pattern. In the case of the Common Tern, a fish eating bird, chromium was found in smaller concentrations than in the Ferruginous Duck which is a species that predominantly feeds on plants of aquatic origin and rarely eats invertebrates (Kiss et al., 1980; Kiss et al., 1984; Kiss et al., 1986; Ciochia, 1992).

The values of chromium in birds were smaller than those found in sediments (Ibram et al., 2019), though larger than the average concentrations found in macroinvertebrates and in most of the studied fish (Burada et al., 2017; Ibram et al., 2019).

Out of all the studied trace elements, manganese was highest in concentration values after zinc. The highest concentrations were recorded for the White Stork (66.541 mg/kg), Western Marsh-harrier (59.037 mg/kg) and Little Egret (64.347 mg/kg) (Fig. 4). For the rest of the species it varied between 18.431 mg/kg and 25.215 mg/kg.

By analysing these differences, we observed that the bird species that feed mainly in the riparian or marsh areas have the highest concentration levels. In contrast, in species that feed in open waters (except the Common Little Bittern), the manganese concentrations were lower.
Manganese is known to be a common element in aquatic ecosystems, occurring in large quantities (Burada, 2015). At concentrations over 0.2 mg/kg, in the presence of oxygen, it precipitates and is deposited in sediments (Allen, 1989), from where it is assimilated in large quantities by aquatic organisms.

In the case of nickel (Fig. 5), much like in the case of manganese, we noted a high accumulation capacity for the species that feed mainly in riparian and marsh areas (White Stork – 6.276 mg/kg, Little Egret – 5.900 mg/kg and Western Marsh-harrier – 4.075 mg/kg). From our results, it seems that both nickel and manganese have a similar concentration distribution among species and it is most likely that they have the same contamination source. The smallest concentration levels of nickel were identified for the Ferruginous Duck (1.391 mg/kg).
The nickel values from sampled feathers are much lower than those found in sediments (Ibram et al., 2019), though relatively similar (with the exception of the White Stork, Little Egret and Western Marsh-harrier that showed higher concentrations) to medium concentrations found in aquatic macroinvertebrates and in most fish species (Burada et al., 2017; Ibram et al., 2019).

Regarding lead, it is important to note that it is an accumulative poison and has no essential biological function, as all of its compounds are toxic. Lead forms strong bonds with enzymes and proteins (e.g. haemoglobin), thus disrupting many metabolic processes (Bolcu & Király, 2012). Lead concentration levels (Fig. 6) varied between 36.474 mg/kg for the Western Marsh-harrier and 0.426 mg/kg for the Ferruginous Duck. Overall, the lead concentrations varied only slightly ($S^2=0.953$).

Considering that the sample size was small and a definitive pattern cannot be observed, preliminary analysis shows that lead levels increase directly in proportion to bioaccumulation along the trophic pyramid. The highest lead concentration was found in the Western Marsh-harrier, a raptor species that feeds mainly on birds and mammals (Ciochia, 1992), species with higher accumulation potentials compared to herbivorous, insectivorous and piscivorous birds.

Thus we can explain a lower lead concentration in the Ferruginous Duck, which is a herbivorous species (Ciochia, 1992). The values of lead concentrations from bird feathers (Fig. 6) were much lower than those from sediments (Ibram et al., 2019), but much higher than those found in fish tissues (Ibram et al., 2017).

![Fig. 6. Lead concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.](image)

The zinc concentrations found in bird feathers (Fig. 7) are higher than those from sediments and much higher than those found in aquatic macroinvertebrates (Ibram et al., 2019).
The highest values, up to 163.237 mg/kg were observed for the Ferruginous Duck, while lowest, at 93.527 mg/kg, were identified for the Common Little Bittern. These high values recorded for Ferruginous Ducks may be caused by the metabolism of some forms of zinc in aquatic vegetation (the basic food of this species), a hypothesis mentioned by Iordache (2009).

CONCLUSIONS

Our investigations regarding heavy metals in samples collected from eight bird species of Community interest revealed inter-specific differences, most likely caused by differences in diet and other ecological features.

Laboratory analyses revealed that the studied species have a high capacity for accumulation of heavy metals and arsenic in their plumage. However, the values were generally lower than those found by other studies to be present in the sediments and water of the DDBR.

Arsenic concentrations varied between 1.610 mg/kg for *Aythya nyroca* and 3.437 mg/kg for *Pelecanus onocrotalus*, whereas variations were minor in the rest of the studied species.

There was a significant difference in the chromium accumulation capacity in medium-sized piscivorous species compared to large species (*Phalacrocorax pygmeus* – 11.063 mg/kg and *Pelecanus onocrotalus* – 12.368 mg/kg).

Out of all the studied elements (with the exception of zinc), manganese had the highest values. The highest concentrations were identified for species that feed in marshy and riparian areas (*Ciconia ciconia* – 66.541 mg/kg, *Circus aeruginosus* – 59.037 mg/kg and *Egretta garzetta* – 64.347 mg/kg).
In the case of nickel, there was also a similarity in storage capacity among the species that predominantly feed in riparian and marshy areas (Ciconia ciconia – 6.276 mg/kg, Circus aeruginosus – 4.075 mg/kg and Egretta garzetta – 5.900 mg/kg).

The lead concentration range varied between 3.474 mg/kg in the case of the Western Marsh-harrier and 0.426 mg/kg for the Ferruginous Duck. The levels of lead increased in proportion to bioaccumulation along the trophic pyramid.

In comparison to background values, zinc concentrations were higher, up to 165.237 mg/kg, as identified in Aythya nyroca, while the lowest, 93.527 mg/kg, was found in Ixobrychus minutus.

We consider it necessary for the future to repeat these analyses on a larger number of birds. With a larger sample we would be able to analyse individual concentrations of trace elements from different areas of the DDBR. In order to have a better understanding of the feather accumulation phenomenon in DDBR, sampling from bird juvenile specimens is recommended.

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The accumulation of heavy metals in the feathers of some wetland birds in the Danube Delta


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EFFECTS OF DIFFERENT LEVELS OF SAVORY
(*SAVUTREA HORTENSIS* L.) POWDER ON PERFORMANCE,
SOME BLOOD BIOCHEMICAL AND INTESTINAL
CHARACTERISTICS OF COBB 500 BROILER CHICKS

YASER RAHIMIAN*, MORTEZA KARAMI**, SAYED MOSTAFA AKBARI***, FARSHID KHEIRI*

In this study we evaluated the effects of Savory powder on the performance, blood biochemical and intestinal characteristics of female Cobb 500 broiler chicks. Total of 240 one day broiler chicks were randomly divided into 4 treatments. Treatments were further divided into 6 replicates. Broilers were fed a basal diet as control group, basal diet with 0.2% Savory powder (S1), basal diet with 0.4% Savory powder (S2) and basal diet with 0.6% Savory powder (S3). The experiment was carried out for 6 weeks. Feed intake (FI) and body weight gain (BWG) was calculated for estimation of feed conversion ratio (FCR). At the end of the experimental period four birds from each replicate were randomly slaughtered to determine carcass characteristics and other parameters. The blood serum samples were subjected to biochemical analysis. For determination of intestinal characteristics and small intestine tissues samples were collected. All data were analyzed as a completely randomized design using General Linear Models (GLM). The significance of the differences among least square means of main effects was tested by Duncan’s new multiple-range test. Data from this study showed BWG and FCR in broilers improved underfed different levels of Savory powder (P≤0.05). There were significant differences between carcasses traits under effect of feed Savory powder levels (p≤0.05). The results of this study showed that serum triglyceride, cholesterol LDL content decreased in groups fed by savory powder and HDL increased significantly (p≤0.05) instead. In addition, villus height decreased in S1 and S2 groups. An increasing crypt depth was seen on S2. According to these data villus widths, epithelium layer and goblet cells increased by using Savory, especially by using S2 (p≤0.05). In conclusion we could demonstrate that the savory powder may be used as ingredient in broilers ration without harming effects on carcass characteristics, blood biochemical parameters, and intestinal morphology of Cobb 500 broiler chicks.

**Keywords:** blood biochemical, performance, intestinal characteristics, Cobb 500 broiler.

**INTRODUCTION**

Natural feed additives had beneficial effect for stimulation and activity of digestive system by improving the diet palatability and enhancing appetite of poultry, thus increasing the amount of feed consumed (Kamel, 2001). Aromatic plants powder, and their associated essential oils or extracts are used as potentially
growth promoters (Dibner & Richards, 2005). In this case, the scientists are working to improve feed efficiency and growth rate of livestock using herbal plants (Bunyapraphatsara, 2007). Savory plant (*Satureja hortensis* L.) is an annual, herbaceous aromatic and medicinal plant belonging to the Lamiaceae family (Zargari, 1997). It is known as summer savory, native to southern Europe, Asia and naturalized in parts of North America (Ghalamkari et al., 2011). It is widely distributed in different parts of Iran as one of the most important of classified twelve *Satureja* species. It’s essential oil contains considerable amounts of two phenolic ketones that is carvacrol (Ultee et al., 1998) and thymol (Ghannadi, 2001). The savory is traditionally used in foods for herbal tea and flavor component and in folk and traditional medicine and to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (Leung & Foster, 1996). Also, Zamani Moghaddam et al. (2007) reported that the beneficial effects of savory in poultry disease treatment. The pharmacological action of herbal extracts and their active materials in humans is also well known, but in animal nutrition the number of experimental studies is relatively low (Faghani et al., 2014), hence the objectives of this study was to explore the potential use of different levels of savory powder as a feed additive and growth promoter, on performance, some blood biochemical and intestinal characteristics in Cobb 500 broiler chicks.

**MATERIAL AND METHODS**

This study was conducted last during 42 days at the poultry farm of the Veterinary College, Islamic Azad University, Shahrekord branch, ShahreKord, Iran during April until June 2017.

**Experimental plan**

A total of 240 commercial (Cobb 500) one days old broiler chicks were divided into four treatment groups and they were further sub-divided into six replicates within 10 birds per each. The savory powder purchased from herbalists, cleaned was mixed and adding different levels to control (basal) diet as four experimental diets as shown in the Table 1. The treatments were as control (basal) diet with no savory powder; basal diet + 0.2% of savory powder (S1), basal diet + 0.4% of savory powder (S2), basal diet+ 0.6% savory powder (S3) respectively. All diets of each period were prepared with the same composition and they were both iso-nitrogenous and iso-caloric. Diets were formulated to exceed the requirements of NRC, 2007 recommendations. Feed and fresh water was provided ad-libitum during this experiment.

**Data collection**

The data on growth performance were collected for the following parameters: body weight gain (g), feed intake (g), feed conversion ratio (g), carcass yield (%),
and edible part weight (g). The body weight of individual bird was recorded on a weekly basis. Daily feed intake was calculated for weekly recorded and then calculated feed conversion ratio (FCR) on base daily feed intake. At the end of growth period, four birds form each replicate (i.e. 24 replicate) were randomly slaughtered for determination of carcass traits and the other parameters. Body sections were separated and weighed as percentage weight. Dressing percentage was calculated free from giblets and the organs were weighed separately as percentage of carcass weight. The point spread, performance index and production efficiency factor were also calculated for evaluating the growth performance. The blood samples were taken from the brachial vein from four birds per replicate and stored at 4°C in refrigerator. The blood serum samples were subjected to biochemical analysis by Pars Azmoon commercial kits. Samples from small intestine tissue were collected for determination intestinal characteristics such as villus height, crypt depth, villus width, epithelium layer and goblet cells. The histomorphometric investigation was performed by light microscopy, and the measurement was done using public domain image analysis software Image J version xx (National Institute of Mental Health, Bethesda, MD, USA).

**Statistical Analysis**

Data were collected and analyzed using as a completely randomized design using the General Linear Models procedure of SAS 9.2. The significance of the differences among least square means of treatments main effects was tested by Duncan’s multiple-range test.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0–7 (days old)</th>
<th>7–14 (days old)</th>
<th>15–29 (days old)</th>
<th>29–42 (days old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grain</td>
<td>52.22</td>
<td>53.30</td>
<td>49.25</td>
<td>43.20</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>37.5</td>
<td>33</td>
<td>27</td>
<td>23.5</td>
</tr>
<tr>
<td>Wheat</td>
<td>6</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>1.60</td>
<td>1.50</td>
<td>1.55</td>
<td>1.40</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.18</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin Premix*</td>
<td>1.25</td>
<td>1</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td>Mineral Premix*</td>
<td>1.25</td>
<td>1</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Calculated nutrient content</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2830</td>
<td>2870</td>
<td>2920</td>
<td>2960</td>
</tr>
<tr>
<td>CP (%)</td>
<td>22</td>
<td>20.5</td>
<td>18.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>1</td>
<td>0.95</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Available Phosphorus (%)</td>
<td>0.50</td>
<td>0.45</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.34</td>
<td>1.20</td>
<td>1.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Methionine+Cystine (%)</td>
<td>0.92</td>
<td>0.85</td>
<td>0.80</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*Supplied per kilogram of feed: 7,500 IU of vitamin A, 2000IU vitamin D3, 30 Mg vitamin E, 1.5 µg vitamin B1, 2.2 Mg B6, 5.5 Mg vitamin K, 5.5 Mg vitamin B2, 1.1 Mg vitamin B1, 40 Mg nicotinic acid, 160 µg vitamin Biotin, 12 Mg Calcium pantothenate, 1 Mg Folic acid, 20 Mg Fe, 71 Mg Mn, 100 µg Se, 37 Mg Zn, 6 Mg Cu, 1.14 Mg I, 400 µg Cu.
RESULTS AND DISCUSSION

Performance

The results obtained from performance of broiler chickens fed by savory powder are shown in the Table 2. Data showed that use of S1, S2 and S3 experimental feed treatments increased feed intake (FI) significantly (P<0.05) compared to control group. Body weight gain and final live weight were also significantly higher in S2 and S3 experimental feed treatments (P<0.05). Amiriandi (2015) reported that adding savory essential oil to the drinking water of chicken in 1–42 days period, this result showed that the live weight gain of chicks was significantly decreased in comparison to the than control group. Additionally, Kamel (2001) and Dibner & Richards (2005) also mentioned that the herbal powder and their essential oil mixture may be considered as a potential growth promoter.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FI (g.d)</th>
<th>BWG (g.d)</th>
<th>FCR</th>
<th>Live weight (g)</th>
<th>Carcass yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>103.20b</td>
<td>48.94b</td>
<td>2.00a</td>
<td>2090.30b</td>
<td>1440.11b</td>
</tr>
<tr>
<td>S1</td>
<td>104.11a</td>
<td>50.21b</td>
<td>1.97ab</td>
<td>2114.40b</td>
<td>1495.00b</td>
</tr>
<tr>
<td>S2</td>
<td>104.24a</td>
<td>52.11a</td>
<td>1.95ab</td>
<td>2168.80b</td>
<td>1516.25a</td>
</tr>
<tr>
<td>S3</td>
<td>104.39a</td>
<td>53.30a</td>
<td>1.92b</td>
<td>2199.41a</td>
<td>1516.14a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.028</td>
<td>0.500</td>
<td>0.023</td>
<td>9.700</td>
<td>7.400</td>
</tr>
</tbody>
</table>

a,b and c means within columns with different superscripts are different (P<0.05).

Overall means in the Table 2 showed the significant difference in body weight among treatments (P≤0.05). Ahmadian-Attari et al. (2011) showed that the herbal can improve growth and have beneficial effect on broilers. Cross et al. (2007) by studying on savory, rosemary, marjoram and their essential oil on growth and digestibility in 7 to 28 days broiler chicks, were suggested that the improvement of body weight gain and feed conversion ration may be due to the active compounds such as cinnamaldehyde and ugenol that they found in this herbal. Lee et al. (2003) showed that the herbal active compounds were caused better efficiency in the utilization of nutrients and better growth and performance. In the present study FCR was at the lowest in broilers fed by the highest savory powder. These results proved that savory though being more effective performed to certain extent and have a great potential to be utilized as an alternative. Data from this study also showed that significant differences for live weight (g) between treatments, and the higher live weight was for S2 and the lesser was for control group (Table 2).

The results of other studied showed that body weight gain, feed conversion ratio and dressing percentages increased (P≤0.05) in birds that supplemented with herb extract than control group birds, also mortalities and sudden deaths were minimized via used of herbal supplementation in birds diets (Kamel, 2001).
Zhang *et al.* (2005) reported that some plants or specific combinations of herbs in formulations may act as antioxidants by exerting superoxide scavenging activity or by increasing superoxide dismutase activity in various tissue sites (Khosravinia *et al.*, 2013). These results are in agreement with researchers who indicated that, addition of medicinal herbal plants had significant effects on improving digestibility coefficient and nutritive values (Shahin *et al.*, 2003).

**Edible Organs**

According to the Table 3 data, using of different levels of savory powder were decreased abdominal fat statistically (p≤0.05). The liver weight was higher when broilers fed by S2 (p≤0.05) and intestine weight were at the highest for S3 group than others.

Table 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Liver (g)</th>
<th>Abdominal Fat (g)</th>
<th>Spleen (g)</th>
<th>Intestine (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.77c</td>
<td>48.45c</td>
<td>1.70b</td>
<td>76.16c</td>
</tr>
<tr>
<td>S1</td>
<td>41.73b</td>
<td>44.71b</td>
<td>1.83a</td>
<td>78.35bc</td>
</tr>
<tr>
<td>S2</td>
<td>43.32a</td>
<td>42.43b</td>
<td>1.80a</td>
<td>80.44b</td>
</tr>
<tr>
<td>S3</td>
<td>44.17a</td>
<td>39.21c</td>
<td>1.89a</td>
<td>83.67a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.500</td>
<td>1.061</td>
<td>0.031</td>
<td>0.980</td>
</tr>
</tbody>
</table>

* and † means within columns with different superscripts are different (P<0.05).

**Blood Biochemical Assay**

Blood biochemical assay showed that the blood triglyceride, cholesterol and LDL content tended to decrease by using savory powder and HDL increased significantly (p≤0.05). The supplemented diets with different levels of savory powder had a beneficial effect on broiler performance and blood plasma cholesterol and glucose. Result of Ebrahimi *et al.* (2013) study showed that lowest cholesterol content was for savory powder and the highest of cholesterol was related to the control (P≤0.05). Inconsistent with the results of this study results (Ghalamkari *et al.*, 2011) showed that the use of savory powder had no significant effect on blood serum biochemical of broilers. The result of our finding are in agreement with those obtained by Lee *et al.* (2003), who found that dietary carvacrol, but not thymol, reduces plasma triglycerides and suggested that carvacrol may have more impact on lipogenesis than on cholesterol biosynthesis. In parallel with other investigation by (Ebrahimi *et al.*, 2013) they noted that the different levels of savory essential oil in drinking water had no effects on blood metabolites. Khalaf *et al.* (2008) showed that the phenolic compounds in some herbs may inhibit lipid per oxidation, scavenge and have the superoxide anion and hydroxyl radical, so they could enhance the activities of detoxifying enzymes such as glutathione-S-transferase (Ultee *et al.*, 1998). For example, Kurucuet *et al.* (2013) mentioned
that the D-limonene (l-methyl-4-(1-methylethenyl)-cyclohexane) is a monocyclic monoterpene component of some herbals have hypo-cholesterolemic effects. The hypo-cholesterolaemic action of herbals is possibly related to its poly-phenolic components (Shahin et al., 2003; Seidavi et al., 2017). Polyphenols have been shown to depress the reverse cholesterol transport, reduce the intestinal cholesterol absorption and even increase bile acid excretion (Faghani et al., 2014).

Table 4
The effects of savory powder levels added as experimental diets on several blood biochemical of broilers (mg. dl)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.26^a</td>
<td>136.02^a</td>
<td>60.15^a</td>
<td>66.14^c</td>
</tr>
<tr>
<td>S1</td>
<td>70.15^b</td>
<td>134.10^a</td>
<td>57.12^b</td>
<td>68.21^bc</td>
</tr>
<tr>
<td>S2</td>
<td>68.15^bc</td>
<td>130.26^b</td>
<td>56.12^b</td>
<td>70.02^b</td>
</tr>
<tr>
<td>S3</td>
<td>66.21^c</td>
<td>128.14^b</td>
<td>54.10^c</td>
<td>73.65^a</td>
</tr>
<tr>
<td>SEM</td>
<td>1.060</td>
<td>1.075</td>
<td>1.017</td>
<td>0.850</td>
</tr>
</tbody>
</table>

^a^b and ^c^ means within columns with different superscripts are different (P<0.05).

According to the Table 5 data, the savory powder affects villus height, crypt depth, villus width, epithelium layer and goblet cells significantly (P≤0.05). The villus height was decreased by using S1 and S2 respectively and the highest crypt depth was related to the S1 and S2 groups. According to our data the villus widths, epithelium layer and goblet cells were increased by using savory powder significantly (P≤0.05).

Intestinal Morphology

Table 5
The effect of savory powder levels added as experimental diets on intestinal characteristics of broilers (Micrometers)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Villus height</th>
<th>Crypt depth</th>
<th>Villus width</th>
<th>Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.70^b</td>
<td>18.65^a</td>
<td>5.76^a</td>
<td>2.21^b</td>
</tr>
<tr>
<td>S1</td>
<td>58.17^b</td>
<td>19.20^a</td>
<td>6.65^a</td>
<td>2.84^a</td>
</tr>
<tr>
<td>S2</td>
<td>60.21^a</td>
<td>20.44^a</td>
<td>7.68^a</td>
<td>2.94^a</td>
</tr>
<tr>
<td>S3</td>
<td>63.50^a</td>
<td>22.55^a</td>
<td>7.87^a</td>
<td>3.10^a</td>
</tr>
<tr>
<td>SEM</td>
<td>1.091</td>
<td>1.076</td>
<td>0.840</td>
<td>0.120</td>
</tr>
</tbody>
</table>

^a^b and ^c^ means within columns with different superscripts are different (P<0.05).

Yeganeparast et al. (2016) showed that most active principles of plant extracts and powders are absorbed in the intestine by enterocytes and readily metabolized by the body and the products of this metabolism are transformed into polar compounds
by conjugation with glucoronate and excreted in the urine. As the active compounds are readily metabolized and have short half-lives, the risk of tissue accumulation is probably minimal.

CONCLUSIONS

According to the results of this study, using of different levels of Savory powder have beneficial effects on performance of Cobb 500 broiler chicks. The savory powder could reduce the blood serum cholesterol and LDL content, but did not affect on HDL levels. In conclusion, we could conclude that the basal diet with 0.6% of savory powder have beneficial effect on performance, some blood biochemical and intestinal characteristics on experimental broiler chicks.

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The paper presents aspects of the life and scientific work of two German biological scientists established in Romania, along with other local people of German origin, thanks to Prince Carol de Hohenzollern-Sigmaringen, brought to lead the country, first as Prince and then King (May 10, 1866) of Romanian Principalities. It also approaches the dynasty of biologists WILHELM KARL (WILHELM CAROL) KNECHTEL, a botanist, specialist in designing and arranging gardens and parks, who had an extensive international experience in the field, but also as an entomologist, as well as his son, WILHELM KARL W. KNECHTEL, born in Romania, the founder of the Romanian school of agricultural entomology. He mainly researched Thysanoptera insects, was a professor of entomology in Bucharest and Chişinău and member of the Romanian Academy.

The thought of venerating prominent people of Romanian biology first led us to a remarkable zoological personality – the entomologist Wilhelm Knechtel. The history of the German origin of this scientist takes us back in time to the story of his father, an equally remarkable biologist of his time, which we are trying to synthesize today from the tangles of information that have emerged over the years.

Preamble

At the end of the 19\textsuperscript{th} century, Romania was a poor, undeveloped and divided country, mainly agricultural, with a rudimentary agriculture, of subsistence and at the will of nature. With the bringing of Prince Carol of Hohenzollern-Sigmaringen (1839–1914) as leader of the country, first as Prince of the Romanian Principalities and then King (May 10, 1866), a number of specialists and businessmen of German origin also came in the Romanian Principalities in order to modernize the different areas of Romania.

The King brought a number of German families into the country [Physician C.F. Witting, who founded vascular surgery in Romania, the great manufacturer Erhard Luther, who built the Romanian Steam Brewery (later the Griviţa Brewery), in 1869], all Protestants, who had commercial interests, but were also in the King’s entourage and they came as friends. Among them there was the young botanist Wilhelm Karl (Wilhelm Carol) Knechtel, Austrian-German, who was a passionate
lover of natural sciences, specialist in designing and arranging gardens and parks, with a vast international experience in the field (Fig. 1). As he said, “no pleasure is more lasting than the pleasure that nature gives us; because it is above all” (“Landscaping Gardens”, 1899).

He was born on August 13th, 1837, from Austrian parents, in a village in Bohemia (Czech Republic), in Burgstein commune, which now belongs to the town of Novy Bor, located between the Lužické Mountains (Horní Lužické) and the Central Bohemian Plateau (České středohoří), a region with a generous nature and a rich biodiversity. He attended courses at the Carolina University in Prague, where he was Professor Kosteletzki’s favorite student, a well-known botanist of the time. At first, Knechtel was hired as part of the maintenance team of the park of Schönbrunn Palace in Vienna.

**At the court of Emperor Maximilian**

Between 1859 and 1860, Archduke Ferdinand Maximilian, a member of the Habsburg-Lorraine Imperial House, hired for the first time the botanist Wilhelm Knechtel as a gardener for his small palace in Lokrum Island (Lacroma) in the Adriatic Sea. Later, for about five years, Knechtel worked at Castle Miramar (in Italian = **sea view**), the main residence of Maximilian from Trieste.

When the Archduke was crowned Emperor of Mexico (1864), Wilhelm Knechtel accompanied him to the American continent, being appointed the “gardener of the court”. An interesting detail is that, from the expeditionary team that accompanied Maximilian to Mexico, there were also two Romanians: Ilarie Mitrea, military physician in the Austrian army and Prince George Bibescu, the son of the former Prince of Wallachia (Gheorghe Bibescu), an officer in French army.

![Botanist Wilhelm Karl (Wilhelm Carol) KNECHTEL](image)

Fig. 1. Botanist Wilhelm Karl (Wilhelm Carol) KNECHTEL
(13.08.1837, Pihlerbaustellen – 22.10.1924, Bucharest)
(photo made at the beginning of 20th century, in a Viennese workshop).
In Mexico City, W. Knechtel designed various gardens, including the magnificent garden on the roof of Chapultepec Castle, the imperial residence of Maximilian, located on Chapultepec Hill (in Aztec *chapoltepec* = *locust hill*). This garden is still admired all over the world.

It is not known whether the time spent there was productive in gardening, but the period was certainly beneficial in terms of... writing! He wrote by hand, in German, the book *Handschriftliche Aufzeichnungen meiner persönlichen Eindrücke und Erlebnisse in Mexiko in den Jahren 1864–1867* / *Personal notes and impressions on the events in Mexico between 1864–1867*, printed in 1905, at the expense of the author, in Karl Bellmann’s printhouse from Prague (Fig. 2). This is a unique document, in which the descriptions of nature are intertwined with numerous events of the tumultuous history of Mexico. The volume represents an European testimony of the different aspects of landscape architecture and daily life at the Mexican Court of the 19th century.

The book was translated into Spanish (*Las memorias del jardinero de Maximiliano. Apuntes manuscritos de mis impresiones y experiencias personales en México entre 1864 y 1867*) and was published in 2012 at the National Institute of Anthropology and History of Mexico (Fig. 3a). The edition also includes a collection of rare photographs, business cards, colour post cards, maps and an extended bibliography.

In 2007 the book was translated into Romanian (Fig. 3b) and published after the copy with a dedication in German, offered by W. Knechtel to Dr. Grigore
Antipa. This precious volume was found thanks to the efforts of Dr. Alexandru Marinescu – historian of biological sciences, who discovered the book among the volumes of Grigore Antipa’s library, which had escaped censorship of the authorities after 1947 directed against the so-called “subversive” literature.

The memoirs of the “royal gardener” include scientific data on plants, vegetation and weather in Mexico and references on plants that the emperor sent from Chapultepec to Miramar and vice versa. During his stay in Mexico, Knechtel discovered new species of plants, conducted speleological research, exploring the Cacahuamilpa cave, located in Sierra Madre del Sur, where he collected insects.

In the pages of the book there are numerous references to curious incidents and anecdotes occurred around the Mexican sovereign, Knechtel witnessing the disintegration of the Mexican Empire. “This book is the testimony of a brave and honest man, who, in the whirlwind of horrific events, was supported by his great passion for nature”, as Alexandru Marinescu writes.

The importance of the notes written by the botanist Knechtel lies in the fact that in his pages we discover the taste and passion that the emperor Maximilian has always shown for “beauty”, architecture and gardening.
Knechtel considered the garden as the recreation of Eden and, at the same time, the expression of power. Maximilian himself considered his gardens a public exhibition of elegance, order and education, and in almost all his residences he worked closely with Knechtel.

But the trip to Mexico ended tragically. Emperor Maximilian was imprisoned and executed by the revolutionaries (1867), Knechtel being forced to return to Europe. His return trip, which he describes in his memoirs, included the stops in Havana, Puerto Rico, Thomas Island, unshipping in Southampton, then the visits to the cities of London, Paris and Strasbourg.

Upon his return to Vienna, Knechtel presented himself as “the one who came back from Mexico”. After his return, Wilhelm Knechtel is rewarded with a pension and sent to the small island of Lacroma, to take care for the imperial property of Franz Josef I of Austria, for two years (Knechtel, 2012).

**In the service of King Carol I of Romania**

After the Mexican experience, at the age of 30, the skilful gardener Wilhelm Knechtel was invited to Romania by Carol I de Hohenzollern, in order to work “in the service of the gardens” of the King, being named “head” of the Royal Gardens, then of the Public Gardens.

He becomes a lecturer in the disciplines Viticulture, Horticulture and Entomology at the Central School of Agriculture in Herăstrău – Bucharest (the future Faculty of Agronomy).

On January 17th 1883, he was appointed Knight of the Romanian Crown Order by Carol I of Romania [Pralong (Coord.), 2013] for “special merits within the Special Commission for the fight against phylloxera”. In 1884, he received the Silver Cup of Honour from King Carol I as a gift.

In Romania, Wilhelm Knechtel had a significant contribution to the modernization of public spaces, having a remarkable activity in the field of landscaping. His achievements include: the arrangement of the Posada-Comarnic Park (for Prince George Bibescu), in 1898, Princess Maria’s tomb, the Botanical Garden, the Cișmigiu and Kiseleff parks, the garden of the Royal Palace in Calea Victoriei Bucharest, the garden of Peleș Palace in Sinaia, etc. Wilhelm Knechtel is one of the most prolific gardeners in Romania in the 19th century, carrying out numerous works (Mexi, 2017, cf. Peleș Museum Archives).

The history of the Peleş Castle garden begins with the construction of the castle in 1873. From the archives it appears that the first gardener of the Royal House of Romania, at the same time the leader of all the arrangements in the royal fields, was Wilhelm Knechtel [(http://arhivadearhitectura.ro/ architects / wilhelm-knechtel /; cf. Dr. Elisabeta Dobrescu, from the Faculty of Horticulture at the University of Agronomic Sciences and Veterinary Medicine of Bucharest) (Mexi, 2017; Soroș, 2016)]. The history of Peleş Castle’s gardens is almost 150 years old and begins with the gardener of King Carol I - Wilhelm Knechtel (Soroș, 2016).
Wilhelm Knechtel, an excellent botanist and a remarkable organizer, was one of the foreign personalities who contributed substantially to the modernization of public spaces in Bucharest. He has performed a remarkable activity not only in the field of landscaping, but also in the one of education.

On March 15, 1888, the Austrian-German gardener born in Bohemia, Wilhelm Knechtel, who had accumulated a great deal of life experience traveling many places around the world, came to Bucharest and permanently settled in Romania, obtaining Romanian citizenship by royal decree.

In the latter part of his life he devoted himself to numismatic study (being one of the founding members of the Romanian Numismatic Society) and published several specialized courses and works, such as:

- Epidemiile insectelor [Insect epidemics]. Tipografia Voința Națională, București, 1894;
- Grădînil[e] peisajere [Landscaping gardens]. Tipografia Gutemberg Joseph Gobel, București, 1899;
- Trandafirul [The Rose]. Tipografia Ziarului Cronica – Thoma Basilescu, București, 1905;
- Curs de Entomologie și Viticultură [Entomology and Viticulture course]. Școala Superioară de Agricultură, Herăstrău, 1905;
- Insectele vătămătoare din România și mijloacele de combatere a lor [Harmful insects in Romania and means to control them]. Editura Albert Baer, București, 1909 (together with his son).

The life and work of this wizard of floral architecture is beautifully synthesized by the title of Erhard F. Knechtel’s paper (2012), Wilhelm Knechtel’s great-grandson - From an apprentice of a Bohemian gardener to the Romanian director of the Royal Garden, Wilhelm Knechtel - a European destiny between the West and East.

He died at the age of 87. His son, the entomologist Wilhelm Knechtel, succeeded in showing him the monograph on the Thysanoptera in Romania, a few days before his death.

Wilhelm Knechtel, the son, zoologist. Biographical incursions

On October 10, 1884, spouses Wilhelm Knechtel and Helene Dierke’s joy was great. Their eldest son, Wilhelm K. Knechtel was born. Here he attended the school. The son could not be less than his father. He was urged by his father to attend the same school – the Higher School of Agriculture in Herăstrău, now the University of Agronomic Sciences of Bucharest.

Ever since his childhood, the young Knechtel loved nature’s beauties (Fig. 4). He remembered with emotion the times when he crossed the paths of the Bucegi Mountains with his father, the guidance received from him leading to his increasing love for the environment, learning from him the mountain flora, habitats, plant associations, insects. He confessed: “My room of my family home was a small museum, on one side the herbarium containing the mountain and
alpine flora and from Bucharest surroundings, on the other side stuffed birds and animals, an insectarium, a collection of minerals and a small beginner's library” (from the speech delivered in the Auditorium of the Romanian Academy, on the 75th anniversary).

Since there was no upper agricultural school in the country at that time, in 1904, W.K. Knechtel was sent to Germany, at the Higher Agricultural School at Stuttgart - Hohenheim (Stanciu, 2016) in Wurttenberg, in order to deepen his studies in this field, agriculture, school which he graduated in 1907. Here he had the privilege of attending lectures in the fields of botany, entomology and pathophysiology, being captivated by the courses held by famous professors of that time: Valentin Haecker, Kirchner, Pompetsky, Schule. They had an overwhelming influence on the preparation and later development of young Knechtel, arousing his interest, passion and love for entomology.

Returning to the country, in 1909, W. Knechtel started to work at the Experimental Station for Tobacco Cultivation, the Department of Phytopathology, where he began research on the thrips (Insecta, Thysanoptera), especially on the species *Thrips tabaci*, known as an important pest in the crops of tobacco.

Fig. 4. Entomologist Wilhelm Karl W. KNECHTEL (28.09/10.10.1884, Bucharest – 8.03.1967, Bucharest).

Between 1912–1914 he attended a specialization course in the field of plant protection in Italy, at Naples, Scafati, then at the Institute of Biology of Berlin - Dahlem, where he developed his knowledge on experimental methods of pest control. Within the period 1916–1918, he held the position of sub-inspector in the field of tobacco culture (Fărcaș & Soran, 1982).
The call of the reunited Bessarabia

After the First World War and the joining of Bessarabia to the mother country, General Ernest Broşteanu, who ruled Chişinău with authority, called for volunteers to occupy the vacant chairs left by the Russian withdrawal. To his appeal, professors I.C. Teodorescu and Wilhelm Knechtel replied, among many others, who go there to teach temporarily, within the period 1918–1920, being professors of entomology and phytopathology at the Viticulture School of Chişinău. Here, Professor Knechtel was entrusted with the management of the Bioentomology Station where he continued his research on the ecology and systematics of Thysanoptera. Also, here he met Matilda, Lucia’s sister, Prof. I.C. Teodorescu’s wife, who accompanied them and whom he married; also in Chişinău, their son Ekkerhardt (Hardy) was born.

Entomologist of the Ministry of Agriculture and Domains

He returned to the country in 1921, when he was called to Bucharest, to the Ministry of Agriculture and Domains, as an entomologist, in a time when, in our country, the research and knowledge on pest biology and the application of the control methods was very low. In the neighboring countries (Hungary, Russia) there was already a tradition of agricultural entomology research. During almost the entire period of activity, W. Knechtel was a consultant in this ministry.

Unlike other scientists, Professor Knechtel was not only limited to theoretical concerns, but also a man of action, an excellent organizer and coordinator, a true mentor. In a difficult historical period, after many difficulties that he overcame only through hard work and devotion, W. Knechtel managed to establish the first research center of the Agricultural Entomology Station in Romania. This station, with a single employee and a single manager, Knechtel himself, initially worked in a shed in Libertăţii Park of Bucharest. Professor Andrei Popovici-Bâznoşanu, who was involved in the beginning of entomological research in Romania (Ciubuc, 2002), was the one who brought Knechtel as an assistant to the Faculty of Natural Sciences in Bucharest. Prof. C. Manolache considered that “We cannot talk about entomological research at the Faculty of Natural Sciences except with the academic Knechtel [...].” In 1924, W. Knechtel was appointed head of the Entomology Department of the University’s Descriptive Zoology Laboratory, position which he held until the foundation of the Institute of Agronomic Research.

Professor M. Ionescu wrote in 1959: “It is not a coincidence that all the staff of assistants of the Laboratory of descriptive zoology went to entomology researches, becoming appreciated entomologists”. In the Entomology Laboratory, eight entomologists worked, doctoral theses were elaborated here, five fascicles of P.R.R. Fauna Series were published – all being related to the beginnings of agricultural entomology research in Romania and to Wilhelm Knechtel’s name.
Hard work and devotion to science

In 1926, W. Knechtel obtained the title of Full Professor at the School of Horticulture, where he taught entomology, for 14 years, without being paid! After the foundation of the Institute of Agronomic Research, in 1929, his professor, Gheorghe Ionescu-Șișești, a colleague of studies at Hohenheim, appointed him coordinator of the Department of Phytopathology, Entomology and Agricultural Parasitology.

In 1940, he was appointed lecturer in the agricultural entomology discipline at the Faculty of Agronomy in Bucharest, where he remained until 1944, when he retired and moved to Sinaia, temporarily retiring from his activity.

After two years, Academician Traian Săvulescu summoned him to Bucharest to work in the newly established teams of the Academy, becoming senior researcher in the Fauna of the P.R.R. group, dealing with thysanopteran insects. Referring to the activity within the group, in the speech given on the occasion of Professor Knechtel’s 75th anniversary, Nicolae Botnariuc said: “Academician Knechtel is a model of discipline and order in zoological work [...] Through his entire complex activity in Fauna R.P.R. team, the academician Knechtel made an essential contribution to the success of this work of national interest”.

The professional prestige amplified by Professor Knechtel’s election as a full member of the Academy, within the Department of Biology and Agricultural Sciences, in 1955, at the proposal of Traian Săvulescu.

Academician Gheorghe Ionescu-Șișești considered him “one of his colleagues of great scientific value and high moral authority”.

Highlights of the scientific activity

Professor Knechtel’s scientific activity was carried out both in the field of applied entomology and theoretical entomology. The main research directions consisted of:

- Faunal, systematic and zoogeographic studies on some groups of insects: Thysanoptera, Vespidae, Bombinae, Coccidae, Formicidae, Aphididae, Orthoptera;
- Research on the biology, ecology and control of some species of insect pests in cereal crops, technical plants, orchards and greenhouses;
- Activities having a technical character and popularization, with an impact in the agricultural field: involvement in effective actions to combat the locust – Locusta migratoria in the Danube Delta; combating orchard pests, mainly turtle lice (Lecanidae); biological control of the woolly louse (Eriosoma lanigerum) using the parasite Aphelinus mali;
- An important objective of the faunistic and systematic researches developed by Professor Knechtel was represented by the study of thysanopteran insects in Romania.
Why did Knechtel choose to study Thysanoptera (thrips)?

Initially, Knechtel published together with his father the work “Harmful insects in Romania and means to control them”, continuing the preoccupations already existing in the family with regard to insects that can cause damage. Although he could have chosen a group of insects easier to work with, Knechtel chose Thysanoptera (thrips), a group of insects difficult to study because of their small size and large number of species. Due to their very small size (usually 0.5–5 mm), their identification requires microscope slides preparation, in a sequence of steps, which requires a lot of time, increased attention and careful observations. However, Knechtel preferred Thysanoptera, which are bioeconomically important insects. His research on thrips was not only theoretical, but also especially practical, applied. Under favorable environmental conditions, thysanopterans have a strong mass development, producing significant damage in cereal crops, in greenhouses, orchards, etc. These thrips can cause atrophy of the spike in grasses, destruction of floral organs and leaves, appearance of stains on leaves and flowers, decreased concentration of substances (cellulose, starch). The appearance of stains on different organs of ornamental plants produced by species of thrips leads to a decrease in their commercial value. It also causes vectorization of tospoviruses (the most harmful plant viruses in the world).

The activity of Prof. W. Knechtel in support of agriculture reflects the spirit of the century on ecology, a spirit triggered by the great scholar Ernst Haeckel (1834–1919) - father of ecology, professor of the brilliant Romanian student Grigore Antipa in Jena. In Romania, Dr. Grigore Antipa carried on the teachings received from his master Haeckel, developing some issues (ichthyology applied to fisheries in Romania, hydrobiology applied to the management of the Danube Delta and the Black Sea, etc.). This encyclopedic biologist initiated the research of the economic aspects of biological productivity, thus being in fact the creator of a new science, which he called bioeconomics, at the interference between ecology and economy. Later, in 1971, the illustrious American economist of Romanian origin Georges Georgescu-Roegen (1906–1994) minutely developed the science of bioeconomy on the basis of thermodynamics.

Probably, the ecological paradigm of bioeconomy was taken over by Prof. Knechtel and applied to the study of Thysanoptera, insects harmful to agriculture, which cause great economic losses by destroying crops. Besides direct damage, thysanopterans can create favorable conditions for further colonization with pathogenic microorganisms. For example, alternariosa (the disease of tobacco seedlings) is caused by the action of several factors: different developmental conditions offered to seedlings, *Thrips tabaci*, *Alternaria* fungus and climatic conditions. Necrosis of plant tissues initially produced by *T. tabaci* constitutes a favorable environment for the further development of *Alternaria* fungus (Knechtel, 1951).
In short, what are thrips

Around 6,000 species of Thysanoptera have been described worldwide, they can be found in all regions, preferably in warm areas. They can reach alpine areas, at the upper limit of the vegetation. In Romania’s fauna 215 species, classified into four families, were reported (Vasiliu-Oromulu, 1998).

The body of these insects consists of three distinct regions: head, chest and abdomen. The head carries a pair of antennas, the mouth cone, two compound eyes with large facets, the ocelli being present only at adults with wings. The buccal apparatus is adapted for stinging and sucking, an interesting feature being the absence of the right mandible. The wings are membranous, elongated, but there are also species in which the wings are reduced or completely missing. As a rule, the wings are bordered with fringes of bristles (Figs. 5–6). The color is pale and transparent at immature forms, while adults are variously colored, in all shades of yellow, brown and black.

From the biological point of view, most thysanopterans are phytophagous. About 40% of the species feed exclusively on fungi, mostly with hyphae, and some with spores. A small number of species are obligatory predators on other small arthropods. Thrips are food for a lot of insects (including other thrips), mites, birds, salamanders and lizards.

Fig. 5. Frankliniella intonsa (Knechtel orig.).

Fig. 6. Thrips tabaci (Knechtel orig.).

In 1937, Knechtel obtained the title of Doctor of Science with the doctoral thesis entitled Study on the distribution of Thysanoptera in Romania. As a result of an intense and meticulous activity, in 1951, Professor Knechtel published the volume Thysanoptera in the series Fauna of P.R.R, in which 152 species from the fauna of Romania are described. 18 species are new to science (Table 1). Subsequently, the complex study of these insects from a taxonomic, biological, ecological point of view and methods of control became his basic concern. The
papers published in the thysanopterological field present descriptions of the species, diagnoses, research on the spread of the species according to the vegetation floors, the influence of the environmental factors on their ecology, the relations of interdependence between thrips and plants.

Table 1

New Thysanoptera species described by Wilhelm Knechtel
(according to Vasiliu-Oromulu, 1998)

<table>
<thead>
<tr>
<th>Family Aeolothripidae</th>
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<tr>
<td>Aeolothrips priesneri Knechtel, 1922</td>
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<td>Aeolothrips verbasci Knechtel, 1955</td>
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<tr>
<th>Family Thripidae</th>
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<tr>
<td>Ereikethrips calcaratus Knechtel, 1960</td>
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<tr>
<td>Eremitothrips manolachei (Knechtel, 1955)</td>
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<tr>
<td>Kakothrips dentatus Knechtel, 1939</td>
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<tr>
<td>Mycterothrips albidicomis (Knechtel, 1923)</td>
<td></td>
</tr>
<tr>
<td>Oxythrips cannabensis Knechtel, 1923</td>
<td></td>
</tr>
<tr>
<td>Oxythrips dentatus Knechtel, 1923</td>
<td></td>
</tr>
<tr>
<td>Oxythrips euxinus Knechtel, 1932</td>
<td></td>
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<tr>
<td>Thrips euphorbiace Knechtel, 1923</td>
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<tr>
<th>Family Phlaeothripidae</th>
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<tr>
<td>Haplothrips floricae Knechtel, 1960</td>
<td></td>
</tr>
<tr>
<td>Haplothrips scyticus Knechtel, 1961</td>
<td></td>
</tr>
<tr>
<td>Hoplothrips absimilis Knechtel, 1954</td>
<td></td>
</tr>
<tr>
<td>Hoplothrips lichenis Knechtel, 1954</td>
<td></td>
</tr>
<tr>
<td>Hoplothrips muscicola Knechtel, 1954</td>
<td></td>
</tr>
<tr>
<td>Hoplothrips quercinus Knechtel, 1935</td>
<td></td>
</tr>
<tr>
<td>Phlaeothrips bacauensis Knechtel, 1948</td>
<td></td>
</tr>
<tr>
<td>Priesneriella clavicornis (Knechtel, 1935)</td>
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Professor W. Knechtel worked on thysanopterans for almost 50 years, collected rich material from Romania and other corners of the world, the results obtained recommending him as one of the most famous specialists in the world in this group, being appreciated and consulted by many scientists outside the country’s borders. The evidence is both the rich material he received from them, to be identified or verified, and the fact that many researchers dedicated a number of species to him, in honour of his name (Melanthrips knechteli Priesner 1936, Haplothrips knechteli Priesner 1923, Amphibolothrips knechteli Priesner, 1936, etc.).

Scientific collection of thysanopterans made by Acad. Knechtel was in the custody of the Institute of Biology Bucharest of the Romanian Academy, in 2010 being transferred, in its original boxes, at “Grigore Antipa” National Museum of Natural History of Bucharest.

This collection of reference for the Romanian entomofauna of Romania was revised by Dr. Liliana Vasiliu-Oromulu, a successor of Prof. Knechtel’s thysanopterological studies (Fig. 7). The collection includes blade-lamella scientific
preparations belonging to the suborders Terebrantia (families Aeolothripidae, Fauriellidae and Thripidae – including 128 species) and Tubulifera (family Phlaeothripidae, represented by 66 species). Out of the total of 3,275 specimens, 177 are type-specimens (3 holotypes, 2 paratypes, 164 syntypes, 8 cotypes), of special scientific value (Stan, 2017).
In addition to Thysanoptera, Prof. Knechtel also dealt with the study of other groups of insects, such as coccids. He highlighted the species present in the Romanian fauna and researched host plants, ecology, biology and control methods.

He also initiated the systematic study of the bumblebee species (Bombinae family) of our fauna, which included research on the intraspecific variability depending on the climatic conditions, the geographical spread influenced by the zonation of the woody vegetation, the results obtained being published in a monograph.

In 1940, in collaboration with Prof. Constantin Manolache, W. Knechtel began the study of aphids, insects with a pronounced polymorphism and which had been researched by Prof. Ioan Borcea. These investigations resulted in the establishment of the systematic position of some species present in the fauna of Romania, the description of new species, information on the ecology and control methods.

Along his long career, W. Knechtel created an impressive collection of formicides (Hymenoptera), and researched chewinglice and orthopterans, on the latter, publishing in collaboration with Prof. A. Popovici-Băznoşanu a volume in Fauna of P.R.R., in which all the species of Orders Saltatoria, Dermaptera, Blattodea and Mantodea are treated. Professor Knechtel contributed to the taxonomic and systematic knowledge of the species from Vespidae family (Vespa germanica and Vespa vulgaris).

Prof. Knechtel and his collaborators’ (Mihail Ionescu, Ecaterina Dobreanu, Constantin Manolache) second major research direction was represented by the studies on the morphology, biology, ecology and control of some pest species. In this respect he carried out a series of researches on the species Thrips tabaci, Phytodecta fornicata, Locusta migratoria, L. caudata, Phlyctaenodes sticticallis, Eulecanium corni, Aspidiotus perniciosus, Claudius pectinicornis, Lema melanopus, etc. bringing valuable contributions to the knowledge of the development cycle, post-embryo stages and number of generations. These investigations were based on careful microscope observations and experiments performed in laboratory and field conditions.

In the papers on the species Phytodecta fornicata (pest of alfalfa crops), published in collaboration with Cornelia Hrisafi, information on the diapause phenomenon is presented. Important contributions are made by Knechtel, along with Prof. Mihail Ionescu, another distinguished Romanian entomologist, on the insect Pyrausta nubilalis, a pest species intensively studied between 1929 and 1932, in Europe and America, within the “International Corn Borer Investigation Association”.

As a representative of Romania, Acad. W. Knechtel has participated in numerous international conferences, congresses and symposiums, among which we mention: The Entomology Congresses from Ithaca, U.S.A. (1929), Paris (1930), Warsaw (1932), Prague (1958). In the summer of 1956, he participated, together with M. Ionescu, in the International Congress of Entomology in Montreal. On this occasion he met eminent Prof. Soren L. Tuxen, director of the Museum of Zoology in Copenhagen and a member of the Standing Committee of the Entomology
Congress. At the invitation of the two Romanian entomologists, Prof. Soren Tuxen paid a visit to Romania, delighted by our country where he met many scientists and culture, among whom Prof. Constantin Motaș was present (Opriș, 2009).

Prof. Knechtel’s scientific achievements have been materialized in more than 120 papers, among which there are:

- Insectele vătămătoare din România și mijloacele de combatere a lor [Harmful insects in Romania and means to control them] (together with his father) (1909);
- Phlyctaenoles strictalis. Un vătămător al tutunului [Phlyctaenoles strictalis. A Tobacco pest] (1915);
- Rolul stațiunilor entomologice [The role of entomological stations] (1928);
- Oekologischzoogeographisches Studium an Coleopteren des Rumänischen Faunengebiets (1944);
- Oekologisch-faunistische Untersuchungen an Thysanopteren Rumäniens (1947);
- Thysanoptera, in the Fauna P.R.R. (1951);
- Hymenoptera, Apinae, in the Fauna P.R.R. (1955);

As a recognition of the value of his scientific activity in the field of entomology, for the contribution made to the development of biological sciences in Romania, in 1951, Prof. W. Knechtel was awarded the “State Prize”, being decorated with different Orders.

Wilhelm K. Knechtel lived in Sinaia, in a building built in 1939, located on Calea Codrului street, no. 34. After retirement, he lived in the silence of the woods of Sinaia, continuing his work, according to his own confessions “in order to finish my work, to leave everything in full order, for the sake of education and discipline in activity received from my parents”. The building was used as a home for his family and as an Entomological Research Laboratory that belonged to the Institute of Biology Bucharest of the Romanian Academy. In 1950, the building was nationalized, but Acad. W. Knechtel continued to live in this building until the end of his life (1967).

He was the first director of the Center for Biological Research in Bucharest, founded in 1957 and transformed into the current Institute of Biology (1960). Even during the three years in which he held the position of Director, Professor Knechtel’s working laboratory was in Sinaia’s house. After his death, in order to continue and develop the scientific activity initiated by him, the house from Sinaia (Fig. 8) became an Ecological Stationary, here carrying out a series of research activities. Until 2006, it in the custody of the Institute of Biology Bucharest.
In 1944, the Knechtel family was struck by terrible news; they lost their son, Ekkehardt, a lieutenant in the Mountain Troops, a tragedy that marked family members for the rest of their lives. Who knows? If this misfortune had not happened, Ekkerhardt might have followed his grandfather’s and his father’s career and skills, benefiting from the impressive library left by them, inaugurating the third generation of the German biology dynasty in Romania.

W. Knechtel loved very much Romania and Romanians. However, from the testimonies of those who knew him closely, after 1944, he suffered because of his German origin, being fired, having no income for a period of time ... I found out late Professor’s sufferings created by the injustices of the communist regime established after the war in Romania by the Soviet winners.

In 1959, one of the authors of this article (Marian - Traian Gomoiu) was freshly employed at the Center for Biological Research of the Romanian Academy, located in Locotenent Ștefan Lemnea street, in some rooms of the former “royal stables”, as it was said at that time. In one of the larger rooms on the floor of the building, there were several tables, usually for young researchers (“the colts of the Academy” – as Prof. Theodor Bușniță called them). A table was more isolated, in front of a window. The occupant of the table was a slightly older gentleman, with a normal height, silent, sober, concerned about his work, imperturbable at the hum of
the room. It was Professor Knechtel, about whose life I would find out little by little, and almost conspiratorially over time. The entire outfit of this scholar was imposing, and we, the youngest, addressed his with the name “Mr. Professor” ... and not using the expression, common that time “Comrade Professor”.

Besides the scientific activity of recognized value, W. Knechtel was also a great admirer of the Romanian folk art and a passionate bibliophile, over the years collecting a rich literature, not only in the scientific field, but also in the artistic one. A large part was donated to the Library of the Romanian Academy and the Central Library of the Institute of Agronomic Research.

On November 2, 1959, Acad. Wilhelm Knechtel was celebrated at the age of 75, in the Auditorium the Romanian Academy, on which occasion honored speeches were given by Acad. Gh. Ionescu-Şişeşti, Prof. C. Manolache, Prof. M. Ionescu and Conf. N. Botnariu. In the speech given by Prof. C. Manolache on this occasion, he said: “You were near the dear microscope and you analyzed diligently and tested tens of thousands of micro and macro-entomofauna preparations from Romania and abroad. You loved a number of other groups of insects with a still mysterious life, the ants and bumblebees once sung so beautifully by Dante in the Divine Comedy”.

In his reply, warmly thanking his colleagues for the words of appreciation addressed to a “modest worker in the field of natural sciences”, Acad. W. Knechtel confessed: “I have received a severe education, directed to work, discipline and order. I led a sober life. I created myself a special world - the world of insects - in which I felt thankfull and happy and where I found the peace of the soul in times of restraint [...]. I fulfilled my dream from a young age. I created a research institution, I trained the scientific staff, I trained working people”.

In 1967, the year in which Acad. W. Knechtel passed away, after a deep suffering, there were almost 60 years since the appearance of his first synthesis paper in the field of agricultural entomology, “Harmful insects in Romania and means to control them”, having as authors W. Knechtel, the father, and W. Knechtel, the son. This paper represented the touchstone for generations of students, pupils, specialists in the field of agricultural entomology.

Over time, detailed appraisals of Professor Knechtel’s scientific achievements were made by those entitled to do so, personalities of Romanian science and culture who have expressed their appreciation and acknowledged the value and merits of the beginnings and development of entomology in our country.

For over half a century dedicated to entomological research, Acad. W. K. Knechtel, the founder of the school of agricultural entomology in our country, served with modesty, devotion and high competence the biological
sciences in Romania. His personality and work represent an example of tenacity, hard work, love for science and will remain forever inscribed in the national scientific heritage.

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**Publications of Wilhelm Karl Knechtel**

1909
1. *Insectele vătămătoare din România și mijloacele de combatere a lor*. Editura Albert Baer, București, 124 pag. (in collaboration with Wilhelm Knechtel father) [*Harmful insects in Romania and means to control them*]

1910

1911
4. Studiu asupra unor specii din ordinul Thysanoptera. *Viața Agricolă*, An II, nr. 6: 434–442 [*Study on some species from order Thysanoptera*]
5. Studiu asupra unor specii din ordinul Thysanoptera. *Viața Agricolă*, An II, nr. 7–8: 522–528 [*Study on some species from order Thysanoptera*]

1914

1915

1919

1920

1921

1922

1923


1924


1927


1928


1929


22. Rolul stațiunilor entomologice. Societatea Agronomilor, Congresul Agricol, 6 pag. [The role of entomological stations]

1930


1931


1932


30. Thysanoptere din România. București, 235 pag. [Thysanoptera from Romania]

1933


1934


34. Thysanopterele din România. Studiu monografic, Buletinul Agriculturii, nr. 24 [Thysanoptera from Romania]

1936


38. Constatări asupra păduchelui țestos de San José. Progresul horticol 1, 2, 24 pag. (in collaboration with C. I. Manolache)

1937

1937
1938
47. Beitrag zur Kenntnis der Mollophagen der Vogelwelt Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XIX, nr. 6–7 (in collaboration with Ion I. Cătuneanu) 
1939
1940
53. Neue Blattlausarten für Rumänien. Beitrag I-IX. *Bulletin de la Section Scientifique*, Tom. XXII, nr. 3, 5; XXIV, nr. 4, 8; XXV, nr. 5; XXVI, nr. 6, 10; XXVII, nr. 7; XXIX, nr. 7 (in collaboration with Constantin Manolache) 
55. *Pemphigus bursarius* L. *Horticultura Românească*, 18, nr. 9–10: 130–134 (in collaboration with Constantin Manolache) 
59. Observații asupra afidului *Doralina pomi* De Geer în România. *Viața Agricolă*, 32, nr. 8, 3 pag. (in collaboration with Constantin Manolache) [Investigations on aphid *Doralina pomi* de Geer in Romania]. 
1942

64. Afide din sere găsite în România. *Horticultura Română*, 20, nr. 1–2: 1–5

**1943**


68. Observații sistematice, ecologice și de combatere la afidele din sere. *Viața Agricolă*, 34, nr. 5: 136–144.

**1944**

69. **Neue Blattläuse für Rumänien.** *Bulletin de la Section Scientifique de l'Académie Roumaine*, 26, nr. 6: 382–392.

70. **Neue Blattläuse für Rumänien.** *Bulletin de la Section Scientifique de l'Académie Roumaine*, 26, nr. 10: 690–702.

71. Beitrag zur Kenntnis der individuellen Zeichnungsvariation bei *Vespa vulgaris* L. *Académie Roumaine, Section Scientifique*, Tom. XXVI, nr. 8, 21 pag.


73. *Dușmanii viței de vie*. Ediția I, București, Editura Universul, 36 pag. (in collaboration with Ioan Martin) [The enemy of vine]

**1945**

74. **Neue Blattläuse für Rumänien.** *Bulletin de la Section Scientifique de l'Académie Roumaine*, 27, nr. 7: 475–485.


76. Beitrag zur Kenntnis der Biologie der Blattwespe *Claudius pectinicornis* Geoffroy. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XXVII, nr. 8: 539–566. (in collaboration with Hrisafi Cornelia)

**1946**


**1947**


**1948**


**1951**


**1953**

83. Constatări asupra păduchelui țestos din San José (*Aspidiotus perniciosus* Comst.). *Revista pomicolă* (in collaboration with Constantin I. Manolache)
1954

1955

1956

1957
94. Variational individuala la unele specii ale genului Formica. In: Omagiu lui Traian Săvulescu, București [Individual variation of some species of the genus Formica]

1959

1960
103. O nouă specie de Thysanoptere. Comunicările Academiei R.P.R., 9, nr. 11: 1325–1328.

1961

1964

1965

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