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MODEST GUȚU, PhD BIOLOGIST-ZOOLOGIST,
AT THE ANNIVERSARY OF 80 YEARS

Happy Birthday, Your Excellency Dr. Modest GUȚU!
If it were possible I would have said, with all certainty,
Happy Birthday, Sir!

I address this unusual appellation in our scientific environments to mark not only the anniversary of the beautiful age, but also the octogenarian's remarkable activity. A scientist full of passion, hardworking, modest, devoted to his profession, who kept beyond the endless and sterile discussions of the colleagues or of the media, whose work tools were the microscope, the patience, the spirit of observation, the discernment, the critical analysis, all used to orientate correct in life and extract a few values from too much sterile. Eighty years of fruitful life, 52 years spent in the temple of nature, the "Grigore Antipa" National Museum of Natural History in Bucharest, the ark that reveals all the jewels of nature and teaches them about the millions of living creatures of Earth and Waters.

I do not know exactly when I first met him, but whenever I made a visit to my master on the knowledge way of ecology of the Black Sea, the Academician Mihai Băcescu, who introduced the carcinological research in the museum he was leading, I stopped for a few moments in my colleague Modest GUȚU's lab. It was

a “hermitage” with two desks, one with the microscope and many small receptacles with precious samples and one with the typewriter, between them being an old swivel chair, from Grigore Antipa’s time, and another ordinary one, for guests, who could hardly fit into so tight a room.

I was going to Modest to change a few words, to a “coffee”, which he was preparing for as a ceremony. And then, we were telling each other what we were doing, which were our progress and plans, which were the hardships, what our families did for us... Once, I was impressed by a true story that I will never forget... His father, who came through the storms of the times from Bessarabia, clandestinely passed the Prut River (border line between Romania and the Soviet Union, after the World War II), one night, only to smoke a cigarette on its invaded land... For me, my colleague who made such a confession, at a time when silence had to be order, remained in my heart. At that time, the memories of Romania’s tragedies, of the Romanian people were forbidden, and the most courageous ones told them whispering, and only when they were very well convinced with whom they were discussing.

The place where his father wanted to smoke is on a Prut River loop (called Zamca, now being a geological reservation), located in the perimeter of his native village, Pererita (documentarily attested in 1623), where he had inherited a piece of land from his parents. It is the same locality where was born and grew up the late poet Grigore Vieru (1935–2009; left for the kingdom of the stars after a tragic car accident while returning from a homage ceremony of the unequalled Romanian poet Mihai Eminescu), whose poems played an important role in reviving the national consciousness of the Romanians in Bessarabia.

MODEST GUȚU’S ORIGIN, WAY OF LIFE AND OF ACKNOWLEDGEMENT

Modest Guțu was born on March 14, 1937 in the town of Lipcani, Hotin County (today, Republic of Moldova). The city, founded in 1699 (but the first historical mention of Lipcani dates back to 1429), had a tumultuous history, as its own people had (pogroms, deportations). Lipcani is located in the north-western corner of the country, on the bank of the Prut River, which is the border between the Republic of Moldova and Romania; the border with Ukraine is a few kilometers to the northwest. The closest urban centers to Lipcani are Cernăuți and Hotin (today in Ukraine), Botoșani and Dorohoi (Romania) and Bălți (Republic of Moldova).

During World War II, March 1944, when the Red Army approached Romania’s borders, his parents fled to the old Kingdom, settling in the village of Miorcani (Botoșani County), located on the right bank of the Prut River; so he avoided his relatives’ fate deported to Siberia in 1940, when Bessarabia was temporarily annexed by the Soviet Union. More than 120 years ago, in Miorcani, the great poet Ion Pillat, academician, essayist and journalist (related to the famous Romanian political

family, Brătianu, who gave some very important personalities for the modern history of Romania), grew up, and later on he came for the holidays. As a matter of fact, the manor house and the outskirts of Miorcani were evoked by Ion Pillat in his poems such as “My Village”, “Evening at Miorcani”, “The House of Walls”, “Travel Journal”.

The child and later the youngster Modest GUȚU attended primary school and gymnasium at the school of the mentioned village, and then he graduated from the theoretical high school in the city of Dorohoi in 1954.

Being interested in physics and mathematics, he enrolled in the admission contest at the Faculty of Electrotechnics in Iași, but after checking his political file, as the authorities did by that time, he was rejected. His only chance to pursue higher education was to attend one of the faculties in the field of agriculture, where there were no “file” restrictions. That’s how he became a student at the Faculty of Horticulture at the Agronomic Institute in Iași.

In the autumn of 1955, he became seriously ill during the agricultural practice and was hospitalized for a few months (October 1955 – February 1956). After healing, he dropped out the courses of the Horticulture Faculty and got hired as a substitute teacher at the Miorcani School.

The persecutions to which his family was subjected, due to the Bessarabia origin, implicitly affected him. Under these circumstances he changed his residence in another part of the country, where his origin could be unnoticed. He managed to become a substitute teacher of agriculture and biology at a school near Bucharest, where he worked for some years. According to the law at that time, he could attend a faculty on extramural courses only if he had a minimum of three years’ seniority in the field in which he was employed. He initially graduated the Pedagogical Institute of three years (the Faculty of Biology and Agricultural Sciences), after which he followed the Faculty of Biology and Geography of the “Babeș-Bolyai” University of Cluj. The bachelor degree exam dealt with the field of invertebrate zoology and was led by the harsh Professor Vasile Radu, Member of the Romanian Academy, who gave him the maximum mark (ten) with congratulations.

In 1978, he enrolled in his doctorate at his mentor, Professor Mihai Băcescu, and passed all his exams and preliminary papers, and later preparing his doctoral thesis in the domain of tanaidacean crustaceans. As it is well known, in Romania at that time, the PhD students, who were not members of the Communist Party, and Modest Guțu wasn’t either, needed a special approval to hold the doctoral thesis, which he did not get. It was only after the events of December 1989 that he could resume the preparation of the PhD thesis, entitled *The morphological and systematic study of the current tanaidaceans (Crustacea) of the suborder Apseudomorpha*, which he finally held (1998) at “Babeș-Bolyai” University in Cluj, with Professor Nicolae Tomescu.

After passing so many adversities during his childhood and first youth, Modest GUȚU finally enters a “normal” rhythm of life and scientific creation.

WHICH ARE THE OCTOGENARIAN'S, DR. MODEST GUȚU'S LANDMARKS OF LIFE?

Dr. GUȚU is a biologist-zoologist, specialist in taxonomy, the main field of activity expanding on morphology, systematics, zoogeography and phylogeny of the tanaidacean crustaceans, as well as on the systematics of some peracarid crustaceans. But, equally, he can be considered a museologist. As a pupil of the school of the late Academician Mihai Băcescu, oceanographer, renowned carcinologist and director of the famous museum inherited from the great scholar Grigore Antipa, Modest GUȚU has the right to successfully practice the two specializations.

He worked at "Grigore Antipa" Museum since March 1, 1965 until March 31, 2002 when he retired on request, as senior researcher, highest grade; but now even if retired, he continues his activity, with or without an employment contract at his place of research.

DR. MODEST GUȚU – AN ILLUSTRIOUS ZOOLOGIST

At 80 years of fruitful life dedicated to science, Dr. Modest GUȚU can be proud of his outstanding results whose importance goes beyond the borders of Romania, including the countries bordered by the world's seas and oceans. The investigated material after which many new taxa have been described originates in the Atlantic Ocean (the Gulf of Mexico, the Bermuda Islands, the Bahamian Archipelago, the coasts of Brazil, etc.), in various shallow water areas of the Indo-West Pacific (Tanzania, Réunion Island, Strait of Malacca, Andaman Sea, Pari and Bunaken Islands, Makassar Strait, Bali Island, Australia, etc.) as well as in the Mediterranean Sea, the Red Sea, the Caribbean Sea, etc., some of these places visited by Dr. GUȚU during some expeditions. He collected the zoological material alone, with a small hand dredger, swimming underwater (in apnea or with scuba), or obtained them from collections of some museums or by the courtesy of his colleagues from abroad: Dr. Hans-Georg Müller (Germany), Dr. Richard Heard, South Mississippi University, Dr. Thomas Iliffe, A & M University at Galveston, Texas (U.S.A.), etc.

Within the "Grigore Antipa" National Natural History Museum, Modest GUȚU has performed numerous tasks specific to museum activity, like guiding, public conferences, educational activities, etc., all achieved successfully, with the imprint and personal charm of the dedicated museum worker. Particularly meritorious, however, was the engagement of Dr. GUȚU on the line of scientific research at the suggestion and under the guidance of Professor Mihai BĂCESCU. The way the Master showed, the knowledge of a small group of crustaceans, Tanaidacea, was a hard way, but it would bring full satisfaction to both the researcher and his mentor over the years.

From the group of crustaceans classified in the Order Tanaidacea, Modest GUȚU focussed, among other things, on Leptocheliidae, a family with many problems, characterized by enigmas and uncertainties. The brief diagnosis of the genus *Leptochelia* (due to the lack of knowledge of females of the type-species and the brief description of the males, plus the high sexual dimorphism as well as many other objective and subjective factors) generated numerous contradictions and confusions in the tanaidologists' world, confusion that has increased with the discovery of new species. This is why species with similar morphological macrostructures but with important different microstructures for the taxonomic classification of these tanaidaceans, ignored by scholars, have been classified in genus *Leptochelia*.

As a result of observations made on a large number of species (males and females), at first sight belonging to the genus *Leptochelia* (type-genus of the family Leptocheliidae), several common microstructures were found, characteristic to the males and females of several species "groups", thus allowing their reclassification into new genera, five of them being new to science (belonging to the subfamily Leptocheliinae). Certainly, Dr. Modest GUȚU successfully responds to the challenges raised by these crustaceans, but his contribution, although it does not solve all the contradictions and confusions in literature, is a major achievement in understanding the importance of micro-features, ignored up to now, for the systematics of the leptocheliids.

Based on personal research, the study of the specialized literature and on the discussions with specialists from abroad, **the tanaidologist Modest GUȚU discovered and described 233 new taxa to science**: 5 families, 9 subfamilies (a subfamily being erected to family rank), one tribe (erected to subfamily rank), 58 genera (some of them being synonymized or unaccepted), 4 subgenera (two erected to genus rank and one synonymized) and 156 species (of which one is synonymized). It is important to mention that **out of the 233 described taxa, 227 are accepted by WoRMS** (World Register of Marine Species), a species, a subgenus, and a few genera being synonymized or unaccepted.

Of the mentioned taxa, Dr. Guțu described as a single author 192 taxa (4 families, 9 subfamilies, one tribe, 48 genera, 3 subgenera and 127 species), 32 (one family, 7 genera and 24 species) in co-operation with researchers from the United States of America, Cuba, Colombia, Thailand and England, as senior author, and only 9 (3 genera, one subgenus and 5 species) as a second author (in collaboration with his mentor, Professor Mihai Băcescu).

Also, he contributed the most to the knowledge of the tanaidaceans of the suborder Apseudomorpha (the field he dedicated most of his activity), discovering and describing 181 new taxa to science (7 families, 10 subfamilies, 43 genera and 121 species) accepted by WoRMS, which represents more than a quarter of the 660 taxa (13 families, 16 subfamilies, 108 genera and about 523 species), as they are known today in the world fauna.

Apart from study on tanaidacean crustaceans of the world fauna, **Dr. GUȚU also has systematic contributions at the level of the superorder Peracarida, describing two new orders to science** (Bochusacea, in collaboration with

Dr. Thomas Iliffe, and Cosinzeaceae), as well as one genus and one species of the new order Bochusacea. The two described orders have generated numerous disputes, being admitted by some carcinologists and rejected by others. **At present, the Order Bochusacea Guțu & Iliffe, 1998 was accepted by WoRMS**, being mentioned in all recent zoology treaties. As a matter of fact, **Dr. GUȚU is the only Romanian zoologist who has described an invertebrate order (Subphylum Crustacea) recognized by WoRMS** and the international scientific community.

Discovering new species of tanaidaceans, Dr. Modest Guțu named them according to the international usage, guided by some features of the analyzed specimens (*bicornis*, *cornicauda*, *longisetosus*, *minimus*, *multiarticulus*, *pigmaeus*, *ornata*, *tetracanthus*, *robustispinosus*, *rectifrons*, etc.), by the origin/geographical area of the new species (*antillensis*, *australianus*, *bahamensis*, *belizensis*, *brasiliensis*, *caribbeanus*, *caymanensis*, *cubensis*, *estafriana*, *estasiatica*, *indonesiana*, *javaensis*, *madagascariensis*, *martinicana*, *mexicanus*, *senegalensis*, *srilankensis*, *sudvestatlantica*, *surinamensis*, *tanzaniana*, *vestafriana*, *vestpacificus*, etc.) or by the names of famous researchers; it is worth mentioning that Dr. GUȚU fulfills a moral and at the same time patriotic duty, naming the species in honor of many Romanian scholars (*antipai* – to Dr. Grigore Antipa, *bacescui* – to Dr. M. Băcescu, *bogoescui* – to Prof. C. Bogoescu, *codreanui* – to Prof. R. Codreanu, *daicovicii* – to Prof. C. Daicovici, *eminescui* – to poet Mihai Eminescu, *negoescuae* – to Dr. Ileana Negoescu, *orghidani* – to Dr. Traian Orghidan, *tomescui* – to Prof. N. Tomescu, *vasileradui* – to Prof. V. Radu, etc.).

We cannot end the presentation of Dr. GUȚU's contributions to the development of crustacean knowledge without mentioning the results of his researches in the field of biology and morphology of tanaidaceans: **he discovered the autotomy in some tanaidaceans**, explaining the causes of this phenomenon, and **highlighted numerous unknown morphological structures** which were the base of the description of some high taxa (genera, subfamilies, families).

All of these contributions are found in more than 600 citations, in more than 190 scientific papers and treatises, published in England, France, Germany, Italy, the Netherlands, Norway, Russia, U.S.A., Brazil, Cuba, Japan, Pakistan, Thailand, Australia, New Zealand, etc.

DR. MODEST GUȚU - OTHER CONCERNS, OTHER ACHIEVEMENTS; ALSO ABOUT CARCINOLOGY AND NOT ONLY...

In his memoir of work, Dr. GUȚU can proudly place other activities fulfilled along the time, wherever he was, besides his major concern as a carcinologist, especially in tanaidaceans:

- advisor for the *European Register of Marine Species* (Paris, 2001);
- drawing up scientific reports on the paper publication at the request of editors of highly prestigious journals (from England, France, the Netherlands, United States of America, Mexico, Japan, Australia, New Zealand, etc.);

- granting, directly or by correspondence, advice to colleagues from abroad (England, Germany, Switzerland, Morocco, the USA, Pakistan, Thailand, Australia, etc.) on morphology and systematics of tanaidaceans;
- holding a series of lectures at the *Systematics and Taxonomy of Crustacean workshop in the Order Tanaidacea*, organized by the Department of Aquatic Science, of the Faculty of Natural Resources (Prince of Songkla University, Hat Yai, Thailand);
- enriching the collections of the “Grigore Antipa” Museum with approximately 12,000 tanaidacean specimens (of more than 200 species of about 85 genera), of which 2,193 specimens are the type material (125 holotypes, 77 allotypes and 1,991 paratypes), registered in the national patrimony in the “treasure” category, which was the basis for describing new taxa for science;
- successfully fulfilling all the tasks that came to him as a museographer, from collection management to holding public conferences.

DR. MODEST GUȚU’S SCIENTIFIC WORK

Dr. Modest GUȚU’s entire work is reflected in his numerous and various publications (see list of published papers). Among the 156 papers, he has published more than 90 original scientific papers, of strict specialty of which about 80 on tanaidaceans of the world fauna, as single author or in collaboration with researchers from Romania, England, Germany, Cuba, Colombia, USA and Thailand.

Of all the papers published by Dr. Modest GUȚU, in particular we note two monographic papers already entered in many libraries of the world: *New Apseudomorph taxa (Crustacea, Tanaidacea) of the World Ocean*, Curtea Veche, 2006 (318 pp.) and *Systematic Novelties of the Enigmatic Universe of the Leptocheliids (Crustacea: Tanaidacea)*, ePublishers, Bucharest, 2016 (205 pp.).

Dr. GUȚU is also a co-author of treatises and catalogues of fauna published abroad: the *Catalogue of Crustacea of Brazil* (Rio de Janeiro, 1998) and the well-known *Traité de Zoologie*, founded by P.-P. Grassé, Vol. VII, Fasc. IIIA, the chapter Order Tanaidacea (Monaco, 1999), in which he is the senior author in collaboration with Jürgen Sieg (it has to be remarked that for the monumental treaty, from the Romanian specialists, also collaborated the late Academician Mihai Băcescu, who co-worked with Dr. Iorgu Petrescu, the chapter Order Cumacea). Dr. GUȚU was also the coordinator of the volume entitled *Results of the Zoological Expedition organized by the “Grigore Antipa” Museum in the Indonesia Archipelago (1991). I. Peracarida (Crustacea)*, published in 1997.

Alongside the original scientific works, Dr. Modest GUȚU’s publication list also includes compilation scientific papers, many of them with original contributions (20), then numerous notes and papers of a museological and popularization character, reviews, as well as 6 outstanding booklets of science popularization for children,

3 books for the science dissemination for the general public (one translated into German), and a travel book.

DR. MODEST GUȚU – TRAVELLER AROUND THE MERIDIANS OF THE WORLD

Among the factors that led to the achievement of remarkable results by Dr. GUȚU are the visits he made to museums, research and universities abroad, as well as his participation in expeditions and campaigns that allowed him collecting rich materials and samples, both for enriching the collections of the "Grigore Antipa" National Museum of Natural History and for detailed studies on crustaceans.

Dr. GUȚU visited and worked at the Institute of Oceanology of Havana, Cuba (1973), the Oceanographic Museum of Monaco (1980, 1987), the Gulf Coast Research Laboratory, of the University of Southern Mississippi, USA (1999), A & M University at Galveston, Texas, USA (1999), the Department of Aquatic Science, of the Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand (2004).

Dr. GUȚU participated in two campaigns in the Caribbean Sea organized by the Institute of Oceanology of Havana, in the Indonesia Archipelago and Brazil, both organized by the "Grigore Antipa" Museum, the Andaman Sea and the Gulf of Thailand, organized by the Prince of Songkla University, Hat Yai (Thailand).

DR. MODEST GUȚU – RECOGNITION AND HONORS

Dr. Modest GUȚU, member of remarkable scientific societies such as The Crustacean Society, S.U.A. and the International Commission for the Exploration of the Mediterranean, Monaco, is appreciated and respected by the scientific world, and received many honors and recognitions.

In the first place there are the taxa dedicated to his honor: one genus and 11 species are named after him by scientists from Australia, England, Cuba, Japan, Russia, USA, Thailand and Romania (see the list).

At the suggestion of the renowned oceanographer and explorer Jacques Yves Cousteau, director of the Monaco Oceanographic Museum at that time, the Academy of Sciences in Paris awarded Dr. Modest GUȚU the "Jules et Mathilde Richard" Award, in 1986, for the discovery in the collections of that museum of some tanaidacean crustaceans of great scientific value, which formed the basis of the description of several new species that were believed to be lost.

In Romania, Dr. GUȚU received the first prize and the "Little Reader's Trophy", in 1977, for the book entitled "Skilled Masters of Animal World" and, in 1982, the same 1st prize for the book "What We Know about Extraterrestrial Life".

Praising Dr. Modest GUȚU for his 80 years of life, highlighting the accomplishments, the places and the people he met in over half a century, not a career but a vocation, I cannot pass his mentor, the late Academician Mihai Băcescu, who, from the very beginning, discovered the qualities of the young museographer and researcher, the one who felt his skills, gave him close guidance and all the confidence, so as he did with many students of his school, like the forefathers who sent him the sacred fire of knowledge.

Happy Birthday, Dr. Modest GUȚU! Happy Birthday, health and achievements!

Marian-Traian GOMOIU
Member of the Romanian Academy

**TANAIDACEAN GENERA AND SPECIES AND OTHER PERACARID CRUSTACEANS
NAMED IN THE HONOUR OF DR. MODEST GUȚU
BY SCHOLARS OF THE UNITED STATES OF AMERICA, AUSTRALIA, CUBA,
ENGLAND, JAPAN, THAILAND, RUSSIA AND ROMANIA**

Genera:

1. *Gutuapseudes* Edgar, 1997 (Tanaidacea).

Species:

1. *Cubanocuma gutzui* Băcescu & Muradian, 1977 (Cumacea);
2. *Sphyrapus gutui* Kudinova-Pasternak, 1985 (Tanaidacea);
3. *Bowmaniella gutzui* Ortiz, 1988 (Mysidacea);
4. *Javanisomysis gutzui* Băcescu, 1992 (Mysidacea);
5. *Katocalliope gutui* Ortiz & Lalana, 1997 (Amphipoda);
6. *Indonesanthura gutui* Negoescu, 1997 (Isopoda);
7. *Tanapseudes gutui* Hansknecht, Heard & Bamber, 2001 (Tanaidacea);
8. *Leptostylis gutzui* Petrescu, 2005 (Cumacea);
9. *Julmarichardia gutui* Ritger & Heard, 2007 (Tanaidacea);
10. *Kalliapseudes gutui* Drumm & Heard, 2011 (Tanaidacea);
11. *Halmyrapseudes gutui* Kakui & Angsupanich, 2013 (Tanaidacea).

**PAPERS PUBLISHED BY DR. MODEST GUȚU
(ALONE AND IN COLLABORATION)**

Original scientific papers:

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3. BĂCESCU, M. & M. GUȚU, 1971 – Contributions à la connaissance du genre *Apseudes* de la Méditerranée: *Fageapseudes* n.g. et *Tuberapseudes* n.ssg. Travaux du Muséum d'Histoire Naturelle "Grigore Antipa", 11: 59–70 (in French).
4. GUȚU M. & D. MANONELI, 1971 – Contributions à la connaissance de la faune du lac Belona (Eforie–Roumanie) avec référence spéciale a certains hydrozoaires (Leptolida). Travaux du Muséum d'Histoire Naturelle "Grigore Antipa", 11: 25–31.
5. GUȚU, M., 1972 – Phylogenetic and systematic considerations upon the Monokonophora (Crustacea, Tanaidacea) with the suggestion of a new family and several subfamilies. Revue Roumaine de Biologie, Serie Zoologie, 17 (5): 297–305.
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7. BĂCESCU, M. & M. GUȚU, 1974 – *Halmyrapseudes cubanensis* n.g., n. sp. and *H. bahamensis* n.sp. brackishwater species of Tanaidacea (Crustacea). Travaux du Muséum d'Histoire Naturelle "Grigore Antipa", 15: 91–101.

8. BĂCESCU, M. & M. GUȚU, 1975 – A new genus (*Discapseudes* n.g.) and three new species of Apseudidae (Crustacea, Tanaidacea) from the Northeastern Coast of South America. Zoologische Mededelingen, Rijksmuseum van Natuurlijke Historie, Leiden, 49 (11): 95–113.
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10. GUȚU M., & O. GOMEZ, 1976 – *Pagurapseudes guitarti*, new species of Tanaidacea (Crustacea) from the Caribbean Sea. Travaux du Muséum d’Histoire Naturelle “Grigore Antipa”, 17:85–91.
11. GUȚU, M., 1977 – Données morphologique comparatives sur *Palaemon elegans* (Crustacea, Decapoda) de la Mer Noire et de l’Ocean Atlantique. Rapp. Comm. Mer Médit., 24 (4): 163–164 (in French).
12. MANOLELI, D. & M. GUȚU, 1977 – Le lac Belona (Eforie Nord – Mer Noire) – l’eutrophisation et l’évolution générale des structures hydrobiologiques. Rapp. Comm. Mer Médit., 24 (6): 101–102 (in French).
13. GUȚU M. & A. MARINESCU, 1979 – *Polydora ciliata* (Polychaeta) perfor le gastéropode *Rapana thomasi* de la Mer Noire. Travaux du Muséum d’Histoire Naturelle “Grigore Antipa”, 20: 35–41.
14. GUȚU, M., 1980a – Recent changes in the Decapod fauna of the Romanian Black Sea littoral. Travaux du Muséum d’Histoire Naturelle “Grigore Antipa”, 21: 103–109.
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CONTRIBUTION TO THE KNOWLEDGE OF THE ANT FAUNA (HYMENOPTERA: FORMICIDAE) OF THE DANUBE GORGES (ROMANIA)

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The paper presents both new and published data on 38 ant species belonging to three subfamilies Formicinae, Dolichoderinae and Myrmicinae. Most of the species are common for the Romanian ant fauna. However, we highlight the presence of *Camponotus tergestinus*, *Crematogaster schmidti* and *Pheidole pallidula*. *Camponotus tergestinus* is a rare species, which was known only from few locations. Until now, *Crematogaster schmidti* was known only from Băile Herculane area whereas *Pheidole pallidula* was previously recorded from Baziaș more than 100 years ago. Insights regarding their biology and Romanian distribution is given.

Keywords: ants, faunistics, *Camponotus tergestinus*, *Pheidole pallidula*, sub-Mediterranean elements.

INTRODUCTION

Faunistical investigations are an important part of the nature conservation assessment. Knowing the overall species distribution represents an important component of this system. The ant fauna of Romania is still poorly understudied, despite recent myrmecological investigations (Markó *et al.*, 2006; Ionescu-Hirsch *et al.*, 2009; Markó *et al.*, 2009; Czekes *et al.*, 2012; Tăușan & Pintilioaie, 2016; Tăușan 2017; Wagner *et al.*, 2017). Altogether, the Romanian myrmecofauna is represented by at least 114 species (Tăușan & Lapeva-Gojnova, 2017; Wagner *et al.*, 2017).

However, the number is rather low compared to that of neighbouring countries (Hungary – 125 species (Csösz *et al.*, 2011), Bulgaria – 175 species (Lapeva-Gjonova *et al.*, 2010, Antonova *et al.*, 2016), and Ukraine – 134 (Czechowski *et al.*, 2012). Therefore, faunistical investigations all around the country may increase the knowledge of species and distribution.

Most of the species are known from several parts of Romania. However, many regions are lacking data (*e.g.* Moldova, Muntenia, Banat) (Markó *et al.*, 2006;

Tăușan, 2017). The Banat region for instances was studied scarcely in the last decades (Markó *et al.*, 2009; Tăușan, 2013).

In our study, we investigated the ant fauna from the Danube Gorges. Early myrmecological data was published more than 80 years ago by Bogoescu (1936). The last myrmecological investigations in the area were carried out more than 40 years ago (Paraschivescu, 1967; 1975) and the check-list for the region consisted of 28 ant species.

MATERIAL AND METHODS

The Danube Gorges lies between two countries: Romania to the north and Serbia to the south. Here, the Danube separates the southern Carpathians from the north-western foothills of the Balkan Mountains. The area is famous for its outstanding biodiversity (Schneider- Binder, 2014).

In contrast with other parts of the Carpathians the Danube Gorges is characterized by a warm climate, sheltering many xerophilous and thermophilous species of Mediterranean, Sub-Mediterranean, Illyric, Balcanic, Pontic-Mediterranean and Pontic-Balcanic affinity (Schneider-Binder, 2014).

The occurrence of such species is connected to several geological (a mosaic of limestone, serpentine, crystalline schists) and climatic characteristics (Posea, 2002). Related to the geomorphological structure and substrate that produces varied soil conditions and the effects of insolation contribute as well to the large variety of site conditions reflected in the occurrence of various macro- and microhabitats, biocoenoses, communities and species (Schneider-Binder, 2014).

Moreover, according to Popa (2003) “*the Iron Gates Natural Park is a key area for geoconservation in Romania, as its geological heritage is among the richest in the South Carpathians. The series of structural units, typical for the South Carpathians, crossed by the Danube, shows unique features from paleontological, structural and morphological points of view*”.

Sampling was carried out in July 2015, directly from the nests, along different habitats along the Danube Gorges between Corinini and Drobeta Turnu-Severin localities. The myrmecological material is deposited in the first author personal collection.

Species identification was carried out using Seifert (2007), Markó *et al.*, (2009) and Czechowki *et al.* (2012). Species ecological characterization was performed based on Karaman (2011), Czechowki *et al.* (2012) and Lapeva-Gojnova & Kiran (2012). The list of species is presented in the results section together with comments regarding their biology and distribution in Romania.

RESULTS AND DISCUSSION

Altogether, we identified 21 species, belonging to three subfamilies (Formicinae, Myrmicinae and Dolichoderinae). Most of the species are common for the Romanian ant fauna. However, we highlight the presence of *Camponotus tergestinus*, *Crematogaster schmidti* and *Pheidole pallidula*.

Camponotus tergestinus is a rare species, which was known only from three locations (Nera Valley, Măcin Mountains and Plopeni) (Ionescu-Hirsch *et al.*, 2009). Besides our finding, *Crematogaster schmidti* was known only from Băile Herculane area whereas *Pheidole pallidula* was previously recorded from Baziaș more than 100 years ago (Markó *et al.*, 2006).

The complete list of species (including published data for the area) is given below, together with records from literature (Table 1).

Table 1

List of species collected in the present study with reference to their ecological preference in terms of temperature and humidity and zoogeographical origin: E – eurytopic; P – polytopic; O – oligotopic; mes – mesohygrophile; hyg-mes – hygro-mesohygrophile; mes-xer – mesohygro-xerophile; mte – mesothermophile; oli-mte – oligo-mesothermophile; mte-ter – mesothermo-thermophile; ter – thermophile; MD – Mediterranean; SP – South Palaearctic; T – Tethyan; ES – Euro-Siberian; EWS – Euro-West-Siberian; EC – Euro-Caucasian; SE – South-European; BM – boreo-montane; WP – West Palaearctic; NP – North-Palaearctic (based on Karaman, 2011; Czechowski *et al.*, 2012; Lapeva-Gjonova & Kiran, 2012; source – * – present in our study).

Species	Zoogeographical element	Ecological characterization			Source	
		Plasticity	Humidity requirements	Temperature requirements		
Subfamily Myrmicinae Lepeletier de Saint-Fargeau, 1835						
1.	<i>Aphaenogaster subterranea</i> Latreille, 1798	MD	O	mes	mte	*; Paraschivescu, 1967
2.	<i>Tetramorium cf. caespitum</i>	SP	P	mes-xer	mte-ter	*; Paraschivescu, 1967
3.	<i>Messor cf. structor</i>	T	S	xer	ter	*
4.	<i>Crematogaster schmidti</i> Mayr, 1853	MD	O	xer	ter	*
5.	<i>Myrmica scabrinodis</i> Nylander, 1846	ES	P	mes	mte	*
6.	<i>Pheidole pallidula</i> (Nylander, 1849)	MD	O	xer	ter	*
7.	<i>Solenopsis fugax</i> (Latreille, 1798)	T	O	mes-xer	ter	Paraschivescu, 1967
8.	<i>Temnothorax unifasciatus</i> (Latreille, 1798)	EC	O	mes-xer	mte-ter	Paraschivescu, 1975
9.	<i>Temnothorax nigriceps</i> (Mayr, 1855)	SE	S	mes-xer	ter	Paraschivescu, 1975
10.	<i>Temnothorax tuberum</i> (Fabricius, 1775)	ES	P	mes	mte	Paraschivescu, 1967

Subfamily Formicinae Latreille, 1809						
11.	<i>Cataglyphis nodus</i> (Brullé, 1832)	EC	O	xer	ter	Bogoescu, 1938
12.	<i>Cataglyphis aenescens</i> (Nylander, 1849)	EC	O	xer	ter	Bogoescu, 1938
13.	<i>Camponotus ligniperda</i> Latreille, 1802	EC	O	mes	mte	*
14.	<i>Camponotus herculeanus</i> (Linnaeus, 1758)	BM	O	mes	oli-mte	Paraschivescu, 1975
15.	<i>Camponotus vagus</i> (Scopoli, 1763)	EWS	O	mes-xer	mte-ter	*
16.	<i>Camponotus aethiops</i> (Latreille, 1798)	WP	O	mes-xer	mte-ter	*, Paraschivescu, 1967
17.	<i>Camponotus tergestinus</i> Muller, 1921	MD	S	xer	ter	*
18.	<i>Camponotus piceus</i> Leach, 1825	MD	S	xer	ter	*, Paraschivescu, 1967
19.	<i>Formica fusca</i> Linnaeus, 1758	NP	E	mes	mte	Paraschivescu, 1975
20.	<i>Formica rufa</i> Linnaeus, 1761	NP	O	mes	mte	Paraschivescu, 1975
21.	<i>Formica sanguinea</i> Latreille, 1798	SP	P	mes-xer	mte-ter	Paraschivescu, 1975
22.	<i>Formica cinerea</i> Mayr, 1853	EWS	O	mes-xer	ter	*, Paraschivescu, 1967
23.	<i>Formica cunicularia</i> Latreille, 1798	EC	P	mes-xer	mte-ter	Paraschivescu, 1975
24.	<i>Formica truncorum</i> Fabricius, 1804	NP	O	mes	mte	*, Paraschivescu, 1975
25.	<i>Formica pratensis</i> Retzius, 1783	SP	P	mes-xer	mte-ter	*, Paraschivescu, 1975
26.	<i>Formica lemani</i> Bondroit, 1917	BM	O	mes	oli-mte	*
27.	<i>Lasius platythorax</i> Seifert, 1991	NP	P	mes	oli-mte	*
28.	<i>Lasius fuliginosus</i> (Latreille, 1798)	EWS	O	mes	mte	Paraschivescu, 1975
29.	<i>Lasius flavus</i> Fabricius, 1781	SP	E	hyg-mes	mte	*, Paraschivescu, 1975
30.	<i>Lasius alienus</i> Foerster, 1850	SP	O	mes	mte	*
31.	<i>Lasius emarginatus</i> Olivier, 1791	EC	O	mes-xer	mte-ter	*
32.	<i>Lasius niger</i> (Linnaeus, 1758)	NP	P	mes	mte	Paraschivescu, 1967
33.	<i>Lasius brunneus</i> (Latreille, 1798)	EC	O	mes	mte-ter	*, Paraschivescu, 1975
34.	<i>Lasius mixtus</i> (Nylander, 1846)	SP	O	mes	mte	Paraschivescu, 1975
35.	<i>Lasius paralienus</i> Seifert, 1992	EC	O	mes	mte-ter	*
36.	<i>Plagiolepis pygmaea</i> (Latreille, 1798)	EC	O	xer	ter	Paraschivescu, 1967
Subfamily Dolichoderinae Forel, 1878						
37.	<i>Tapinoma erraticum</i> (Latreille, 1798)	T	S	xer	ter	Paraschivescu, 1967
38.	<i>Dolichoderus quadripunctatus</i> (Linnaeus, 1771)	EWS	O	mes	mte-ter	Paraschivescu, 1967

Concerning the humidity requirements, the species covered a wide spectrum of preferences, ranging from mesohygrophilous species such as *Myrmica scabrinodis*, *Lasius alienus*, and *L. brunneus* to xerophilous species such as *Cataglyphis aenescens*, *C. nodus* and *Messor* cf. *structor*. Most of the species are thermophilous and mesothermo-thermophilous. More than half of the identified species were oligotopic.

The region climatic influences and typical vegetation supported the occurrence of sub-Mediterranean elements such as *Aphaengaster subterranea*, *Crematogaster schmidti*, *Pheidole pallidula*, *Camponotus piceus* and *C. tergestinus*.

A detailed overview of the identified species concerning their biology and distribution in Romania is given below.

SUBFAMILY MYRMICINAE

1. *Aphaenogaster subterranea*

Biology. It occurs in warm and moderately humid deciduous forests, mainly nesting in the ground, under stones or in decaying wood (Czechowski *et al.*, 2012).

Distribution in Romania: The species is known from several localities (Markó *et al.*, 2006; Tăușan *et al.*, 2011), probably more common than the data shows.

2. *Crematogaster schmidtii*

Biology. Usually it nests at the base of trees and shrubs, in cavities of trees and sedges or in dead wood. When nests are disturbed workers emerge in great numbers and are very aggressive (Karaman 2010).

Distribution in Romania. Few localities are known for the species (Markó *et al.*, 2006).

3. *Myrmica scabrinodis*

Biology. A polytopic species preferring humid habitats. It requires high insolation but is very tolerant of soil moisture, avoiding xerothermal places. It occurs both in open areas and forests (Czechowski *et al.*, 2012). It is often found in peat bogs (Czekes *et al.*, 2012).

Distribution in Romania. It is a common species, with many localities from Transylvania (Markó *et al.*, 2006; Czekes *et al.*, 2012).

4. *Messor cf. structor*

Biology. It is a granivorous and frugivorous species, occurring in grasslands with rich seed vegetation (Czechowski *et al.*, 2012).

Distribution in Romania. The species from the complex (Schlick-Steiner *et al.*, 2006) are mainly known from Dobrogea Region and scarcely from other parts of the country (Markó *et al.*, 2006).

5. *Pheidole pallidula*

Biology. It nests on arid areas or sunny borders of wood and on sunny slopes facing south or west with low vegetation density (Detrain 1990).

Distribution in Romania. Few localities are known for the species, mainly restricted to the southern part of the country (Markó *et al.*, 2006).

6. *Solenopsis fugax*

Biology. A thermophilic and quite xerophilic oligotope, occurring in dry habitats, grasslands and light sunny forests (Czechowski *et al.*, 2012).

Distribution in Romania. It's a common species (Markó *et al.*, 2006).

7. *Temnothorax nigriceps*

Biology. A thermophilic and quite xerophilic stenotope, occurring in xerothermal grasslands. It prefers sun-exposed rocky habitats with sparse vegetation (Czechowski *et al.*, 2012).

Distribution in Romania. Few localities are known for this species (Markó *et al.*, 2006).

8. *Temnothorax tuberum*

Biology. It prefers forests, but also met in warm and moderately dry stony open places. It nests mainly in the ground, often around a plant root, under moss, under small stones or in rock crevices, sometimes in decaying wood (Czechowski *et al.*, 2012).

Distribution in Romania. Few localities are known for this species (Markó *et al.*, 2006).

9. *Temnothorax unisfasciatus*

Biology. It occurs mainly in deciduous forests, inhabiting also other light forests, and occurs even in various dry open habitats. Nests mainly in dead dry branches of trees and in empty stems of herbs, under bark, in rock crevices, under stone and patches of lichens (Czechowski *et al.*, 2012).

Distribution in Romania. Few localities are known for this species (Markó *et al.*, 2006).

10. *Tetramorium cf. caespitum*

Biology. It's a quite thermophilic and semixerophilic polytope of dry sun-exposed habitats sparingly covered with herbs (both open and forest); especially common in sandy soils in plains (Czechowski *et al.*, 2012).

Distribution in Romania. Probably the most common ant species in Romania. However, based on recent findings (Wagner *et al.*, 2017) at least four species from this complex occur in Romania. A morphometrical analysis must be carried out to establish what species was sampled in the Danube Gorges.

SUBFAMILY FORMICINAE

11. *Camponotus piceus*

Biology. It occurs mainly in xerothermal grasslands. It prefers steppes and open dry mountain slopes-rarely found in light and dry forests (Czechowski *et al.*, 2012).

Distribution in Romania. A rather common species in Romania (Markó *et al.*, 2006, Markó *et al.*, 2009).

12. *Camponotus vagus*

Biology. It's a rather thermophilic oligotope of coniferous, occurring in light and warm pine forests, where it occurs first of all in open places, especially old clearings. It may be found also in such places in mixed and deciduous forests (Czechowski *et al.*, 2012).

Distribution in Romania. It's a common species (Markó *et al.*, 2006, Markó *et al.*, 2009).

13. *Camponotus herculeanus*

Biology. An oligotope of warm and moderately humid deciduous forests, nesting in the ground, under stone, in decaying wood, rarely in litter (Czechowski *et al.*, 2012).

Distribution in Romania. It's a common species (Markó *et al.*, 2006, Markó *et al.*, 2009).

14. *Camponotus ligniperda*

Biology. It occurs mainly in deciduous forests. Yet, it may be also found in mixed and coniferous forests, and even in open habitats sparsely overgrown with shrubs or single trees. It nests in dry stumps, in the ground under wood, under stones or in rock crevices – apparently in soil, but always in connection with wood (Czechowski *et al.*, 2012).

Distribution in Romania. Maybe the most common species (Markó *et al.*, 2006, Markó *et al.*, 2009).

15. *Camponotus tergestinus*

Biology. Little is known about the species' ecology and life-history. According to Ionescu *et al.* (2009), this species is linked with oak forests and warm climate.

Distribution in Romania. Scarce data is available regarding this species (Ionescu-Hirsch *et al.*, 2009).

16. *Camponotus aethiops*

Biology. It inhabits xerothermal grasslands and shrub areas. It nests in the soil (Markó *et al.*, 2009).

Distribution in Romania. It is a rather common species (Markó *et al.*, 2006, Markó *et al.*, 2009).

17. *Cataglyphis nodus*

Biology. The species can be active at temperatures of 45°C in steppe habitats (Agosti, 1990).

Distribution in Romania. It is restricted to the southern part of the country (Markó *et al.*, 2006).

18. *Cataglyphis aenescens*

Biology. Similar to *C. nodus* (Agosti, 1990).

Distribution in Romania. It is restricted to the southern part of the country, yet the species is known from few localities (Markó *et al.*, 2006).

19. *Formica lemani*

Biology. It is a boreo-montane, quite oligothermic oligotope of mountain meadows, both moist and wet, but met also in forest glades (Czechowski *et al.*, 2012).

Distribution in Romania: a common species, yet not very abundant (Markó *et al.*, 2006).

20. *Formica pratensis*

Biology. A species included into wood ants, although it is a quite thermophilic polytope of dry, predominantly open habitats. It can be found in meadows, pastures and steppes, clearings in forests and sparse forests (Czechowski *et al.*, 2012).

Distribution in Romania: the most common *Formica* s. str (Markó *et al.*, 2006).

21. *Formica cinerea*

Biology. It prefers dry open habitats and forests; it occurs in sunny sandy sites, bare or overgrown with sparse herb vegetation, from coastal and inland dunes to open

light pine forests. Nests are deep and widely spread underground. Aggressive ants that live largely by predation, though also intensely tending aphids (Czechowski *et al.*, 2012).

Distribution in Romania. One of the most common *Formica* (*Serviformica*) species (Markó *et al.*, 2006).

22. *Formica cunicularia*

Biology. It prefers rather open habitats, from sandy dunes, limestone slopes and gypseous hills through meadows and pastures to forest glades, forest edges and sparse dry forests. Nests, frequently with fairly large soil mounds, in the ground, sometimes under stones (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

23. *Formica fusca*

Biology. It occurs in various habitats from dunes and dry sun-exposed slopes of limestone hills through meadows, mid-forest glades and young growth to mires and dense, humid forests with thick undergrowth. Nests, occasionally with soil mounds, are constructed in the ground, under stones, in decaying tree stumps, among decaying litter, even in wet tufts of moss (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

24. *Formica sanguinea*

Biology. It occurs in dry habitats both in woodlands and open areas, such as clearings, forest edges and roadsides. Nests most often in decaying tree stumps, covered around with dry plant material or constructed in the ground often under stones (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

25. *Formica truncorum*

Biology. A wood ant species (outside the *F. rufa* group), being fairly thermophilic associated mainly with coniferous and mixed forests, though also found in deciduous one and in mires. Nests, in decaying tree stumps, partly covered with loose dry plant material (Czechowski *et al.*, 2012).

Distribution in Romania. Few localities are known for this species (Markó *et al.*, 2006).

26. *Formica rufa*

Biology. A wood ant species. It occurs in coniferous and mixed forests (including deciduous ones, nesting in sunny places and in glades, along forest edges and forest, but met also in shaded places (Czechowski *et al.*, 2012).

Distribution in Romania. Few localities are known for this species despite available data. This is due to, most likely, the misidentification of *F. polycytena* (Markó *et al.*, 2006).

27. *Lasius mixtus*

Biology. It prefers humid habitats, both open (meadows, pasture) and wooded (light forests) (Czechowski *et al.*, 2012).

Distribution in Romania: few locations are known for this species (Markó *et al.*, 2006; Tăușan, 2017).

28. *Lasius niger*

Biology. It occurs in open habitats (dry and semidry grasslands), very common in various anthropogenic environments, found also in fairly light dry forests; it avoids shaded woodland. Nests in the ground, often under stones (Czechowski *et al.*, 2012).

Distribution in Romania. The most common *Lasius sp* in the country (Markó *et al.*, 2006).

29. *Lasius brunneus*

Biology. It occurs in deciduous forests, in decaying parts of living deciduous trees, under bark and in the wood, from the underground parts of the trunk to the main boughs. Very timid non-aggressive ants; foragers avoid open spaces (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

30. *Lasius platythorax*

Biology. In comparison with *L. niger*, it prefers more humid sites, being a quite oligothermophilic polytope of forests, but also in wet open habitats, especially mires. It tends to avoid anthropogenic sites. Builds nest in organic substrate, most frequently in dead wood (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

31. *Lasius emarginatus*

Biology. A quite xerophilic and one of the most thermophilic species of the subgenus *Lasius s. str.* in the Central-European; it occurs in dry forest and grasslands, especially of rocky sun-exposed habitats with sparse herb vegetation (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

32. *Lasius paralienus*

Biology. A quite thermophilic oligotope of dry grasslands, especially those on limestone substratum. Ants are hardly aggressive, even when the nest is in danger (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

33. *Lasius fuliginosus*

Biology. A fairly thermophilic oligotope of deciduous forest, encountered also in mixed and coniferous forests and in parls and old orchards. A dendrobiont that nests in cavities under the trunk and roots of usually living trees, both deciduous and coniferous or in holes at the base of the trees. The empty spaces are filled with carton nests of chewed wood impregnated with honeydew and reinforced by hyphae of myrmecophilic fungi (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

34. *Lasius flavus*

Biology. A fairly thermophilic ubiquist (eurytope), yet preferring open and sunny habitats. In especially high densities it occurs in meadows and pastures, where

nests with big soil mounds render cultivation and mowing difficult. Mounds are overgrown with moss, herbs and grasses (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

35. *Lasius alienus*

Biology. A fairly thermophilic oligotope of dry habitats, typical of grasslands, open rocky areas, sun-exposed forest edges and sparse warm forest, especially oak one; it prefers soils on limestone substratum. Nests, occasionally with small mounds, are built in the ground, under stone and pieces of wood (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant species (Markó *et al.*, 2006).

36. *Plagiolepis pygmaea*

Biology. Prefers open, xerothermous habitats where it nests in the soil or under the rock (Moscaliuc, 2009).

Distribution in Romania. Few localities are known for this species (Markó *et al.*, 2006).

SUBFAMILY DOLICHODERINAE

37. *Dolichoderus quadripunctatus*

Biology. A dendrobiotic species, an oligotope of warm, mainly deciduous forests. Nests in dead parts of living trees, under the bark or in dead tree trunks up to a height of several metres, and also in wooden constructions and even old stone walls. It occurs in sun-exposed wooded places-in forest edges, parks orchards, etc. (Czechowski *et al.*, 2012).

Distribution in Romania. A common ant specie, yet not abundant (Markó *et al.*, 2006).

38. *Tapinoma erraticum*

Biology. A stenotope of xerothermal grasslands (steppes, dry sunny meadows, open mountain slopes), especially with limy subsoil. Nests usually in the soil, occasionally with small mineral or organic mounds, under stones, rarely in dry empty plant stems (Czechowski *et al.*, 2012).

Distribution in Romania. A common species (Markó *et al.*, 2006).

CONCLUSIONS

Based on our findings, the current check-list consists of 38 ant species. Out of this, 11 species are new for the area, namely: *Camponotus ligniperdus*, *C. vagus*, *C. tergestinus*, *Crematogaster schmidtii*, *Formica lemani*, *Lasius platythorax*, *L. alienus*, *L. emarginatus*, *L. paralienus*, *Pheidole pallidula* and *Myrmica scabrinodis*.

Our results support that high diversity potential of the area and more such faunistical surveys may enrich the species knowledge in the area.

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DIVERSITY OF LAND SNAIL FAUNA IN CAPREI AND RÂMEȚ GORGES NATURE RESERVES (TRASCĂU MOUNTAINS, ROMANIA)

VOICHIȚA GHEOCA

The land snail fauna of limestone areas is particularly rich, because of the favorable conditions in this type of habitats. The paper presents the land snail fauna of two less studied natural reserves located in the Trascău Mountains, Caprei and Râmețului Gorges. A number of 57 land snail species were identified. Most of the snails were found in the forest habitat, where the presence of calcium in the substrate is completed by a high level of humidity and the diversity of microhabitats. The malacofauna of limestone walls is less diverse, but the species present here are developing large populations. Five endemic species were found, among which are the door snails *Alopia bielzi tenuis* and *Alopia livida iulii*.

Keywords: land snails, endemic species, diversity, Râmeț Gorges, Caprei Gorges, Trascău Mountains.

INTRODUCTION

Besides their cultural and historical values, the karst areas and the caves developed here are extremely valuable natural resources, hosting a wide variety of often unique ecological niches (Pipan & Culver, 2013), and therefore sheltering a large biodiversity including high species endemism (Culver & Sket, 2000).

Land snail communities are particularly rich on limestones as they generally require large amounts of calcium for their shells and eggs (Kerney & Cameron, 1979; Gärdenfors, 1992; Nekola, 1999; Horsák, 2006). Snails contribute significantly to the general biodiversity of the limestone areas, and are important contributors to invertebrate biomass, by developing large populations.

The Western Carpathians – Apuseni Mountains – include some of the most interesting karst areas in Romania. Many of them are well studied but some still remain with very poor reference regarding different groups. Besides the classical malacological works describing the malacofauna of Romania or Transylvania, including informations regarding the Apuseni Mountains (Bielz, 1867; Kimakowicz, 1890; Rotarides, 1930; Wagner, 1942; Grossu, 1981, 1983, 1986, 1987), more recently there are several papers concerning mostly the land snails of their western part (Bába & Sárkány-Kiss, 1999 a, b; Bába & Sárkány-Kiss, 2001; Domokos & Vánca,

2005; Domokos & Lennert, 2007; Lengyel & Páll-Gergely, 2010). The only recent paper focusing on the land snails of Trascău Mountains is that of Bába and Sárkány-Kiss (1998) regarding Cheile Turzii, while data from their southern part is included in the study of Cameron *et al.* (2011) regarding the forest land snails of Transylvania.

This paper is focusing on the land snail fauna of two limestone gorges located in the eastern and southern part of the Trascău Mountains, with poor previous specific reference regarding the land snail fauna.

MATERIAL AND METHODS

THE STUDY SITE

Cheile Râmețului Nature Reserve located in the central-eastern part of the Trascău Mountains, Central Romania consists of Jurassic limestone dominated by two limestone massives, Uzmezeu in north and Fundoi in south (Fig. 1). They are the remains of an old limestone plateau, in which the Geoagiu Valley has dug a very picturesque key (Fig. 2). The tourist pressure is relatively low in the area because of the distance from urban areas and the difficult access.

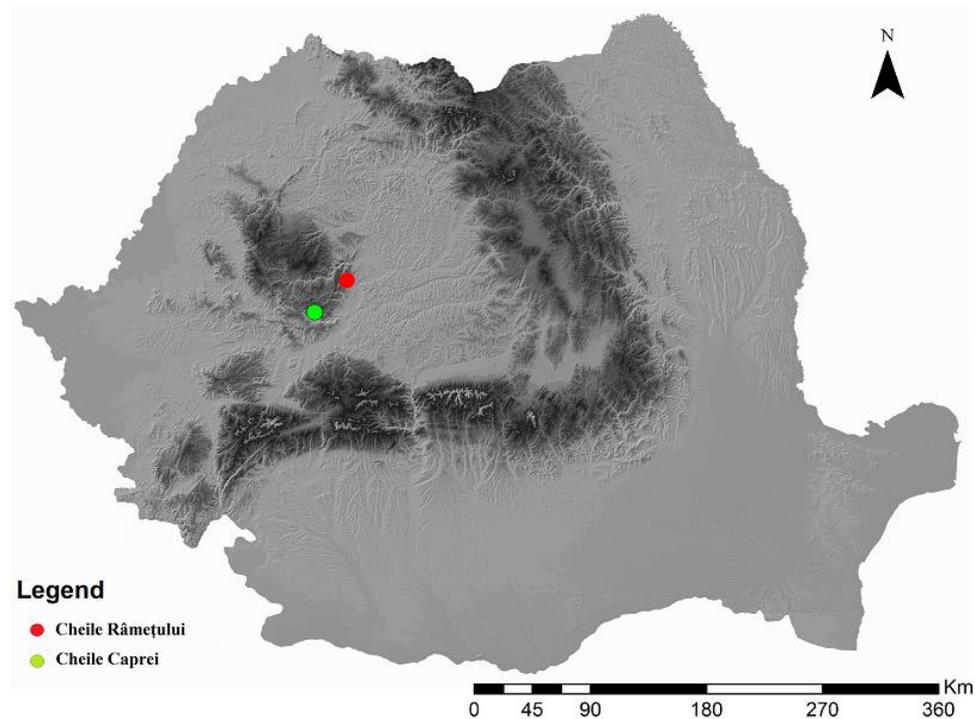


Fig. 1. The location of the study area (Râmeț Gorges and Caprei Gorges Nature Reserves).

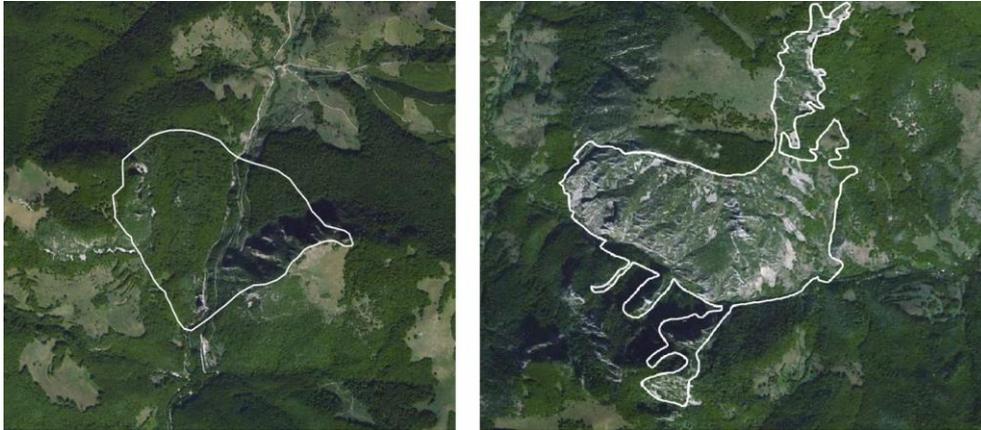


Fig. 2. The limits of Caprei Gorges Nature Reserve on the Feneș River valley (left) and Râmeț Gorges Nature Reserve on the Geoagiu River (right).

Cheile Caprei Nature Reserve (110 ha) is located in the southern part of the Trascău Mountains (Figs. 1–2). The reserve is also named Cheile Feneșului after the Feneș River that crosses the gray Jurassic limestones. On the west side, the reservation's boundary consists of the Dâmbăului plateau, with an altitude of 1200–1300 m, while at its eastern limit stays the Corabia massif. The relief reflects the difference in hardness of these geological formations and explains the two isolated rocks formed at the southern end of the keys, with heights of 67 and 75 m, which are called Pietrele Caprei (the goat's rocks), which give the name of the reserve (Cheile Caprei – the Goat's Gorges).

SAMPLING

The study was carried out in 2016 and 2017. Samples were taken in both locations from two habitat types, limestone walls and forest of *Fagus sylvatica* and *Carpinus betulus*.

The snails were collected by visual searching and for the microsnails leaf litter and soil samples were taken. Surface leaf litter and soil was collected after sieving through a 1 cm net. The litter samples were dried in laboratory, fractionated by sieving and sorted under a stereomicroscope. All the snails were preserved in 70% ethanol.

The snails were identified in the laboratory to species according to Kerney & Cameron (1979), Grossu (1981, 1983, 1985, 1987) and Welter-Schultes (2012). Nomenclature follows Fauna Europaea (Bank, 2017).

RESULTS AND DISCUSSION

A number of 57 land snail species were identified in the area of the two limestone gorges. The species list is presented in the Table 1.

Table 1

The list of land snail species identified in Caprei and Râmeț Gorges

	Family/Species	Caprei Gorges		Râmeț Gorges	
		forest	limestone walls	forest	limestone walls
	Family Aciculidae				
1.	<i>Platyla polita</i> (Hartmann, 1840)	x	x		
2.	<i>Platyla banatica</i> (Rossmässler, 1842)			x	x
3.	<i>Platyla perpusilla</i> (Reinhardt, 1880)	x	x	x	x
	Family Carychiidae				
4.	<i>Carychium tridentatum</i> Müller, 1774	x		x	x
	Family Cochlicopidae				
5.	<i>Cochlicopa lubrica</i> (Müller, 1774)	x			
6.	<i>Cochlicopa lubricella</i> (Rossmässler, 1834)		x		
	Family Pyramidulidae				
7.	<i>Pyramidula pusilla</i> (Vallot, 1801)	x	x		x
8.	<i>Pyramidula rupestris</i> (Draparnaud, 1801)				x
	Family Vertiginidae				
9.	<i>Truncatellina cylindrica</i> (A. Ferussac, 1807)	x	x	x	
10.	<i>Vertigo alpestris</i> Alder, 1838	x			
11.	<i>Vertigo pusilla</i> O.F. Müller, 1774			x	x
12.	<i>Vertigo pygmaea</i> (Draparnaud, 1801)			x	
	Family Pupillidae				
13.	<i>Pupilla muscorum</i> (Linnaeus, 1758)	x			
14.	<i>Pupilla triplicata</i> (Studer, 1820)			x	
15.	<i>Pupilla alpicola</i> (Charpentier, 1837)			x	
	Family Chondrinidae				
16.	<i>Granaria frumentum</i> (Draparnaud, 1801)		x		x
17.	<i>Chondrina arcadica</i> subsp. <i>clienta</i> (Westerlund, 1883)		x		x
18.	<i>Chondrula tridens</i> O.F. Müller, 1774			x	
	Family Orculidae				
19.	<i>Sphyradium doliolum</i> (Bruguere, 1792)	x	x	x	x
20.	<i>Orcula doliolum</i> (Draparnaud, 1801)				x
21.	<i>Orcula jetschimi</i> M. Kimakowicz, 1883	x			
	Family Strobilopsidae				
22.	<i>Spelaediscus triarius</i> (Rossmässler, 1839)	x	x	x	x
	Family Valloniidae				
23.	<i>Vallonia costata</i> (O.F. Müller, 1774)	x			x
24.	<i>Vallonia excentrica</i> Sterki, 1893				x
	Family Enidae				
25.	<i>Merdigera obscura</i> (O.F. Müller, 1774)	x			
26.	<i>Mastus bielzi</i> (M. von Kimakowicz, 1890)	x		x	

	Family Punctidae				
27.	<i>Punctum pygmaeum</i> (Draparnaud, 1801)	x		x	
	Family Vitrinidae				
28.	<i>Vitrina pellucida</i> (O.F. Müller, 1774)	x		x	x
	Family Pristilomatidae				
29.	<i>Vitrea transylvanica</i> (O.F. Müller, 1774)	x		x	x
30.	<i>Vitrea diaphana</i> (Studer, 1820)			x	
	Family Oxychilidae				
31.	<i>Aegopinella pura</i> (Alder, 1830)		x	x	
32.	<i>Aegopinella epipedostoma</i> (Fagot, 1879)	x		x	x
33.	<i>Oxychilus glaber</i> (Rossmässler, 1835)	x	x	x	x
34.	<i>Oxychilus draparnaudi</i> (H. Beck, 1837)	x	x	x	
35.	<i>Carpathica calophana</i> (Westerlund, 1881)	x		x	
	Family Euconulidae				
36.	<i>Euconulus fulvus</i> (O.F. Müller, 1774)	x			
	Family Clausiliidae				
37.	<i>Alopioides livida iulii</i> (Wagner, 1913)		x		
38.	<i>Alopioides bielzii tenuis</i> (Bielz, 1861)				x
39.	<i>Cochlodina laminata</i> (Montagu, 1803)	x			
40.	<i>Cochlodina orthostoma</i> (Menke, 1828)	x		x	
41.	<i>Cochlodina marisi</i> (Schmidt, 1868)			x	x
42.	<i>Ruthenica filograna</i> (Rossmässler, 1836)	x	x	x	x
43.	<i>Clausilia dubia</i> Draparnaud, 1805	x	x	x	x
44.	<i>Laciniaria plicata</i> (Draparnaud, 1801)	x		x	
45.	<i>Vestia elata</i> (Rossmässler, 1836)	x		x	
46.	<i>Vestia turgida</i> (Rossmässler, 1836)	x			
47.	<i>Pseudalinda stabilis</i> (Pfeiffer, 1847)	x		x	
48.	<i>Bulgarica cana</i> (Held, 1836)	x			
49.	<i>Bulgarica vetusta</i> (Rossmässler, 1836)	x		x	x
	Family Bradybeidae				
50.	<i>Fruticicola fruticum</i> (O.F. Müller, 1774)	x		x	
	Family Hygromiidae				
51.	<i>Trochulus bielzi</i> (Bielz, 1860)	x		x	
52.	<i>Euomphalia strigella</i> (Draparnaud, 1801)	x			
	<i>Perforatella dibothrion</i> (M. von Kimakowicz, 1884)	x			
53.	<i>Monachoides vicinus</i> (Rossmässler, 1842)	x		x	
	Family Helicidae				
54.	<i>Isognomostoma isognomostomos</i> (Schroter, 1784)	x		x	
55.	<i>Faustina faustina</i> (Rossmässler, 1835)	x	x	x	x
56.	<i>Drobacia banatica</i> (Rossmässler, 1838)	x		x	
57.	<i>Helix pomatia</i> Linnaeus, 1758	x			

Among the 57 land snail species, 47 species were present in Caprei Gorges, and 43 in Râmneț Gorges. A number of 29 species are common for the two studied areas, species characteristic for limestones or typical forest species. Since the area of exposed limestone cliffs in Râmneț Gorges is much more extensive than in Caprei Gorges, the diversity of limestone snails is higher, species like *Pyramidula rupestris*, *Pupilla triplicata*, *Orcula dolium*, *Vallonia excentrica* were found only here.

Higher diversity was found in forest compared to limestone habitats. Since the conditions are more favorable for the presence of land snails, some rock dwelling species cohabit here with typical forest species. Five endemic species were identified in the area: *Mastus bielzi*, *Orcula jetschini*, *Cochlodina marisi*, *Alopi* *bielzi tenuis* and *Alopi* *livida iulii*. Each of the gorges has its own endemic *Alopi* species, *Alopi* *bielzi tenuis* is present in Râmeș Gorges, while *Alopi* *livida iulii* inhabits Caprei Gorges. This endemic door snails are developing large populations living in the crevices of the limestone walls and feeding on algae.

The land snail fauna of Cheile Turzii, in the north-eastern area of the Trascău Mountains is richer, as reported by Baba & Sarkany (1998). The authors found there 55 species, to which are added those mentioned in the previous works, some of them having undergone over the time changes in nomenclature or being currently synonyms with other species. However, some species found in the eastern and southern area of Trascău Mountains, Râmeșului and Caprei Gorges are not present in Cheile Turzii. Such are *Bulgarica cana*, *Bulgarica vetusta*, *Vitrea transsylvanica*, *Pyramidula pusilla* and *Platyla banatica*.

CONCLUSIONS

The limestone area of the Trascău Mountains shelter a rich malacofauna, including endemic and protected species as is the case of *Drobacia banatica*, the Banat rock snail, one of the four land snail species present in Romania that is included in Annex II of EC Habitats Directive. Although the area is not subject of a very severe human disturbance, because of the fact that the gorges are difficult to access and not very popular, there are some potential threats that can subject the land snails. It is the case of rock climbing that is allowed on the two limestone blocks located at the entrance of the Caprei Gorges, the same area where *Alopi* *livida iulii* was found. This activity could severely menace the surviving of this subspecies in the area.

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NEW RECORD IN ROMANIAN DANUBE DELTA
PART AS AN EXTENSION IN THE LOWER DANUBE AREA
OF THE NON-NATIVE BRYOZOAN *PECTINATELLA*
MAGNIFICA (LEIDY, 1851)

AUREL NĂSTASE, SILVIU COVALIOV, MIHAI DOROFTEI,
GEORGE ȚIGANOV, VASILE OȚEL

The water flow of the Danube River brings in the Danube Delta a lot of solid silt, big quantities of waters, but sometimes new living organisms. So, in 2016 it was first recorded in freshwater of the Romanian Danube Delta part a new non-native species *Pectinatella magnifica* (Leidy, 1851), a colonial organism, bryozoan, after that in 2017 more colonies of individuals were found in the Danube Delta Biosphere Reserve (DDBR).

Keywords: new record, Romanian Danube Delta, bryozoan, *Pectinatella magnifica*.

INTRODUCTION

The Chilia, Sulina and Sfântu Gheorghe arms of the Danube River are major paths which through the river transport water and solid flow across the delta towards the Black Sea. Before branching at “Ceatal” Chilia, multiannual mean Danube flow is estimated at 6515 m³/s (Driga; 2004, Gâștescu & Știucă, 2008). According to the same authors, in the last century, the water flow in Chilia arm has decreased from 72% (1910) to about 54% possible less, at the beginning of the new millennium. The flow share of Tulcea arm increased from 28% to actually 46% or more after some unpublished scientific reports: less to Sfântu Gheorghe arm (from 20% to 25%), but especially because of the Sulina arm (from 8% to 23%, due to its continuous correction and dredging). The water flow from river discharge in the 3 units of the Danube Delta (Letea, Caraorman and Dranov units) about 5%, fuelling the lakes complexes (Bondar, 1994; Driga, 2004; Gâștescu & Știucă, 2008).

The consequences of biological invasions can be diverse, interconnected and complex (Zorić *et al.*, 2015). Invaders can alter fundamental ecological properties, such as the dominant species in a community, the productivity and nutrient cycling, and thereby they can modify the structure and functioning of the ecosystem (Mack *et al.*, 2000). The anthropogenic impact on the distribution of plants and animals is considered to be one of the major threats to biodiversity (Grigorovich, 2003). Aquatic ecosystems are not an exception when this aspect of disturbance is

considered. The ballast waters of ships, deliberate fish stocking and aquaculture are potential means of introduction of non-native species.

The constructions of artificial channels, that connect previously geographically isolated river basins, facilitate the intensive dispersal of species and greatly contribute to the spread of non-native taxa (Leuven *et al.*, 2009). This scenario has occurred at different sections along the Danube River. The river belongs to the Southern Invasion Corridor that links the Black Sea Basin with the North Sea Basin via the Danube and Main-Rhine Canal (reopened in 1992).

This corridor is one of the four principal routes for entry of invasive non-native aquatic organisms into Europe (Panov *et al.*, 2009). This complex system of interconnected river basins and artificial channels (the Danube Delta, the Danube River, the Main – Danube Canal, the Main River, and the Rhine River) facilitates the spread of non-native taxa in both downstream and upstream directions throughout the Danube River Basin. The Danube River and its main tributaries are also exposed to aquatic invasions, *e.g.* the rivers Sava (Paunović *et al.*, 2008; Žganec *et al.*, 2009), Tisa (Tomović *et al.*, 2013) and Velika Morava (Tomović *et al.*, 2012; Zorić *et al.*, 2013).

Despite intensive research, it is still not possible to assess the real consequences of aquatic invasions and to provide effective solutions for proper management, especially in the case of large and complex systems such as the Danube River. A certain amount of progress has been achieved in evaluating the pressures of biological invasions on particular aquatic assessment units (Olenin *et al.*, 2007; Arbačiauskas *et al.*, 2008; Panov *et al.*, 2009; Tricarico *et al.*, 2010).

However, considerable efforts still need to be undertaken in order to fully understand invasion processes (Zorić *et al.*, 2015).

The freshwater species *Pectinatella magnifica* (Leidy, 1851) (Bryozoa: Phylactolaemata: Plumatellida) is a non-native taxon exhibiting considerable long-distance spread, well away from its natural distribution range. This taxon is native to the eastern part of North America (from Ontario in Canada to Florida in the United States of America) (Zorić *et al.*, 2015).

However, nowadays it can be found in other parts of the USA (Balounová *et al.*, 2013). Its presence has been reported from several European countries, including Germany (Kraepelin, 1887; Grabow, 2005), France (Rodriguez, Vergon 2002; Devin *et al.*, 2005; Nott Enghem, 2009), Czech Republic (Opravilova, 2005, 2006; Balounová *et al.*, 2011), Poland (Balounová *et al.*, 2013), Austria (Bauer *et al.*, 2010), Hungary (Szekeres *et al.*, 2013), Ukraine (Aleksandrov *et al.*, 2014) and from Asia Minor (Lacourt, 1968). In the Ukrainian part of the Danube Delta the abundance of *P. magnifica* is found mainly associated with *Phragmites australis* reedbeds that line the river (Aleksandrov *et al.*, 2014). It is believed that the species was introduced to Europe in the 19th century. First it was reported in Hamburg in 1883 (Bernauer & Jansen, 2006).

The riverbed of the Danube at the sites (Figs. 1–2) where the magnificent bryozoan (Aleksandrov *et al.*, 2014; Zorić *et al.*, 2015) was recorded consisted

predominantly of silt-clay and very fine sand substrate (mineral substrate classification according to Verdonschot (1999): grains not visibly perceptible; < 0.125 mm). The bank area at the sites was characterised by dense associations of aquatic vascular macrophytes.

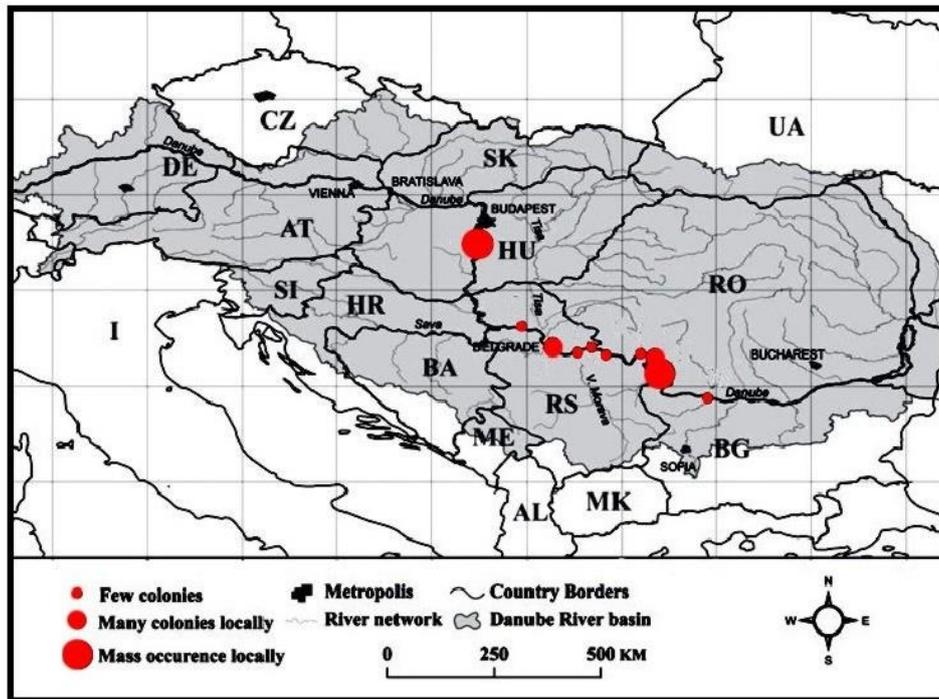


Fig. 1. Map showing the sites along the River Danube with records of *Pectinatella magnifica* (after Zorić *et al.*, 2015).

The magnificent bryozoan is a colonial organism with ciliated tentacles that are attached to a large gelatinous mass (Pennak, 1989; Wood, 2010). The typical size of the colonies is between 10 and 20 cm, while the diameter of large colonies can be up to two meters. It feeds on diatoms, green algae, cyanobacteria, non-photosynthetic bacteria, dinoflagellates, rotifers, protozoa, small nematodes and microscopic crustaceans (Callaghan & Karlson, 2002). As in all bryozoan species, the life cycle of *P. magnifica* includes both sexual and asexual reproduction. During favourable temperature conditions (in temperate climate zone between May and June (Rodriguez & Vergon, 2002), *P. magnifica* reproduces sexually. Asexual reproduction includes simple bulking and formation of new individuals, but also formation of statoblasts that enable survival during unfavourable conditions, at lower temperature and during periods of draught. *Pectinatella magnifica* is a thermophilous species. The details of its life cycle, including literature reviews, are given in Rodriguez & Vergon (2002).

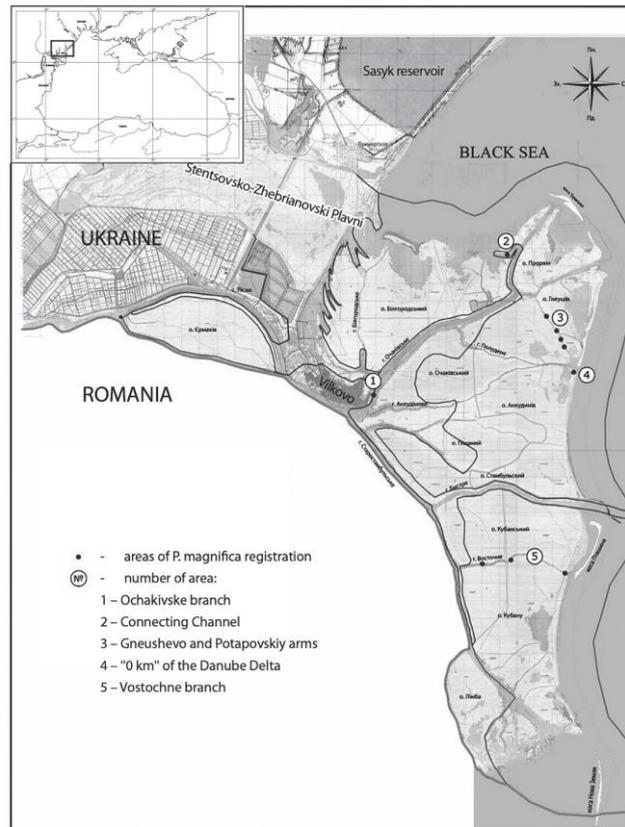


Fig. 2. Schematic Map showing the sites in Lower Danube in Ukrainian Danube Delta part (Kiliya branch) with records of *Pectinatella magnifica* (after Aleksandrov et al., 2014).

Close to Lower Danube River and Danube Delta always a new species stay to enter with water flow or with other vectors. For example, already adapted are some non-native species in Danube Delta Biosphere Reserve condition like: plants (*Amorpha fruticosa*, *Elodea canadensis*, plus other 54 plant species), molluscs (*Corbicula fluminea*, *Anodonta woodiana* and other 4 molluscs species), Decapoda-Crustacea species *Eriocheir sinensis* (Oțel, 2003–2004), fish species *Pseudorasbora parva*, *Hypophthalmichthys molitrix*, *H. nobilis*, *Ctenopharyngodon idella*, *Liza hematocheila*, *Lepomis gibosus*, *Percarina demidoffi* and the newest recorded species *Perccottus glenii* (recorded in 2007 by Năstase, actually acclimatised in the Danube Delta) fish species escaped from aquaculture, accidentally introduced or naturally entered. Also, *Phasianus colchicus*, bird and *Ondatra zibetica*, *Nyctereutes procyonoides* mammals are now found in the fauna of the Danube Delta.

The objective of this paper is to present first record of *P. magnifica* in the Romanian Danube Delta part based on the 2016 survey and image from territory, also many other colonies of *P. magnifica* were found in 2017.

MATERIAL AND METHODS

STUDY AREA AND SAMPLING PERIOD

Study area represents inferior sectors of the Danube River and lakes or canals from the Danube Delta. Sampling with direct observations was performed in the period May–September 2016, further more in 2017, but also a close relation with local's peoples and delta enthusiasts was very important in finding species first time.

The sampling methods for the Danube Delta include also collaborations with locals or environmental cares, which in *Pectinatella magnifica* case was beneficial to observe the species.

TAXONOMY AND ECOLOGY

The scientific name of species used is according to International Code of Zoological Nomenclature (ICZN).

RESULTS AND DISCUSSION

In the summer of 2016 a new species for Danube Delta – colonies of *Pectinatella magnifica* were found by chance by a local people, from Iacob Lake (Roşu-Puiu lakes-complex, the Danube Delta).

The freshwater bryozoan *P. magnifica* (Fig. 3) was recorded first time in the Danube Delta in Iacob Lake in the summer of year 2016 (Fig. 4), more individuals were found in Cazanele Channel in 2017 (Figs. 4–5).



Fig. 3. Colony of *Pectinatella magnifica* on the submerged stem of *Trapa natans* (the Danube Delta, Iacob Lake).

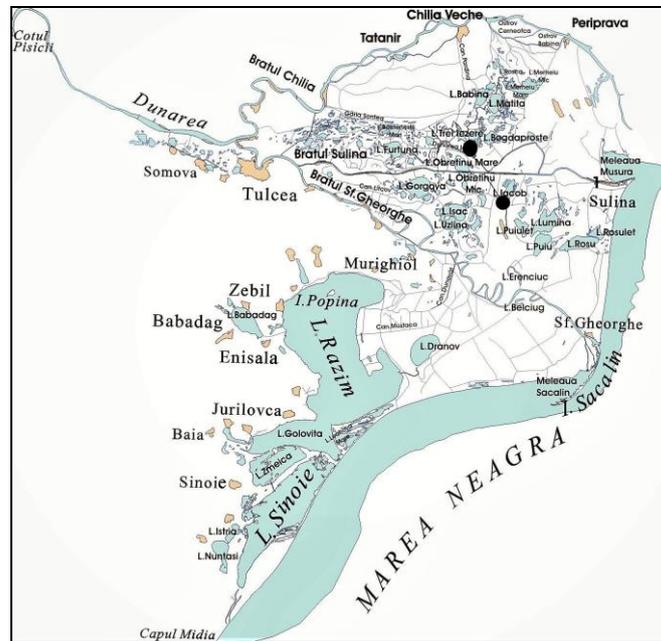


Fig. 4. Romanian Danube Delta part: the place (Iacob Lake) where *P. magnifica* was first recorded in 2016 (Southern black dot), more individuals were found in Căzânele Channel and neighborhood in 2017.



Fig. 5. Colony of *Pectinatella magnifica* stick on the submerged parts of some macrophytes, usually reed (Danube Delta, Căzânele Channel) observed in 2017.

The colonies were found on aquatic macrophytes (mostly *Trapa natans* species, as *Trapa natans* Kárpáti, 1963) (Figs. 3–4) and woody debris of reed, submerged in the water (Figs. 4–5), mostly along the shore in channel or shallow lake (0.5–1.5 m deep). The recorded colonies were formed near the surface of the water, up to a depth of 15–30 cm.

The size of the colonies ranged between 10–15 cm in diameter.

Since the initial detection of the magnificent bryozoan in the Rackeve-Soroksar Danube River side arm in 2011 (Szekeres *et al.*, 2013), it rapidly colonised a 900 km-long stretch of the Danube River. The organism is already a well-established inhabitant of the entire length of the Rackeve-Soroksar Danube River arm (Szekeres *et al.*, 2013) and Zorić *et al.* (2015) data has confirmed the frequent appearance of extensive colonies of *P. magnifica* in the most downstream stretch of the side arm, immediately upstream from the lock.

Since its introduction to Europe in the 19th century, *P. magnifica* has invaded many parts of Europe (Kraepelin, 1887; Lacourt, 1968; Rodriguez & Vergon, 2002; Devin *et al.*, 2005; Grabow, 2005; Opravilova, 2005, 2006; Nott Enghem, 2009; Bauer *et al.*, 2010; Balounova *et al.*, 2011; Aleksandrov *et al.*, 2014; Zorić *et al.*, 2015) and Asia Minor (Lacourt, 1968). The species was given as a present also in Romania (Lacourt, 1968), data taken from Chirică (1906) (first record of species in Romania in Jijia river) and Căpușe (1962) (in Greaca Lake, near Danube, actually dry lake); other authors who have systematically dealt with bryozoan were Băcescu & Skolka O. (1982, 1983) (data taken from both Romanian authors Chirică and Căpușe), last record of species belonging to Cogălniceanu Dan 2012 (between discharging the river Nera and Orșova in the Danube), personal communication to Skolka Marius, whose thinking is that species has “in jumps” development, the appearance of large and visible colonies being favored by certain external factors like temperatures. The species has also spread in North America, and is now found in Canada (Benson & Cannister, 2014), Texas (Neck & Fullington, 1983) and in 18 lakes in the Pacific Northwest, including the states of Idaho, Oregon and Washington (Marsh & Wood, 2002).

Based on Zorić *et al.* (2015) results, as well as on recent studies of other authors (Opravilova, 2005; 2006; Devin *et al.*, 2005; Grabow, 2005; Nott Enghem, 2009; Bauer *et al.*, 2010; Balounová *et al.*, 2011; Szekeres *et al.*, 2013) it can be speculated that this species is becoming increasingly common in areas outside its range.

The possible reasons for this species’ invasiveness are related to its autoecological characteristics and changes of its freshwater habitats (Zorić *et al.*, 2015).

The results of Zorić *et al.* (2015) suggest that the changes in habitats and reduced flow regimes provided favourable conditions for invasion by *P. magnifica*. Aside from habitats that are typical for this species (reservoirs) fish ponds and other aquatic habitats with altered hydrological conditions are also potentially suitable recipient ecosystems for the magnificent bryozoan.

Aquaculture (Seo, 1998; Nott Eghem 1999) and zoochory, dispersal of statoblasts by birds (Oda, 1974) are likely vectors for the spread of this invasive species.

The effect of the magnificent bryozoan on native ecosystems is still unknown. Mass occurrence of *P. magnifica* is suggested to improve water quality during the initial period of colonisation of new habitats (Zorić *et al.*, 2015). Wood (2010) described increased transparency of water due to removal of suspended particles as a result of the feeding of individual zooids as a long term effect of colonisation. This in turn establishes conditions for increased algal production, which can severely affect the functionality of the aquatic ecosystem.

With regard to a more direct impact on humans, mass occurrence of the magnificent bryozoan has been reported to clog the drainage systems and water pipes in North America, and to cause unpleasant smell when large colonies remain in dried out areas after water level drawdown (Wood, 2010). But on the other hand, according to the experimental data obtained by Pejin *et al.* (2016), *P. magnifica* methanol extract may be considered as a good resource of novel natural products with potent antibiofilm activity against the bacterium (*Pseudomonas aeruginosa* PAO1) well known for its resistance.

Authors' opinion of coming in the Danube Delta Biosphere Reserve from Europe of *P. magnifica* is because of disperse species with solid flows transport by Danube's waters among the same native condition for species, into a general global warming of climate.

CONCLUSIONS

New record in 2016 for Romanian part of the Danube Delta is non-native colonial bryozoan *Pectinatella magnifica* (Leidy, 1851). Future studies are needed to determine the impact this species has on the Danube Delta ecosystems.

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A DECISION-SUPPORT MANAGEMENT SYSTEM DESIGNED FOR *EUDONTOMYZON DANFORDI* REGAN, 1911 POPULATION OF UPPER TÂRNAVA MARE RIVER

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DORU BĂNĂDUC*

The ADONIS: CE free software has been used for one protected fish species, *Eudontomyzon danfordi* Regan, 1911, to create and adapt an on-site (upper Târnava Mare River) support-system model for local management decision-making. Habitat requirements and indicators of good protection condition have been examined, pressures and threats to these fish species have been identified, and management proposals have been recommended. This adapted management system permits an on-site and species applicable management elements implementation for the local lotic habitats recovery.

Keywords: Carpathian lamprey, habitat requirements, human impact, management, Transylvania, Romania.

INTRODUCTION

The waters of Romanian Carpathians high-medium altitude streams and rivers are generally of excellent to very good and good characteristics where the human impact is not critical (Breabăn & Romanescu, 2014; Romanescu *et al.*, 2016).

Nowadays, fish are one of the most worthwhile conservative and economic animal groups which are influenced by both natural conditions and a high number of human activities effects (Lenhardt *et al.*, 2016; Radhi *et al.*, 2017; Năstase & Oțel, 2017; Khoshnood, 2017; Balasaheb *et al.*, 2017; Florea, 2017).

The European Union component states gave their agreement in 1992 for the Habitats Directive, to admit different species of European Community concern to prosper, in compliance with the responsibility to protect the species and habitats belonging to this Directive (Annex 2), by conserving their status (*, 1992).

This aim of this study was to develop a model for a management tool for one of the most valuable conservation fish species of the Târnava Mare upper sector, the Carpathian lamprey. In nature protection, modelling is regularly used to obtain a “general image” of distinctive systems and/or actions within certain areas. The components of the modelling process support discerning distinguishable levels of

species and their habitat management. The use of ADONIS: CE free software, can design models that support a local adapted management structure. The models target three central functional areas, relevant for environment protection: 1) to validate the existing state, 2) to determine the consequences of changing, and 3) to recommend a programme to improve the actual state in a needed way. In the end, different diagrams can be produced to reveal the significant elements of management. (Hall & Harmon, 2005)

MATERIAL AND METHODS

The streams and rivers of the Târnava Mare Watershed occupy the interior area of the Romanian Carpathians. With a watershed of 3,606 km² and a length of 221 km (Badea *et al.*, 1983), Târnava Mare River is one of the principal rivers of Transylvania.

Eudontomyzon danfordi Regan, 1911 (Fig. 1), is one of the most elusive protected fish species of the Romanian Carpathians; usually specific designed management plans for this species populations are missing, introducing the need for new management elements (Bănăduc, 2011).



Fig.1. *Eudontomyzon danfordi*.

The condition of this fish species population was evaluated based on elements containing: the dimension of fish populations; the dimension of the population distribution in the researched area; the balanced distribution of the sampled individuals in age categories; and maximum and minimum numbers of this fish species in fish communities. The habitat necessities, pressures and threats on *Eudontomyzon danfordi* were researched in relation with their ecological status, the relations among them and the conservation context of this species.

A flexible management model was created to bring together a fitting management plan that would determine the preservation of the researched fish species, with an accent on needed processes. The ADONIS: Community Edition (ADONIS: CE) free software, produced by the Business Object Consulting (BOC) Group, was used in this study. This free software is in an advantageous form of ADONIS with some restrictions (compared to the commercial version). It uses a Business Process Model and Notation (BPMN), a standardized modelling terminology that holds up recognizable processes structure. ADONIS:CE is commonly used as an access point to Business Process Management. These processes can be modelled using compatible notation. (**)

RESULTS AND DISCUSSION

IDENTIFIED HUMAN PRESSURES AND THREATS

In the researched upper Târnava Mare River sector, from its springs area to downstream Zetea Dam lake, based on the Biotic Integrity Index for Carpathian river assessment score values (Bănăduc & Curtean-Bănăduc, 2002) the local Carpathian lamprey ecological state varies significantly, this index scores vary from 45 – excellent (excellent, comparable to pristine conditions, exceptional assemblage of fishes) to 10 – poor (very few species and individuals present, tolerant species dominant).

In the studied area, the following significant pressures and threats on *Eudontomyzon danfordi* were identified, as a cumulative result the local natural fish associations of the trout lower zone and grayling and Mediterranean barbel zone were partially changed or replaced by modified fish association (Bănărescu, 1964; Bănăduc, 2005) condition induced by the human impact. These principal pressures and threats are: the typical habitats change or loss, the lotic system sectors continuum fragmentation provoked by the hydrotechnical works, the disorganized and sometimes lawless stocking and restocking, poaching, the organic pollution of water, the logging of lotic systems riparian trees vegetation, and the expansion of some invasive/more tolerant fish populations (*Squalius cephalus*, *Gobio gobio*, *Barbus meridionalis*, etc.), species that are not naturally a prey target for *Eudontomyzon danfordi*.

IDENTIFIED SPECIFIC REQUIREMENTS

The researched fish species required: relatively high level of water sectors; relatively variable water flow sectors; relatively fast speed of water flow sectors; relatively moderate to low speed water flow sectors; cold water; oxygenated water; stones/rocky river bed sectors; sandy-muddy river bed sectors; heavy shadowed banks (Bănărescu, 1969).

Last but not least, the lack or low basin lotic systems sectors connectivity, chaotic restocking, poaching and non-native species presence have highly negative importance.

PROPOSED SPECIFIC HABITAT INDICATORS

In the researched mountain river sectors, the principal habitat indicators are recommended in this paper as argumentation for the presence/absence and relative abundance of *Eudontomyzon danfordi*: relatively high level of water (70%); relatively variable water flow (70%); relatively fast speed of water flow (60%); relatively moderate to low speed water flow (40%); cold water (90%); oxygenated water (90%); stones/rocky river bed (70%); sandy-muddy river bed (30%); heavy shadowed banks (90%); lotic systems connectivity (100%); chaotic stocking and restocking (0%); poaching (0%); non-native fish species presence (0%).

MANAGEMENT MEASURES

According with this suggested model we proposed that the most influential management elements are: preservation of the lotic systems natural hydrology, preservation of the natural morphology of the lotic system and its banks; no riverbed mineral exploitation should be allowed in the studied area; preserving the vegetation of the basin on the banks riverine valley slopes; a ban on the disposing of any type of waste in streams and rivers; keeping a permanent high/medium level of the water on river sectors markedly in drought cold and/or warm seasons based on avoidance of high water derivations; decreasing water organic pollution.

All the hydrotechnical works should have species adapted fish pass facilities, stocking and restocking should be rigorously guarded from the scientific point of view, poaching should be banned, and non-native species should be diminished or eradicated through targeted fishing.

ADJUSTED MODEL FOR THE SITE MANAGEMENT

The modeling of the species *Eudontomyzon danfordi* was designed using three processes (Fig. 2): the presentation of the species *Eudontomyzon danfordi* (Fig. 3), the possible habitat indicators (Fig. 4) and the management measures to be taken for the species to ensure its existence (Fig. 5).



Fig. 2. The processes of *Eudontomyzon danfordi* model.

MODEL DESCRIPTION

The process from which it was started is "Species *Eudontomyzon danfordi*" (Fig. 3), which specifies: habitat type, critical habitat requirements, pressures and threats from people, as well as possible indicators measured and analyzed on the ground (they were modelled as a sub process to understand and visualize them more easily). After the indicators have been completed, a decision is made to check whether the indicators ensure the favorable conservation status of the species. If it is fulfilled, the Yes branch of the decision (variable: Favorable_conservation_state='YES', probability: 0.15%) then follows the activity "Human pressures and threats" and the process is closing. If the conservation status is not favorable, the No branch (variable: Favorable_conservation_state='NO', probability: 0.85%), then return to the activity "Specific requirements" and once again go through the "Indicators of *Eudontomyzon danfordi*" subprocess.

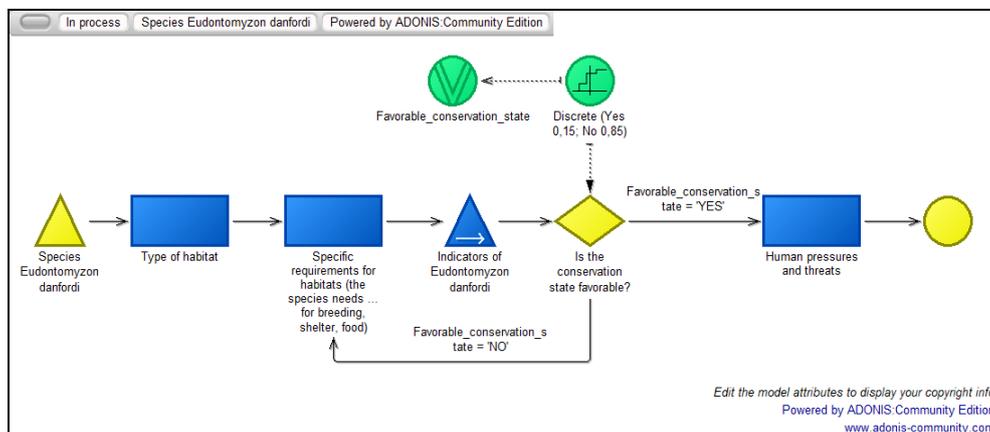


Fig. 3. *Eudontomyzon danfordi* species – basic process.

The "Indicators of *Eudontomyzon danfordi*" sub process (Fig. 4) goes through all the indicators outlined above and highlights through decisions the likelihood that

they will meet the favorable conservation status of the *Eudontomyzon danfordi* species. The percentage was determined by field measurements, comparing the current state with favorable conservation status. If we go through all the decisions on the “YES” branch (selection of variables: probability: probability:

High_level_of_water='YES',	probability:	0.71%;
Natural_relatively_cool_water='YES',	probability:	0.66%;
Stones_rocky_river_bed='YES',	probability:	0.85%;
Sandy_mud_river_bed='YES',	probability:	0.99%;
Heavy_shadowed_banks='YES',	probability:	0.55%;
Lotic_systems_connectivity='YES',	probability:	0.02%;
Non_native_fish_species='YES',	probability:	0.50%

then the species is in a favorable conservation status and the process ends with the “Implementation of an integrated monitoring system” activity.

If the indicators do not fulfill the favorable conservation status, the branch of “NO” (variables:

High_level_of_water='NO',	probability:	0.29%;
Natural_relatively_variable_water_flow=' NO',	probability:	0.29%;
Natural_relatively_fast_speed_water_flow=' NO',	probability:	0.15%;
Natural_relatively_moderate_to_low_speed_water_flow=' NO',	probability:	0.25%;
Natural_relatively_cool_water=' NO',	probability:	0.34%;
Natural_relatively_oxygenated_water=' NO',	probability:	0.34%;
Stones_rocky_river_bed=' NO',	probability:	0.15%;
Sandy_mud_river_bed=' NO',	probability:	0.01%;
Heavy_shadowed_banks=' NO',	probability:	0.45%;
Lotic_systems_connectivity=' NO',	probability:	0.98%;
Chaotic_restocking=' NO',	probability:	1%;
Poaching=' NO',	probability:	1%;
Non_native_fish_species=' NO',	probability:	0.50%),

then the “Management measures” subprocess (Fig. 5) is called, after which it returns to check the indicator, forming a loop. It can only come out of the loop when that indicator ensures the conservation status of the species.

The last subprocess (Fig. 5) is structured using ten activities presenting the management measures that should be considered for the welfare of the *Eudontomyzon danfordi* species. These have been outlined above.

Concluding, the model of *Eudontomyzon danfordi* species provides the presentation of the species characteristics, the visualization of the collected data, shows the possible habitat indicators and their percentage, as well as the management measures that should be taken into account for species preservation. An overview of the model can be seen in Fig. 6.

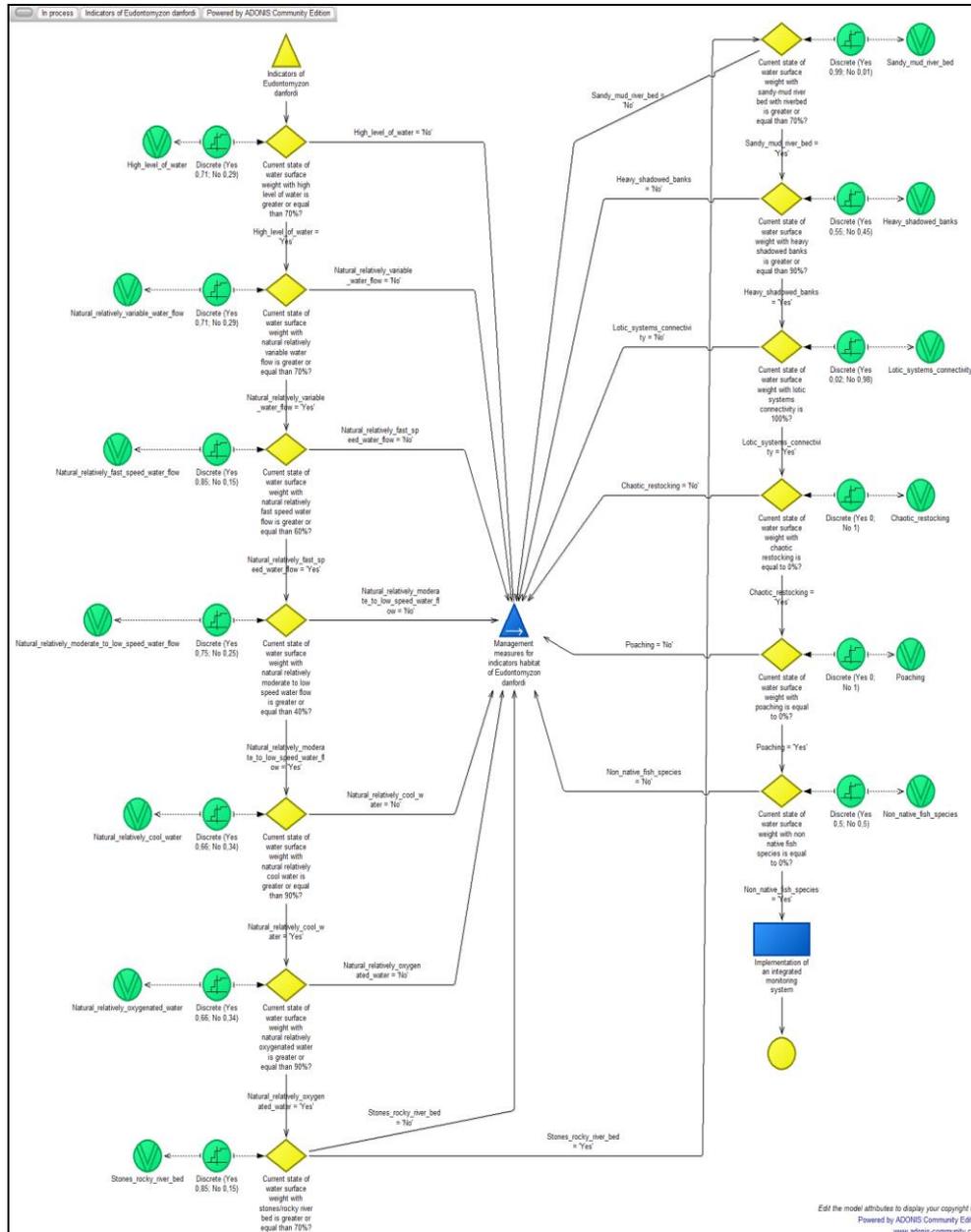


Fig. 4. Critical requirements for *Eudontomyzon danfordi* species – subprocess.

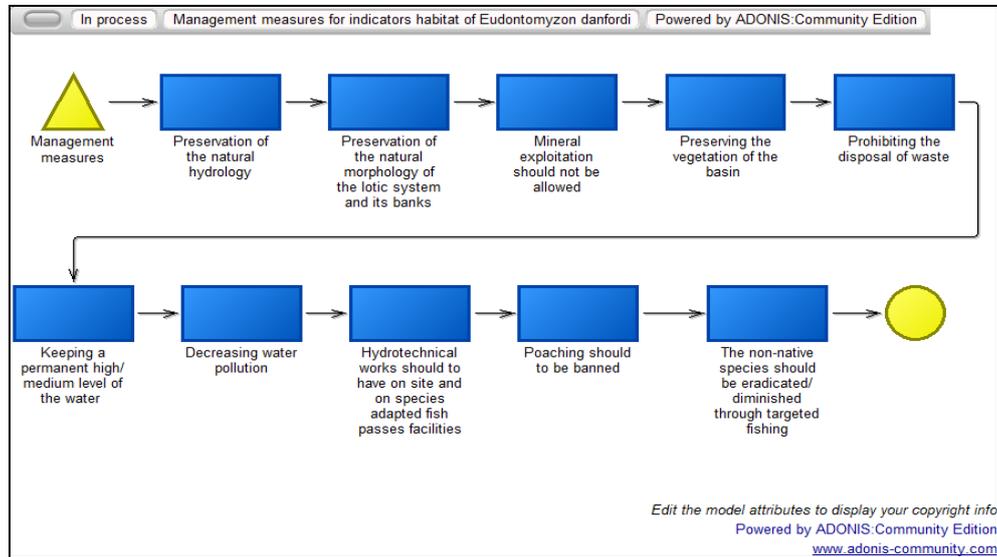


Fig. 5. Management measures for indicators of *Eudontomyzon danfordi* – subprocess.

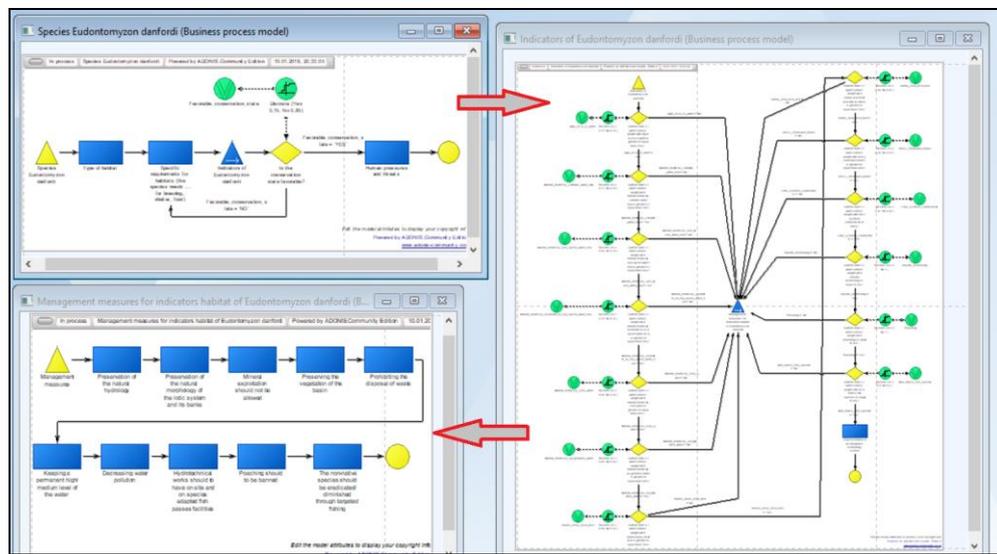


Fig. 6. Overview of *Eudontomyzon danfordi* model.

CONCLUSIONS

The preeminent pinpointed pressures and threats on *Eudontomyzon danfordi* researched populations ecological status in upper Târnava Mare River are change or loss of typical habitats, lotic system sectors continuum fragmentation, fish stocking and restocking, poaching, pollution, logging, and invasive/more tolerant fish species.

Necessary management measures for *Eudontomyzon danfordi* are: preservation of the natural hydrology and geomorphology; no riverbed mineral exploitations; vegetation protection; a ban on the disposing of any type of waste in streams and rivers; keeping a permanent high/medium level of the water on river sectors; decreasing water pollution. All the hydro technical works should be adapted with local fish fauna passes upstream and downstream; stocking and restocking should be rigorously scientifically supervised, poaching should be banned, and the non-native species should be diminished by targeted fishing.

The ADONIS: CE software was used in this research to design a management model for *Eudontomyzon danfordi*, a valuable fish species from conservation point of view. This management model contains the main requirements for lotic habitat, and the indicators that highlight good ecological conditions – the management measures, and the threats and pressures which influence this fish species. We advocate that this management instrument be used to design a much complex management model for all the fish fauna in the region.

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LENGTH-WEIGHT RELATIONSHIP OF *TILAPIA ZILLII* AND *SAROTHERODON GALILEAUS* REARED IN CAGES IN ODEDA LAKE, OGUN STATE, NIGERIA

IYABODE OLUSOLA TAIWO*, OLANIYI ALABA OLOPADE**

Comparative study was conducted to evaluate the growth performances of *Tilapia zillii* and *Sarotherodon galileaus* fingerlings caged reared using length-weight (LW) relationship technique. The water quality parameters were also assessed in the cages and outside the cages. The mean value of pH (7.03) inside the cages were slightly higher than outside the cage (6.80) similar results were observed for water temperature, ammonia and Total dissolved solid. The mean value of dissolved oxygen (9.17mg/L) and conductivity (1024 microfarads) inside cages were lower than outside the cages 11.07mg/L and 1356 microfarads respectively. The length-weight relationship showed that weight of the fish species increased with total length in both species. The length – weight relationship equations were given as follows: *Tilapia zillii* $\text{Log } W = -0.34 + 1.21 \text{Log } L$; $R^2 = 0.985$; *Sarotherodon galileaus* $\text{Log } W = -0.32 + 1.21 \text{Log } L$; $R^2 = 0.959$. *Sarotherodon galileaus* grew better and faster than *Tilapia zillii* indicating that *Sarotherodon galileaus* species are preferable for cage culture than *Tilapia zillii*. Odeda Lake contains water of acceptable quality suitable for cage culture.

Keywords: Cage culture, Length-weight relationship, water quality, Odeda Lake.

INTRODUCTION

Cage culture is not new in Nigeria. However, the practice has remained at the experimental level and unpopular among Nigerian fish farmers. There are encouraging studies on the viability of cage culture in Nigeria (Otubusin, 1989; Otubusin & Olatunde, 1992). Otubusin (1997) reported on cage culturability of some commercial important fish Viz. *Ctharinus citharus*, *Oreochromis niloticus*, *Clarias gariepinus*, *Sarotherodon galilaeus*, *Heterobranchus bidorsalis*, *Tilapia zillii*, *Alestes dentex* and *Distichodus rostratus* in Lake Kainji, Nigeria. All these scientific contributions have not translated to large scale cage culture either at the subsistence or commercial levels despite the vast Nigerian aquatic medium of numerous water bodies like rivers, streams, lakes reservoirs, flood plains, irrigation canals and coastal swamps which offer great potentials for cage culture in Nigeria.

In countries such as Nigeria which have similar socio-economic standards, cage culture has provided spectacular opportunities for creation of employment and large scale fish production. It is acknowledged that research in aquaculture is not always sufficiently geared towards ensuring commercial viability of aquaculture or benefits to end users. Williams *et al.* (1983) report that research into cage culture has been limited mainly because large scale open pond culture was more economically viable and, therefore, it received most of the research focus.

However, Beveridge (1987) opines that cage culture offers the farmer a chance to utilize the existing water resources which in most cases have only limited use for other purposes. This flexibility makes it possible to exploit underused water resources to produce fish. It could be operated in any type of aquatic environments. It is cheaper and more affordable than other forms of aquaculture practices.

A clear understanding of the growth of farmed fish is necessary for accurate prediction levels. The length-weight relationship has also a biological basis as it depicts the pattern of growth of fishes. Its importance is pronounced in estimating the average weight at a given group (Beyer, 1987) and in assessing the relative well-being of a fish population (Bolger & Connolly, 1989). Nevertheless, the biology of many tilapines in natural systems is well documented (Fryer & Iles, 1972; De Silva, 1985; Tudorancea *et al.*, 1988; Stewart, 1988; Getachew and Fernando, 1989; Robotham, 1990; Gómez-Márquez *et al.*, 2003). There have been significant developments in the farming of tilapias globally, even in Nigeria. Due to the increasing commercialization and continual growth of the tilapia industry, the tilapia fish commodity is the second most important farmed fish globally, next to carps and it is also the most important aquaculture fish species of the 21st century with about 98% of tilapia produced in these countries grown outside their original habitats (Shelton, 2002). The fish is being farmed in about 85 countries worldwide (FAO, 2016).

However, information on the length-weight relationship of tilapia species in cage culture is limited. The objectives of this study were: (i) to determine the length – weight relationship of both *Sarotherodon galileaus* and *Tilapia zillii* cultured in cages and (ii) to determine the effects of the water quality parameters on the growth rate of fish.

MATERIALS AND METHODS

Six cages were constructed with aluminium pipes and synthetic net, each with a dimension of 1m × 1m × 1m. Fingerlings of *Sarotherodon galileaus* and *Tilapia zillii* were purchased from fishermen at Odeda fish farm. They were then acclimatized in floating net cages for two weeks before they were stocked for monoculture trials. 50 fingerlings with mean weight $3 \pm 0.13\text{g}$ were stocked per cage, three cages for *Tilapia zillii* and three cages for *Sarotherodon galileaus* respectively.

The fishes were fed twice daily, in the morning and the evening at (5%) of their body weight. The proximate analysis of feed was 42% crude protein, crude fat 13%, crude fibre 1.8%, ash content 7.4%, phosphorus 1%, vitamin A1500iu/kg, Vitamin D3 2000iu/kg, Vitamin E 200mg/kg, Vitamin C 159mg/kg and Cu 5mg/kg.

At the end of the experiment (12 weeks), all fish from each experimental cage were counted. The total length was measured using a measuring board to the nearest cm. Each fish sample was weighed using a spring platform scale to the nearest gram. The length-weight (log-transformed) relationships were determined by linear regression analysis and scatter diagrams of length and weight were plotted.

Water temperature, dissolved oxygen, pH, ammonia, total dissolved solid and conductivity were measured weekly following standard methods (APHA, 2005).

Pearson moment correlation test was used to establish the relationship between the water quality parameters inside and outside the cage in Odeda Lake and its effect on the growth of fish.

RESULTS

WATER QUALITY

The results of the physicochemical parameters of the water samples inside and outside cages are presented in Tables 1 and 2. The mean pH varied between 6.7 and 7.03 inside the cages while outside the cages the pH ranged from 6.5 to 7.0. The mean temperature of the water inside the cages varied between 29°C and 30°C and was slightly higher than outside the cages with mean values of 27 to 28°C. The DO ranged from 9.0 to 9.5 mg/L inside the cages while outside the cages the values were slightly stable and higher (11–11.07 mg/L) than inside the cages. The mean values of the total suspended solids (TSS) ranged from 124–190 ppm inside the cages and 60–80 ppm outside the cages.

The mean values of the ammonia oscillated between 0.9241 mg/L and 1.0822mg/L inside the cages and also between 0.93 mg/L and 1.0092 mg/L for outside the cages. Conductivity values inside and outside the cages were constant 1024 microfarads and 1386 microfarads, respectively.

The effect of water quality parameters on one another showed that a significant ($P<0.05$) relationship existed between temperature and dissolved oxygen. There was a significant ($P<0.05$) relationship between the pH level and dissolved oxygen. pH had a negative non-significant relationship with temperature and dissolved oxygen indicating that the higher the temperature, the lower the pH. In summary, all parameters were within the range for normal growth expected conductivity values recorded during the study.

The minimum and maximum observed Total length of *S galileaus* was 16.50 and 16.75 cm respectively and the weight ranged from 3–75 g. The log transform of the

LWR is presented in Fig. 1 and expressed as: $\text{Log } W = -0.34 + 1.21 \text{Log } L$ ($r = 0.959$) for *S. galileaus*. The TL measurements of *T. zillii* specimens ranged from 16.20 to 16.35cm and the weight ranged from 2 to 65 g. The length-weight relationship (Fig. 2) is expressed as: $\text{Log } W = -0.32 + 1.21 \text{Log } L$ ($r = 0.985$) for *T. zillii*. Positive allometric growth (b) existed between the two species even though low values of $b = 1.21$ were exhibited by both species. High regression coefficient values of $r = 0.959$ and 0.989 were recorded respectively by *S. galileaus* and *T. zillii*.

Table 1

Water Quality Parameters inside the Cage

Week	Temperature (°C)	Dissolved Oxygen (mg / litre)	pH	[x1.216] Ammonia (mg / litre)	Totally dissolved solid (ppm)	Conductivity (microfarads)
2	30	9.0	7.3	0.9241	190	1024
4	29	9.5	7.1	1.0822	176	1024
6	30	9.0	6.7	1.0822	124	1024
8	30	9.0	7.3	0.9241	190	1024
10	29	9.5	7.1	1.0822	176	1024
12	30	9.0	6.7	0.9241	190	1024
Mean	29.7	9.17	7.03	1.00	174.3	1024

Table 2

Water Quality Parameters outside the Cage

Week	Temperature (°C)	Dissolved Oxygen(m/ L)	pH	[x1.216] Ammonia (mg / litre)	Totally dissolved solid(ppm)	Conductivity (microfarads)
2	27	11.0	7.0	0.8512	60	1356
4	27	11.0	6.9	1.0092	70	1356
6	28	11.2	6.5	1.0092	80	1356
8	27	11.0	7.0	0.8512	60	1356
10	27	11.0	6.9	1.0092	70	1356
12	28	11.2	6.5	0.8512	60	1356
Mean	27.3	11.07	6.80	0.93	66.7	1356.

Inside the cages the total dissolved solid showed a significant and positive correlation with dissolved oxygen ($r = 0.03$, $P < 0.05$) while dissolved oxygen showed a significant and negative correlation with totally dissolved solid ($r = 0.01$, $P < 0.01$) (Table 3). While outside the cages pH and totally dissolved solid showed a significant and negative correlation with dissolved oxygen ($r = -0.98$ and 0.01 respectively, $P < 0.01$) (Table 4).

Table 3

Pearson Correlation of Water Quality Parameters inside the Cage

	Temperature (°C)	Dissolved Oxygen (mg / litre)	pH	[x1.216] Ammonia (mg / litre)	Totally dissolved solid (ppm)				
Temperature (°C)	0.00	-0.12	-0.06	0.10	1.00				
Dissolved Oxygen (mg / litre)	0.00	0.12	0.06	-0.10	-1.00**	1.00			
pH	-0.47	-0.51	-0.41	-0.42	-0.19	0.19	1.00		
[x1.216] Ammonia (mg / litre)	-0.10	0.07	-0.09	-0.24	-0.71	0.71	-0.27	1.00	
Totally dissolved solid (ppm)	0.14	0.03*	0.19	0.23	-0.05	0.05	0.58	-0.67	1.00

* P < 0.05

**P < 0.01 level

Table 4

Pearson Correlation of Water Quality Parameters outside the Cage

	Temperature (°C)	Dissolved Oxygen (mg / litre)	pH	Ammonia (mg / litre)	Totally dissolved salt (ppm)				
Temperature (°C)	0.414	0.41	0.344	0.404	1				
Dissolved Oxygen (mg / litre)	0.414	0.41	0.344	0.404	1.000**	1			
pH	-0.452	-0.47	-0.389	-0.419	-0.98**	-1.000**	1		
[x1.216] Ammonia (mg / litre)	-0.098	0.07	-0.088	-0.238	-0	0	-0.154	1	
Totally dissolved solid (ppm)	-0.131	0.02	-0.158	-0.256	0.316	0.32	-0.414	0.89	1

* P < 0.05

**P < 0.01 level

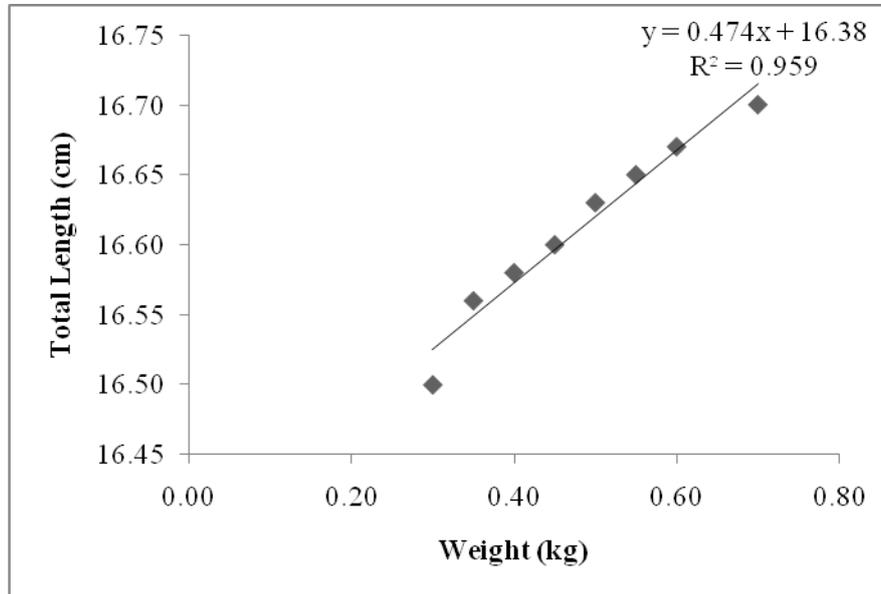


Fig. 1. Total Length – Weight relationship of *Sarotherodon galileus*.

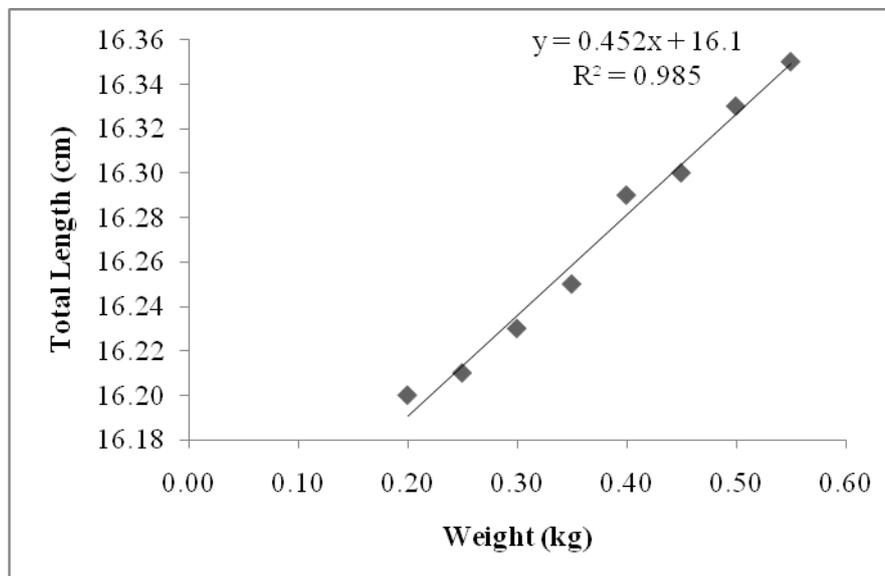


Fig. 2. Total Length – Weight relationship of *Tilapia zillii*.

DISCUSSION

Growth is a specific adaptive property, ensured by unity of the species and its environment (Nikolsky, 1963). In this study both *Sarotherodon galileaus* and *Tilapia zillii* showed a positive allometric growth pattern with the *b* values of 1.21 obtained for the two species. Positive allometric growth implies the fish becomes relatively stouter or deeper-bodied as it increases in length (Riedel *et al.*, 2007). Thus, when *b* is not equal to 3, allometric pattern of growth occurs, which could be positive if >3 or negative if <3 . The result obtained on *T. zillii* is below the *b* values recorded from the recent works of Haruna (2006) and Bala *et al.* (2009) from the Magaga Lake and Kano Daberam reservoir in Katsina state respectively. Imam *et al.* (2010) recorded a 'b' value in *T. zillii* of 1.53 and 2.5 for wet and dry seasons respectively. The coefficient in the present study indicated that there was a high degree of correlation between total length and body weight in the two fish species. This was in conformity with the observations of Taiwo & Odunaiya (2004). The results obtained indicated that *S. galileaus* has a faster and better growth rate than *T. zillii*.

The water quality of the lake under study was within the recommended limits for the culture of this type of fish (*Tilapia* sp.). The mean pH values both inside and outside the cages varied between 6.80 and 7.30 indicating that the lake was moderately alkaline and was within the range of pH known for most lakes and streams of the world (Welch, 1952). The electrical conductivity was constant both inside (1024 microfarads) and outside the cages (1356 microfarads). This result was above the maximum limit of 1000.00 $\mu\text{S}/\text{cm}$ specified by WHO and Nigerian standard for drinking water quality (WHO, 2004; NSDW, 2007). The mean values of ammonia inside the cages were slightly higher than outside the cages. Ammonia was higher at fish culture site due to feces released by the fish (Nyanti *et al.*, 2012). However, the mean values of ammonia both inside cages and outside were within the range as suggested by Boyd & Tucker (1998). The mean totally dissolved solid value was higher in cages (174.3 ppm) than outside the cages (66.7 ppm). This could be attributed to the application of artificial feed inside the cages. Higher value of TSS in the cage culture site was due to the fish excretion and excess fish feed (Boyd, 2004). The result in this study falls within the WHO recommended value of 1000.00 mg/L and 500.00 mg/L of the National standard for drinking water quality (WHO 2004; NSDW, 2007). The mean dissolved oxygen value was higher outside the cages (11.0 mg/l) than inside the cages (9.17 mg/l). The lower DO at some aquaculture sites is mainly caused by consumption of DO by microorganisms in decomposition of organic matter (Yee *et al.*, 2012). Karnatak & Kumar (2014) reviewed that localized water quality problems, particularly low dissolved oxygen, are common in cage culture.

High water quality observed presently was also reported by Akinbuwa (1999), Obodai & Waltia (2003) and Komolafe & Arawomo (2008) in Erinle, Tono and Osinmo reservoirs.

CONCLUSIONS

The study has revealed the growth rate of *Sarotherodon galileaus* and *Tilapia zillii* in cage culture. It was proved from this study that *S. galileaus* grew better than *T. zillii* indicating that *S. galileaus* species are preferable to *T. zillii* species in cage culture. This study has revealed variations in some water quality parameters which were due to high metabolic activities by the fish inside the cage than outside the cage. The effect of the water quality parameters on *S. galileaus* and *T. zillii* growth rate was investigated and was proved that it had no significant effect on their growth rate thus indicating that Odeda Lake is likely to be highly productive and confirmed to be very suitable for aquaculture and agricultural purposes. It was also found that most of the physio-chemical parameters were in conformity with the recommended values for tropical waters.

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HISTOPATHOLOGICAL ALTERATIONS IN THE DIGESTIVE SYSTEM OF *RUTILUS FRISII KUTUM* (KAMENSKY, 1901) FRY AFTER EXPOSURE TO ATRAZINE HERBICIDE

ZAHRA KHOSHNOOD

To investigate the histopathological effects of a most widely used herbicide, atrazine in Caspian kutum fry, *Rutilus frisii kutum*, fish (3.5 cm TL and 2.6 g BW) were exposed to a sublethal concentration of 12.47 mg/L ($\frac{1}{2}$ LC50) for 96h. Acute exposure of *R. frisii kutum* fry to atrazine causes some alterations $\frac{1}{2}$ in the digestive system and the liver of the fry. The most significant alterations were necrosis of intestinal epithelial cells at the apical and basal parts, detaching of epithelial cells from the basement membrane, degeneration of the apical sides of the intestinal folds, hyperplasia in intestinal epithelial cells and hyperplasia and hypertrophy of the goblet cells of the intestine. In hepatic tissue the most significant alterations were dilution of sinusoids, necrosis, vacuolation and increasing the intercellular spaces in hepatocytes, picnotic nuclei of hepatocytes and degeneration of adipose tissue of the liver. Atrazine could affect the nutritional ability and osmoregulation process of the fry by causing histopathological changes in the digestive system even at sublethal concentration and acute exposure.

Keywords: Caspian kutum, atrazine, digestive system, liver.

INTRODUCTION

The pollution effects on fish are main scientific issues (Monte-Luna *et al.*, 2016; Bănăduc *et al.*, 2016; Khoshnood, 2017). The widespread use of chemical agents as pesticides and herbicides, to control the plague and weeds every year, does not necessarily translate to ecological crisis, but there has been considerable discussion in both the scientific literature and the lay press regarding the possibility that environmental chemicals, through their effects on endocrine function, are responsible for a number of reproductive and developmental anomalies in a wide range of wildlife species, from invertebrates through fish, reptiles, birds and mammals, and even including humans (Cooper & Kavioc, 1997).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), which is one of the most widely used herbicides, has been widely applied in agricultural and forestry fields. Due to its relatively high aqueous solubility and high mobility, atrazine can be transported to groundwater by infiltration or to surface waters by water runoff, thus entering aquatic environment easily (Graymore *et al.*, 2001),

therefore, it is more frequently detected in groundwater and surface water than any other herbicides in many countries (Ta *et al.*, 2006). It has been clear that estuaries and coastal marshes are vulnerable to atrazine contamination because they receive waters carrying agricultural pesticides from upland sources (de Lorenzo *et al.*, 2001).

Since atrazine is most commonly found in lakes, rivers and streams, different aquatic species are at particular risk. Various laboratory and ecological applied field studies have shown that atrazine adversely affects multiple biological processes, including growth, metabolism, immune and endocrine system function, in several species of frogs and fish (Srinivas *et al.*, 1991; Freeman & Rayburn, 2005; Houck & Sessions, 2006; Forson & Storfer, 2006; Rymuszka *et al.*, 2007; Fatima *et al.*, 2007; Nieves- Puigdoller *et al.*, 2007; Rowe *et al.*, 2008). However, fish are not usually target organisms for pesticides, and specific knowledge about negative effects of pesticides in the field is still considered sparse. Surprisingly, only a few studies have shown that fish, inhabiting natural freshwater ecosystems, may be affected by unintentional spreading of pesticides (Bálint *et al.*, 1997; Csillik *et al.*, 2000).

Herbicides are often regarded as relatively harmless to fish. Direct effects caused by, for example, the herbicide atrazine are scarce. Fish also can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem (Lakra & Nagpure, 2009).

Early developmental stages are considered to be one of the most sensitive stages in the fish life cycle to the toxic effects of chemical contaminants (Weis & Weis, 1987). Short-term sublethal effects on the growth, behavior or osmotic control may affect the survival of these critical stages and impact population recruitment (Houde 1987, 1989; Sclafani *et al.*, 1997; Alvarez & Fuiman, 2005).

Rutilus frisii kutum (Kamensky, 1901) is one of the native, and commercial fish species of the Caspian Sea. It is a migratory anadromous fish spawning from March to April (Sharyati, 1993; Razavi, 1995) on aquatic weeds and graveled and sandy substrates in rivers and lagoons (Abdoli, 1999). Decline in the stocks and catch of Caspian kutum was caused by overfishing, excessive catches of adults, increased pollution, overexploitation of sands and sediments of the Caspian Sea, and the construction of bridges and dams that alter or blocked the natural spawning grounds (Azari Takami *et al.*, 1979; Emadi, 1979). For several years, starting from 1925, artificial breeding raised larvae for release in the 10 most important rivers. According to the Iranian Fisheries Organization's report, more than 150 million juveniles are being released into the Caspian Sea annually (Iranian Fisheries Organization, 2006).

In order to investigate the toxic effects of atrazine herbicide on *Rutilus frisii kutum*, histopathological alterations of the digestive system have been studied. Results of the present study would be useful as basic data for related studies on environmental monitoring on atrazine contamination.

MATERIALS AND METHODS

FISH AND EXPERIMENTAL DESIGN

Caspian kutum, *Rutilus frisii kutum*, fingerlings were obtained from Shahid Ansari Fish Proliferation and Culture Center, Rasht, Iran. Mean total length and mean body weight of fingerlings were 3.5 cm and 2.6 g respectively. Following the determination of 96 h-LC50 of atrazine for the fingerlings (Khoshnood *et al.*, 2014), a sub lethal concentration was determined as $\frac{1}{2}$ LC50 (12.47 mg/L). Atrazine was dissolved in distilled water, filtered and added to the aquarium following the method of Pluta (1989).

Fish were exposed to this sub lethal concentration for 96h in triplicate group of 30 fish each (~0.43 g/L biomass) in glass aquaria, in the laboratory conditions. One triplicate group of the fingerlings was held in clean water as the control group. No mortality was observed during the experiments in all experimental groups. The water parameters monitored daily through the experiment for all experimental groups using Eutech instruments, pcd650 and the values were as follows: temperature: $14.5 \pm 0.5^\circ\text{C}$, pH: 7.6 ± 0.1 , dissolved oxygen: 8.5 ± 0.5 mg/L, and the photoperiod was 12h:12h lightness and darkness. Water quality conditions (pH, temperature and O_2) did not differ among treatments, and water did not change during the experiment.

HISTOLOGY

For histological studies, fish were hypothesized and immediately immersed into Bouin's fixative for 24 hours, washed and dehydrated in an ascending series of ethanol for embedding in Paraffin (Merck). Following embedment in Paraffin, transversal and longitudinal sections of 6 μm were cut on a Leica RM2255 microtome and collected on glass slides and stained with Haematoxylin and Eosin (Martoja & Martoja-Pierson, 1967; Khoshnood, 2015a). Histopathological alterations detected in the digestive system of fingerlings were recorded as present or absent and expressed as a percentage of fish affected (prevalence) per experimental group (10 fish each). The slides were studied by the means of knowing that they belong to each experimental group (Khoshnood, 2015b).

CHEMICALS

Two experimental groups for atrazine were investigated in triplicate series of: control group with nominal concentration of 0 ppm (at the beginning of the experiment: $t=0$), and atrazine exposed group with nominal concentration of 12.47 ppm (at the beginning of the experiment: $t=0$), both for 96h. The water of both experimental groups was analyzed after 24h of exposure period of 96h for assessing the real concentration of atrazine ($t=24$).

For analysis of the atrazine concentration in experimental groups, sampled water was transferred into a glass bottle which contains 10ng of $^{13}\text{C}_{12}$ PCB-101 to

assess the extraction efficiency. In order to perform extraction, dichloromethane with the volume of about 25% of sample solution was used and the method has been replicated 3 times. A combination of extracts has been reduced to approximately 50 μL and then addition of internal standard: tris (4-chlorophenyl) methane (TCPMe, 100 $\text{pg}/\mu\text{L}$) as internal standard.

Concentration analysis has been conducted using gas chromatography (GC) equipped with a DB-5MS capillary column coupled to a Varian Saturn 2000 ion trap mass spectrometer (MS) by a transfer line kept at 300°C. The carrier gas was Helium (flow rate, 1.0 mL/min). The electron impact for ionization was 70eV and the ion trap was operated in MS–MS mode. To calculate the atrazine concentrations, sample response relative to the one of $^{13}\text{C}_{12}$ PCB-101 in the same sample was considered. Four point calibration curve was considered for relative response factor and $^{13}\text{C}_{12}$ PCB-101 and TCPMe were kept at constant concentration of 100 $\text{pg}/\mu\text{L}$. Correction of the atrazine concentrations was calculated on the basis of the recovery of the surrogate compound. The quantification limit was 0.003 ng/L and the precision of analysis was 6%. Limit of quantification was 0.003 ng/L for atrazine and analytical precision was 6% (Khoshnood, 2015b).

RESULTS

CHEMICALS

Measured atrazine concentrations at $t=0$ (Table 1) were within 85–105% of the nominal concentrations. Fish were exposed to nearly constant atrazine concentrations over the bioassay period. Variations of atrazine concentration within each 24-h period were of the same amplitude as the day to day variation at $t=0$. In the control group, atrazine was occasionally detected in trace amounts ($<0.01 \mu\text{g}/\text{L}$).

Table 1

Nominal and measured atrazine concentrations in exposure solution at the beginning ($t=0$) and the end ($t=24$) of 24-h laboratory exposure periods

Experimental Group	Nominal Concentrations (ppm)	Measured Concentrations (ppm)	
		$t=0$	$t=24$
Fingerling	12.47	12.32	12.28

HISTOLOGICAL STRUCTURE OF DIGESTIVE SYSTEM IN *R. FRISII* KUTUM

Results showed that the primary parts of the digestive system of *R. frisii kutum* are oral cavity and pharynx which lined up by stratified squamous epithelium with numerous goblet cells and taste buds (Fig 1a). Pharynx bears 4 pharyngeal teeth at the lower part and a horny pad at the upper side (Fig 1a).

Esophagus was short with thick muscular layer, and in its epithelium numerous goblet cells and taste buds were observed (Figs. 1a and 1b).

No definite stomach was observed in the digestive system and esophagus has straightly ended up to the intestine (Fig. 2a). At the primary part of the intestine the folding was more than in the ending part, where the lumen got wider and called the rectum (Fig. 2a). The intestine was lined up with a simple columnar epithelium and pear shape goblet cells (Figs. 2b and 2c).

The liver was large and it was observed almost all along to the digestive system except for the oral cavity and pharynx. The most significant cells of the liver were hepatocytes with a central nucleus (Figs. 2a and 2d).

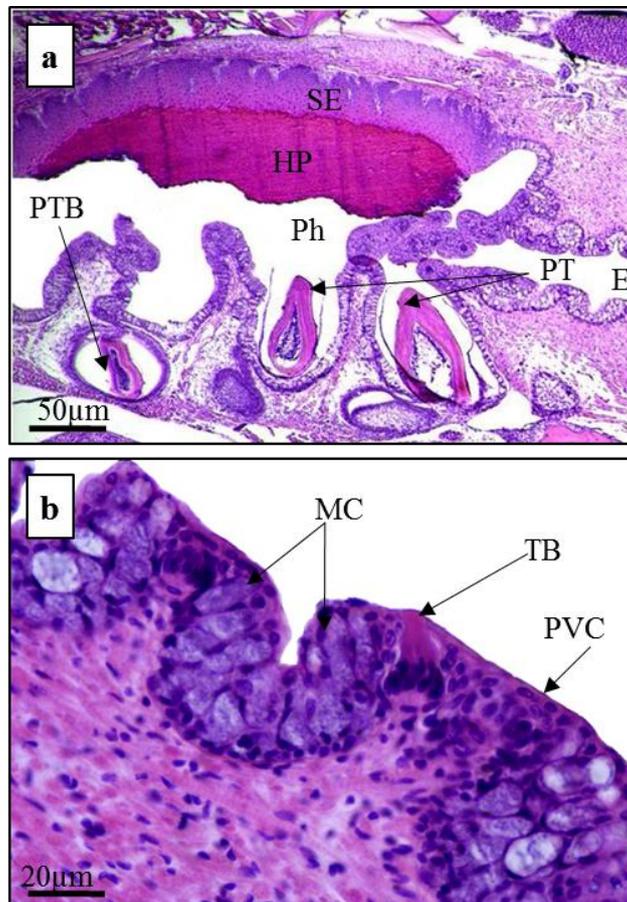


Fig. 1. Normal histological structure of the primary part of the digestive system in *Rutilus frisii kutum* fry. Digestive system begins with oral cavity (not shown), Esophagus (a) and pharynx (a), all lined up by stratified squamous epithelium with numerous goblet cells and taste buds (a). Pharynx bears 4 pharyngeal teeth at the lower part and a horny pad at the upper side (a). Esophagus was short with a thick muscular layer, and in its epithelium numerous goblet cells and taste buds were observed (a and b).

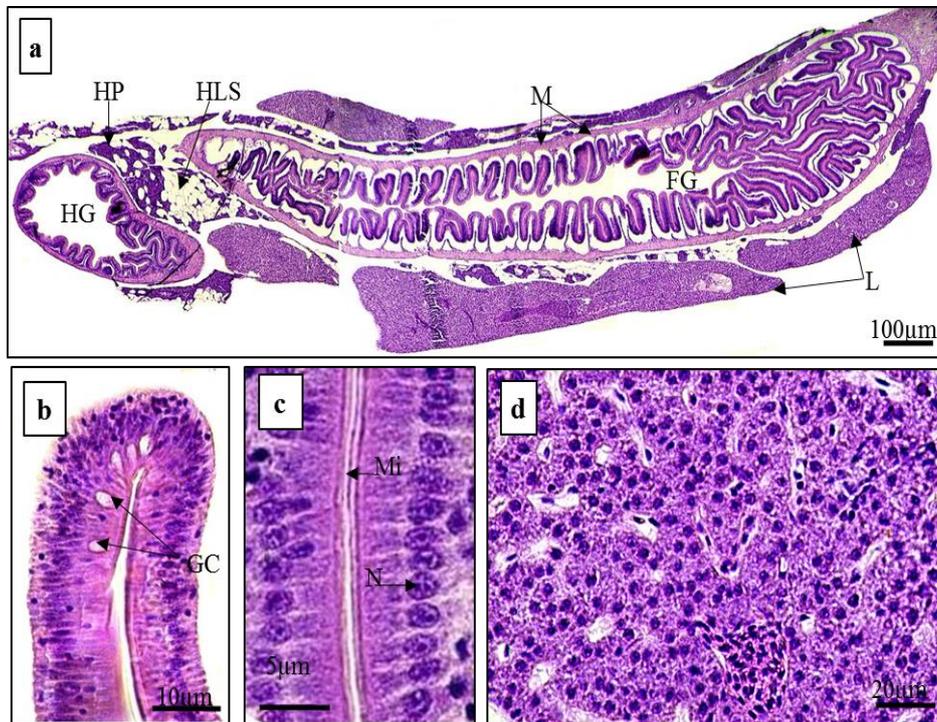


Fig. 2. Normal histological structure of the intestine and liver in *Rutilus frisii kutum* fry. Primary part of the intestine with more folding (a) and the ending part, where the lumen got wider and called the rectum (a). Intestine was lined up with a simple columnar epithelium and pear shape goblet cells (b and c). Liver was observed almost all along to the digestive system except for the oral cavity and pharynx (a). The most significant cells of the liver were hepatocytes with a central nucleus (a and d).

HISTOPATHOLOGICAL ALTERATIONS OF THE DIGESTIVE SYSTEM

Results showed that in atrazine exposed fish, the most significant alterations in the digestive system were as follows: necrosis of intestinal epithelial cells at the apical and basal parts (Fig. 3a), detaching of epithelial cells from the basement membrane (Figs. 3b and 3c), degeneration of the apical sides of the intestinal folds (Fig. 3c), hyperplasia in intestinal epithelial cells (Fig. 3d) and hyperplasia and hypertrophy of the goblet cells (Fig. 3e).

In the liver, the main histopathological alterations were dilution of sinusoids (Fig. 4a), necrosis, vacuolation and increasing the intercellular spaces in hepatocytes (Figs. 4b and 4c), picnotic nuclei of hepatocytes (Fig. 4c) and degeneration of adipose tissue of the liver (Fig. 4d).

Histopathological alterations detected in the digestive system of fingerlings were recorded as present or absent and expressed as a percentage of fish affected (prevalence) per experimental group (10 fish each). Observers were aware to which

experimental group each slide belonged (Fig. 5). Results of the quantitative observation of the histopathological alterations in the digestive system of fingerlings showed that the most significant alterations were detaching of epithelial cells, hyperplasia of epithelial cells and necrosis of epithelial cells (Fig. 5).

In the liver, the main histopathological alterations were dilution of sinusoids (Fig. 4a), necrosis, vacuolation and increasing the intercellular spaces in hepatocytes (Figs. 4b and 4c), picnotic nuclei of hepatocytes (Fig. 4c) and degeneration of adipose tissue of the liver (Fig. 4d).

Histopathological alterations detected in the digestive system of fingerlings were recorded as present or absent and expressed as a percentage of fish affected (prevalence) per experimental group (10 fish each). Observers were aware to which experimental group each slide belonged (Fig. 5). Results of the quantitative observation of the histopathological alterations in the digestive system of fingerlings showed that the most significant alterations were detaching of epithelial cells, hyperplasia of epithelial cells and necrosis of epithelial cells (Fig. 5).

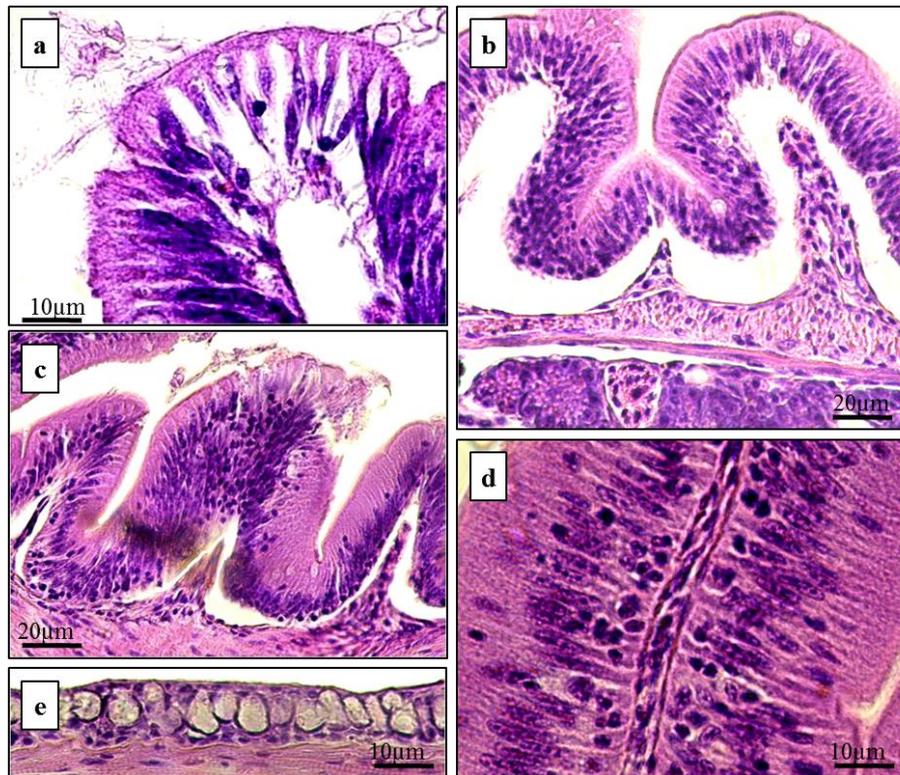


Fig. 3. Histopathological alterations of the digestive system of the *Rutilus frisii kutum* fry after exposure to atrazine herbicide. Necrosis of intestinal epithelial cells at the apical and basal parts (a), detaching of epithelial cells from the basement membrane (b and c), degeneration of the apical sides of the intestinal folds (c), hyperplasia in intestinal epithelial cells (d) and hyperplasia and hypertrophy of the goblet cells (e).

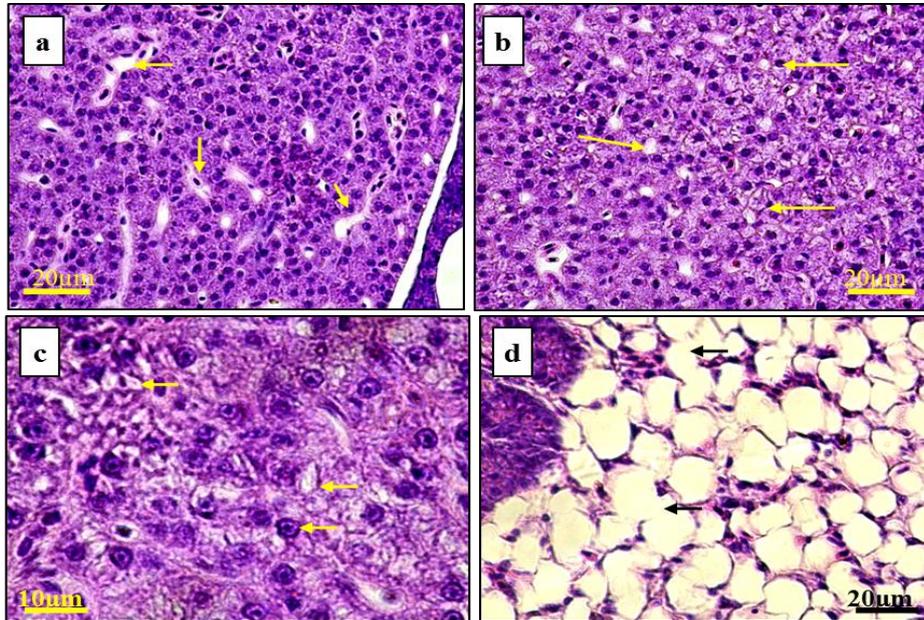


Fig. 4. Histopathological alterations of the liver of the *Rutilus frisii kutum* fry after exposure to atrazine herbicide. Dilution of sinusoids (a), necrosis, vacuolation and increasing the intercellular spaces in hepatocytes (b and c), picnotic nuclei of hepatocytes (c) and degeneration of adipose tissue of the liver (d).

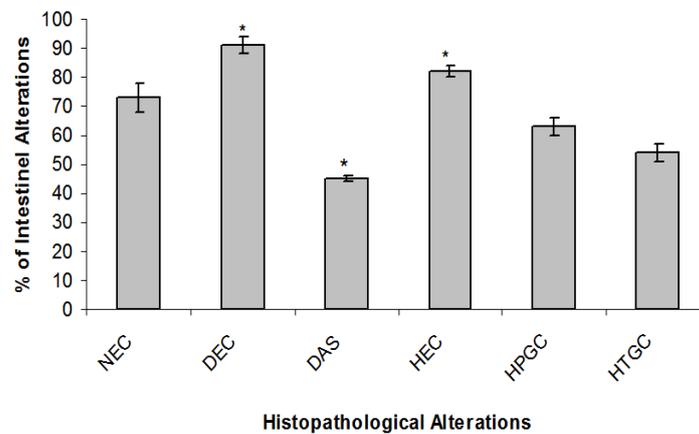


Fig. 5. Prevalence (%) of digestive system histopathological alterations in *Rutilus frisii kutum* fingerlings after exposure to atrazine herbicide. Alterations marked with (*) were significantly different from other values ($p < 0.05$). Values are mean \pm SE.

Abbreviations: NEC: Necrosis of Epithelial Cells; DEC: Detaching of Epithelial Cells; DAS: Degeneration of Apical Side; HEC: Hyperplasia of Epithelial Cells; HPGC: Hyperplasia of Goblet cells; HTGC: Hypertrophy of Goblet cells.

DISCUSSION

The digestive system is a multifunctional organ in fish. Besides the main duty of digestion and nutrients uptake, excretion of some wastes, regulation of water and ions, and detoxication in relation with liver are other important functions of the digestive system. Because the digestive system is receiving the surrounding water (through swallowing of food), it could easily get affected by the pollution (Au 2004) and any damages to this organ can cause nutritional and osmoregulatory problems (Sindermann, 1979). Until now different investigations on the effects of xenobiotics on fish digestive system were performed (Chakrabarti & Sinha, 1987; McCarthy & Fuiman, 2008; Senapati *et al.*, 2009) but data on the effects of herbicides on fish digestive system are scarce and there are few researches on the effects of atrazine. Different investigations on the effects of herbicides were performed, for example, Senapati *et al.* (2012) showed that exposure of *Anabas testudineus* with Almix herbicide caused the necrosis of the apical parts of the squamous epithelial cells of buccal cavity, pharynx and esophagus, disorder of the columnar epithelial cells of the intestine, increasing in mucus content of the goblet cells and necrosis of the intestinal folds. Another investigation on glyphosate herbicide, on *Channa punctatus* showed necrosis and pathological alterations in the digestive system (Senapati *et al.*, 2009). Apical parts of the epithelial cells have a vital role in absorption of nutrients and minerals and it is protected by a mucus layer. This mucus layer also play a role as a lubricating layer for passing foods along the alimentary canal (Sinha & Chakrabarti, 1986; Chakrabarti & Sinha, 1987). Due to these facts, necrosis at the apical parts of the epithelial cells could lead to disorder in nutrient uptake, and further necrosis by eliminating the mucus layer (Senapati *et al.*, 2012). Previous studies suggested that alterations in order of the columnar epithelial cell of the intestine and hyperplasia of these cells are protective mechanisms of the intestine against the pollutants (Tuvikene *et al.*, 1999). It seems that the increase in number of the mucus cells and their contents observed in the present study, appeared due to the protective duty of mucus layer for the digestive system which was previously seen in different species exposed to pollutants (Au, 2004).

Necrosis of the columnar epithelial cells of the intestine which was observed in the present study could affect the nutritional abilities of the Caspian kutum fry. Previous studies showed that due to the high activity of these cells in transporting ions, water and nutrients, these cells are vulnerable in front of the pollutants and easily become necrotic (Sindermann, 1979; Tuvikene *et al.*, 1999; Au, 2004).

The liver is the most important organ for biodeformation of the pollutants, removing of hazardous heavy metals, and storage of some nutrients and metabolism of sexual hormones (Au, 2004). Various investigations were performed on the liver cell and tissue alterations of different fish species in case of exposure to a wide range of pollutants (GlobalTox, 1997; Khoshnood *et al.*, 2010). Most of

the pollutants were changed to non-toxic forms by liver special enzyme system, but sometimes this process can make cell or tissue damages in different levels dependent on the concentration and toxic levels of the pollutant in the liver (Au, 2004). Vesiculated hepatocytes, necrosis in adipose tissue of the liver and necrosis in some hepatocytes were the most significant alterations observed in Caspian kutum fry after exposure to atrazine. It has been clear that liver is extremely sensitive to environmental pollution, and due to the natural ability of hepatic cells for concentrating the absorbed pollutants, these cells were faced higher amounts of the hazards compared to other cells of the body (Au, 2004). Generally hepatic alterations are not specific for defining pollutants and besides some of the hepatic alterations have only occurred in specific species, for example, exposure to PAHs, PCBs, DDTs, Chlordane and Dieldrin which cause a wide range of hepatic alterations in English sole, *Pleuronectes vetulus*, like neoplasm hepatocytes, megalocytic hepatocytes, polymorphic nucleus hepatocytes and vacuolated hepatocytes, but in winter flounder, *Pleuronectes americanus*, exposure to PAHs, DDTs or chlordane significantly caused vacuolated and non-neoplastic increases of the hepatocytes, and non-specific necrosis in hepatocytes (Meyers & Hendricks, 1985; Johnson *et al.*, 1992). The histopathological alterations observed in the present study in the liver of the Caspian kutum fry after exposure to atrazine were previously observed in some other fish species exposed to different kinds of pollutants, for example exposure of *Ophiocephalus striatus* with cadmium chloride (Bais & Lpkhande, 2012), exposure of *Salmo trutta* and *Barbatula barbatula* with pesticides, PAHs and ammonium (Gernhöfer *et al.*, 2001), exposure of Nile tilapia, *Oreochromis niloticus* with roundup herbicide (Jiraungkoorskul *et al.*, 2002), and exposure of *Heteropneustes fossilis* with cypermethrin (Joshi *et al.*, 2007). Comparison between the results of the present study with previous data on the effects of various pollutants on hepatic tissue showed that histopathological alterations of the liver were not specific to pollutant and similar alterations could be observed under the effects of a wide range of pollutants. Results of the present study also showed that it is toxic enough and can produce enormous alterations in the liver of the fry even at sublethal concentration. Results of the previous studies on the toxic effects of atrazine showed that atrazine could have an inhibitory effect on the main hepatic enzymes of the glyconeogenesis (such as hexokinase, glycogen synthase and glucokinase) and lead to lose weight (Curic *et al.*, 1999). Histopathological alterations also reported in the hepatic tissue of the zebra fish, *Danio rario*, exposed to atrazine (Yuanxiang *et al.*, 2011), include changes in the protein content of hepatocytes too. Chronic exposure to atrazine also caused changes in lipid metabolism and insulin resistance (Lim *et al.*, 2009). It is suggested that all these hepatic alterations are dependent on a wide range of cellular biochemical processes in response to oxidative stress, oncogenesis, etc.

CONCLUSIONS

Outcomes of this study revealed that significant degradations in vital fish (Caspian kutum fry) organs of the experimental model such as digestive tract and liver tissues could happen due to acute (short-term) exposure to a sublethal concentration of commercial atrazine herbicide, even though the fish is not a target organism for such substance.

The tissue damages in this case were almost severe so one could conclude that such damages could have resulted in malfunction of the alimentary canal and intoxication duty of the liver and also in nutrition problems and toxicity of the environmental contaminations which finally ended up with mortality at long time (chronic) exposure.

The results also showed that sublethal concentration of atrazine even at acute exposure could affect the liver and make some tissue damage and alterations in this vital organ. Due to the natural responsibility of the liver in intoxication with toxins, drugs, contaminations, etc. it would not be unexpected that probably this organ received a higher concentration of atrazine compared to other internal organs, and for such reason, the tissue alterations were severe in the liver. The severe tissue alterations in the liver could lead to malfunction of this organ for intoxication and influence the whole body of the organism at chronic exposure.

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