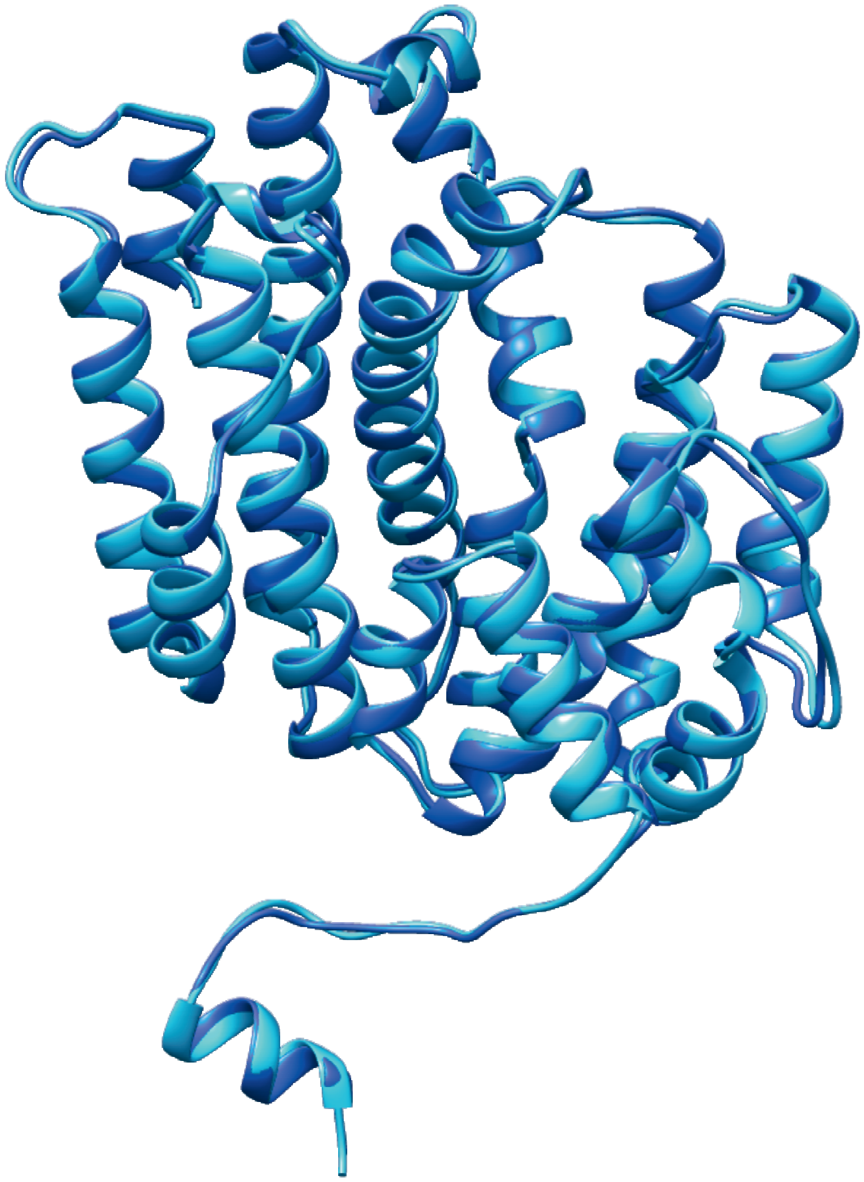
STUDIA UNIVERSITATIS
BABEŞ-BOLYAI



BIOLOGIA

1/2020

STUDIA
UNIVERSITATIS BABEŞ-BOLYAI
BIOLOGIA

1 / 2020
January - June

YEAR Volume 65 (LXV) 2020
MONTH JUNE
ISSUE 1

PUBLISHED ONLINE: 2020-06-30
PUBLISHED PRINT: 2020-06-30
ISSUE DOI:10.24193/subbbiol.2020.1

STUDIA UNIVERSITATIS BABEŞ-BOLYAI

BIOLOGIA

1

STUDIA UBB EDITORIAL OFFICE: B.P. Hadeu no. 51, 400371 Cluj-Napoca, Romania, Phone + 40 264 405352, www.studia.ubbcluj.ro

SUMAR – CONTENTS – SOMMAIRE – INHALT

REGULAR ARTICLES

All authors are responsible for submitting manuscripts in comprehensible US or UK English and ensuring scientific accuracy.

Original pictures on front cover: Superimposed 3D structure of the SSL-1 enzyme from *Botryococcus terribilis* AICB 872 strain, initial (dark blue) and refined (turquoise). © Tiberiu Szőke-Nagy, Alin Sebastian Porav & Nicolae Dragoș

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 5-18)
doi:10.24193/subbbiol.2020.1.01

In silico modeling and analysis of squalene synthase-like 1 (SSL-1) enzyme from green microalga *Botryococcus terribilis* AICB 872

Tiberiu Szöke-Nagy[1,2], Sebastian Alin Porav[1,2] and Nicolae Dragoș[1,3]✉

[1] Babeș-Bolyai University, Faculty of Biology and Geology, Cluj-Napoca, Romania;
[2] Department of Molecular and Biomolecular Physics, National Institute of R&D of Isotopic and Molecular Technologies, Cluj-Napoca, Romania;
[3] Institute of Biological Research, branch of the National Institute of R&D for Biological Sciences Bucharest, Cluj-Napoca, Romania;
✉ ***Corresponding author, E-mail: dragos.nicolae@ubbcluj.ro.***

Abstract. The genus *Botryococcus* contains a small number of green, colonial algae, with some taxa still uncertain. *B. braunii* is the most extensively studied species of the genus because of its hydrocarbon oils which can be used as an alternative energy source. Some *B. terribilis* AICB strains were previously described showing their ability to synthesize C30–C32 botryococcenes similar to those produced by chemical race B of *B. braunii* strains. The present study aimed to investigate the structural features of SSL-1 enzyme involved in the biosynthesis of presqualene diphosphate from *B. terribilis* AICB 872, and its functional conservation by means of computational proteomics and molecular biology techniques. Using PCR amplification we obtained a 3811bp contig containing the sequence of SSL-1 gene. The homology modeling analysis revealed the presence of alpha helical structures and a small beta sheet which are forming the SSL-1 catalytic core. Coil structures and both N and C terminus regions of the protein are characterized by highly disordered structural fragments. Finally, our data integrated within the available information in the literature allowed us to presume that the formation of presqualene diphosphate, the first step of hydrocarbon biosynthesis in *B. terribilis* strain occurs in a similar fashion with that described in *B. braunii*

Keywords: *Botryococcus terribilis,* squalene synthase-like 1, 3D structure prediction, AICB strain, biofuel.

Introduction

Botryococcus is a genus of green, colonial microalgae, consisting of 14 taxa according to AlgaeBase (Guiry and Guity, 2019), including *B. braunii* Kützing and *B. terribilis* Komárek & Marvan. *B. braunii* is the most extensively studied species of the *Botryococcus* genus because of its hydrocarbon-biosynthesis property.

Strains of *B. braunii* can synthesize large amounts of liquid hydrocarbons oils which can be used as an alternative energy source to fossil fuels (Al-Hothaly, 2018). *B. braunii* strains produce and accumulate various types of hydrocarbons, being classified in four chemical races (A, B, L and S).

Race A produces n-alkadiene and n-triene (Metzger *et al.*, 1986; Metzger and Largeau, 2005). Race B produces botryococcenes (Metzger *et al.*, 1985; Metzger and Largeau, 2005), squalenes and their methylated derivatives (Huang and Poulter, 1989; Achitouv *et al.*, 2004). Chemical race L produces C40 lycopadiene and small amounts of lycopatriene (Metzger and Casadevall, 1987; Metzger *et al.*, 1990). Recently, the lycopaoctaene production by lycopaoctaene synthase (LOS) has been reported in *B. braunii* Songkla Nakarin strain (Thapa *et al.*, 2016). Race S produces epoxy-n-alkane and saturated n-alkane (Kawachi *et al.*, 2012).

Niehaus *et al.* (2011), isolated the squalene synthase-like 1 (SSL-1) cDNA by screening the cDNA library with a radiolabeled *Botryococcus* squalene synthase probe under low stringency hybridization conditions. Further, by combining transcriptomic data from two independent projects and with deep mining computational screening another two SSL were discovered, namely SSL-2 and SSL-3. SSL-1, SSL-2 and SSL-3 enzymes are involved in the last steps of botryococcenes and squalenes biosynthesis pathway. SSL-1 catalyzes the formation of presqualene diphosphate (PSPP) intermediate by a head-head condensation of two moieties of farnesyl diphosphate (FPP). PSPP being further converted to squalenes (SSL-2) or botryococcenes (SSL-3). Squalene biosynthesis was observed under co-expression of SSL-1 and SSL-2, similar to botryococcenes biosynthesis under SSL-1 and SSL-3 co-expression.

The squalene synthase (SQS) genes were described among different taxa. For example, the overexpression of SQS in medicinal plants *Panax ginseng* (Lee *et al.*, 2004), *Eleutherococcus senticosus* (Seo *et al.*, 2005) and *Salvia miltiorrhiza* (Rong *et al.*, 2016) leads to enhanced biosynthesis of triterpenes and phytosterols. Human SQS is a microsomal enzyme involved in the first step of cholesterol biosynthesis (Park *et al.*, 2014).

The importance of studying the SSL enzymes from different strains of *Botryococcus* resides in the ability of these algal species to synthesize and secrete significant extracellular quantities of hydrocarbons similar to fossil fuels, the best-studied species of this genus from this point of view being *B. braunii* Showa (Hillen and Wake, 1979; Peterson *et al.*, 2008).

The major goal of this work was to investigate the structural and functional relationship between SSL-1 enzyme from *B. terribilis* AICB strain and *B. braunii* using molecular technique and in silico tridimensional structure prediction and analysis of amino acids.

Materials and methods

Strain cultivation

B. terribilis AICB 872 strain was isolated from Tăureni fishpond, Mureș County, Romania. This strain is deposited in the Algal and Cyanobacterial Culture Collection (AICB) at the Institute of Biological Research, Cluj-Napoca, Romania (Dragoș *et al.*, 1997). The algal culture was grown on BBM medium, under continuous irradiation (approx. 150 µmol photons $m^{-2}s^{-1}$), at 25°C±2°C, in 100 mL Erlenmeyer flask. The algal biomass was freshly harvested before every experiment, in the exponential growth phase by centrifugation at 10.000 x g for 2 minutes.

Genomic DNA (gDNA) purification

gDNA was isolated and purified from algal biomass using ZR Soil Microprobe DNA MiniPrep™ Kit (Zymo Research Corp., Irvine, CA, USA), according to the manufacturer's protocol. gDNA quantification was performed on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

PCR amplification, isolation and sequencing of SSL-1 gene

The PCR was carried out using SSL1-F2 and SSL1-R3 primer pairs published in a previous paper (Szoke-Nagy *et al.*, 2015), as follows: each 20 µL reaction volumes containing 1U of Thermo Scientific™ DreamTaq™ DNA Polymerase in 2 µL of the manufacturer`s buffer, 0.2 mM dNTPs, 0.3 µM of each primer, and approximately 20 ng of gDNA template. The PCR was performed in a TProfessional TRIO Termocycler (Biometra, Göttingen, Germany). PCR cycling conditions were: initial denaturation at 95°C for 5 min followed by 34 cycles of denaturation at 95°C for 50 s, primer annealing at 59°C for 55 s, extension at 72°C for 2 min and a final extension at 72 °C for 10 min.

The PCR products were verified by electrophoresis on a 1% agarose gel in 1 X TAE running buffer, stained with ethidium bromide (1 µg mL^{-1}), and visualized on a UVP transilluminator (Analytik Jena AG, Germany). PCR products were purified from agarose gel with GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by a commercial company (BaseClear B.V., Leiden, The Netherlands), with specific primers. SSL-1 gene was partially sequenced (3811 bp) using the primer walking technique with internal newly designed primers (data not shown).

The obtained SSL-1 fragments ranging from 700 to 1100 bp were manually corrected for mismatches and ambiguous nucleotides using Chromas Lite 2.1.1 software (Technelysium Pty, South Brisbane, Australia). Gene contig was generated using Contig editor from GeneStudio Pro 2.2.0.0 (GeneStudio Inc., USA).

3D structure prediction methodology

The obtained nucleotide sequence was virtually translated into amino acid sequences by Translate tool from ExPASy (Gasteiger *et al.*, 2003). The ORF (Open reading frame) showing long-chain amino acid sequence was subjected to NCBI protein BLAST and further used for protein structure prediction.

Phyre2 web portal (Lawrence *et al.*, 2015) was used to generate a primary 3D structure form the selected ORF. According to Lawrence *et al.*, (2015) the core method of Phyre2 for generating a 3D model consists of four stages: i) gathering homologous sequences using HHblits (Remmert *et al.*, 2012) to generate the evolutionary profile of amino acid residues from query sequence; ii) fold library scanning, data obtained in stage 1 was converted to a hidden Markov model (HMM) and subjected to HHsearch (Söding, 2005) for HMM-HMM matching against a database of known structures; iii) loop modeling using cyclic coordinate descent (Canutescu and Dunbrack, 2003); iv) side chain placement using R3 fitting protocol (Wei and Sahinidis, 2006).

Protein structure obtained from Phyre2 was subjected to quality assessment by RAMPAGE - Ramachandran plot analysis (Lovell *et al.*, 2003), ProSA-web Z-score (Wiederstein and Sippl, 2007) and MolProbity global score (Chen *et al.*, 2009).

Further, PREFMD (Protein REFinement via Molecular Dynamics) (Heo and Feig, 2018) was run locally for protein structure refinement. After refinement, the obtained model was subjected to another step of energy minimization process by employing the steepest descent algorithm with Charmm36 force field (Huang and MacKerell, 2013) and was allowed to run until it converged to machine precision or to a maximum force on each atom less than 100 kJ mol^{-1} nm^{-1}. For this step, we used the GROMACS (Abraham *et al.,* 2015) software package with GPU acceleration on a Linux workstation. The refined 3D model obtained at the end of the protocol was subjected to another round of quality assessment and validation.

Finally, the refined protein structure was used for subsequent analysis, including: multiple sequence alignment using ClustalW from Mega7 (Kumar *et al.*, 2016); identification of functional regions and calculating of evolutionary conservation by ConSurf (Ashkenazy *et. al.*, 2016), ligand binding site prediction by P2Rank (Krivák and Hoksza, 2018; Jendele *et al.*, 2019). UCSF CHIMERA was used for protein visualization and analysis (Pettersen *et al.,* 2004).

Results

Molecular analysis

Total DNA extracted from *B. terribilis* AICB 872 strain was 387.7 ng μL^{-1}. Nucleic acids purity was estimated by analysing the A_{260}/A_{280} and A_{260}/A_{230} ratios. The A_{260}/A_{280} for DNA isolated using ZR Soil Microprobe DNA MiniPrep™ Kit was 2.10 and A_{260}/A_{230} ratio was 1.68. The isolated gDNA was further subjected to gel electrophoresis to verify the integrity of extracted DNA (Fig. 1A). DNA gel electrophoresis yielded a strong band above the 10000 bp marker band and a faintly smear consisting of DNA fragments under 500 bp.

PCR amplification of SSL-1 gene using gDNA as template and SSL1-F2 and SSL1-R3 primer pair, revealed a band of approx. 3500 bp. The PCR product was isolated and sequenced. SSL1-F2 (820 bp) and SSL1-R3 (777 bp) fragments were subjected to NCBI Nucleotide BLAST. SSL1-F2 nucleotide sequence BLAST report showed a high percentage of identity with 57-172 and 173-333 regions from *Botryococcus braunii* squalene synthase-like 1 mRNA (HQ585058.1); similar SSL1-R3 showed 100% identity with 951-1058 and 1059-1114 region from the same mRNA sequence of *B. braunii*. Further, we designed a new primer pair (data not shown) and used the primer waking techniques to partially sequence the SSL-1 gene. Finally, using Contig editor from GeneStudio Pro we managed to assemble a partial SSL-1 contig of 3811 bp. The partial CDS of SSL-1 was obtained by multiple alignments with similar squalene synthase CDS fragments in MEGA 7 and BLAST from NCBI.

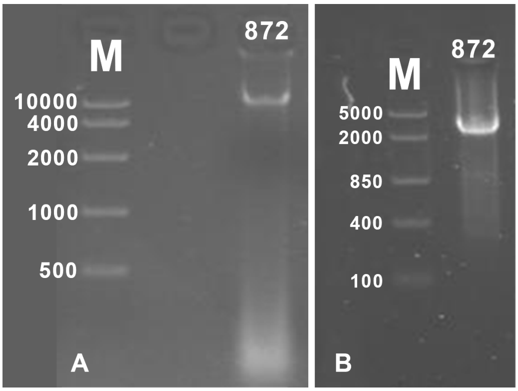


Figure 1. Gel electrophoresis of gDNA extracted from *B. terribilis* AICB 872 (A) and PCR product amplification of SSL-1 gene using SSL1-F2 and SSL1-R3 primer pair (B). DNA sample was electrophoresed on 1.6% (A) or 1% (B) agarose gel in 1X TAE Buffer. M - FastRuler High Range (A) / Middle Range (B) DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA).

Based on Phyre2 secondary structure and disorder prediction (Fig. 2), the SSL-1 of *B. terribilis* AICB 872 strain consists of 18 α-helix structure (70% of total protein structure) and a short β-sheet at Met216 and Phe217 residues position. The C-terminal transmembrane domain was not observed according to Phyre2 secondary structure prediction. Based on domain prediction, high hits were obtained with the chain C of *Trypanosoma cruzi* squalene synthase (PDB: 3wcc) and chain A of human squalene synthase (PDF: 1ezf).

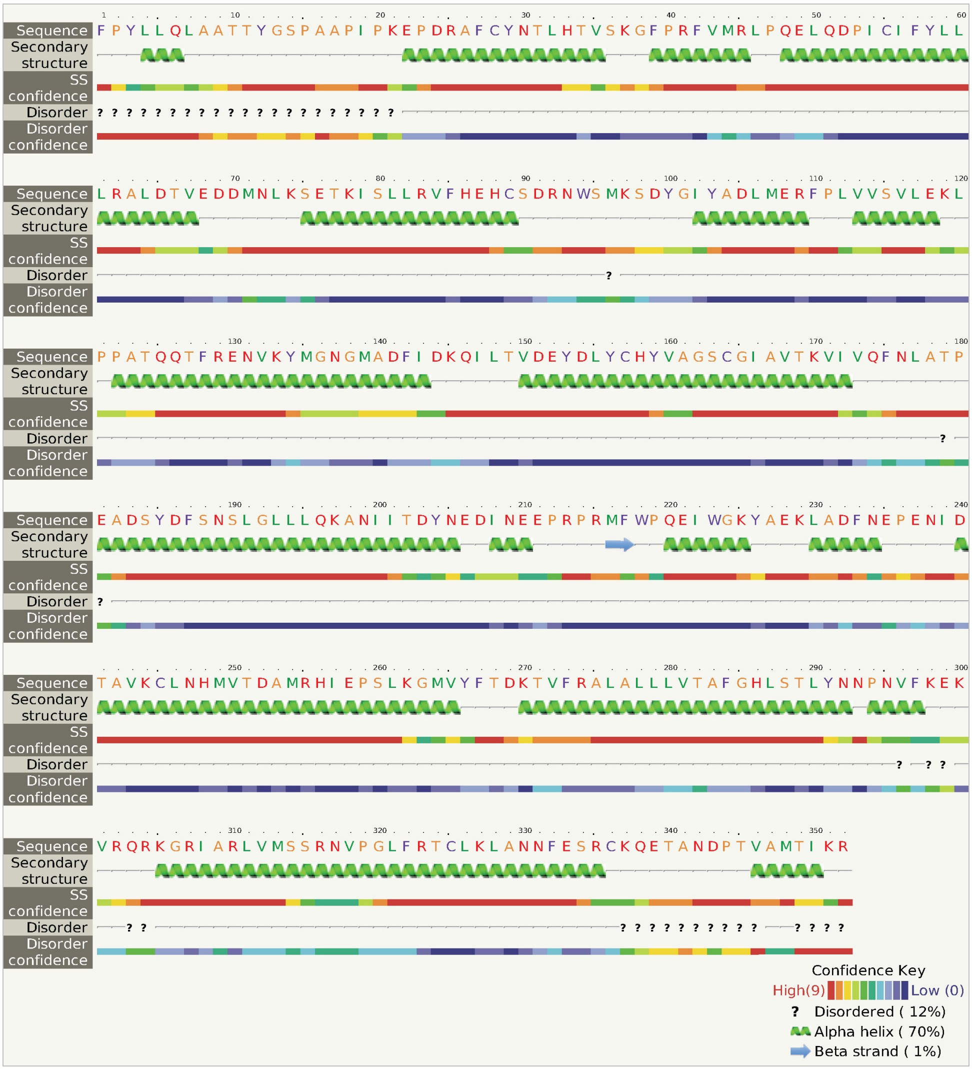


Figure 2. Protein secondary structure and disorder prediction of SSL-1 enzyme of *B. terribilis* AICB 872 strain generated by Phyre2.

Structure prediction and refinement.

The 3D structure of squalene synthase-like 1 from AICB 872 was generated by Phyre2 using the PDB structures of 3wcc chain C and 1ezf chain A as templates. The squalene from *Trypanosoma cruzi* 3wcc was found to be most similar to our protein, having a sequence identity of 38%. Based on these two templates 329 aa were modeled with 100% confidence while 23 residues were modeled by *ab initio*. Before further analysis of the protein structure, we performed a refinement step using PREFMD protocol followed by energy minimization in GROMACS.

Based on model quality score for both unrefined and refined structures, the refined model was found to be more accurate based on various parameters including MolProbity, Ramachandran plot and Prosa Z score. Protein dihedral angle plotted as a Ramachandran diagram (Fig. 3) showed better stereochemical properties for the refined model with 96% of the residues in the favoured regions while for the initial model just 92% of the residues were found in this region. The amino acid residues Thr30, Leu65, Ser117, Gly120, Glu137, Leu139, Pro199, Glu229, Met235, Ala246, Asn257 and Lys317 from the initial structure, Tyr3, Lys21, Tyr100 and Arg213 from the refined structure, respectively were found in the disallowed region of the Ramachandran map.

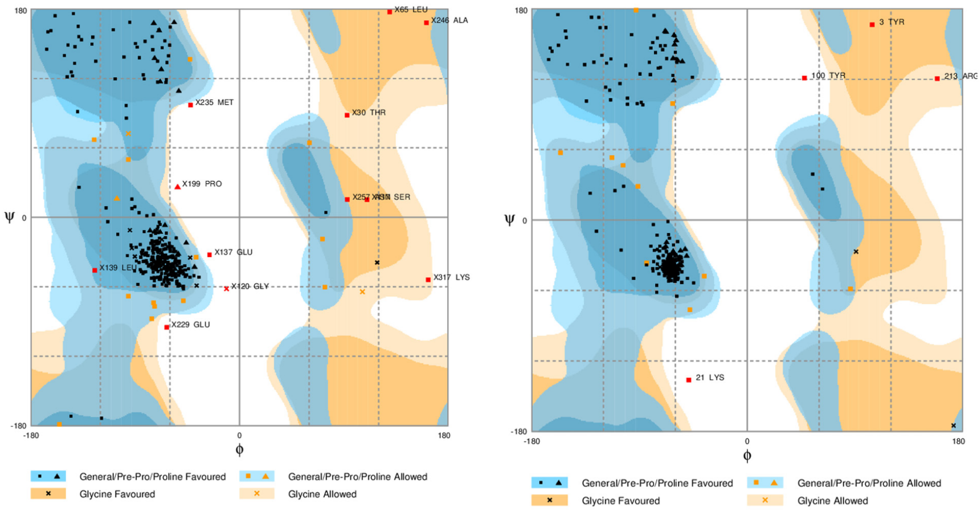


Figure 3. RAMPAGE - Ramachandran plot of both initial (left) and refined (right) SSL-1 protein structure from *B. terribilis* AICB 872 strain.

The overall quality score calculated by ProSA for the two models were very similar 9.23 for initial model and 8.98 for the refined, respectively. Graphic representation of the Z-score (Fig. 4) in the context of all known, experimentally determined protein structures, is placing the models in the characteristic range for native proteins with similar molecular weight. A more drastic improvement of the refined model was observed based on MolProbity, where the overall score was 1.66 with 0 clashes between atoms, 0 bad bonds and 60 bad angles compared to the crude model that has a general quality score of 3.02 with 126 clashes, 21 bad bonds and 135 bad angles. The only score better for the crude model consists of 92% favoured rotamers over 85%.

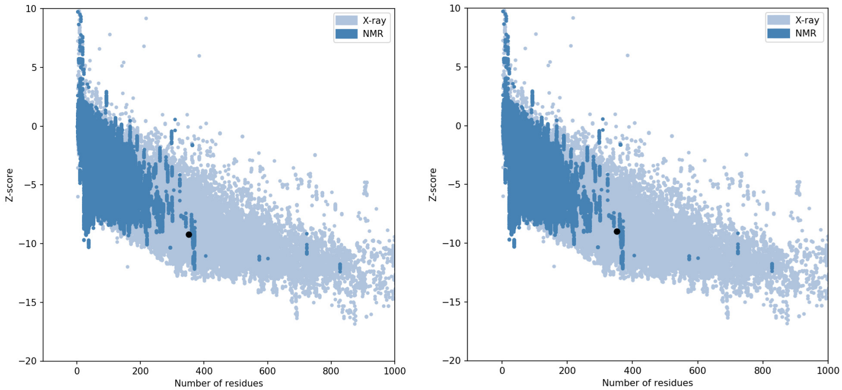


Figure 4. ProSA-web Z-score graphic representation of initial SSL-1 (left) and refined (right) from *B. terribilis* AICB 872 strain.

Visual analysis and comparison of the two models by superimposing them with MatchMaker from UCSF Chimera showed good alignment of all a-helices, with no visible modifications. Most differences between the models are among coil structures (Fig. 5) and both N and C terminus regions of the protein. These regions, as shown by 2D structure prediction, are characterized by the presence of highly disorder structural fragments. The results are not unexpected, because the disordered regions usually present intrinsic flexibility, which is problematic even for experimentally methods not just for computational methods. Also, these fragments were not covered by the template provided, the residues being modeled *ab initio.*

We used the refined model to predict the ligand-binding site and to identify the amino acids that form the pocket. This analysis was performed by P2Rank with default settings. We predicted 5 pockets, of which only one had high confidence score (>50). The predicted pocket is comprised of 48 residues being located in the middle part of the protein (Fig. 6 centre). Also, based on the protein conservation analysis done with ConSurf, the predicted pocket is localized in a well-conserved region (Fig. 6 left). Based on these facts we concluded that this pocket is responsible for specific binding of the ligand. The ligand prediction based on the most conserved binding site revealed two possible candidate: PS7 and FPS - Farnesyl thiopyrophosphate.

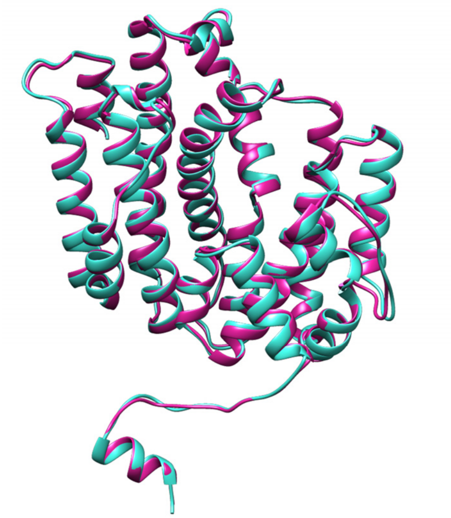


Figure 5. The superimposed 3D structure of both initial (magenta) and refined (turquoise) model of the SSL-1 enzyme from *B. terribilis* AICB 872 strain. The superimposed 3D structure was obtained using UCSF CHIMERA.

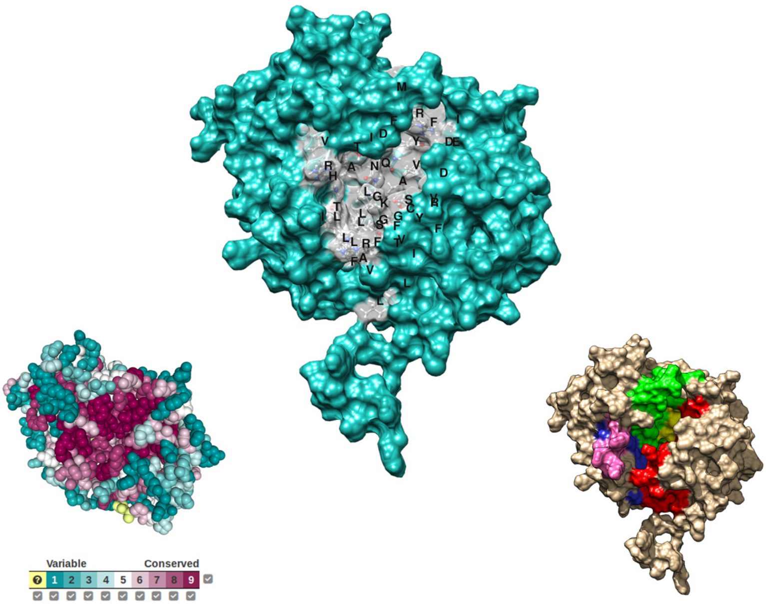


Figure 6. Amino acid residue conservation according to ConSurf (left), predicted pocket from P2Rank (centre) and conserved domain (Lee & Poulter, 2008) (right) representation of the SSL-1 enzyme from *B. terribilis* AICB 872 strain. Domain I – red, Domain II – yellow, Domain III – green, Domain IV – blue, NADPH binding motif – pink.

Multiple sequence alignments were performed as previously described (Szoke-Nagy *et al.*, 2015) and four conserved domains, two Mg^{2+} binding motifs and one NADPH binding motif were also identified in SSL-1 enzyme from *B. terribilis* AICB 872 strain (Fig. 6 right). Domain I and III presented each other one aspartate-rich motif being possibly involved in substrate binding. The conserved domains and motifs were previously described and identified by Gu *et al.* (1998), Pandit *et al.* (2000), and Lee and Poulter (2008).

Discussion

Squalene synthase-like proteins are enzymes involved in hydrocarbon biosynthesis in *B. braunii*, being isolated and described for the first time by Niehaus *et al.* (2011). Hydrocarbon biosynthesis in *B. braunii* Showa starts with a head-head condensation of two moieties of FPP to form an intermediate PSPP, a reaction catalysed by SSL-1, further squalenes are produced by rearrangement of PSPP by SSL-2 in an NADPH-dependent reaction, while SSL-3 converts PSPP to botryococcenes.

In this study, we identified and partially sequenced SSL-1 gene (3811 bp) from a local strain of *Botryococcus terribilis* AICB 872 using previously-designed primer pairs. The stain *B. terribilis* AICB 872 was isolated from a fishpond located in Mureș country from the region of Transylvania, Romania. The homology modeling analysis of SSL-1 enzyme from *B. terribilis* AICB 872 revealed the presence of 18 alfa helix structures and a small beta-sheet. Based on model quality score for both unrefined and refined structures, the refined model was found to be more accurate having less amino acid residues in the disallowed region of Ramachandran plot, and a better Z-score. The superimposed 3D structure of both SSL-1 enzymes from *B. braunii* and *B. terribilis* AICB 872 models showed that the coil structures and both N and C terminus regions of the protein are characterized by the presence of highly disordered structural fragments. Based on the most conserved binding site two possible ligand candidates were identified as FPS and PS7.

The 3D structure of squalene synthase-like enzymes from *B. braunii* was previously investigated by Bell *et al.* (2014) and Elumalai *et al.* (2018). Bell *et al.* (2014) used tridimensional structure prediction and directed mutagenesis of SSL-1 and SSL-3 from *B. braunii* Showa and for a better understanding of 1′–1 and 1′–3 linkages specificity for PSPP rearrangement, also they attempted to identify the functional residues and domain responsible for this critical step.

The hydrocarbon content of *B. terribilis* AICB strains was published for the first time by Hegedűs *et al.* (2014), showing the ability of *B. terribilis* AICB strains to synthesize C_{30}–C_{32} botryococcenes similar with those produced by chemical race B of *B. braunii* strain. Our observations related to the similarities between SSL-1 from both *B. terribilis* AICB 872 and *B. braunii* unravelled the high degree of conservation of the catalytic situs from both strains. On these facts, we believe that the first step of hydrocarbon biosynthesis starting from two moieties of FPP is similarly in both species. Until these findings, the hydrocarbon biosynthesis was limited to one species, being described only at *B. braunii*.

Conclusions

In the present work, we successfully identified and sequenced the partial SSL-1 gene (3811 bp) encoding squalene synthase-like 1 enzyme from *B. terribilis* AICB 872 strain using PCR assay with previously described primer pairs.

The procedures for *in silico* tridimensional structure prediction and analysis of amino acids sequence from *B. terribilis* AICB 872 strain revealed high-quality tridimensional structure which can be used for structure prediction for other SSL enzymes.

The homology modeling analysis of tridimensional structure reveals a high degree of conservation of the catalytic situs between the predicted structure of SSL-1 enzyme from *B. terribilis* AICB 872 strain with those obtained by Elumalai *et. al.* (2018) and SSL-1 from *B. braunii*. Moreover, hydrocarbon content of *B. terribilis* AICB strains published by Hegedűs *et al.* (2014), which reveals the ability of *B. terribilis* AICB strains to synthesize C_{30}–C_{32} botryococcenes similar with those found in chemical race B of *B. braunii*, we presume that the first step of hydrocarbon biosynthesis in *B. terribilis* occurs similar way with the one described in *B. braunii* strains.

REFERENCES

Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindahl, E. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, 1-2, 19-25.

Achitouv, E., Metzger, P., Rager, M.N., & Largeau, C. (2004). C31 - C34 methylated squalenes from a bolivian strain of *Botryococcus braunii*. *Phytochem*., 65(23), 3159-3165.

Al-Hothaly, A. K. (2018). An optimized method for the bio-harvesting of microalgae, *Botryococcus braunii*, using *Aspergillus* sp. in large-scale studies. *MethodsX*, 5, 788-794.

Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., & Ben-Tal, N. (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Res.*, 44:W344-W350.

Bell, S. A., Niehaus, T. D., Nybo, E., & Chapell, J. (2014). Structure-function mapping of key determinants for hydrocarbon biosynthesis by squalene and squalene synthase-like enzymes from the green alga *Botryococcus braunii* race B. *Biochemistry*, 53(48), 7570-7581.

Canutescu, A. A., & Dunbrack, R. L. (2003). Cyclic coordinate descent: a robotics algorithm for protein loop closure. *Protein Sci.,* 12(5), 963–972.

Chen, V. B., Arendall III, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapra, G. J., Murray, L. W., Richardson, J. S., & Richardson, D. C. (2009). MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D*, 66(Pt 1), 12-21.

Dragoș, N., Peterfi, L. Ș., Momeu, L., & Popescu, C. (1997). An introduction to the algae and the culture collection of algae – At the Institute of Biological Research Cluj-Napoca. Cluj University Press, Cluj-Napoca, 267 pp.

Elumalai, S., Sangeetha, T., & Rajesh Kanna, G. (2018). *In silico* modeling and characterization of squalene synthase and botryococcene synthase enzymes from a green photosynthetic microalga *Botryococcus braunii. J. Pet. Environ. Biotechnol.*, 9, 3.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.*, 31(13), 3784-3788.

Gu, P., Ishii, Y., Spencer, T. A, & Shechter, I. (1998). Function-structure studies and identification of three enzyme domain involved in the catalytic activity in rat hepatic squalene synthase. *J. Biol. Chem.*, 273(20), 12515-12525.

Guiry, M. D., & Guiry, G. M. (2019). AlgaeBase, World-wide electronic publication, National University of Ireland, Galway, http://www.algaebase.org/

Hegedűs, A., Mocan, A., Barbu-Tudoran, L., Coman, C., Drugă, B., Sicora, C., & Dragoș, N. (2014). Morphological, biochemical, and phylogenetic assessments of eight *Botryococcus terribilis* strains collected from freshwaters of Transylvania. *J. Appl. Phycol.*, 27(2), 865-878.

Heo, L., & Feig, M. (2018). PREFMD: a web server for protein structure refinement via molecular dynamics simulations. *Bioinformatics*, 34(6), 1063-1065.

Hillen, L. W., & Wake, L. V. (1979). Solar oil [microform]: liquid hydrocarbon fuels from solar energy via algae, First National Conference of Australian Institute of Energy, The University of Newcastle, 5th-9th February.

Hunag, J., & MacKerell, A. D. (2013). CHARMM36 all-atom additive protein force field: validation based on comparison to NMR data. *J. Comput. Chem.*, 34(25), 2135-2145.

Huang, Z., & Poulter, C. D. (1989). Tetramethylsqualene, a triterpene from *Botryococcus braunii* var. Showa. *Phytochem*., 28(5), 1467-1470.

Jendele, L., Krivák, R., Škoda, P., Novotný, M., & Hoksza, D. (2019). PrankWeb: a web server for ligand binding site prediction and visualization, *Nucleic Acids Res.*, 47(W1), W345-W349.

Kawachi, M., Tanoi, T., Demura, M., Kaya, K., & Watanabe, M. M. (2012). Relationship between hydrocarbons and molecular phylogeny of *Botryococcus braunii*. *Algal Res.*, 1(2), 114-119.

Krivák, R., & Hoksza, D. (2018). P2Rank: machine learning based tool for rapid and accurate prediction of ligand binding sites from protein structure. *J. Cheminformatics*, 10(1), 39.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33(7), 1870-1874.

Lawrence, A. K., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. E. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.*, 10(6), 845-858.

Lee, S., & Poulter, C. D. (2008). Cloning, solubilization, and characterization of squalene synthase from *Thermosynechococcus elongates* BP-1, *J. Bacteriol.,* 190(11), 3808 – 3816.

Lee, M. H., Jeong, J. H., Seo, J. W., Shin, C. G., & Kim, Y. S. (2004). Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. *Plant Cell Physiol.*, 45(8), 976- 984.

Lovell, S. C., Davis, I. W., Arendall III, W. B., de Bakker, P. I. W., Word, J. M., Prisant, M. G., Richardson, J. S., & Richardson D. C. (2003). Structure validation by Calpha geometry: phi,psi and Cbeta deviation. *Proteins*, 50(3), 437-450.

Metzger, P., & Largeau, C. (2005). *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biot.*, 66(5), 486-496.

Metzger, P., Allard, B., Casadevall, E., Berkaloff, C., & Coute, A. (1990). Structure and chemistry of a new chemical race of *Botryococcus braunii* that produces lycopadiene, a tetraterpenoid hydrocarbon. *J. Phycol.*, 26(2), 258-266.

Metzger, P., & Casadevall, E. (1987). Lycopadiene, a tetraterpenoid hydrocarbon from new strains of the green alga *Botryococcus braunii*. *Tetrahedron Lett.*, 28(34), 3931-3934.

Metzger, P., Templier, J., Largeau, C., & Casadevall, E. (1986). An *n*-alkatriene and some *n*-alkadienes from the A race of the green alga *Botryococcus braunii*. *Phytochem*., 25(8), 1869-1872.

Metzger, P., Casadevall, E., Pouet, M. J., & Pouet, Y. (1985). Structures of some botryococcenes: branched hydrocarbons from the B race of the green alga *Botryococcus braunii*, *Phytochem*., 24(12), 2995-3002.

Niehaus, T. D., Okada, S., Devarenne, T. P., Watt, D. S., Sviripa, V., & Chappell, J. (2011). Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii*. *PNAS*, 108(30), 12260-12265.

Pandit, J., Danley, D. E., & Schulte, G. K. (2000). Crystal structure of human squalene synthase. A key enzyme in cholesterol biosynthesis. *J. Biol. Chem.,* 275(39), 30610-30617.

Park, J., Matralis, A. N., Berghuis, A. M., & Tsantrizos, Y. S. (2014). Human isoprenoid synthase enzymes as therapeutic targets. *Front. Chem.*, 2: 10.3389/fchem.2014. 00050.

Petersen, H. I., Rosenberg, P., & Nytoft, H. P. (2008). Oxygen groups in coals and alginite-rich kerogen revisited. *Int. J. Coal Geol.*, 74(2), 93-113.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera – a visualization system for exploratory research and analysis. *J. Comput. Chem.*, 25(13), 1605-1612.

Remmert, M., Biegert, A., Hauser, A., & Söding, J. (2012). HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. *Nat. Methods*, 9, 173–175.

Rong, Q., Jiang, D., Chen, Y., Shen, Y., Yuan, Q., Lin, H., Zha, L., Zhang, Y., & Hunag, L. (2016). Molecular cloning and functional analysis of squalene synthase 2(SQS2) in *Salvia miltiorrhiza* Bunge. *Front. Plant Sci.*, 7:1274.

Seo, J. W., Jeong, J. H., Shin, C. G., Lo, S. C., Han, S. S., Yu, K. W., Harada, E., Han, J. Y., & Choi, Y. E., (2005). Overexpression of squalene synthase in *Eleutherococcus senticosus* increases phytosterol and triterpene accumulation. *Phytochem*., 66(8), 869-877.

Söding, J. (2005). Protein homology detection by HMM-HMM comparison,. *Bioinformatics*, 21(7), 951–960.

Szoke-Nagy, T., Hegedűs, A., Baricz, A., Chiriac, C., Szekeres E., Coman, C., & Dragoș, N. (2015). Identification, isolation and bioinformatic analysis of squalene synthase-like cDNA fragments in *Botryococcus terribilis* AICB 870 Strain. *Studia UBB Biologia*. LX(1), 23-37.

Thapa, H. R., Naik, M. T., Okada, D., Takada, K., Molnár, I., Xu. Y., & Devarenne, T. P. (2016). A squalene synthase-like enzyme initiates production of tetraterpenoid hydrocarbons in *Botryococcus braunii* Race L. *Nat. Commun.*, 7:e11198.

Wei, X., & Sahinidis, N. V. (2006). Residue-rotamer-reduction algorithm for the protein side-chain conformation problem. *Bioinformatics*, 22(2), 188–194.

Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res*., 35, W407-W410.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 19-28)
doi:10.24193/subbbiol.2020.1.02

Diversity of aphids (Hemiptera: Aphididae) in oases agro-ecosystem: seasonal dynamics and host plants

Nacima Deghiche Diab[1,2,3]✉, Lahcen Deghiche[1,2] and Mohammed Belhamra[1,2,3]

[1] Agronomic Department, University of Mohammed Khaider, Biskra, Algeria;
[2] Laboratory of Ecosystem Diversity and Dynamics of Agricultural Production Systems in Arid areas, University of Mohammed Khaider, Biskra, Algeria;
[3] Center for Scientific and Technical Research on Arid Regions (CRSTRA);
✉ ***Corresponding author, E-mail: diab_nassima@yahoo.fr.***

Abstract. During the study conducted at the palm grove of Ziban oases from June 2017 until May 2018 and from 48 samples yellow water traps, a total of 969 aphids specimens were captured from 36 spontaneous plants identified at Ain Ben Noui palm grove, Biskra. Over 11 aphid species were identified, which 5 were qualified as accidentals and 3 were respectively accessories and constant species. The most abundant species were *Myzus persicae* (26.32%) and *Aphis gossypii* (21.05%). *Myzus persicae* (Sulzer, 1776) was observed on 9 spontaneous plants, *Aphis fabae* (Scopoli, 1763) was associated to 12 spontaneous plants, whereas *Aphis craccivora* (Koch, 1854) was recorded on 6 spontaneous plants. The most important richness of winged aphids in palm grove was recorded during the spring season (April = 65 individuals; March = 58 individuals) where temperature average was between 17.5°C, 25°C and level rains (May=49.53mm; March=11.67mm).

Key words: Aphids, Biskra, diversity, host plants, palm groves.

Introduction

Aphids belonging to Insecta class, Hemiptera order, Aphididae family, encompass about 4700 species in the world (Remaudière and Remaudière, 1997; Blackman and Eastop, 2000). Their ecology presents a particular phenomenon, winged that constitutes the dispersal form and skills form specialized in colonization of plants. Because aphids have a good biological model for habitat selection (Pettersson *et al.*, 2007), in addition host plants have a great impact on their diversification, aphids follows an annual cycle between a primary host (cultivated crops) and a secondary host (spontaneous plants). Spontaneous plants are favoured secondary hosts during bad times and in absence of crops

(Guzand Kilincer, 2005). Oases ecosystems are favourable home of wild flora associated to cultivated vegetation that occupy up to three levels (Diab and Deghiche, 2013; 2016, Deghiche-Diab, 2015). In order to have an idea about aphid population that inhabit oasis ecosystem, this study have for objective to assess the diversity of aphids, abundance and their habitats diversity according to wild plants.

Material and methods

Study area

Located in the municipality of El Hadjeb, at 8 km west of the capital of Biskra province, Ain Ben Noui (34° 48 '21.68' 'N and 05 ° 39'24.72' 'E) palm grove is a part of the experimental station of the Technical Institute for Saharan Agriculture Development (Fig. 1), it covers an area of 21.5 hectares. Situated in an arid area, the period of study was characterized by cumulative precipitation 49.25mm, the average of the maximum temperature 28.9°C and the minimal temperature 17°C (www.tutiempo.net).

Figure 1. Plot survey and pitfall traps location in palm grove (Google earth).

Sampling methods

Our survey was performed from the same sampling areas for both aphids and spontaneous plants but obviously applying different protocols. Aphids were sampled weekly using 3 yellow traps placed in diagonal and spaced far enough to collect specimens (Benkhellil, 1991). Visual observations of spontaneous plants were performed from 4 belt transect (Holt *et al.*, 2013) with 50m length and escaped 50m (Fig. 1). Collected specimens were separated using magnifier binocular in laboratory. Samples were identified to genus and species for the majority specimens. They were stored in collection boxes and kept in the entomology laboratory.

Ecological Analysis

To characterize aphids and host plants from spontaneous plants in Ziban palm groves, we use of environmental indices; total wealth (S) and average (Sm = Σ S / N) (Ramade, 1983), the relative abundance (AR or F = 100 or x /N) (Dajoz, 1971), density (D=N/P) Occurrence and Dominance (Dajoz, 1985), Shannon index (H '= $\log_2$ -Σ pi) (Blondel, 1973) and evenness ($E = \frac{H'}{H'_{max}} = \frac{H'}{\log 2S}$) for both (Blondel, 1975). To describe aphid species captured we also classified aphid species according to their host specificity. Data on species structure, abundance, diversity and differences in community composition at each month were analysed using PAST (Paleontological Statistics; Version 2.17) software. The relationships between insect diversity parameters were verified using Pearson correlation tests for each month.

Results and discussions

Aphid diversity and abundance

During the trapping season over 48 weeks, 969 individuals aphids were collected, including 11 genera and 11 species (Tab. 1)**.** The study reveals the presence of 11 species of winged aphids distributed in one family (Aphididae), 1 (one) subfamily (Aphidinae) and 2 (two) tribes. With regard to the tribes listed, it appears that Aphidini tribe was best represented (6 species) in palm groves, whereas Macrosiphini tribe was represented with 5 species (Tab. 1). Aphis genus was the most represented in different sites in palm grove with 4 species followed by *Rhopalosiphum* genus with only 2 species, the other genus were represented only with 1 species for each.

Results of few studies carried out across Biskra region indicate a richness that oscillates between 3 species (Saighi *et al*., 2005), 18 species (Ben Abba and Bengouga, 2007) and 30 species (Laamari *et al*., 2010). In total, from latest survey at Ziban palm groves: Menacer (2012), Deghiche Diab (2009), Deghiche et *al*., (2015), Deghiche Diab et *al*. (2015a,b) and Deghiche *et al*., (2020) listed 33 species of aphids in Ziban oases. The most represented aphid species in oasis ecosystem were (Fig. 2): *Myzus persicae* Sulzer, 1776 (26.32%), *Aphis gossypii* Glover, 1877 (21.05%), *Brachycaudus helichrysi* Kaltenbach, 1843 (19.9%.) and *Aphis fabae* Scopoli, 1763 (10.22%). Those represented with less diversity were; *Rhopalosiphum maidis* Fitch, 1856 (1.14%), *Aphis nerii* Fonscolombe, 1841 (1.55%) and *Uroleucon sonchi* Linnaeus, 1767 (2.37%).

Table 1. Aphid species collected from Ziban palm grove (2017-2018).

Family	Sub-family	Tribes	Genus	Species
Aphididae	Aphidinae	Aphdini	*Aphis*	*Aphis craccivora* Koch, 1854
				Aphis fabae Scopoli, 1763
				Aphis nerii Fonscolombe, 1841
				Aphis gossypii Glover, 1877
			Rhopalosiphum	*Rhopalosiphum maidis* Fitch, 1856
				Rhopalosiphum padi Linnaeus, 1758
		Macrosiphini	*Brachycaudus*	*Brachycaudus helichrysi* Kaltenbach, 1843
			Brachyunguis	*Brachyunguis harmalae* B. Das, 1918
			Hyperomyzus	*Hyperomyzus lactucae* Linnaeus, 1758
			Myzus	*Myzus persicae* Sulzer, 1776
			Uroleucon	*Uroleucon sonchi* Linnaeus, 1767

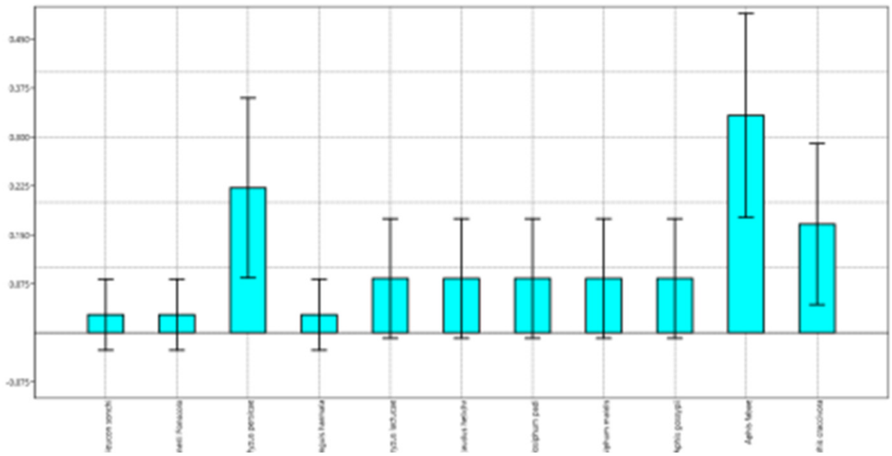


Figure 2. Aphid species richness under Ziban palm groves.

The presence of those species as indicated Robert & Rouze-Jouan (1976) can be related to several factors and parameters gathered in a single environment such as our oasis system, climatic (temperature, humidity) or biological (host plants and the parasite procession).The height number of *Aphis gossypii, Myzus persesea* in palm grove were also indicated during three years of study by Deghiche Diab *et al*., (2015a), Deghiche-Diab *et al*., (2015b) worked in oases of Ziban. Moreover, Menacer (2012), found higher rates of *Myzus persicae* species and *Aphis gossypii*.

Another study, Ghalbi and Mouada (2008), carried out on a field of barley and beans in Sidi Okba region (Biskra), note high rates of *Hyperomyzus lactucae, Myzus persicae* and *Aphis craccivora* species among the 7 species collected.

Constancy

Obtained results on constancy analyses indicate that *Aphis fabae* (Scopoli 1763) *Aphis gossypii* (Glover 1877) and *Myzus persicae* (Sulzer1776) were classified as constants species, whereas *Aphis craccivora* (Koch 1854), *Rhopalosiphum maidis* (Fitch, 1856), *Brachyunguish armalae* (B.Das, 1918), *Aphis nerii* (Fonscolombe, 1841) and *Uroleucon sonchi* (Linnaeus, 1767) were accidental species, identified in our study (Fig. 3) and also reported by Frah (2009) in apple orchards in Batna. Species *Rhopalosiphum padi* (Linnaeus, 1758), *Brachycaudus helichrysi* (Kaltenbach, 1843) *Hyperomyzus lactucae* (Linnaeus, 1758) were accessories species in palm groves (Fig. 3).

The large presence of constant species in palm grove can be explained by polyphagous species living on large number of cultivated species and hosts plants belonging to different wild flora families (Hullé *et al.*, 1999; Evelyne *et al.*, (2011), from which *Myzus persicae* only have about 875 secondary host plants. Of these is highlighted *Rhopalosiphum padi*, one of the accessory species, because their presence can be related to the senescence of wild grasses responsible for the massive formation of these emigrant adults and cultivated barley plots in palm groves. During our survey rare species were not found, which could be a result of the sampling design (borders were avoided).

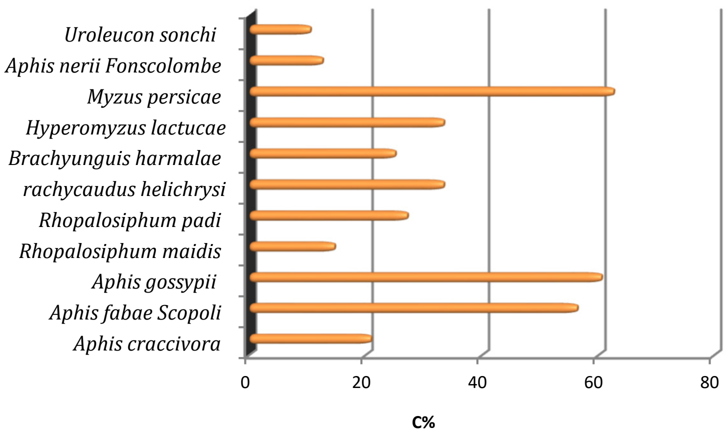


Figure 3. Constancy scale of aphids collected in palm groves.

Shannon diversity index

Value of Shannon diversity index (2.19 bits) calculated for sampled species in palm groves with yellow traps was considered high (Tab. 2); it means that palm groves had height diversity of aphid species. The evenness value (0.63) calculated showed equilibrium with aphid species between them.

Table 2. Total wealth, Shannon diversity index and evenness value for aphid species in palm groves.

Wealth (S)	Evenness value (E)	Shannon diversity (H')	**H MAX**
11	**0.63**	**2.19**	**3.45**

A total of 34 associations were established with 11 species of aphids and 36 spontaneous plants found under palm groves agro-ecosystem (Fig. 4). The largest number of associations were recorded with *Aphis fabae* (12 associations) and *Mysus persicae* (9 associations), followed by *Aphis craccivora* (6 associations) and *Brachycaudus helichrysi* (4 associations) wheels, *Aphis gossypii*, *Rhopalosiphum padi* and *Rhopalosiphum maidis* (3 associations), *Hyperomyzus lactucae* (2 associations), *Aphis nerii*, *Uroleucon sonchi* and *Brachyunguis harmalae* (1 association).

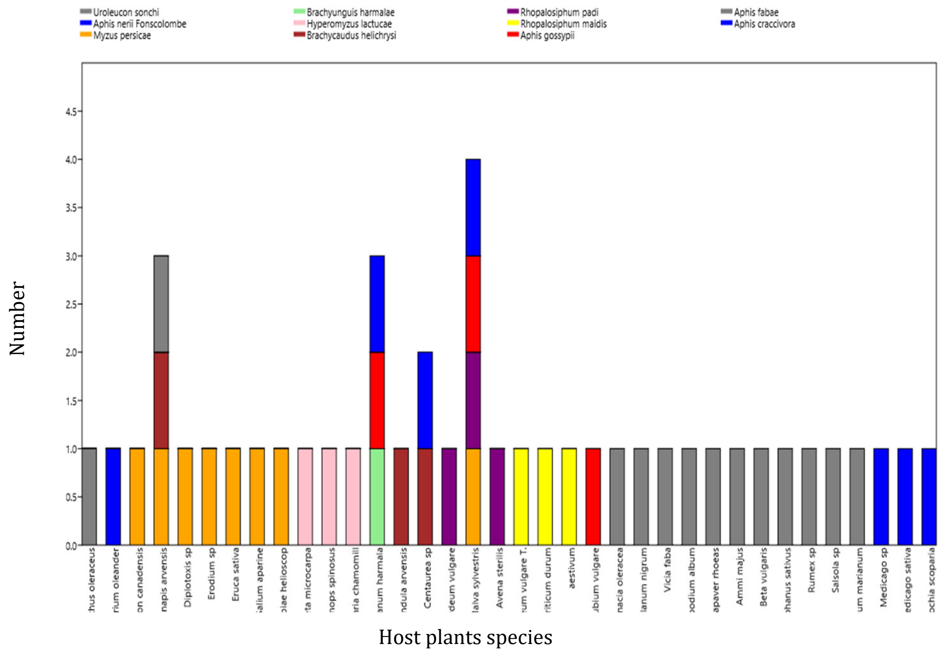


Figure 4. Aphid species association according to their host plants.

According to Hullé *et al*. (1999), *Hyperomyzus lactucae* found in the Mediterranean region is a holocyclic and diocidal aphid. Its secondary hosts are mostly from *Sonchus* genus Asteraceae family, whereas *Aphis craccivora* can be found on species belonging to different families from Asteraceae, Cucurbitaceae, Fabaceae and Solanaceae family (Evelyne *et al.,* 2011). In addition our results were confirmed by Hullé *et al*., (1999), as reported that *Aphis fabae* species have above 200 host plants from which species from Fabaceae, Chenopodiceae, Asteraceae, Brassicaceae botanical families. Laamari *et al*., (2011) in study conducted in two types of ecosystem recorded also 16 associated plants to *Myzus persicae*, 15 plant species with *A. fabae* and *A. craccivora* with only 10 plant species in Biskra region.

We analysed aphid species based on their host plant specificity polyphagous (living on plants from different families), oligophagous living on a reduced number of host plants) and monophagous (on plants of the same family) (Resh and Cardé, 2009). The half of recorder aphid species in palm grove were monophagous aphid species with (45.45%), polyphagous species was least-frequently observed (36.36%) and the oligophagous species were represented only by 18.18%. Among the polyphagous aphids collected we remark: *Aphis craccivora, Aphis fabae, Aphis gossypii* and *Myzus persicae.* The monophagous species were *Rhopalosiphum maidis, Aphis nerii, Uroleucon sonchi, Brachyunguis harmalae* and *Hyperomyzus lactucae.* The oligophagous species were; *Rhopalosiphum padi* and *Brachycaudus helichrysi.*

Distribution of aphid species according to climate conditions

The most important richness of winged aphids in palm grove was recorded during spring season with 65 individuals (April) and 58 individuals (March). During this period the temperature average raised 17.5°C, 22.4 and 25°C respectively during March, April and May, during this period the level rains noted was the highest (May= 49.53mm; March=11.67mm) which favours an important vegetative cover (Fig. 5).

Another peak of aphid activity, but less important, was recorded during autumnal period where temperatures were (Sept= 28; Oct= 22.5; Nov= 11.9 °C) and level rain does not exceed 10mm. A very low percentage was recorded during summer period (July=4; August = 0) and autumn (Sept=6). In addition, during our survey, relative humidity average varies between 24.00 and 55.6%. December, January and February were the wettest, while June, July and August were the driest.

Apparently, the important activity of adults during April and May can be attributed to spread of most species in order to settle on cultivated crops or weeds. It can be also related to temperature that had great importance on aphids activity, life cycle, fertility (Robert, 1980), they can reach their maximum development between 18 until 24°C (Leclant, 1970). In addition, as reported

Bouchet *et al.*, (1981), aphids were not able to fly below 13 ° C and died below 5 ° C that explain their number decrease during summer and earlier autumn period. Similarly Bonnemaison (1962) noted that frequent rainfall and high relative humidity decrease the fertility of aphids and increase their mortality. Rainfall had less importance on aphid activities (Robert and Rouze-jouan, 1978) the small quantities recorded during spring period did not disrupt the flight activity of adults but their fertility (Bonnemaison, 1962) and can destroy proportion of wingless and adults.

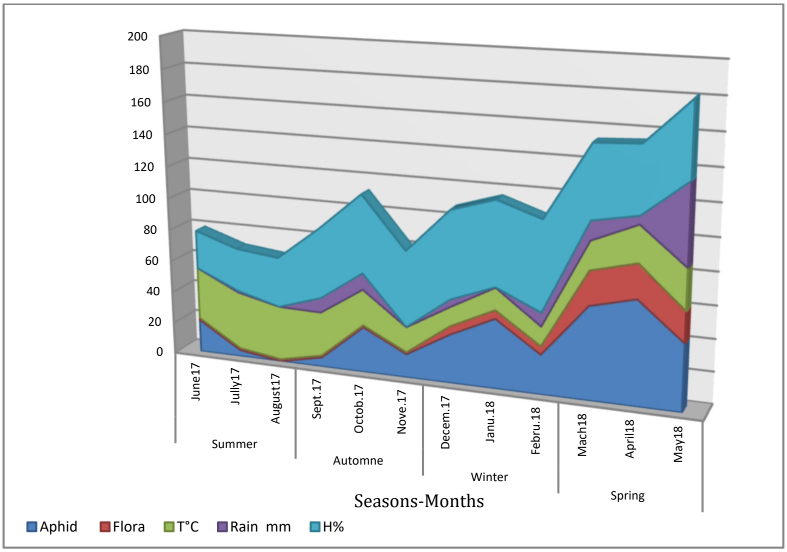


Figure 5. Evolution of aphids and thier host plants according to climat conditions of palm grov during 2017-2018.

Conclusion

At Ain Ben Noui palm grove, 11 aphids species were captured from 36 spontaneous plants identified. The most abundant species were *Myzus persicae* (26.32%) and *Aphis gossypii* (21.05%). *Myzus persicae* (Sulzer, 1776) was observed on 9 spontaneous plants, *Aphis fabae* (Scopoli, 1763) was associated to 12 spontaneous plants, whereas *Aphis craccivora* (Koch, 1854) was recorded on 6 spontaneous plants.

Obtained results from survey conducted in chosen Ziban palm grove for studying dynamic of aphids and their associated host plants, indicate important relations between aphid species and spontaneous flora (weeds, wild flora) inhabiting. Those associations can be useful in future by establishing a program pest control under palm grove.

Acknowledgements. Authors are thankful to personnel of the Laboratory of Ecosystem Diversity and Dynamics of Agricultural Production Systems in Arid areas, University of Mohammed Khider, Biskra, Algeria for their help and determination of species.

REFERENCES

Ben Abba, C., & Bengouga, K. (2007). Contribution à l'étude qualitative des pucerons (Homoptera, Aphididae) sur l'orge et la fève dans la région de Biskra. *Mémoire Ing. Agro., Dép. Agro.*, Univ. Biskra, 100 pp.

BenBenkhelil, M.L. (1991). Les techniques de récolte et de piégeage utilisées en entomologie terrestre. *Ed. OPU* Alger, pp. 66.

Blackman, R.L., & Eastop, V.F. (2000). Aphids on the World's Crops-An identification and information guide*. Ed. Ltd JWS and Natural History Museum*, London, pp. 466.

Blondel, J. (1975). L'analyse des peuplements d'oiseaux, éléments d'un diagnostic écologique: la méthode des échantillonnages fréquentiels progressifs (E.F.P.) *Terre et vie* XXIX, 533-589.

Blondel, J. (1979). Biogéographie et écologie, *Ed. Masson*, France, pp. 173.

Blondel, J., Ferry, C., & Frochot, B. (1973). Avifaune et végétation. Essai d'analyse de la diversité. *Alauda*, 4, 63-84.

Bonnemaison, L. (1962). Les ennemis animaux des plantes cultivées et des forêts. Tome II. *Ed. S.E.P.*, Paris, pp. 605.

Bouchet, F., Bayou, F., Mouchart, A., Costes, J.P., Lescar, L., Devriendt, M., Dedryver, C. A., Lapierre, H., Moreau, J.P., Chambon, J.P., Brenian, D., Lamessilaire, C., Roque, B., Lechapt, G., & Martin, M. (1981). Les pucerons des céréales à paille. In : *Journées d'étude et d'information sur les pucerons des cultures*, 2,3 et 4 mars 1981, Paris: 117-122.

Dajoz, R. (1971). Précis d'écologie. *Ed. Dunod*, Paris pp. 434.

Dajoz, R. (1985). Précis d'écologie. *Ed. Dunod*, Paris pp. 505.

Deghiche-Diab, N. (2009). Inventory of insects in the oases of Ziban, Biskra- Algeria. Thesis Master of Science, Iam -Bari, Italy. pp. 82.

Deghiche-Diab, N., Deghiche, L., & Belhamra, M. (2015b). Inventory of Arthropods in an agro-ecosystem Ziban oasis, Ain Ben Noui, Biskra, Algeria. *Journal of Entomology and Zoology Studies*. 3(4), 229-234.

Deghiche-Diab, N., Prcelli, F., & Belhamra, M. (2015a). Entomofauna of Ziban oasis. *Journal of Insect Science*. *Oxford*. 15(41).

Deghiche-Diab, N., Belhamra, M., Deghiche, L., & Boultif, M. 2020. Cartography and distrubution of insects species according to habitats diversity in Ziban Biskra-Algeria. *Munis Entomology & Zoology*, 15 (2), 412-421.

Diab, N., & Deghiche, L., (2016). La flore spontanée des Oasis des Ziban*. Éditions Universitaires Européennes*. pp. 300.

Données climatiques: Algérie (2018) [Accessed Dec 2018) *https://fr.tutiempo.net.*

Farah, N. (2009). Entomofaune, impact économique et bio-écologie des principaux ravageurs du pommier dans la régions des Aurès. Thèse Doctorat, Université de Batna. pp. 204.

Google earth V6.2.2.6613.DigitalGlobe . http://www.earth.google.com[DECec, 2018].

Güz, N., & Kılınçer, N. (2005). Aphid Parasitoids (Hymenoptera: Braconidae: Aphidiinae) on Weeds from Ankara, Turkey. *Phytoparasitica*, 33 (4), 359-366.

Hammer, Ø., Harper, D.A.T., & Ryan P.D. (2001). Past: Paleontological Statistics Software Package for Education and. Data Analysis. *Palaeontologia Electronica* http://palaeo-electronica.org.

Holt, B.G., Rioja-Nieto, R., MacNeil, M.A., Lupton, J. & Rahbek, C. (2013). Comparing diversity data collected using a protocol designed for volunteers with results from a professional alternative. *Methods in Ecology and Evolution* 4, 383–392.

Hullé, M., Chaubet, B., Dedryver, C.-A. & Turpeau-Ait Ighil E. (2011). Les pucerons des grandes cultures: Cycles biologiques et activités de vol. *Ed. Quae*, Paris, pp. 36.

Laamari, M., Jousselin, E., & Coeur d'acier, A. (2010). Assessment of aphid diversity (Hemiptera: Aphididae) in Algeria: a fourteen-year investigation. *Entomologie faunistique Faunistic Entomology* 62 (2), 73-87.

Laamari, M., Tahar Chaouche, S., Benferhat, S., Abbès, B.S., Merouani, H., Ghodbane, S., Khenissa, N., & Stary, P. (2011). Interactions tritrophiques: plante-puceron-hyménoptère parasitoïde observées en milieux naturels et cultivés de l'Est algérien. *Entomologie faunistique, Faunistic Entomology* (63)3, 115-120.

Leclant, F. (1970). Les aphides et la lutte intégrée en verger. Bulletin technique d'information. La lutte intégrée en vergers. *Ed. Ministère de l'agriculture*, 249, pp. 261.

Leclant, F. (1981). Les effets nuisibles des pucerons sur les cultures. In : *Journées d'études et d'information. Les pucerons des cultures*. Paris 2, 3 et 4 mars 1981: 37-55

Menacer, S. (2012). Influence des facteurs microclimatiques de la palmeraie sur la diversité du peuplement aphidien dans la région de Biskra. *Mémoire de Magister. Inst. Nat. Agro. El Harrach*. pp. 100.

Pettersson, J. W. F., Tjallingii Hardie, J. (2007). Host-plant Selection and Feeding. Aphids as Crop pests. H. F. van Emden and R. Harrington. Cambridge, CAB International: 87-113.

Ramade, F. (1983). Eléments d'écologie. Ecologie fondamentale. *Ed. Mc Graw-Hill*, Paris. 11, pp. 397.

Remaudière, G., & Remaudière, M. (1997). Catalogue of the world's Aphididae (Homoptera Aphidoidea). *INRA Ed.*, Paris, pp. 473.

Resh, V. H., & Cardé, R. T. (2009). Encyclopedia of Insects, 2^{nd} Ed. pp. 798-800.

Robert, Y. (1980). Recherche sur la biologie et l'écologie des pucerons en Bretagne. Application à l'étude épidémiologique des viroses de la pomme de terre. Thèse doctorat Scie., Rennes, pp. 242.

Robert, Y., & Rouze-Jouan, J. (1976). Activité saisonnière de vol des pucerons (Hom. Aphididae) dans l'ouest de la France. Résultats de neuf années de piégeage. *Ann. Soc. Ent. Fr. (N.S)* 12(4), 671-690.

Robert, Y., & Rouze-Jouan, J. (1978). Recherches écologiques sur les pucerons *Aulocathum solani* Kith., *Macrosiphum euphorbiae Thomas* et *Myzus persicae* Sulz. Dans l'ouest de la France. Etude de l'activité de vol de 1967 à 1976 en culture de pomme de terre. *Ann. Zool. Ecologia Animale*, 10(2), 171-185.

Saighi, S., Doumandji, S., Aifaoui, O., & Haichar, M. (2005). Contribution à l'inventaire de l'entomofaune dans la région de Biskra. In: *Actes des journées internationales sur la désertification et le développement durable*, du 10 au 12 juin 2006. CRSTRA et Univ. de Biskra : 239-248.

Turpeau-Ait Ighil, E., Dedryver, C.-A., Chaubet, B., Hullé, M. (2011). Les pucerons des plantes maraîchères. Cycles biologiques et activités de vol. ACTA –*INRA Ed.*, pp. 136.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 29-36)
doi:10.24193/subbbiol.2020.1.03

Distribution and characteristic habitat of *Convolvulus persicus* L. in South-East Romania: threats and protection solutions

Valentin Adrian Kiss[1,2]✉, Paul-Marian Szatmari[1,3]

[1] *Babeş-Bolyai University, Faculty of Biology and Geology, Cluj-Napoca, Romania;*
[2] *Babeş-Bolyai University, Student College for Advanced Performance Studies, Cluj-Napoca, Romania;*
[3] *Biological Research Center, Botanical Garden "Vasile Fati" Jibou, Jibou, Romania;*
✉ **Corresponding author, E-mail: kissvalentinadrian@gmail.com.**

Abstract. *Convolvulus persicus* is a critically endangered species, endemic to the embryonic shifting dunes of Caspian Sea and Black Sea. The distribution of this species in Romania includes also the sand dunes on the seashores of Sfântu Gheorghe and Sulina. This paper presents new data about the endangered species *Convolvulus persicus* regarding the distribution and the effective population size in Romania. The conservation status of the embryonic shifting dunes, found in the mentioned sites in 2019, was reviewed, along with the threats for both the habitat and the species. Several protection methods were proposed.

Keywords: conservation, critically endangered species, endemic.

Introduction

Some of the main features of the Anthropocene era are the decline of biodiversity and the high rate of species extinction (Waters *et al.,* 2016). Coastal areas with habitats suitable for *Convolvulus persicus* are constantly modified, destroyed or reduced by human pressures, but also by natural processes, such as coastal erosion and dramatic changes in beaches caused by sea level fluctuations (Golubtsov and Lee, 1997, Golitsyn *et al.,* 1998, Ignatov et al., 1983, Beni *et al.,* 2013). *C. persicus* is a pioneer species for habitat 2110 (Strat and Holobiuc, 2018), forming associations with high importance due to its status as critically endangered species (CR) in Romania (Dihoru and Negrean, 2009) and its limited distribution (Făgăraș and Jianu, 2015).

The bibliography mentions Sfântu Gheorghe and Sulina among the places where populations of *Convolvulus persicus* exist (Oprea, 2005; Dihoru and Negrean, 2009). This study establishes the approximate distribution of the population in the two mentioned sites and at the same time evaluates the habitat status, to identify the possible threats and to propose some methods of protection for both the habitat and *Convolvulus persicus*.

Materials and methods

The paper examines the bibliography regarding the distribution of this species, its biology and ecology, and also the references and the management plans regarding the habitat 2110. In addition, during the period 3-11 July 2019, field trips were made, in which we marked with the GPS (GPSmap 61s GARMIN) approximately the extreme limits where the phytoindividuals of *C. persicus* were located both on Sfântu Gheorghe and Sulina beach. Using ArcGis-ArcMaps, a Stereo 70 projection map with the approximate population distribution was created. At the same time, we consulted the *List of activities to indicate the impacts, respectively of the current and past pressures and of the future threats* and evaluated them on the ground. The scale on which the estimation of the population numbers of *C. persicus* was made is taken from the management plan of *ROSCI0073* from 2011 (Management plan ROSCI0073, 2011).

Study area

The Black Sea coast of Romania covers 256 km and is divided into two major sections: the northern part, with sandy beaches in the deltaic area and the southern part, composed largely of cliffs with soft rocks (Strat and Holobiuc, 2018). The pressures are exerted more strongly in the southern part, being more pronounced in the metropolitan area of Constanța, the ancestral form of the beaches being irreversibly changed, along with the habitats present there (Strat and Holobiuc, 2018).

Sulina is the most eastern city in Romania, located in the coastal area of the Danube Delta. It is crossed by the Sulina canal, one of the three channels of the Danube. Sulina beach is located south of the Sulina canal and responsible for the beach management are the City Council and the Danube Delta Administration (Făgăraș and Jianu, 2015). The beach of Sfântu Gheorghe, 7.5 km long, is located in the central part of the Danube Delta coast (Vespremeanu and Preoteasa, 2006). Most of the economic activities in Sulina and Sfântu Gheorghe are represented by fishing and tourism.

Convolvulus persicus – biology and ecology

C. persicus (Fig. 1) is distinguished from the rest of the species of the genus *Convolvulus* by its woolly appearance, with the sudden axis from which flowering stems rise. The leaves are elliptic-oblong and short petiolate, its corolla (Fig. 1 right) is white (Ciocârlan, 2009; Sârbu *et al.*, 2013; Dihoru and Negrean, 2009). The flowers are bisexual and 4 lobes bloom from bottom to top on the stem. The fruits are capsules with 2-3 seeds. (Strat and Holobiuc, 2018). *C. persicus* is amphimyctic, apomictic by vegetative reproduction and polycormia (Dihoru and Negrean, 2009).

Figure 1. *Convolvulus persicus* L

It is a psammophile, xeromezophile plant that prefers dry, neutral and poor sands in nitrogen (Dihoru and Negrean, 2009). It forms populations that develop in the form of large colonies, the accompanying species being mainly confined to the periphery of the association. *C. persicus* forms the association *Convolvuletum persici* Borza, 1931 (Făgăraș and Jianu, 2015). The floristic composition of the association is poor in species (37 taxa). The following species from the orders *Festucetea vaginatae* and *Festuco-Brometea* were mostly present: *Alyssum borzaeanum, Alyssum hirsutum, Bromus tectorum, Secale sylvestre, Medicago falcata, Silene conica, Astragalus varius, Euphorbia seguieriana, Centaurea arenaria* subsp. *borysthenica, Stachys atherocalyx, Scabiosa argentea, Ephedra distachya, Linaria genistifolia, Silene borysthenica, Sideritis montana, Marrubium peregrinum, Senecio vernalis, Cynanchum acutum, Papaver rhoeas, Cerastium pumilum, Polytrichum piliferum, etc.* (Management plan ROSCI0073, 2011).

Habitat 2110 – Embryonic shifting dunes

Coastal formations represent the first stages of the formation of dunes (Fig. 2), consisting of undulating or elevated sand surfaces of the upper beach, or a seaward edge at the base of the high dunes (Gafta *et al*., 2008).

Structure: Phytocenoses are structured on two levels: the shortest is composed of annual species such as: *Bromus tectorum, Secale sylvestre, Plantago arenaria, Apera maritima*, which use sand moisture during the spring, and ends its vegetative cycle at the onset of the dry season. In addition to the annual species, some psammophile, perennial plants make up the upper floor, such as: *Leymus sabulosus, Agropyrum junceum, Centaurea arenaria* subsp. *borysthenica, Gypsophila perfoliata, Artemisia arenaria, Corispermum nitidum, Eryngium maritimum* (Doniță *et al.,* 2005).

Floristic composition

Edifying species: *Leymus sabulosus*, *Artemisia arenaria, Agropyron junceum.*
Characteristic species: *Artemisia arenaria, Leymus sabulosus, Agropyron junceum*.
Other important species: *Centaurea arenaria* subsp*. borysthenica, Gypsophila perfoliata, Eryngium maritimum, Cakile maritima* subsp. e*uxina, Secale sylvestre, Astrodaucus littoralis, Euphorbia seguieriana, Bromus tectorum, Salsola soda, Crambe maritima* (Doniță *et al.,* 2005).

Figure 2. Habitat 2110 – Sfântu Gheorghe beach

Results and discussions

Distribution and state of the population of C. persicus

Sfântu Gheorghe beach has a size of 60.02 hectares, among them, the populations of *C. persicus* identified in the field campaigns carried out within the POCU project (Fig. 3) occupy 2.76 hectares (population A) and 0.03 hectares (population B). It can be seen from figure 3 that there is a fragmentation of 1,048 km between the two populations. This fragmentation is due to the presence of the arranged beach and the commercial areas (bars and restaurants).

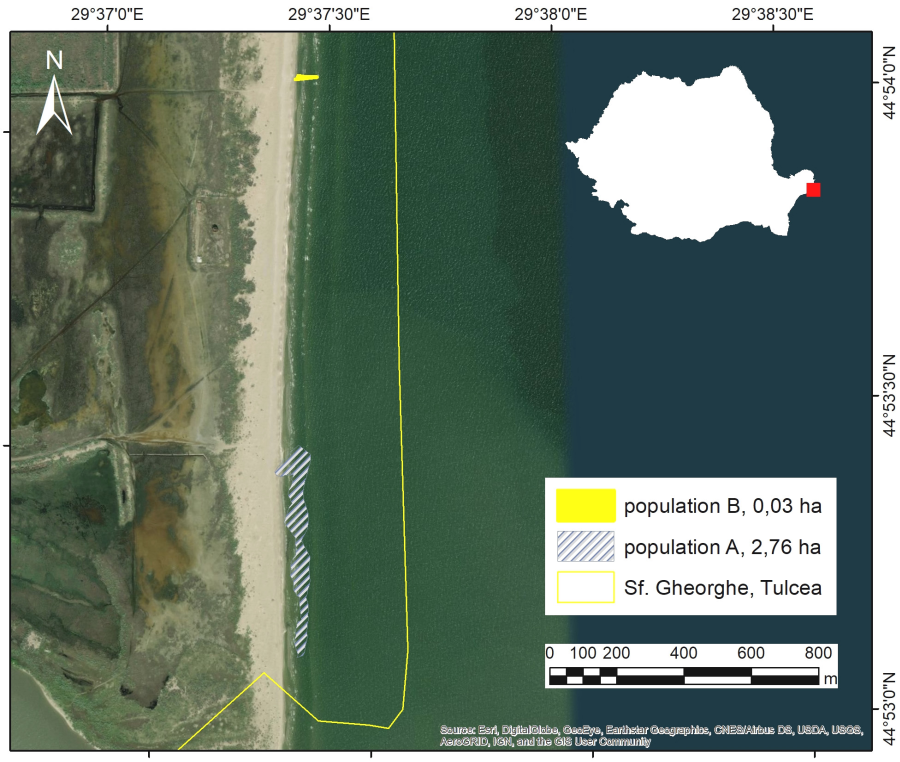


Figure 3. Stereo 70 projection map - Populations of *C. persicus* (population A – blue and white and population B – yellow) on Sfântu Gheorghe beach identified in 2019.

The risk factors for the populations of *C. persicus*, both on Sfântu Gheorghe and Sulina beach are: tourism, plastic pollution, irresponsible breeding of animals (cows and horses), use of pesticides, appearance of paths and trails, dispersed habitation, harvesting flowering phyto-individuals, outdoor sports and recreational activities and developing resting complexes. At the same time, the covering of embryonic shifting dunes with steppe species, the solification and humification of the sands, the penetration of problematic native species, the extension of bushes on the sand dunes and their ruderalization were observed.

Current status of habitat 2110 – Embryonic shifting dunes

A number of 16 threats were identified (Tab. 1), but the main problems for the habitat are of anthropic origin, being represented by the presence of bars and beaches, macro-pollution with plastic and micro plastic (Fig. 4), irresponsible growth of livestock and outdoor recreational activities. The conservation state of the population on the beach of Sfântu Gheorghe is good, having average population numbers (Medium effective population size). This is due to the not very large number of tourists who frequent the beach. In contrast, on the beach of Sulina we could not identify any phytoindividual (Fig. 5 and Tab. 1).

The embryonic sand dunes are widespread on the beach of Sfântu Gheorghe, the present habitat being in a good, semi-natural state, the anthropic impact on it being low.

The habitat is also in a semi-natural state, but the current anthropogenic impact on it is of medium intensity. Sulina beach is much more populated than Sfântu Gheorghe beach, and with the increased number of tourists, the number of risk factors on the habitat increases. Three risk factors not encountered on Sfântu Gheorghe beach were identified: beach cleaning, improving access in the area and extinction of *C. persicus* species (Fig. 5).

Figure 4. Sulina beach - Terrestrial macro-pollution with plastic and micro plastic represented by cigarette butts.

Figure 5. Sulina Beach - *Eryngium maritimum* indicates the presence of habitat 2110, which is in a state of degradation, due to the grazing of horses and cows (left) and the presence of commercial areas (right).

Table 1. Threats identified in the field for *C. persicus* and habitat 2110: list of activities to indicate the impacts, respectively of the current and past pressures and of the future threats (** present only on Sulina beach)

CODE	IMPACT
A04.01.01	cattle grazing
A04.01.03	horse grazing
D01.01	paths, trails, routes for cycling
D05**	improving access in the area
E02.05	other industrial or commercial areas
E03.01	storage of household waste
F04	sampling of land plants in general
F04.02.01	manual assembly
G01.02	walking, horseback riding and non-motorized vehicles
G01.03.	motor vehicles
G01.08.	other outdoor sports and recreational activities
G05.05**	cleaning the beaches
H05.01	garbage and solid waste
I02	problematic native species
J03.02	reducing connectivity between habitats due to anthropogenic causes
M02.03.**	decline or disappearance of species

The following proposed protection methods could prevent the extinction of the remaining population of this critically endangered species:

- Restricting *C. persicus* populations with an electric fence to prevent horses and cows from settling.
- Location of information panels about *C. persicus* near the populations
- Carrying out and publishing studies on *C. persicus* for a better understanding of the ecology and biology of the species
- Prohibition of outdoor recreational activities on the area of *C. persicus* populations
- Periodic greening (garbage cleaning) on the beach

Drafting of tourist materials that promote and present the need for protection of this species and its close connection with habitat 2110.

Conclusions

Although *C. persicus* is an endangered species, at present, the population numbers and the habitat to which it is closely linked have a good state. Instead, the anthropization, the excessive tourism and the effects that the man produces in the natural habitats can lead in the future to the extinction of this species, thus, the majority of the risks for the species and the habitat are mainly of anthropogenic origin.

Aknowledgments. The authors would like to thank Dr. Florin Crișan for his suggestions and Dr. Andrei Crișan for his guidance and help generating the distribution map in ArcMaps. This study was carried out within the project *Competences adapted to the labor market for students from the Faculties of Chemistry and Chemical Engineering, Biology and Geology and Physics* PROJECT POCU/90/6/13/6/14/109175.

REFERENCES

Beni, N. A., Lahijani, H., Harami, M. R., Arpe, K., Leroy, S. A., & Mmarriner Nreimer, P. (2013). Caspian sea-level changes during the last millennium: historical and geological evidence from the south Caspian Sea. *Clim. Past*, *9,* 1645–1665.

Ciocârlan, V. (2009), *Flora ilustrată a României. Pteridophyta et Spermatophyta,* Ed. Ceres, Bucureşti, pp. 1141.

Dihoru, G., & Negrean, G. (2009). Cartea roşie a plantelor vasculare din România. *Ed. Academiei Române*, pp. 630.

Doniţă, N., Popescu, A., Păucă-Comănescu, M., Mihăilescu, S., & Biriş, I.-A. (2005). Habitatele din România (*Habitats in Romania*). *Ed. Tehnică – Silvică*, pp. 57.

Făgăraș, M., & Jianu L. D. (2015). Phytocenoses with Convolvulus Persicus L. on the Western Coast of the Black Sea. *Journal of environmental protection and ecology* 17(4), 1294-1304.

Gafta, D., Mountford, O., Alexiu, V., Anastasiu, P., Bărbos, I. M., Burescu, P., Coldea, G., Drăgulescu, C., Făgăraș, M., Goia, I., Groza, G., Micu, F., Mihăilescu, S., Moldovan, O., Nicolin, A. L., Niculescu, M. M., Oprea, A., Oroian, S., Comănescu, M. P., Sârbu, I., & Șuteu, A. (2008). Manual de interpretare a habitatelor Natura 2000 din România. *Ed. Risoprint*, pp. 25.

Golitsyn, G. S., Ratkovich, D. Y., Fortus, M. I., & Frolov, A. V. (1998). On current rise of the Caspian sea level. *Water Resources, 30*, 177–185.

Golubtsov, V. V., & Lee, V. I. (1997). On possible change of the Caspian sea level. *Hydrometeorology and Ecology, 2*, 97-102.

Ignatov, Y. I., Kaplin, P. A., Lukyanova, S. A., & Solovieva, G. D. (1983). Evolution of the Caspian Sea coasts under conditions of sea-level rise. *Journal of Coastal Research*, *9*, 104-111.

Management plan ROSCI0073, (2011), Plan de management ROSCI0073 „DUNELE MARINE DE LA AGIGEA".

Oprea, A. (2005). Lista critică a plantelor vasculare din România, *Ed. Univ. „Al. I. Cuza"*, pp. 283.

Sârbu, I., Ştefan, N., & Oprea, A. (2013), Plante vasculare din România: determinator ilustrat de teren, *Ed. Victor B Victor,* Bucureşti, pp. 1320.

Strat, D., & Holobiuc, I. M. (2018). The occurrence and conservation status of *Convolvulus persicus* L. on the western Black Sea coast – Romania. *Acta zool. bulg*., Suppl. *11*, 125-132.

Vespremeanu, S. A., & Preoteasa, L. (2006). Beach-dune interactions on the dry-temperate Danube delta coast. *Geomorphology, 86*(3), 267-282.

Waters, C. N., Zalasiewicz, J., Summerhayes, C., Barnosky, A., Poirier, C., Galuszka, A., & Wolfe, A. P. (2016). The Anthropocene is functionally and stratigraphically distinct from the Holocene. *Science, 351*, 6269.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 37-48)
doi:10.24193/subbbiol.2020.1.04

Evolutionism and creationism in the social mentality – a study case in the Romanian population

Ioana-Mirela Szekely[1]✉ and Alexandru Nicolae Stermin[1]

[1] Babeş-Bolyai University, Faculty of Biology and Geology, Department of Taxonomy and Ecology, Cluj-Napoca, Romania;
✉ ***Corresponding author, e-mail: ioanasze@gmail.com.***

Abstract. Due to the dynamic of our ever-evolving society, basically, population is now divided in two categories: one with conservative values (religion) and the other one focused on exploring the unknown (science). The aim of our study is to analyze the proportion of the two types of perspectives in the Romanian social mentality and to evaluate the participants' attitude towards science or religion subjects. In the period between November 2016 and March 2017, questionnaires have been distributed to 400 people from Cluj-Napoca (N-W Romania) and Petroșani (S-W Romania). The questionnaire consisted of a field which evaluates personal data and a second part of 21 questions. Some of the most important results of the study included: 56% of those are religious, 71% believe in God, 55% believe that a supernatural force controls the universe, 44% consider it necessary to teach evolution in schools and 57% consider it necessary to teach religion in schools. The percentages for conservative values (religion) is 56% and those who are focused on exploring the unknown (science) is 29%.

Keywords: evolutionism, creations, conservative society, religion.

Introduction

As we know, our species has evolved and adapted through time. Evolution happened gradually; humans started as hunters-gatherers and then became farmers, which led to physical, behavioral, social and cognitive changes. It has not stopped here, as we will see further developments (Losos *et al*., 2014).

The hunter-gatherers were the first to begin a form of "society", because they had a socio-political structure, divided into men and women (Bondarenko *et al.,* 2002). The structure evolved in time and become the society that we know today, with a dynamic evolution. In the last century, we observed an "explosion" of industrial, scientific and technological development, which evolved based on the existing domains (Arthur and Polok, 2006). At the same time, technology gave us a feeling of power and control which helped us overcome the feeling of uselessness (Barbour, 1990).

Along with the technological era, society began to separate into two categories of values: conservative and avant-garde. One of the leading factors of this segregation was the exponential growth that mass-media, religion and culture have known recently (Hoover and Clark, 2002).

Conservative people are the ones that hardly accept facts, those who see everything in black and white, without in-betweens (Watson *et al.,* 1999). They hardly accept new concepts and ideas, like those related to evolution and the beginnings of life on Earth. In this sphere is included religion with its fixed concepts that haven't changed in centuries, even though science has evolved and it's providing more and more facts.

People with avant-garde values have free, open thinking. They are the ones that give credit to scientific facts for explaining phenomena and subjects that past generation did not understand. They also understand that everything evolves, such as bacteria becoming resistant to antibiotics (e.g. *Staphylococcus aureus* becoming increasingly resistant to methicillin) (Nadelson and Hardy, 2015). As previously stated, the society has split in two groups: the religious group and the group that accepts evolutionism.

As far as we know, in Romania, there have not yet been carried out studies to explore the proportion of those categories. As an overview, the Romanian population has 21.5 million inhabitants, 86.5% are Orthodox, 5% Greek Catholics, while the rest have other religious beliefs. In this context, the aims of our study are: (i) to evaluate the percentages of those two views (evolutionism and creationism) distribution in the evaluated Romanian group, (ii) explore the participants' attitude towards science or religion subjects, analyze them in the social- cultural context and (iii) compare the results with available ones from other studies performed on different communities.

Materials and methods

Between November 2016 and March 2017, 400 subjects were questioned in the study. The questionnaire, distributed printed or online, consisted of a field containing personal data: name and surname (was optional, initials of the name could be used), age, occupation, etc., and one section containing 21 questions - 13 questions oriented towards religion and 8 related to science.

For comparing our results, we used studies belonging to the Gallup Institute. The questionnaires used were:

- Global index of religion and atheism
- In the US, 42% Believe Creationist View of Human Origins (Newport, 2014).

- Evolution, Creationism, Intelligent Design
- On Darwin's Birthday, only 4 in 10 Believe in Evolution (Newport, 2009).
- Americans Weigh in on Evolution vs. Creationism in Schools (Carlson, 2005).

Global index of religion and atheism: This study was made in 2012 in 57 countries around the world, with a sample of more than 50,000 people, both women and men. There was also presented data on Romania, where 1050 people were interviewed, between November 26th and December 5th. The countries where the study was conducted are: China, Japan, Romania, Iceland, Australia, Germany, France, Czech Republic, Iraq, Kenya, Macedonia, Fiji, Armenia, Brazil, Peru, Moldova, Columbia, India, Nigeria, Turkey. The data gathered in Romania only aimed at presenting a percentage not to analyze the factors behind the numbers.

In the U.S., 42% of Believe Creationist View of Human Origins was realized for 1,028 adults, aged 18 and older, living in all 50 U.S. states and the District of Columbia. In this study, the questions were based on facts about evolution, e.g.: "Human beings have developed over millions of years from less advanced forms of life, but God guided this process", "Human beings have developed over millions of years from less advanced forms of life, but God had no part in this process". The results showed that 40% believe that God is the explanation of the origins of life on Earth.

Evolution, Creationism, Intelligent Design: was conducted between 3-7 May 2017 in the U.S., with 1011 people questioned.

On Darwin's Birthday, only 4 in 10 believed in Evolution: was done in U.S. with 1,018 people on February 6th-7th, 2009. In this study, people were asked if they believe in evolution and facts related to it. Only 39% of Americans say they "believe in the theory of evolution," while a quarter say they do not believe it, and the remaining 36% don't have an opinion either way.

Americans Weigh In on Evolution Creationism in Schools: conducted on 21-23 March 2005 with 1001 participants. The overall result of the study is that the percentage of Americans wanting to have creationism taught in schools may seem relatively modest at 30%, but still significantly larger than the percentage of Americans wanting evolutionism taught to their children.

Results

Age: the majority of interviewees are young people (ages between 16 – 22, 66%; 23 – 30, 10 %) (Fig. 1). This will be visible in the percentage of some questions, such as those related to scientific topics. But also, the fact that they are so young means that they can be easily manipulated or feel compelled to act or behave as "normal" for either society (of religious-conservative values), or their family, even if the answers are confidential.

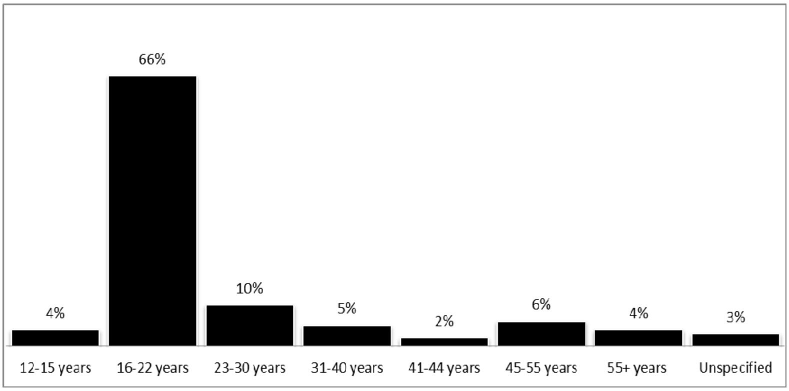


Figure 1. Age percent of interwied people.

Another influencing factor is that teenagers have the tendency to spend more time in groups, including church groups (Cheadle and Schwadel, 2012).

Education level: there is a link between the education level and the way of understanding and accepting evolution (Heddy and Nadelson, 2013), which can be seen in the answers given to some questions of the study.

The fact that the percentages were equal for high school and university (Fig. 2) gives us two points of view about certain topics like evolution, religion, life and death, etc.

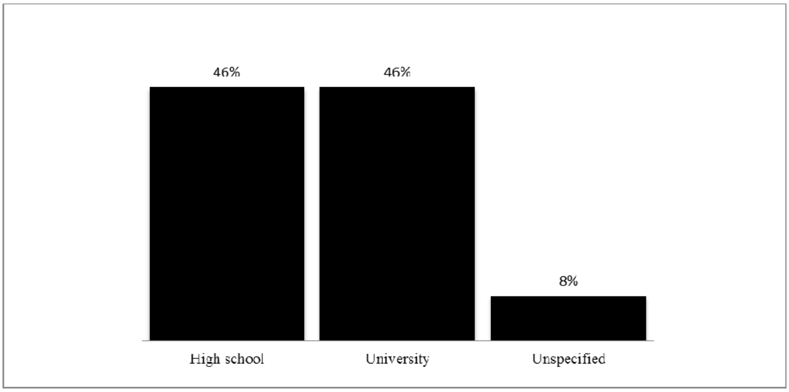


Figure 2. Level of education of interviewed population.

The education level is important because people having better studies can take decisions on their own and they analyze the information given to them before drawing conclusions.

Background. The fact that most of those who have taken the questionnaire are born in urban regions, can be seen in their responses to questions concerning science ("Do you like to keep up with the scientific topics?", "Do you consider yourself a modern, open minded person?"), as they have easier access to information (in Romania the countryside is underdeveloped, providing lesser access to Internet and public libraries).

We can observe for those from the urban areas that there is a higher rate of university attendance due to several social (Fig. 3), economic factors, etc., while in the rural areas just a few have graduated or followed college-level education, these may graduating high-schools or technological schools having levels up to 10 classes.

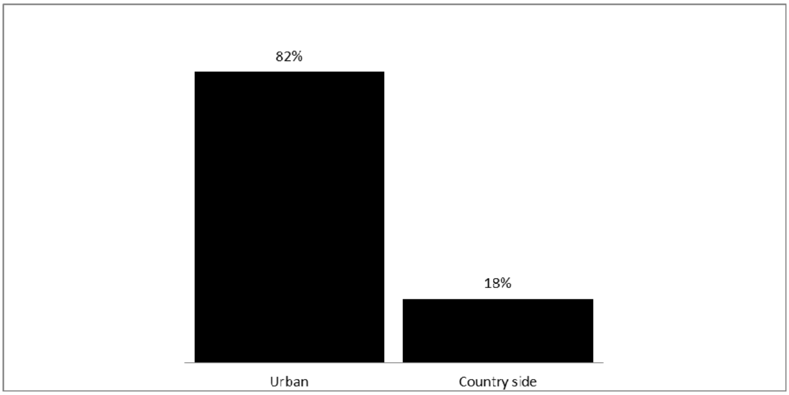


Figure 3. The backround of interwied people.

We analyzed the answers of the most relevant questions:

Question: *Are you a religious person?*

56% of people answered affirmatively (Fig. 4), which is not surprising, since most of the interviewees are teenagers or young people that haven't come out of the family environment, where the influence of their parents is stronger. This issue was discussed by Deborah Kelemen in "Are Children Intuitive Theists?", where she supports the idea that at the age of 5, children can make the difference between natural and supernatural events (Keleman, 2004). Thus, the children know that God is a supernatural being, but because of the massive institutionalization of religion, children are likely to view religious rules as relatively coherent (Guthrie, 2002).

Another reason for the high percentage is the marketing over religion. Today's religions must compete with more fun and recreational activities (Einstein, 2008). In Romania, a cult of religious icon depicting saints has evolved recently, and this is associated with religion and belief. Religion brings people together and creates links between people (Heddy and Nadelson, 2013).

According to the results of the “Religion and atheism” study conducted by Gallup International during 2005-2012, which involved questioning 1050 individuals, there has been an increase in the number of religious people. Romania ranked 6th in the most religious countries of the world, and we also rank high among the countries with the highest percentage of religious people.

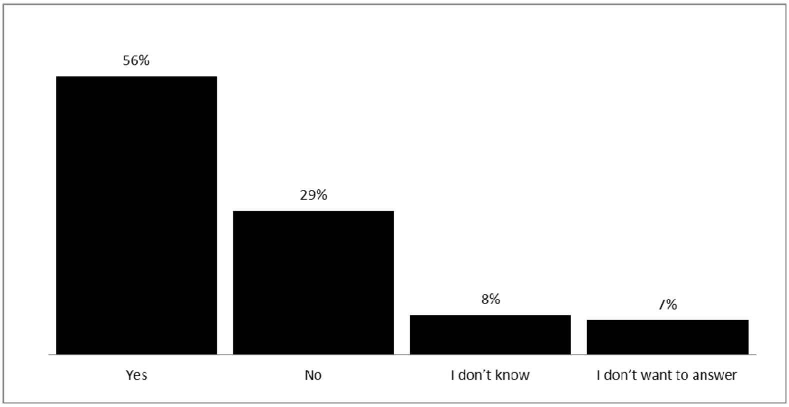


Figure 4. Answers to: “Are you a religious person? “

Question: *Do you believe in God?*

Those who have chosen “Yes” (Fig. 5) are raised or forced to believe in God and at the same time inherited the concept of God (Alper, 2008). Much of the present population comes from the countryside and that influences current thinking (in particular subjects like religion). People also have designed their “personal God” to motivate their choices in life (Van Ments *et al.,* 2018). This is another factor that can explain the high percentage for the affirmative answer.

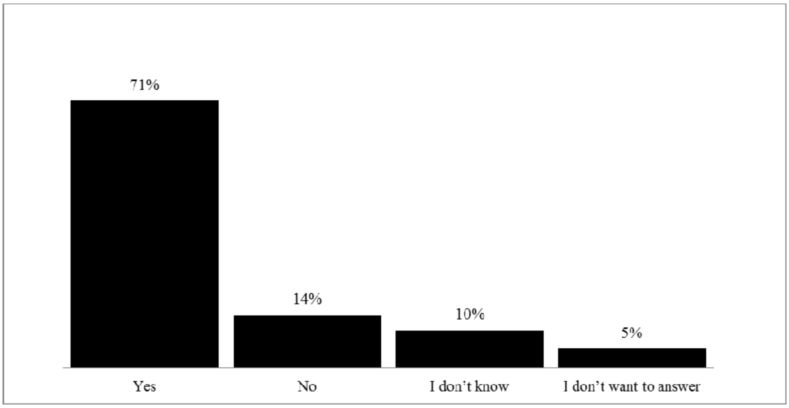


Figure 5. The percentage for those who believe in God.

We can also see that there is also 10% that chose "Do not know" as answer, percentage that belongs to the agnostic category, thinking that it is impossible to really know if God exists (Scott, 2009).

As we previously mentioned, Romania was ranked 6th among the most religious countries in the world, and since then, no relevant increase of the number of atheists was recorded, showing the rigidity that churches exercise on their followers.

Question: *Do you agree with the existence of a supernatural force that controls the Universe?*

This question was not used just for verification for the previous question, but it goes along with the theory of Intelligent Design. This theory states that evolution was controlled by a "smart designer" and evolution did not happen chaotically or accidentally, as natural selection shows, instead having a precise and certain creator (Coyne, 2015). The fact that 55% responded affirmatively to this question confirm what figure number 6 indicates: this concept is quite widespread (thanks to the media, literatures etc) (Fig. 6).

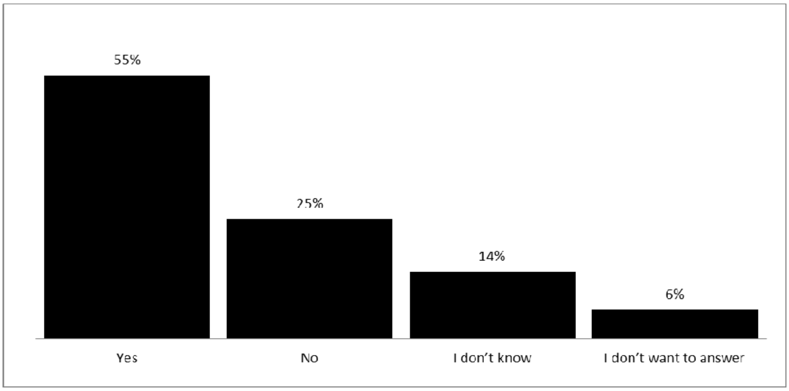


Figure 6. Answers for: "Do you agree with the existence of a supernatural force that controls the universe?"

The fact that there is a 29% difference between the answers to "Do you believe in the existence of God" and "Do you agree with the existence of a supernatural force that leads the universe?" indicates that people still need to believe in something.

Question: *Do you believe in natural selection?*

Due to the fact that we have evolved from many points of view (social, economic, informational, technological, educational, etc.), people have a more open way of thinking and they accept new ideas like natural selection much easier. The percentage of those who chose "No" and "I don't know" are similar (Fig. 7), meaning that people are either uninformed or they don't want to accept that fact. Even though 71% believe in God (Fig. 5), 61% of respondents also believe in natural selection (Fig. 7). These results, from a scientific perspective may be contradictory. The "intelligent design theory" may explain the compatibility between God faith and natural selection. This was supported by Wallace's statement which said that humans evolved through natural selection, just as every animal, but God gave them a soul and also by Pope Pius XII (Singer, 2006).

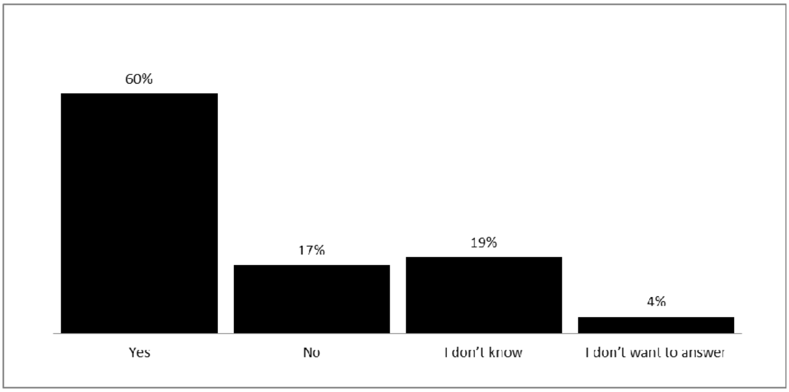


Figure 7. The percentage of those who believe in natural selection.

From the study conducted by Gallup in February 2009 in which the 1018 interviewees answered to the question "Do you believe in evolution?", results that the high school students don't believe in evolution (21%) A higher percentage for the same answer is seen in those with higher education (71%).

Question: *Do you consider it necessary to teach evolutionism in primary and high schools?*

We know that the teaching of evolutionism in Romanian education (and also in other countries) is still a controversial topic. Evolution is a concept of biology, and many college students do not accept this because of their religious beliefs (Barnes and Brownell, 2016). This can be seen quite well through the fact that the percentage of those responding affirmatively (44%) is small (not even 50%) (Fig. 8). A cause for this is also the parents' mindsets, who often blame or straight refuse to let teachers explain evolutionism in school. For most countries, religiosity is negatively associated with evolution (Heddy and Nadelson, 2013).

In the study conducted by Gallup, "Evolution, Creationism, Intelligent Design", the question: "Do you think each of the following explanations about the origin and development of life on earth should or should not be taught in public school science classes, or are you unsure?", evolutionism has the highest positive percentage - 61% (U.S. is well developed compared to Romania). The percentage for "No, should not" (20%) and "Unsure" (19%) are close, proving their uncertainty towards that subject.

In the "Americans Weigh in on Evolution vs. Creationism in Schools" study, conducted by Gallup with a total of 1001 people between March 21th-23th 2005, the ones that graduated high school (37%) said that they would feel disturbed to teach only evolutionism in school, but for the postgraduate level the situation is exactly the opposite, 35% refusing to teach creationism alone. This indicates that people with higher level of education give more credit to scientific explanations on the dilemmas of existence.

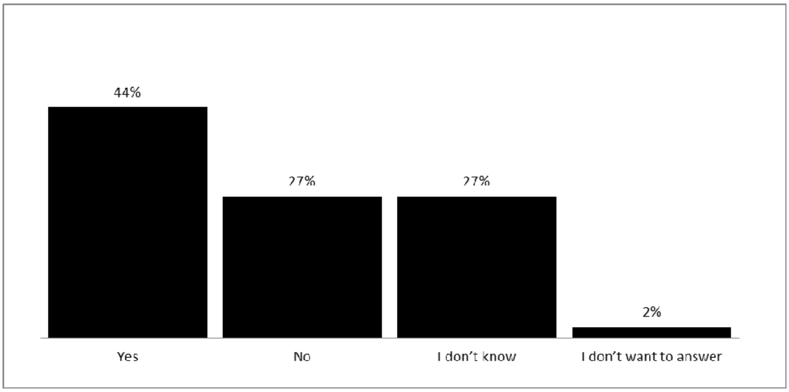


Figure 8. Opinion of the interviewed population on teaching evolution.

Question: *Do you think you understand the concept of natural selection?*

In the answers for this question, a link between birthplace (rural or urban) and level of study can be seen (in Romania, the schools from rural regions are poorly equipped compared to the urban areas), due to the access to information. The 68% that answered positively (Fig. 9), denotes the fact that young people are interested in knowing the meaning of life and its scientific explanation.

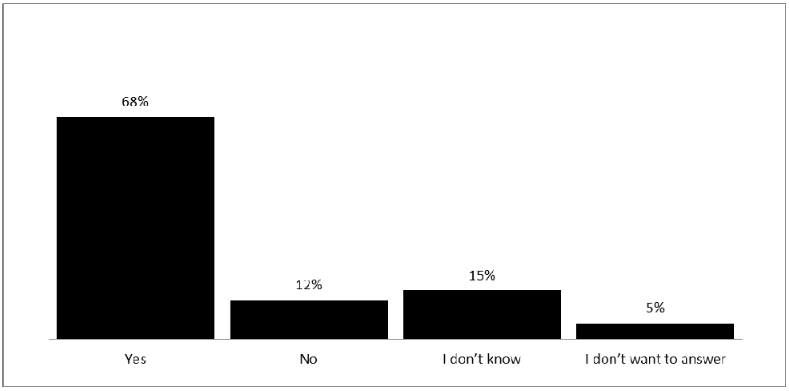


Figure 9. The percentage of those who understand natural selection.

A small percentage belongs to those that chose to answer “Do not know” (perhaps due to several factors like family, entourage of friends etc).

For the question posed by the Gallup website in the study entitled "Evolution, Creationism, Intelligent Design" – “How familiar would say you are with each of the following explanations about origin and development of life on earth?” we can see an increase in the familiarity of the American population with the idea of evolution because in June 2007 there were 41% while in May 2014 it was 42%.

We can notice a decrease for familiarity of creationism (from 50% to 38%), attributed to the evolution of technology and ease of access to information.

Question: *Do you consider it necessary to teach religion in primary and high schools?*

We conducted the study in a country under a strong religious influence (especially Orthodox), and this was reflected in the percentage of affirmative answers (57%) (Fig. 12). Some of the factors that influenced this percentage are family (in each family there is at least one religious parent who sees teaching religion as an absolute necessity for education), and age (those that answered the questionnaire, have recently graduated or currently follow high-school or college-level education - they consider religion to be a non-essential subject for their studies).

Question: *Name a personality who has contributed to religion/ theology/ mysticism.*

Answers: Arsenie Boca - 32%, Jesus - 13%, Virgin Mary - 13%, Luther King - 3%, The Pope - 11%, people mentioned in the Bible - 2%, Buddha - 3%, Other saints - 6%, Unspecified - 16%, I don’t know - 0%, Another - 1%.

Due to the fact that Romania is a predominantly Orthodox country, with a percentage of 86.45% out of the whole population that declared their belief (Opaschi, 2014) , this greatly influenced the choices of the answers. This is visible in the fact that “Arsenie Boca” has a 32% share, much higher than “Jesus”, with only 13%. This result may suggest the poor quality of religious education, focused more on spectacles and miraculous on a local scale and less on the universal values of the Orthodoxy.

Question: *Name a personality that has contributed to science/ biology/physics.*

Answers: Emil Racoviță - 2%, Albert Einstein - 20%, Gregor Mendel - 4%, Nikola Tesla - 4%, Charles Darwin - 7%, Thomas Edison - 2%, Isaac Newton - 11%, Stephen Hawking - 5%, Another - 19%, I don’t know - 3%, I don’t want to answer- 1%, Unspecified - 22%.

The first thing that we can see is the difference between the percentages of the "I don't know" answers for personalities that contributed to religion (0%) and the same answer for this question (3%). This is due to the fact that in Romania religion is taught since first grade, while science is only taught from sixth grade.

Conclusions

Following the analysis of the answers given by the respondent positively to the question "Are you a religious person?" (56%) and "Do you belive in God?" (71%) we conclude that the majority of Romanians are religious and believe in the theory of creationism. But we can see that there is an openness to science from those who said they believed in natural selection – 60%.

Is should also be noted that the current young generation has more interest and gives more credit to the scientific explanations and they have a much more open attitude to science, as 44% of them consider it necessary to teach evolutionism in schools.

People's attitude towards religion has remained unchanged along the centuries. They are guided by standards of religion in their everyday life and are against other ideas or ideologies that are not the same as theirs and that can be seen by the 57% who consider it necessary to teach religion in schools.

Even though, the study has reduced number of questionnaires and a narrow age range of the subjects, it brings new and valuable information about the mentality's dynamics related to evolutionism and creationism in a part of the Romanian society.

REFERENCES

Alper, M. (2008). The "God" Part of the Brain: A Scientific Interpretation of Human Spirituality and God. *Sourcebooks*, Inc., Illinois.

Arthur, W. B., & Polak, W. (2006). The Evolution of Technology within a Simple Computer Model. *Complexity at Large*, Volume 11, Issue 5, USA.

Barbour, I. (1990). Religion in an Age of Science (Gifford Lectures 1989-1991, Vol 1), Harpercollins, New York.

Barnes, M. E., & Brownell, S. E. (2016). Practices and Perspectives of College Instructors on Addressing Religious Beliefs When Teaching Evolution. *CBE- Life Sciences Education*, Vol. 15.

Bondarenko, D. M, Grinin, L. E., & Korotayev, A. V. (2002). Alternative Pathways of Social Evolution and Basic Principles of Culture Organization, Russian State University for the Humanities, Moscow.

Carlson, D. K. (2005). Americans Weigh In on Evolution vs. Creationism in Schools. Responses vary by religiosity, education, ideology [Accessed 18 March 2019] https://news.gallup.com/poll/16462/americans-weigh-evolution-vs-creationism-schools.aspx.

Cheadle, J. E., & Schwadel, P. (2012). The ‘friendship dynamics of religion,’ or the ‘religious dynamics of friendship’? A social network analysis of adolescents who attend small schools, Social Science Research, Volume 41, Issue 5.
Coyne, J. A. (2015). Faith Versus Fact: Why Science and Religion Are Incompatible. Ed. *Viking*, New York.
Einstein, M. (2008). Brands of Faith-Marketing Religion in a Commercial Age, Routledge, New York.
Evolution, Creationism, Intelligent Design [Accessed 18 March 2019] https://news.gallup.com/poll/21814/evolution-creationism-intelligent-design.aspx
Global index of religion and atheism (2012) [Accessed 18 March 2019] https://sidmennt.is/wp-content/uploads/Gallup-International-um-tr%C3%BA-og-tr%C3%BAleysi-2012.pdf .
Guthrie, S. (2002). Animal animism: Evolutionary roots of religious cognition, Current approaches in the cognitive science of religion. *Continuum,* New York.
Heddy, B. C., & Nadelson, L. S. (2013). The variables related to public acceptance of evolution in the United States, Evolution: Education and Outreach, Vol. 6, Issue 3.
Hoover, S. M., & Clark, L. S. (2002). Practicing Religion in the Age of the Media: Explorations in Media, Religion, and Culture. *Columbia University Press*, Columbia.
Keleman, D. (2004). Are Children “Intuitive Theists”? - Reasoning About Purpose and Design in Nature. *Sage journal*, Volume 15, Number 5.
Losos, J. B., Baum, D. A., Futuyma , D. J., Hoekstra H. E, Lenski, R. E., Moore, A. J., Peichel C. L., Schluter D., & Whitlock M. C. (2014). The Princeton Guide to Evolution. *Princeton University Press*, New Jersey.
Nadelson, L. S., & Hardy, H. H. (2015). Trust in science and scientists and the acceptance of evolution, Evolution: Education and Outreach, Volume 8, Issue 9.
Newport, F. (2009). On Darwin’s Birthday, Only 4 in 10 Believe in Evolution. Belief drops to 24% among frequent church attenders, [Accessed 18 March 2019] https://news.gallup.com/poll/114544/darwin-birthday-believe-evolution.aspx.
Newport, F. (2014). In U.S., 42% Believe Creationist View of Human Origins. Americans' views related to religiousness, age, education [Accessed 18 March 2019] https://news.gallup.com/poll/170822/believe-creationist-view-human-origins.aspx.
Opaschi, V. (2014). Statul și cultele religioase, Secretariatul de Stat pentru Culte, București.
Scott, E. C. (2009). Evolution vs. Creationism: An Introduction, Greenwood Press, London.
Singer, P. (2006). Tratat de Etică. *Ed. Polirom*, Iași.
Van Ments, L., Roelofsma, P., & Treurcorresponding, J. (2018). Modelling the effect of religion on human empathy based on an adaptive temporal–causal network model. Computational Social Networks.
Watson, P. J., Morris, R. J. , Hood, R. W., Jr. , Miller, L., & Waddell, M. G. (1999). Religion and the Experiential System: Relationships of Constructive Thinking with Religious. *The International Journal for the Psychology of Religion*, Volume 9, Issue 3.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 49-60)
doi:10.24193/subbbiol.2020.1.05

Last meal: food composition of road-killed *Lacerta viridis* (Reptilia: Lacertidae) from Romania

Alexandra-Roxana-Maria Maier[1], Achim-Mircea Cadar[1] and Severus-Daniel Covaciu-Marcov[1]✉

[1] *University of Oradea, Faculty of Informatics and Sciences, Department of Biology, Oradea Romania;*
✉***Corresponding author. E-mail: severcovaciu1@gmail.com.***

Abstract. Food composition of road-killed *Lacerta viridis* was studied on 41 individuals from the scientific collection of the University of Oradea, Romania. They were collected from different roads in the country between 1998-2019. All lizards had stomach contents. Although in most cases the stomach contents were partially digested, the preys could be identified at a taxonomical level comparable with the one achieved in other lizard feeding studies. The analysed *L. viridis* individuals had consumed 275 preys, which belong to 25 taxa, as well as inorganic elements and vegetal remains. The most important preys were Orthoptera, Araneidae and Coleoptera. The differences in lizards` food composition according to geographic origin were not significant. Our results confirm that this species food composition can be studied on road killed individuals, but also proves the uniformity of its food, at least in the Balkan Peninsula.

Keywords: road ecology, feeding, European green lizard, region, invertebrates, human impact.

Introduction

Nowadays, road mortality affects countless animals, including lizards (e.g. Meek, 2009; Tok *et al.,* 2011; Mollov *et al.,* 2013; D'Amico *et al.,* 2015; Covaciu-Marcov *et al.,* 2017). The European green lizard *Lacerta viridis* (Laurenti, 1768) is a species of a relatively large size (Fuhn and-Vancea, 1961). In Europe, it is distributed in the south-eastern part of the continent (Sillero *et al.,* 2014). Similarly to many other lizard species, *L. viridis* is frequently killed by cars on roads in different regions of its distribution range, but the number of road-killed individuals is generally low (e.g. Tok *et al.,* 2011; Kambourova-Ivanova *et al.,* 2012; Cicort-Lucaciu *et al.,* 2016; Rassati, 2016; Ciolan *et al.,* 2017). Analysing the food composition of road killed lizards in a relatively good condition had started many years ago (Angelici *et al.,* 1997). Road killed amphibians were also used in feeding studies (Kolenda *et al.,* 2019). Recent data confirms that road mortality studies and the victims of this anthropogenic impact can provide numerous useful information (see in: Schwartz *et al.,* 2020). Also, recent studies confirm the importance of museum preserved individuals even for clarifying the status of extinct taxa, with an unclear status for a long time (e.g. Kehlmaier *et al.,* 2020). The food composition of *L. viridis* was studied in the past using preserved individuals from museum (Sagonas *et al.,* 2018) or university collections (Mollov *et al.,* 2012). Other reptiles from different collections were also analysed from this point of view (e.g. Castilla *et al.,* 1991; Mollov, 2010; Mollov and Petrova, 2013; Sagonas *et al.,* 2015). The feeding ecology of *L. viridis* is still not studied comprehensively (e.g. Korsós, 1984; Mollov *et al.,* 2012; Sagonas *et al.,* 2018). In Romania, to our knowledge, there is only old information on this subject in a volume of the Romanian Fauna referring to reptiles (Fuhn and Vancea, 1961). However, data do exist about lizards` diet, but for other species (e.g. Castilla *et al.,* 1991; Hodar *et al.,* 1996; Angelici *et al.,* 1997; Gvoždík and Boukal, 1998; Sagonas *et al.,* 2015). Starting from the information presented above, we presumed that *L. viridis* individuals killed by vehicles in Romania can offer information about the species` feeding traits. Since in the last several years some road-killed *L. viridis* individuals were collected for educational purposes and were stored in the collection of the Department of Biology, Faculty of Informatics and Sciences, University of Oradea, we could use them in this study. Therefore, the objectives of this study were: **1.** to establish the food composition of the species *L. viridis* killed by cars in Romania, **2.** to observe if there are different food consumption patterns of this species in different areas of the country.

Material and methods

We analysed the stomach content of 41 *L. viridis* specimens, collected between 1998-2019. Initially, the lizards were collected without any intention related to feeding study, but for educational purposes, and they were road-killed specimens identified by chance in different areas of Romania. However, in recent years, as we initiated studies on road mortality (Cicort-Lucaciu *et al.,* 2012, 2016; Ciolan *et al.,* 2017; Covaciu-Marcov *et al.,* 2017; Teodor *et al.,* 2019; Ile *et al.,* 2020) and because of the multiple uses of road traffic victims have started to appear (e.g. Kolenda *et al.,* 2019; Schwartz *et al.,* 2020), we paid more attention to these corpses. The lizards come from 12 counties of Romania (Bihor, Arad, Satu-Mare, Alba, Hunedoara, Caraș-Severin, Mehedinți, Gorj, Dolj, Ialomița, Tulcea, Constanța), including the western, central (Transylvania) and southern part (including Dobruja). Seven individuals collected long time ago for educational purposes did not have data about the region of origin. The rest of 34 lizards had labels with the collecting date, locality and county. The specimens were preserved in alcohol and they are deposited in the scientific collection of the Department of Biology, Faculty of Informatics and Sciences, University of Oradea.

The preserved lizards were dissected, and their stomach was extracted. Although we also tried to analyse the intestinal and cloacal content, the preys were too digested to be determined. The stomachs were dissected in a Petri dish, and the preys were identified using a stereomicroscope, as in other cases (e.g. Angelici *et al.,* 1997; Mollov, 2010; Sagonas *et al.,* 2018). Preys were determined to higher taxonomic level (family, order, etc), as many of them were in an advanced degree of digestion. This assignment to taxonomic groups was also used in other studies on lizards` feeding pattern (e.g. Castilla *et al.,* 1991; Angelici *et al.,* 1997; Mollov *et al.,* 2012; Sagonas *et al.,* 2015, 2018). The data has been processed both for the total and according to the region of origin (northwest, southwest and southeast). We calculated the average number of preys/specimen, percentage abundance and frequency of occurrence for each taxa. The prey diversity was calculated with the Shannon index (Shannon, 1948), the homogeneity with the Pielou index (Pielou, 1966). The significance of differences between the feeding of lizards from the three zones was calculated with help of the Kruskal Wallis test. The calculations were made in Microsoft Office Excel and Past software (Hammer *et al.,* 2001).

Results

None of the lizards were with empty stomachs. The 41 individuals of *L. viridis* had consumed 275 preys in total, from 25 invertebrate taxa (Tab. 1). The average number of prey / individual was 6.70, the number of preys consumed by an individual varied between 1 and 17. Besides preys of animal origin, in the stomachs of the lizards we found vegetal fragments and inorganic particles (Tab. 1). The largest percentage abundance had Orthoptera (19.27%) and Araneida (18.90%). Among Orthopterans, alongside plenty grasshoppers and locusts in a very advanced digestion stage, we identified one *Gryllotalpa* sp. The third place by percentage abundance was occupied by undetermined Coleoptera (15.27%). But if we take the Coleoptera families altogether, their percentage exceeds 25%, thus, this taxon becomes the most favoured by lizards. By frequency, the first place is occupied by Araneida, who were consumed by 68.29% of lizards, followed by Coleoptera and Orthoptera, both with a frequency of 60.97%. Vegetal fragments were found in 70.73% and inorganic elements in 17.07% of the stomachs.

Table 1. Percentage abundance, frequency of occurrence, Shannon diversity and Pielou homogeneity of prey taxa consumed by *L. viridis* in Romania (NW – northwestern Romania, SW – southwestern Romania, SE – southeastern Romania, * includes both individuals from the three geographic regions and the seven specimens which could not be assigned to any region).

	Percentage abundance (%)				Frequency of occurrence (%)			
	NW	SW	SE	Total*	NW	SW	SE	Total*
Vegetal fragments					80.00	69.23	50.00	70.73
Inorganic elements					6.66	30.76	16.66	17.07
Gastropoda	-	2.27	-	0.72	-	15.38	-	4.87
Isopoda	-	-	6.45	2.18	-	-	16.66	7.31
Diplopoda	2.10	1.13	-	1.09	13.33	7.69	-	7.31
Araneida	20.00	22.72	9.67	18.90	73.33	76.92	33.33	68.29
Odonata	-	-	3.22	0.36	-	-	16.66	2.43
Dermaptera	-	-	-	0.36	-	-	-	2.43
Orthoptera	22.10	12.50	12.90	19.27	66.66	53.84	50.00	60.97
Homoptera	2.10	-	-	0.72	13.33	-	-	4.87
Heteroptera	6.31	5.68	16.12	7.63	26.66	30.76	50.00	34.14
Coleoptera, Silfidae	-	-	-	0.36	-	-	-	2.43
Coleoptera, Scarabeidae	-	-	-	1.09	-	-	-	4.87
Coleoptera, Cicindelidae	1.05	-	-	0.36	6.66	-	-	2.43
Coleoptera, Curculioniae	-	-	3.22	1.09	-	-	16.66	4.87
Coleoptera, Carabidae	4.21	2.27	3.22	3.27	20.00	15.38	16.66	19.51
Coleoptera, Chrysomelidae	1.05	-	-	0.36	6.66	-	-	2.43
Coleoptera, Cerambicidae	3.15	3.40	-	2.54	6.66	23.07	-	12.19
Coleoptera, Elateridae	1.05	2.27	-	1.09	6.66	15.38	-	7.31
Coleoptera (larvae)	1.05	-	-	0.36	6.66	-	-	2.43
Coleoptera (undeterm.)	10.52	20.45	16.12	15.27	53.33	61.53	66.66	60.97
Lepidoptera	1.05	1.13	-	1.09	6.66	7.69	-	7.31
Lepidoptera (larvae)	11.57	6.81	12.90	8.00	40.00	15.38	50.00	29.26
Diptera, Brahicera	2.10	7.95	9.67	4.36	13.33	30.76	50.00	21.95
Diptera, Nematocera	1.05	3.40	3.22	1.81	6.66	7.69	16.66	7.31
Hymenoptera, Formicidae	8.42	7.95	3.22	7.27	40.00	23.07	16.66	31.70
Hymenoptera (undeterm.)	1.05	-	-	0.36	6.66	-	-	2.43
Shannon diversity	**2.34**	**2.26**	**2.29**					
Pielou homogeneity	**0.81**	**0.85**	**0.92**					

The food diversity was H=2.46, and the homogeneity J'=0.76. From the 34 individuals which could be assigned to a geographical region, the majority came from north-western Romania, then from the south-western- and only a small number from the south-eastern part of the country (Tab. 1). The number of preys and the number of consumed taxa varied from region to region. The largest number of preys was consumed in south-western Romania, and the largest number of taxa was consumed in north-western Romania (Tab. 1), but, the number of taxa / lizards was high in the south-eastern region. Differences between regions were observed also in the vegetal and inorganic elements consumption. Thus, vegetal remains were ingested more frequently by lizards from the north-western region, and the inorganic elements by lizards from the south-western region (Tab. 1). In each region, Orthoptera and Coleoptera registered high percentage abundance, but the taxa percentage abundance varied (Tab. 1). In the case of frequency of occurrence, also Orthoptera and Coleoptera were consumed in each region by at least 50% of the lizards. Nevertheless, in the north-western and south-western regions, spiders registered a frequency of occurrence above 70% (Tab. 1). The highest food diversity was registered in the north-western region and the lowest in the south-western region, where also the number of lizards was small (Tab. 1). The differences of the food consumed by *L. viridis* between the three studied regions were not significant (p=0.11).

Discussions

The European green lizards killed by cars in Romania can be used to establish the food composition of this species, like in the case of some related lizard (e.g. Angelici *et al.,* 1997), or snakes species (e.g. Daltry *et al.,* 1998; Brito, 2004). Because the analyzed lizards were not collected for food composition studies, generally their preservation was not done immediately. However, their stomach contents, although relatively digested, could be determined at least to a taxonomic level comparable with other lizard food composition studies (e.g. Korsós, 1984; Angelici *et al.,* 1997; Mollov *et al.,* 2012; Sagonas *et al.,* 2015, 2018). Nevertheless, the stomach contents level of digestion probably depends less on the time between collecting and preservation of lizards and more on the time elapsed between killing and collecting. Even in the case of lizards from museum or university collections, the taxonomic level of prey identification was not more detailed (e.g. Mollov *et al.,* 2012; Sagonas *et al.,* 2015, 2018). The number of road killed *L. viridis* individuals observed in the last years was far higher than the one analysed here, because most lizards were repeatedly crushed by cars, and thus could not be used for studies. For example, in some road mortality studies (Cicort-Lucaciu *et al.,* 2016; Ciolan *et al.,* 2017) all road killed *L. viridis* found were too crushed by cars to be analysed. Probably, the road killed individuals in good condition were hit only once. In some individuals the head was squashed, as in other species (Jackson and Lemn, 2009). Monitoring roads surrounded by favourable habitats for this species would be useful from this perspective. At least on this way, road-kills could offer information on the ecology of some species, moreover because the intensity of this anthropogenic impact is increasing (e.g. Philcox *et al.,* 1999; Kazemi *et al.,* 2016; Hill *et al.,* 2020).

The 41 road-killed European green lizards gave us information about their last meal. Their food consisted entirely from invertebrates, although some large sized Lacertidae also feed on small vertebrates (e.g. Busack and Visnaw, 1989; Castilla *et al.,* 1991; Angelici *et al.,* 1997; Christopoulos *et al.,* 2020). Generally, the food of this species consisted in invertebrates, while the consumed taxa are almost the same (e.g. Mollov *et al.,* 2012; Sagonas *et al.,* 2018). However, because in the case of Coleoptera we generally managed to determine the family, the number of prey taxa and the food diversity were higher than in other cases (Sagonas *et al.,* 2018). At least two important prey taxa for the European green lizards from Romania (Coleoptera and Orthoptera) were also important in Greece and Bulgaria, although in those cases Araneida had lower percentage abundance (Mollov *et al.,* 2012; Sagonas *et al.,* 2018). The number of preys/individual was higher than in other cases (Mollov *et al.,* 2012). As well as in Romania, vegetal elements were found in the stomachs of the green lizards in other regions too (Sagonas *et al.,* 2018). This indicates that the species diet does not differ much from region to region, at least in the Balkan Peninsula, *L. viridis* mainly eating the available insects. Coleoptera had the largest number of species on the planet (e.g. Radu and Radu, 1967; Nielsen and Mound, 1999), thus the fact that they were consumed in large number was not surprising, as they are important prey for other lizards too (e.g. Castilla *et al.,* 1991; Pérrez-Melado *et al.,* 1991; Burke and Mercurio, 2002; Sagonas *et al.,* 2015). Orthoptera are insects that prefer dryer steppe areas (e.g. Radu and Radu, 1967), and *L. viridis* also likes a dryer substratum with vegetation, mainly composed of shrubbery and bushes (e.g. Fuhn and Vancea, 1961; Korsós, 1984; Strijbosch *et al.,* 1989; Heltai *et al.,* 2015), but also move frequently in grassy areas for feeding (Arnold, 1987). Thus, the contact between predator and prey is facilitated, a fact that explains the high consumption of Orthoptera by *L. viridis*. At the same time, the large size of the European green lizards facilitates the consumption of Orthoptera, as in the case of large sized amphibians (Cicort-Lucaciu *et al.,* 2013). Nevertheless, there are some differences in this species feeding between different areas. Thus, sometimes inorganic elements were not recorded (Sagonas *et al.,* 2018), although in Romania they were relatively frequently consumed.

Although *L. viridis* seems to be more trophic specialized than *L. agilis*, in Hungary it also consumes a large number of Coleoptera, even if caterpillars were its main food (Korsós, 1984). Caterpillars were also consumed in Romania, but in a lower percentage abundance and frequency of occurrence, although they are slow prey, which can be easily captured by lizards. Even if it seems that *L. viridis* adults avoid eating small prey like ants (Sagonas *et al.,* 2018), we found ants only in the stomachs of large lizards. *L. viridis* individuals that feed on ants consumed inorganic elements too (pebbles). Probably inorganic elements were ingested by accident, because *L.viridis* is a lizard that only uses its jaws to catch the prey-(e.g. Bels and Goosse, 1990; Urbani and Bels, 1995). Thus, it is difficult for lizards to separate the ants from the substratum on which they were captured. Unlike this, locusts were probably captured from the vegetation, not with the substratum. The consumption of inorganic elements was also registered in other lizard (e.g. Roth, 1971; Castilla *et al.,* 1991) and amphibian species diet (e.g. Cicort-Lucaciu *et al.,* 2011, 2013). The consumption of vegetal remains by *L. viridis* seems to be recorded only recently (Sagonas *et al.,* 2018), albeit in other species it was well-known (e.g. Busack and Visnaw, 1989; Castilla *et al.,* 1991; Hodar *et al.,* 1996; Sagonas *et al.,* 2015). Although it seems that *L. viridis* juveniles consume vegetal elements intentionally (Sagonas *et al.,* 2018), it is possible that they are ingested accidentally, just like the inorganic elements, together with the prey, as it was previously indicated in amphibians (e.g. Mollov, 2008; Cicort-Lucaciu *et al.,* 2013).

The food composition of *L. viridis* is alike the one consumed by other large lizards, like *L. trilineata* (Mollov and Petrova, 2013; Sagonas *et al.,* 2015), which consume even vegetal elements (Sagonas *et al.,* 2015). Also, in Italy *L. bilineata* consumed numerous Coleoptera, but in that case there were some differences, because the percentage abundance of Isopoda was higher (Angelici *et al.,* 1997) than in Romania. This is probably a consequence of a different region and period, because in some cases some amphibians, for example, consume a large number of isopods (e.g. Covaciu-Marcov *et al.,* 2012; Çiçek *et al.,* 2017; Pafilis *et al.,* 2019). Probably the different period affects the percentage of Orthoptera too, as the previous study was done between March and July (Angelici *et al.,* 1997), and most of our lizards were collected in summer and the beginning of autumn. In a smaller sized lizard (*L. agilis*), even if the study was made in summer, the percentage abundance of Orthoptera was low, but Coleoptera registered a higher percentage (Gvoždík and Boukal, 1998). However, in autumn, *Lacerta lepida* from Spain feed almost only on Orthoptera and Coleoptera (Castilla *et al.,* 1991), although this species generally consumes numerous Coleoptera (Busack and Visnaw, 1989; Castilla *et al.,* 1991). Even smaller lizards consumed predominantly Coleoptera (Pérrez-Melado *et al.,* 1991). Most preys consumed by *L. viridis* were common taxa, well represented in their habitats. Beside the prey taxa, in the stomachs of two lizards we found eight nematodes. Probably they were parasites, because this species is known to be infested by different types of Nematodes (Yildirimhan *et al.,* 2020).

Although in other cases there were seasonal variations in lizards feeding (e.g. Castilla *et al.,* 1991; Sagonas *et al.,* 2015, 2018), we analysed only few specimens, almost all collected in summer and early autumn, thus we did not register seasonal variations of feeding. As well as other related species, like *L. trilineata* (Sagonas *et al.,* 2015), *L. viridis* seems to change its food composition depending on habitats, fact indicated by the slight differences between the three regions of origin. In the case of another lizard species, *L. agilis*, it seems that specimens from a southern, warmer area are more active than those situated in more northern areas with less warm days (Pačuta *et al.,* 2018). Such differences probably exist between the three regions of Romania, but they are too small to modify noticeably the feeding pattern of *L. viridis*.

REFERENCES

Angelici, F.M., Luiselli, L., & Rugiero, L. (1997). Food habits of the green lizard, *Lacerta bilineata*, in central Italy and a reliability test of faecal pellet analysis. *Ital J Zool*, 64, 267-272.

Arnold, E.N. (1987). Resource partition among lacertid lizards in southern Europe. *J Zool*. 1, 739-782.

Bels, V.L., & Goosse, V. (1990). Comparative kinematic analysis of prey capture in *Anolis caroliensis* (Iguania) and *Lacerta viridis* (Scleroglossa). *J Exp Zool,* 255, 120-124.

Brito, J.C. (2004). Feeding ecology of *Vipera latastei* in northern Portugal: ontogenetic shifts, prey size and seasonal variations. *Herpetol J*, 14, 13-19.

Burke, R.L., & Mercurio, R.J. (2002). Food habits of a New York population of Italian Wall Lizards, *Podarcis sicula* (Reptilia, Lacertidae). *Am Midl Nat*, 147, 368-375.

Busack, S.D., & Visnaw, J.A. (1989), Observations on the natural history of *Lacerta lepida* in Cádiz Province, Spain. *Amphibia-Reptilia*, 10, 201-213.

Castilla, A.M., Bauwens, D., & Llorente, G.A. (1991). Diet composition of the lizard *Lacerta lepida* in central Spain. *J Herpetol*, 25(1), 30-36.

Christopoulos, A., Zogaris, D., Karaouzas, I., & Zogaris, S. (2020). A predation case of *Anguis graeca*, Bedriaga, 1881 (Squamata: Anguidae) by *Lacerta trilineata* (Squamata: Lacertidae) from Central Greece. *Herpetology Notes*, 13, 105-107.

Cicort-Lucaciu, A.Ş., Cupşa, D., Ilieș, D., Ilieș, A., Baiaș, Ș., & Sas, I. (2011). Feeding of two Amphibian species (*Bombina variegata* and *Pelophylax ridibundus*) from artificial habitats from Pădurea Craiului Mountains (Romania). *North-West J Zool*, 7(2), 297-303.

Cicort-Lucaciu, A.Ş., Covaciu-Marcov, S.-D., Bogdan, H.V., & Sas, I. (2012). Implication upon herpetofauna of a road and its reconstruction in Carei Plain natural protected area (Romania). *Ecologia Balkanica*, 4(1), 99-105.

Cicort-Lucaciu, A.-Ş., Pelle, C., & Borma, I.T. (2013). Note on the food composition of a *Pelophylax ridibundus* population from the Dubova locality region, south-western Romania. *Biharean Biologist*, 7(1), 33-36.

Cicort-Lucaciu, A.-Ş., Sas-Kovács, I., & Covaciu-Marcov, S.D. (2016). Non road human influence upon road mortality on three secondary roads in the Vâlsan River protected area, Romania. *Oltenia, Studii și Comunicări, Științele Naturii*, 32(2), 99-106.

Ciolan, E., Cicort-Lucaciu, A-Ş., Sas-Kovács, I., Ferenți, S., & Covaciu-Marcov, S.-D. (2017). Wooded area, forest road-killed animals: Intensity and seasonal differences of road mortality on a small, newly upgraded road in western Romania. *Transport Res D - Tr E*, 55, 12-20.

Covaciu-Marcov, S.-D., Ferenți, S., Cicort-Lucaciu, A-Ş., & Sas-Kovács, I. (2012). Terrestrial isopods in the diet of two amphibian species (*Epidalea viridis* and *Pelobates syriacus*) from Dobruja, Romania. *Entomologica Romanica,* 17, 5-11.

Covaciu-Marcov, S.-D., Puskás, A., Pop, A.N., Târț, M., & Ferenți, S. (2017). Road-killed amphibian and reptiles on a local road in a protected area in western Romania. *Acta Zool Bulgar*, 69(1), 115-120.

Çiçek, K., Koyun, M., & Tok, C.V. (2017). Food composition of the Near Eastern Fire Salamander, *Salamandra infraimmaculata* Martens, 1885 (Amphibia: Urodela: Salamandridae) from Eastern Anatolia. *Zool Middle East*, 63(2), 130-135.

Daltry, J.C., Wüster, W., & Thorpe, R.S. (1998). Intraspecific Variation in the Feeding Ecology of the Crotaline Snake *Calloselasma rhodostoma* in Southeast Asia. *J Herpetol*, 32(2), 198-205.

D'Amico, M., Román, J., de los Reyes, L., & Revilla, E. (2015). Vertebrate road-kill patterns in Mediterranean habitats: Who, when and where. *Biol Conserv*, 191, 234-242.

Fuhn, I., & Vancea, Şt. (1961). "Fauna R.P.R.", vol. XIV, Fascicola 2, Reptilia. *Editura Academiei R. P. R.*, Bucharest, 352 pp.

Gvoždík, L., & Boukal, M. (1998). Sexual dimorphism and intersexual food niche overlap in the sand lizard, *Lacerta agilis* (Squamata: Lacertidae). *Folia Zool*, 47(3), 189-195.

Jackson, R., & Lemm, J.M. (2009). Stomach content note for a road-killed *Varanus spenceri*. *Biawak*, 3(1), 18-20.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 9.

Heltai, B., Sály, P., Kovács, D. & Kiss, I. (2015). Niche segregation of sand lizard (*Lacerta agilis*) and green lizard (*Lacerta viridis*) in an urban semi-natural habitat. *Amphibia-Reptilia*, 36(4), 389-399.

Hill, J.E., DeVault, T.L., & Belant, J.L. (2020). Research note: A 50-year increase in vehicle mortality of North American mammals. *Landscape Urban Plan*, 197, 103746.
Hodar, J.A., Campos, F., & Rosales, B.A. (1996). Trophic ecology of the Ocellated Lizard *Lacerta lepida* in an arid zone of southern Spain: relationship with availability and daily activity of prey. *J Arid Environ*, 33, 95-107.
Ile, G.A., Maier, A.R.M., Cadar, A.M., Covaciu-Marcov, S.D., Ferenți, S. (2020). Dead snakes and their stories: morphological anomalies, asymmetries and scars of road killed *Dolichophis caspius* (Serpentes, Colubridae) from Romania. *Herpetozoa*, 33, 77-85.
Kambourova-Ivanova, N., Koshev, Y., Popgeorgiev, G., Ragyov, D., Pavlova, M., Mollov, I., & Nedialkov, N. (2012). Effect of traffic on mortality of amphibians, reptiles, birds and mammals on two types of roads between Pazardzhik and Plovdiv region (Bulgaria) – preliminary results. *Acta Zool Bulgar*, 64(1), 57-67.
Kazemi, V.D., Jafari, H., & Yavari, A. (2016). Spatio-temporal patterns of wildlife road mortality in golestan national park – north east of Iran. *Open Journal of Ecology,* 6, 312-324.
Kehlmaier, C., Zinenko, O., & Fritz, U. (2020). The enigmatic Crimean green lizard (*Lacerta viridis magnifica*) is extinct but not valid: Mitogenetics of a 120-year-old museum specimen reveals historical introduction. *J Zool Syst Evol Res*, 58, 303-307.
Kolenda, K., Kuśmierek, N., Kadej, M., Smolis, A., & Ogielska, M. (2019). Road-killed toads as a non-invasive source to study feeding ecology of migrating population. *Eur J Wildlife Res*, 65, 55.
Korsós, Z. (1984). Comparative niche analysis of two sympatric lizard species (*Lacerta viridis* and *Lacerta agilis*). *Vertebrata Hungarica,* 22, 5-14.
Meek, R. (2009). Patterns of reptile road-kills in the Vendée region of western France. *Herpetol J*, 19, 135-142.
Mollov, I. (2008): Sex Based Differences in the Trophic Niche of *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Anura) from Bulgaria. Acta Zoologica Bulgarica 60(3): 277-284.
Mollov, I. (2010). A contribution to the knowledge of the trophic spectrum of the Slow Worm (Anguis fragilis L., 1758) (Reptilia: Anguidae) from Bulgaria. *ZooNotes*, 9, 1-4.
Mollov, I., & Petrova, S. (2013). A contribution to the knowledge of the trophic spectrum of three lacertid lizards from Bulgaria. *J Bio Sci Biotech*, 2(1), 57-62.
Mollov, I., Boyadzhiev, P., & Donev, A. (2012). Trophic Niche Breadth and Niche Overlap Between Two Lacertid Lizards (Reptilia: Lacertidae) from South Bulgaria. *Acta Zool Bulgar*, Supplement 4, 133-140.
Mollov, I.A., Kirov, K.H., Petrova, S.T., Georgiev, D.G., & Velcheva, I.G. (2013). Assessing the influence of the automobile traffic on the amphibians and reptiles in the buffer zone of Biosphere Reserve "Srebarna" (NE Bulgaria). *Ecologia Balkanica*, 5(2), 31-39.

Nielsen, E.S., & Mound, L.A. (1999). Global diversity of insects: the problems of estimating numbers, In: *Nature and Human Society: the Quest for a Sustainable World*, Raven, P.H., Williams, T. (Eds.), pp. 213-222.

Pačuta, A., Žagar. A., Kočiková, B., Mijláthová, V., Mihalca, A.D., & Majlát, I. (2018). Time matters. Locomotor behavior of *Lacerta viridis* and *Lacerta agilis* in an open field maze. *Acta Ethol*, 21, 91-99.

Pafilis, P., Kapsalas, G., Lymberakis, P., Protopappas, D., & Sotiropoulos, D. (2019). Diet composition of the Karpathos marsh frog (*Pelophylax cerigensis*): what does the most endangered frog in Europe eat? *Anim Biodiv Conserv*, 42(1), 1-8.

Pérrez-Melado, V., Bauwens, D., Gil, M., Guerrero, F., Lizana, M., & Ciudad, M.-J. (1991). Diet composition and prey selection in the lizard *Lacerta monticola*. *Can J Zool*, 69, 1728-1735.

Philcox, C.K., Grogan, A.L., & MacDonald, D.W. (1999). Patterns of *Lutra lutra* road mortality in Britan*. J Appl Ecol*, 36, 748-762.

Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, 13, 131-144.

Radu, V.G., & Radu, V.V. (1967). Zoologia nevertebratelor, vol. 2, Editura Didactică și Pedagogică, București, 708 pp.

Rassati, G. (2016). Road mortality of amphibians and reptiles along two roads in the Carnis Alps (Friuli, north-eastern Italy) before and after asphalting. *Atti Mus Civ Stor Nat Trieste*, 58, 161-170.

Roth, V.D. (1971). Food habits of Ditmars' horned lizard with speculations on its type locality. *Journal of the Arizona Academy of Sciences*, 6, 278-281.

Sagonas, K., Pafilis, P., Lymberakis, P., & Valakos, E.D. (2015). Trends and patterns in the feeding ecology of the widespread Balkan green lizard *Lacerta trilineata* (Squamata: Lacertidae) in insular and continental Greece. *North-West J Zool*, 11(1), 117-126.

Sagonas, K., Valakos, E.D., Lymberakis, P., & Pafilis, P. (2018). Traits of reproduction and feeding of the Green Lizard, Lacerta viridis (Laurenti, 1768), at the southern edge of its distribution. *Herpetozoa*, 30 (3/4), 115-129.

Schwartz, A.L.W., Shilling, F.M., & Perkins, S.E. (2020). The value of monitoring wildlife roadkill. *Eur J Wildlife Res*, 66, 18.

Shannon, C.E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27, 379-423.

Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P.-A., Crnobrnja Isailovic, J., Denoël, M., Ficetola, G.F., Gonçalves, J., Kuzmin, S., Lymberakis, P., de Pous, P., Rodríguez, A., Sindaco, R., Speybroeck, J., Toxopeus, B., Vieites, D.R., & Vences, M. (2014). Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia*, 35, 1-31.

Strijbosch, H., Helmer, W., & Scholte, P.T. (1989). Distribution and ecology of lizards in the Greek province of Evros. *Amphibia-Reptilia*, 10, 151-174.

Teodor, L.A., Ferenţi, S., & Covaciu-Marcov, S.D. (2019). Weevils die in vain? Understanding messages from road-killed weevils (Coleoptera: Curculionoidea). *Coleopts Bull*, 73(2), 359-368.
Tok, C.V., Ayaz, D., & Çiçek K. (2011). Road mortality of amphibians and reptiles in the Anatolian part of Turkey. *Turk J Zool*, 35(6), 851-857.
Urbani, J.-M., & Bels, V.L. (1995). Feeding behaviour in two scleroglossan lizards: *Lacerta viridis* (Lacertidae) and *Zonosaurus laticaudatus* (Cordylidae). *J Zool*, 236(2), 265-290.
Yildirimhan, H.S., Karaman, D., & Bursey, C.R. (2020). Helminth fauna of the European Green Lizard, *Lacerta viridis* (Laurenti, 1768), from Bursa, Turkey. *Comp Parasitol*, 87(1), 56-67.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 61-82)
doi:10.24193/subbbiol.2020.1.06

Macrozoobenthic invertebrate assemblages in rivers Șușița and Sohodol (Gorj County, Romania) and their indicator value for the water quality characterization

Diana Cupșa[1]✉, Antonia Cirle[1], Alexandra-Roxana-Maria Maier[1], Achim-Mircea Cadar[1] and Ilie Cătălin Telcean[1]

[1] *University of Oradea, Department of Biology, Oradea, Romania;*
✉ ***Corresponding author, E-mail: dcupsa@uoradea.ro.***

Abstract. The present research evaluates the macrozoobenthic invertebrate assemblages from two rivers situated in the Jiu River basin. The invertebrates were collected from three sample sites on Sohodol River and five sample sites on Șușița River. The sample sites were situated along the two rivers which flow almost along their entire length through a forested area. In the case of Sohodol River we have encountered anthropic activities related to seasonal tourism, but no localities or industrial activities were observed in the area. The aim of the study was to establish besides the structure of the macrozoobenthic invertebrate assemblages, the water quality using BMWP score and also to evaluate the human impact on the macrozoobenthos in Sohodol River and the differences between the macrozoobenthic assemblages in the two rivers caused by natural or anthropic factors.

Keywords: macrozoobenthic invertebrates, diversity indexes, water quality, feeding categories.

Introduction

Macrozoobentic invertebrates form assemblages with a high importance in the ecological characterisation of the ecological status of the surface waters wordlwide (Azrina *et al,* 2005; Opreanu, 2010; Varnosfaderany *et al.,* 2010; Moya *et al*., 2011; Sinitean and Petrovici, 2012; Mesa *et al*., 2013; Macedo *et al*., 2016; Young *et al*., 2014). They are sensitive to the variations of the physico-chemical paramethers of the water due to natural (Longing *et al*., 2010; Nguyen *et al*., 2014; Macedo *et al*., 2016) or anthropogenic causes (Varnosfaderany *et al.,* 2010; Cuffney *et al.,* 2010; Buss and Vitorino 2010; Sharifinia *et al.,* 2012; Ortiz *et al.*, 2013). Macrozoobenthic invertebrate assemblages reflect the disturbances produced by the human activities on river environments (Azrina *et al.,* 2005; Heino *et al.*, 2007; Fishar and Williams, 2008; Harrgrave, 2010; Li *et al.,* 2012). Nowadays at global level the rivers are among the most affected ecosystems by various anthropic pressures such as pollution, hydrotechnical constructions, sand and gravel extraction, (Faria *et al.,* 2006; Cuffney, *et al.,* 2010; Alvial *et al.,* 2012). In Romania the most important pollutant sources of the surface waters are represented by the domestic untreated or insufficient treated waste water effluents, the sand and gravel extraction, hydrotehnical floodind protection constructions, or microhydropower systems constructions (Opreanu, 201; Șerban and Ionuș, 2011; Sinitean and Petrovici, 2012; Truță, 2014; Cismașiu *et al.,* 2017; Zanfir *et al.,* 2019). These phenomena affect the structure of the macrozoobenthic invertebarate assemblages in terms of density, species structure, the ratio of different feeding groups, the ratio of different tolerant taxonomic groups. (Pîrvu and Pacioglu, 2012; Mititelu *et al.,* 2012; Cioboiu *et al.* 2017). The ratio of different feeding groups is influenced by the food resource availability in the riverbed. The increased nutrient content will allow a higher number of autotrophic groups development and this will modify the ratio of different feeding groups (Mesa *et al.,* 2013; Marcovic *et al.,* 2015). Physico-chemical paramethers disturbances can also be reflected in the structure of the macrozoobenthic invertebrate assemblages (Sharifinia *et al.,* 2012; Hedrick *et al.,* 2013; Young *et al.,* 2014; Chang *et al.,* 2014; Marcovic *et al.,* 2015). The water quality influence the number of the pollution tolerant groups, the most sensitive groups density in the water will decrease as the water quality diminish (Varnosfaderany *et al.,* 2010; Li *et al,* 2012; Sidagyte *et al.*, 2013; Chang *et al.,* 2014).

There are several researches upon the macrozoobenthic invertebrate assemblages and the effect of pollution or of different anthropic activities in the Jiu River Basin (Onciu *et al.,* 2007; Cupșa *et al.,* 2010; Dumbravă-Doboacă and Petrovici, 2010; Șerban and Ionuș, 2011; Mititelu *et al.*, 2012; Cioboiu and Cismașiu, 2016; Cioboiu *et al.,* 2017; Cismasiu *et al.,* 2017; Cioboiu and Cismașiu,2018; Zanfir *et al*., 2019) as well as in other rivers from Romania (Momeu *et al.,* 2009; Badea *et al,* 2010; Răescu *et al.,* 2011; Marin *et al.,* 2011; Benedek *et al.,* 2013; Stoica *et al.,* 2014; Popescu *et al.,* 2016; Marin *et al.,* 2018).

The aim of our research was to investigate the structure of the macrozoobenthic invertebrates assemblages from two rivers in the Jiu River basin: Șușița Seacă and Sohodol. These rivers have a series of similar hydrological characteristics: riverbed width and depht, flow value, substrate, physico-chemical parameters, structure of the neibouring ecosystems, but different human impact.

Șușița Seacă is a tributary of Jiu River, it flows through a beech forest, an area with no human activities, there are no localities in the investigated sector, only a forest road along the riverbanks. Sohodol River is a tributary of Tismana, in it's upstream sector it flows through a beech forest where a touristic camp is found, in the lower sector it passes an open landscape, than a gorge area. Along this river portion during summer we observed touristic activities especially one day visitors and a few tent campers.

Because the two rivers pass through almost the same type of environments but they are subject to different human activities we wanted to investigate how the macroozoobenthic invertebrates assemblages are structured and if they show differences caused by the natural or anthropic factors.

Materials and methods

Study area

Șușița seacă is formed by the confluence of Straja and Măcriș rivulets at an altitude of 1400 m and flows into the Jiu at an altitude of 178 m. The total lenght of the river is 37 km and a surface of the river cathment of 234 km^2 (Administrația Națională Apelor Române - Cadastrul Apelor – București). It is situated in an area with no human activities, no localities. The river flows along a forest road used especially for forest exploitation in a beech forest ecosystem. The forest ensures a covering of the riverbed of 75-90% in the upstream area. The width of the river is between 5-10 m and a depht of 10 - 50 cm, in some places upstream with 1m deep pools. The riverbed is hard formed mainly by rocks and boulders in the upstream sectors and rocks, peebles and sand in downstream portions. The vegetation in the riverbed is absent except the first sample site where some groups of *Fontinalis* moss on the rocks. The bioderm is lacking on the upstream sites or it is very scrace downstream, in the sites with slower flow of the river small quantities of sediment deposits made up mainly by tree leafs from the nearby forest. In some portions antierozional concrete sills can be found in the riverbed.

Sohodol river originates form Vâlcan Mountains from an altitude of 680 m and it flows in River Tismana close to the Godinești village at an altitude of 180 m. The lenght of the river is 14 km with an area of the catchment system of 66 km^2 (Administrația Națională Apelor Române - Cadastrul Apelor – București). It passes through an area of upstream course covered by beech forests and in the lower sector it passes a more open area where nearby small pastures are present. Upstream there is a camp area frequently visited by turists and the middle and downstream sectors are also visited by tourists for the spectacular gorges situated here. The width of the river is between 5-6 m upstream and 10-12 m downstream. In the upper sector it has a turbulent flow and it has antierozional concrete sills in the riverbed. The riverbed in this portion is covered by stones and pebbles. In the downstream sector the width of the riverbed is greater, hard bottomed, covered with a small amount of bioderm.

Water and macroinvertebrate sampling

The samples were collected from five sample sites on the river Șușița and 3 sample sites from river Sohodol, during 2019 summer, at low water flow period.

Water samples were taken from each sampling site. The following paramethers were determined on the field using a portable WTW 410i multiparameter: water temperature, conductivity, pH, dissolved oxygen content, total dissolved solids (TDS).

From each sample site three samples were taken. The samples were collected with a Surber sampler with a sampling surface of 0,1 m^2, and a 250 μm mesh size. The samples were fixed in the field in 4% formalin in plastic bags, labeled and transported in laboratory. In the lab the samples were sorted using a 10x – 40x magnifying stereomicroscope. The sorted samples were stored in 80% ethanol and determined to family, genus or species level using specific keys (Aubert, 1959; Ujhelyi, 1959; Steinmann, 1968; Elliot *et al*, 1988; Solem and Gullefors, 1996; Bouchard, 2004).

Data analysis

The commonly used non-parametric community structure indices were calculated: number of individuals (N), number of taxa (S), the abundance of taxa (% taxon), the proportion of Ephemeroptera, Plecoptera and Trichoptera (% EPT), the proportion of Chironomida (% Chironomida), EPT/Ch value, diversity indexes (Margalef, Berger-Parker, Simpson, Shannon-Wiener, Pielou).

The value of BMWP scoring for each sample sites was calculated by adding the individual scores of the families (Armitage *et al*., 1983).

The degree of similarity between the macroinvertebrate assemblages was tested on the basis of Jaccard simmilarity, Bray-Curtis simmilarity, one way analysis of variance (ANOVA). Kruskal-Wallis test for equal medians and Mann Whitney test were conducted to test significant difference of assemblages between sites. All statistical analysis was performed using the SPSS software (version 10).

Results

The values of the physico-chemical paramethers

The physico-chemical parameters from the analyzed rivers sample sites were relatively constant in values. The pH had alkaline values in both rivers in each sample site. In the case of Sohodol the pH values were more constant than in Șușița (Tab. 1).

The temperatures were relatively low different from the air temperatures situated between 28-32^{0}C during sampling. The lowest temperature was 13.5^{0}C on Șușița at sample site Su4, after the confluence with a rivulet; the highest temperature in Șușița river was registered in the last sample site from downstream Su5 - 21^{0}C (Tab. 1). In Sohodol river the lowest temperature was recorded in the first sample site from upstream So1 – 14.7^{0}C and the highest downstream at So3 - 16^{0}C (Tab. 1).

The amount of the dissolved oxygen was lower in Șușița (8.46 mg/L - Su 1) in the upstream sites than in Sohodol (9.42 mg/L – So1) (Tab. 1). The measured conductivity values were lower in Șușița (85-134 µS/cm) than in Sohodol (200-204 µS/cm) (Tab. 1). The values of total dissolved solids (TDS) were also lower in Șușița (43-68 mg/L) than in Sohodol (92-110 mg/L) (Tab. 1).

The river is narrower in the case of Șușița (6.5 m – 7.5 m) than in Sohodol (5.5 m – 11 m) and deeper in Șușița (20 cm – 55 cm) compared with Sohodol (20 cm – 25 cm) (Tab. 1).

Table 1. The mean values of the physico-chemical parameters at the investigated sites.

Sample sites	pH	T (^{0}C)	Dissolved O_2 (mg/L)	Conduc tivity (µS/cm)	TDS (mg/L)	River width (m)	Water depth (cm)
Su 1	7.93	14.3	8.46	85	43	6.5	55
Su 2	7.83	14.5	8.89	87	46	5.5	30
Su 3	7.91	14.4	8.96	95	48	6.5	20
Su 4	8.12	13.5	9.43	133	65	7.5	25
Su 5	8.66	21	9.01	134	68	3.5	30
So 1	8.35	14.7	9.42	200	92	11	20
So 2	8.50	15	8.75	202	97	5.5	15
So 3	8.47	16	8.65	204	110	11	25

The macrozoobenthic invertebrate assemblages

We have collected a number of 34 taxonomic groups from river Susita and 30 from Sohodol. The maximal number on sample sites was 24 (Su4) in Susita and 26 (So3) in Sohodol, and the minimal number 21 (So2) in Sohodol and 13 (Su1) in Susita (Tab. 2).

The most of the groups were represented by aquatic insect larva (Ephemeroptera, Odonata, Plecoptera, Trichoptera, Diptera) or adults (Coleoptera) in both investigated rivers. Beside the insect groups we have found also Turbelariata, Oligochaeta, Gastropoda, Gammarida, species in each river and in some sample sites from Șușița River also Nematomorpha specimens in small numbers.

The total number of collected specimens was 2812 (1450 from Sohodol and 1362 from Șușița). The number of specimens was between 41 (Su1) - 384 (Su4) in the case of Șușița river and 390 (So2) – 562 (So1) in Sohodol river (Tab. 2).

The percent of the taxa, was calculated as the ratio between the number of taxa found in one sample site and the number of taxa found in the whole investigated river section. The obtained values were between 38.24 (Su1) and 70.59 (Su4) in the case of Șușița river and between 70 (So2) and 86,67 (So3) in Sohodol river. The percent of Ephemeroptera, Plecoptera and Trichoptera (% EPT) was situated between 60.16% (Su4) and 69.28% (Su5) and the percent of Chironomida larva (%Ch) between 10.24 (Su5) and 26.83 (Su1) so the EPT/Ch ratio varied between 2.36 (Su1) and 6.77 (Su5) (Tab. 2).

In Sohodol river % EPT varied between 62.10 (So1) and 73.59 (So2), the % Ch between 2.05 (So2) and 7.83 (So3) so the EPT/Ch ratio has much higher values in Sohodol between 8.9 (So3) and 35.9 (So2) than in Șușița (Tab. 2).

The biodiversity indexes have very close values in the two investigated water courses and also between the sample sites in each river. Margalef index was between the maximal values of 3.87 (Su4) and 4.03 (So3) and minimal values of 3.22 (Su3) and 3.35 (So2) (Tab. 2). The Berger-Parker index was maximal 0.4 at Su3 and 0.37 So 3, and minimal 0.17 at Su2 and 0.28 So1 respectively (Tab. 2). The Simpson index has the maximal values 0.21 in Su3 and 0.19 in So3, and the minimal values 0.09 Su2 respectively 0.14 So1. The Simpson diversity was the highest 0.96 at Su2 and 0.86 So 2 and the lowest 0.79 in Su3 and 0.81 in So3 (Tab. 2). The Shannon-Wiener index was situated between 2.7 at Su2 and 2.18 at So 1, and 2.09 at Su3 and 2.12 at So2. The evenness (Pielou index) has also very uniform values between the sample sites and the two investigated rivers with maximal values of 0.87 at Su2 and 0.77 So3 and minimal values of 0.7 at Su3 and So2 (Tab. 2).

The feeding groups were present in different proportions in both rivers except the parasites which were represented by Nematomorpha specimens found only in Șușița River. From all feeding groups we have highlighted the proportion of collectors/gatherers with higher proportions in Sohodol between 57.47% (So1) and 66.67% (So2) and lower in Șușița between 19.51% (Su1) and 52.41% (Su5) (Tab. 2). The proportion of shredders was between 1.41% (So3) and 3.33% (So2), and between 0 (Su1) and 8.79 (Su2) in the case of Șușița River (Tab. 2).

The values of the BMWP score were higher in Sohodol 112 (So1) and 140 (So3) and much more variable in Șușița between 50 (Su1) and 114 (Su4) (Tab. 2). The values of the BMWP score were obtained especially from the presence of the sensitive groups of Ephemeroptera, Plecoptera and Trichoptera in almost all investigated sample sites.

Table 2. The values of the number of individuals (N), taxa (S), the abundance of taxa (% taxon, S/total number of taza from the investigated river*100), the proportion of Ephemeroptera, Plecoptera and Trichoptera (% EPT), the proportion of Chironomida (% Chironomida), EPT/Ch value, diversity indexes (Margalef, Berger-Parker, Simpson, Shannon, Pielou) % of collector/gatherer and % of shredder feeding groups, value of BMWP scoring for each sample site

Indexes	Su 1	Su 2	Su 3	Su 4	Su 5	So 1	So 2	So 3
N	41	239	366	384	332	562	390	498
S	13	22	20	24	23	23	21	26
% taxa	38.24	64.71	58.82	70.59	67.65	76.67	70	86.67
% EPT	63.41	66.53	65.85	60.16	69.28	62.10	73.59	69.68
% Ch	26.83	15.06	10.93	21.88	10.24	2.31	2.05	7.83
EPT/Ch	2.36	4.42	6.02	2.75	6.77	26.88	35.90	8.90
Margalef	3.23	3.83	3.22	3.87	3.79	3.47	3.35	4.03
Berger Parker	0.27	0.17	0.40	0.28	0.32	0.28	0.32	0.37
Simpson index	0.15	0.09	0.21	0.15	0.14	0.14	0.18	0.19
Simpson diversity	0.85	0.91	0.79	0.85	0.86	0.86	0.82	0.81
Shannon	2.18	2.70	2.09	2.31	2.45	2.38	2.12	2.25
Pielou	0.85	0.87	0.70	0.73	0.78	0.76	0.70	0.77
% collectors /gatherers	19.51	38.91	31.69	30.47	52.41	57.47	66.67	64.46
% shredders	0	8.79	1.09	0.78	4.52	2.49	3.33	1.41
BMWP score	50	104	96	114	104	112	116	140

CCA between the physico-chemical parameters and the diversity indexes in the sample sites of the two investigated rivers showed a weak negative correlation between pH and number of taxa, Shannon-Wiener and Pielou indexes and a weak positive correlation with the number of individuals (Fig. 1).

The conductivity is strong negatively correlated with the number of individuals and weak positive correlation with number of taxa, Shannon-Wiener and Pielou indexes, the dissolved oxygen values are weak positively correlated with all indexes, total dissolved solids (TDS) are strongly positive correlated with the number of individuals and weak with number of taxa, Shannon-Wiener and Pielou indexes, the riverbed width is weak positive correlated with the number of individuals, strong negative correlated with the number of taxa and Pielou index and weak negatively correlated with Shannon_Wiener index (Fig. 1). The riverbed depth is strong positively correlated with the number of individuals, taxa and Pielou index and weak positive correlation with the Shannon-Wiener index (Fig. 1).

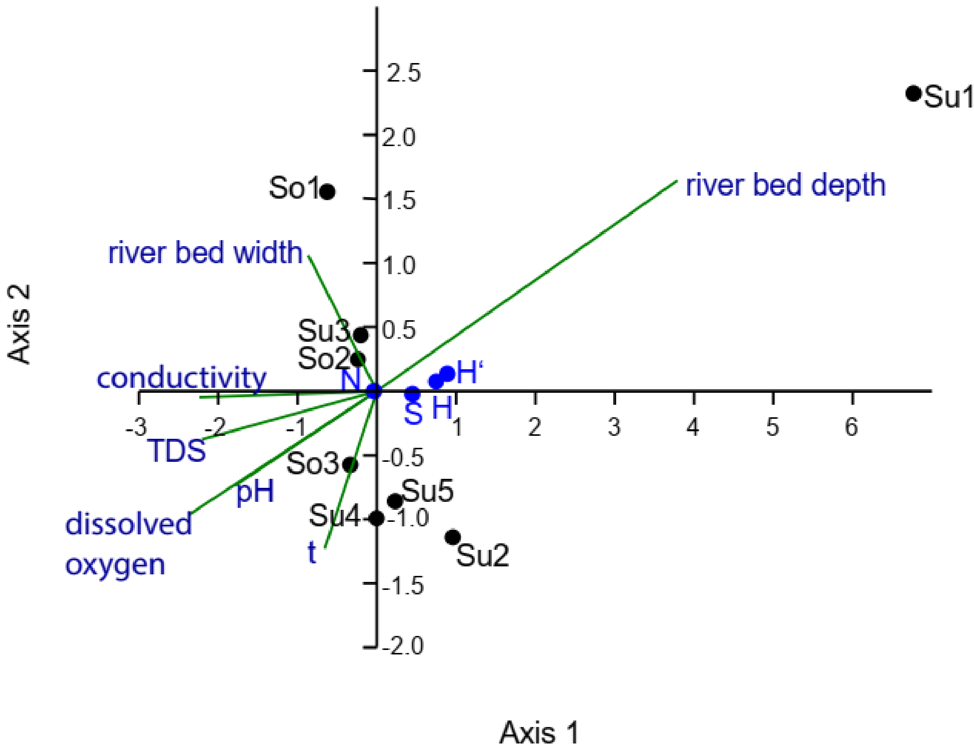
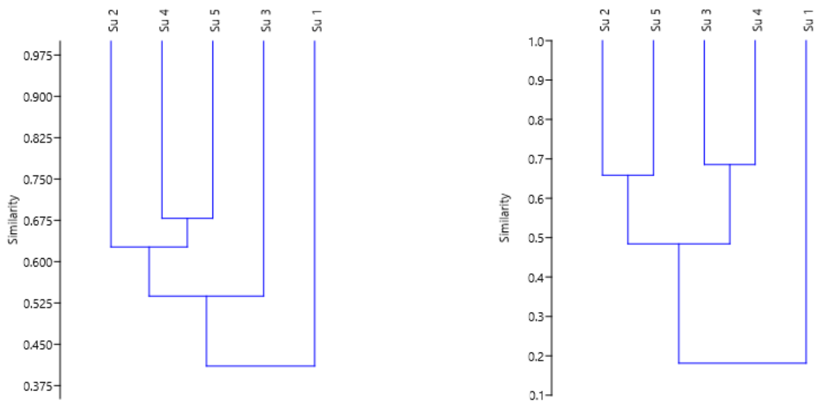


Figure 1. The result of the canonical correspondence analysis (CCA) based on the diversity indexes values with respect to environmental variables. Green lines represent the environmental variables, the blue circles the indexes and the black circles the sample sites. N= number of individuals, S= number of taxa H= Shannon-Wiener index, H`= Pielou index.

The Jaccard correlation index between macrozoobenthic invertebrate communities in river Şuşiţa was the highest between sample sites Su4 and Su5, 0.68 respectively 0.67, and with Su2 (0.49 and 0.66). The lowest value of the Jaccard index was between the Su1 sample site and the rest of the sites (Fig. 2A).

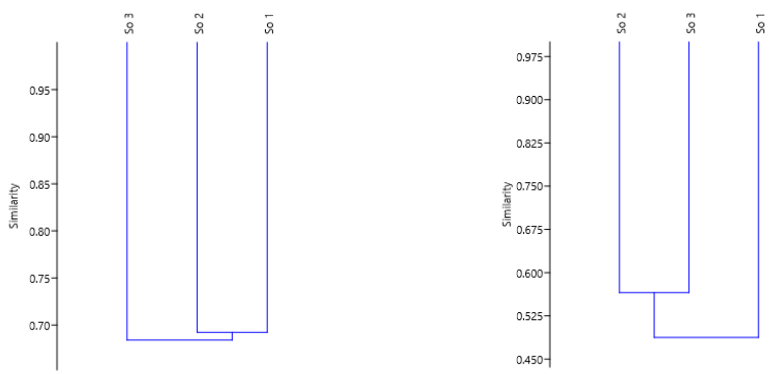
The Bray Curtis index shows the lowest similarities between the Su2 and Su5 0.66 macrozoobenthic communities on one hand and Su3 and Su4 0.68 on the other hand. These two communities form a separate branch of the cladogram on one side outstanding from the community of Su1 sample site which form another branch of the cladogram, results which look very alike as those obtained from the Jaccard index (Fig. 2B).



A **B**

Figure 2. Jaccard (A) and Bray-Curtis (B) similarity indexes between the macrozoobenthic communities along Șușița River

Between the macrozoobenthic communities along Sohodol river the Jaccard similarity index was the highest between So1 and So2 0.69 and lower with So3, but above 0.67 so a much higher similarity than in the case of Șușița river communities. According to the Bray-Curtis similarity the communities from So2 and So3 where less similar between each-other 0.57 and more with So1 0.48-0.50 (Fig. 3).



A B

Figure 3. Jaccard (A) and Bray-Curtis (B) similarity indexes between the sample sites along Sohodol River

One way ANOVA was calculated in case of macrozoobenthic invertebrate groups between the sample sites and showed no significannt differences between the communities from Șușița ($p=0.169$), from Sohodol ($p=0.797$) and from both rivers ($p=0.266$). Kruskal-Wallis test for equal medians have shown significant differences between the medians of the samples in Șușița ($p=0.0115$) and between the median of all samples ($p=0.0255$).

Table 3. The values of Mann-Whittney test for all communities from the sample sites on the two investigated rivers.

Sample sites	Su 1	Su 2	Su 3	Su 4	Su 5	So 1	So 2	So 3
Su 1		**0.004**	**0.023**	**0.002**	**0.002**	**0.002**	**0.019**	**0.001**
Su 2	**0.004**		0.620	0.876	0.851	0.594	0.645	0.519
Su 3	**0.023**	0.620		0.520	0.501	0.342	0.965	0.276
Su 4	**0.002**	0.876	0.520		0.992	0.773	0.497	0.679
Su 5	**0.002**	0.851	0.501	0.992		0.748	0.506	0.655
So 1	**0.002**	0.594	0.342	0.773	0.748		0.361	0.890
So 2	**0.019**	0.645	0.965	0.497	0.506	0.361		0.272
So 3	**0.001**	0.519	0.278	0.679	0.655	0.890	0.272	

The values of Mann Whitney test between the sample sites showed significant differences between the communities Su1 from Șușița river and the other macrozoobenthic invertebrate communities from the two investigated rivers ($p<0.05$). The rest of the sample sites from both rivers do not show significant differences in their macrozoobenthic communities according to this test $p>0.05$ (Tab. 3).

Discussion

The pH values of the two investigated rivers are alkalinne which is common for the unpolluted rivers and streams in temperate climate (Challar *et al*., 2011; Sharifinia *et al*., 2012; Li, *et al*., 2012; Nguyen *et al*., 2014). The lower pH in Șușița river in the upstream sample sites is due to probably to the higher content of SO_4 measured in these sample sites. The SO_4 ions combine with water and increase acidity of the environment. The SO_4 content is higher in Șușița than in Sohodol river probably due to a different lithoological substrate, considering that the river flows very close to the Rovinari mining basin. In the area there are lignite resources and this type of coal have a high content in sulfur compounds (Cristesco and Sandor, 1929; Iamandei and Diaconu, 2013). We asume that the SO_4 comes from natural sources because the riverbed in the investigated area is hard with very low amount of organic deposits and content, the temperature is low, the disolved oxygen has a high value and the water has a rapid flow, so the bacterial decomposing activities which can increase the SO_4 content (Ruuskanen et al., 2018) are almost absent.

The temperatures have low values at the sample sites situated in the forrested regions, where the water surface is shadowed by the forrest and the water remains cool. Downstream in the sample sites which are exposed to the sunlight the water tempertaure is higher, but compared to the air temperature is lower. In these areas the insolation period is short during the day because both rivers flow through a hilly region with high slopes close to the riverbed. These slopes shadow the river for the most part of the day.

The oxygen content of the two rivers corresponds to the normal values for a hilly water course, on a hard substrate and slightly or not affected by human activities (Chadar *et al.,* 2011; Sharifina *et al.,* 2012; Pîrvu and Pacioglu, 2013; Nguyen *et al.,* 2014).

The values of the water conductivity are lower in Șușița River than in Sohodol based on the different ions content of the two water courses. Both rivers have values which are easily tolerated by the macrozoobenthic invertebrates and similar to those of other rivers which flow on silicious substrates (Benetti *et al.,* 2012; Marcovic *et al.,* 2015).

The higher values of TDS in Sohodol can result in part from the diffuse organic pollution produced by human activities in the area and by the allohtone substances washed by the high level waters from the river banks (Chadar *et al.,* 2011; Chang *et al.*, 2013)

The number of taxonomic groups was slightly higher (34) number in Șușița River than 30 în Sohodol. In both rivers we have found the important groups of macrozoobenthic invertebrates characteristic for this type of water courses (Turbelariata, Oligochaeta, Gastropoda, Gammarida, Ephemeroptera, Odonata, Plecoptera, Coleoptera, Trichoptera, Diptera). In Șușița we have also find Nematomorpha specimens, which are aquatic only in the adult period of their life and can be find in the rivers only during summer months by chance due to their short adult stage and their low density (Hanelt and Janovy, 1999). In Șușița we have found one specimen of Heteroptera in the last sample site from the downstream (Su5) and 4 specimens of Gomphidae (Odonata) at sample site Su3. The Odonata specimes were found in a sample site where that water flow is slower and these insect larvae can find an adequate environment for their development (Kalkman et al., 2008; Dijkstra and Kalkman, 2012). Also here they have enough preys to be able to ensure their food necessities. The Heteroptera was found in sample site Su5 which is situated closer to other water bodies and to localities, they usual install in perturbed water bodies (Tchakonté *et al.,* 2015).

We observed also some differences concerning the presence of different families from order Ephemeroptera and Trichoptera, for example in Șușița we have found Leptophlebia specimens and in Sohodol Ephemera specimens. Leptophlebia species species which have a tolerance value of 10 according to BMWP scoring so they are characteristic to very good quality waters (Azrina *et al.,* 2005; Li *et al.,* 2012). Ephemera species are burrowing larvae so they need soft sediments at least near to the riverbanks to be able to settle in a stream (Lee *et al*., 2008).

The smallest number of taxonomic groups (13) and also specimens (41) were found in the first sample site from upstream of Șușița River. At this sample site the water flow is turbulent, with high velocity, the riverbed is covered by boulders and big stones which have no bioderm on their surface. These conditions do not allow a high number of species to settle in that type of riverbed because they haven't got enough food resources and they are exposed to downstream drift during high water levels after heavy precipitations (Li *et al.,* 2012).

The number of specimens/sample sites vary between normal values for this type of rivers and substrate (Momeu *et al.* 2009; Răescu *et al.,* 2011; Benedek *et al.,* 2013). The hard bottom of the rivers and the low amount of the sediments allow a small number of specimens to develop in the benthos due to a relatively scarce trophic offer.

The % of taxa on sample sites was high between 70%-86,67%, these values indicate a high stability of the environmental conditions, stability which ensure the same structure of the macrozoobenthic assemblages. (Hedrick *et al.,* 2010; Benetti *et al.,* 2012). The only sample site with a lower % of taxa is the first site from upstream Șușița 38,24%. Here the assemblage of the macroinvertebrates is less stable due to the caracteristics of the river in this portion mentioned above.

The % EPT was high in every sample site above 60% which reflect together with the low %Ch and high EPT/Ch ratio the fact that the water has a good quality in both rivers in each sample sites (Dumbravă-Dodoacă and Petrovici, 2010; Mititelu *et al*., 2012; Sinitean and Petrovici, 2012; Chang *et al.,* 2013). Although the Ephemeroptera species are considered to be very sensitive species to disturbances and Chironomids are considered as organic pollution indicators, there are species in the case of both groups which do not respect these general rules (Li *et al.,* 2012; Nguyen *et al.* 2014). There are several Ephemeroptera species which can tolerate moderate pollution (Moja *et al.,* 2011; Li *et al.,* 2012; Chang *et al.,* 2013) and also Chironomid species which are very sensitive to the water quality (Moja *et al.,* 2011).

The value of the Margalef index was relatively constant between the sample sites and slightly lower in Șușița compared to Sohodol River. The values of the index are obtained based on the total number of species and total number of individuals/sample. Due to this fact there is a linear relationship between the species richness (S) and the value of Margalef index (Gamito, 2010) (Tab. 2).

The Berger-Parker index have the highest values in sample sites So3 and Su3. This index has high values in sites dominated by the most common species (Olszewski, 2007) and it's increasing values can be a measure of an increasing disturbance (Caruso *et al.,*2007). In the sample sites which have the highest value of the index the assemblage is dominated by common species for this river sector such as Gammaridae, Baetidae and Hydropsychidae in Șușița and Gammarida and Baetida in Sohodol.

Simpson index and Simpson diversity shows high diversity in each sample site but because this index is not so sensitive to the less abundant species in the sample their value do not reflect the complete picture of the biodiversity from a sample site (Keylock, 2005). The Shannon-Wiener index has values between 2,7 and 2,09 showing a high diversity in each sample sites for both rivers at numerical values which are common to this type of river sectors (Azrina *et al.,* 2005; Sharifinia *et al.,* 2012; Mesa *et al.,* 2013; Benedek *et al.,* 2013).

The evenness (Pielou index) also have high values in each sample site which reflects together with Shannon-Wiener diversity index the existence of well-established benthic invertebrate assemblages in undisturbed or slightly disturbed environments (Azrina *et al.,* 2005; Mesa *et al.,* 2013; Benedek *et al.,* 2013).

The proportion of different feeding groups can be a measure of the perturbance of the river environment (Mesa *et al.*, 2013). Collectors/gatherers and shredders are the major primary consumers in forest streams which ensure the link between the organic input and the predator groups (Cheshire *et al.*, 2005). Shredders prefer leaf detritus colonized by microbial and fungal organisms which usually occur in river sectors from forest areas and collectors/ gatherers prefer fine detritus especially sediment related detritus but in some extent also transported detritus (Cummins, 1979). In wooded streams the ratio between shredders and collectors/gatherers during summer season was 0,125 and the annual mean ratio was 0,6 (Cummins, 1979). In our study the ratio between these two feeding groups varied between 0,02 and 0,04 for Sohodol showing a scarce resource of leaf litter in the water although it flows through a forested area. Despite this the leaf litter can't accumulate in the substrate because the drift of the vegetal detritus during high water flows in the spring period. In Șușița River the ratio between the two feeding groups was between 0,23 and 0. The Su2 sample site had the highest ratios due to the particularities of the riverbed. It is covered by stones and pebbles, between these the leaf litter can remain captive even at high flowing rates and allow the shredders to install. At normal flows the water has a laminar flow which allows the fallen leaves to sink to the substrate easily that in the first sample site. At the first sample site from upstream Su1, the riverbed is covered by boulders and stones, the water has a turbulent flow and it washes away the leaves fallen from the trees near the riverbanks, that is why we didn't find any shredder specimens.

The small proportion of shredders is also frequent in tropical streams but the cause of their scarcity is the low quality of the leaf litter which contain a lot of tanin which makes it difficult to decompose (Oliveira and Nessimian, 2010; Mesa *et al.,* 2013).

The water quality was asses using BMWP score. The water quality is very good in each sample site except Su1 where the water quality is medium corresponding to a BMWP score 50. This site is situated in an unimppacted area, but because of the hydrological caracteristics, the macrozoobenthic assemblage is not very stable and diverse, beeing frequently washed out by the turbulent water flow especially after heavy precipitations. So the apparently lower water quality is given in fact by the unstable environment which cause a perturtance in the assemblage. Other authors (Moja *et al.,* 2011) also showed the fact that the BMWP score do not reflect the absolute quality of the water because it is based on family level scoring of the tolerance values. The tolerance values at family level can represent intermediate values of the species tolerance (Armitage *et al.,* 1983) so the indices at family level can over or underestimate the tolerance level of the species (Varnosfaderany *et al*., 2010). However the indces at family level is used because the species identification is a very time consuming procedure and sometimes taxonomic experts for each group are not available. The value of the BMWP score give enough accuracy to be largely used for water quality assessment in several countries worldwide both in Europe: UK (Armitage *et al.,* 1983), Greece (Artemiadou and Lazaridou, 2005), Italy (Solimini *et al.,* 2000), Poland (Czerniawska-Kusza, 2005), Spain (Zamora-Munoz *et al.,* 1995), Portugal (Faria *et al.,* 2006), in Asian countries: Thailand (Mustow, 2002), Malaysia (Azrina *et al.,* 2006), Hindu and Kush-Himalaya region (Ofenböck *et al.,* 2008), African countries: Egipt (Fishar and Williams*,* 2008), South American countries: Brazil (Silveira *et al.,* 2005), North-American and (Ruiz Picos *et al*., 2017), Central American ones: Costa Rica (Sedeño-Díaz et al., 2012)

The weak negative correlation between pH and number of taxa, Shannon-Wiener and Pielou indexes shows that the pH value is an important limitative factor for the macroinvertebrate assemblages structure (Marcovic *et al.,* 2015). They are sensitive to the variation of the pH values especially when it exceed the tolerance limits for some species. In the investigated rivers the pH tend to have a more alkalinic value due to the natural mineralization of the water and very low microbiological activity. The pH and TDS shows also a weak positive correlation with the number of individuals which increase together with the decrease of the species number according to the area-species curve theory (Williams, 1964).

The conductivity has a weak positive correlation with number of taxa because conductivity usually is positively correlated with the majority of the nutrients in the water (Chalar *et al.,* 2011). The nutrients to some extent are necessary for community development in a water body especially in the case of the autotrophic species which represent trophic base for benthic invertebrates.

The weak positive correlation between the riverbed width and the number of individuals as well as the strong positively correlation between the riverbed depth and the number of individuals is according to the area-species curve theory (Williams, 1964). Especially is the river width comes also with a great diversity of microhabitats, it creates favorable environmental conditions for a great diversity of invertebrates. The depth in the investigated rivers remain below 1 m, so it not represents a limiting factor for the development of the macrozoobenthic invertebrates.

The riverbed width is negative correlated with Pielou and Shannon_ Wiener index because the multitude of microhabitat encountered with the increasing value of river width decrease the evenness in the river sector. These two diversity indexes are positively correlated with the river depth because in the investigated rivers the depth variance is not very high (Tab. 1) so this factor do not create as much microhabitat types as the variation of the river width.

Jaccard similarity index shows the highest similarity between sample sites 4 and 5 from Șușița River which are the last two sites from downstream, they have the most similar hydrological parameters so their assemblages look very much alike. The next site with high similarity with the above mentioned is Su2 characterized by similar hydrology. The Su3 site situated between Su2 and Su 4 has a more turbulent flow and shallower water. These characteristics determine the establishment of a more different macrozoobenthic assemblage than in the above mentioned sites. The first site from upstream Su1 is less similar with the others because has the most turbulent flow, deeper water and a substrate dominated by boulders, here the assemblage is made up by a very few number of species and individuals due to frequent disturbances caused by the water volume variation determined by the rainfall volume.

In Sohodol River the most similar are the sample sites So1 and So2 situated closer to each other than So3 to So1. This similarity is according to the gradual variation of the macrozoobenthic assemblages along a water course (Răescu *et al.,* 2011; Benedek *et al.,* 2013). The first two sample sites from upstream even if they suffer some anthropic impact due to the camping site near So1, are more similar, because they have almost the same width, the river flows through a forested area in these sectors, unlike So3 which flows through an open sector, which is more exposed to the sun during daytime.

The Bray Curtis index shows two different clusters of sites with high difference between their assemblages structure in Șușița River Su2 and Su5 one hand and Su3 with Su4 one the other hand. These two groups of sites differ a lot in their hydrological characteristics that's why they develop slightly different macroinvertebrate assemblages. Su1 sample site forms another branch of the cladogram also according to this index due to the reasons mentioned above.

In Sohodol River the Bray Curtis index revealed the same similarities between the sample sites as Jaccard index, namely less similar are sample sites So2 and So3 than So1 and So2.

The one-way ANOVA showed no significant differences between the sample sites, those are situated in the same hydrological basin, the Jiu River basin, Șușița being a first rank tributary and Sohodol a second rank tributary of this river. They flow through similar habitats represented by deciduous beach forests, with minimal human impact in the case of Șușița River and a slight human impact generated by seasonal touristic activities in the case of Sohodol River. In this situation the macroinvertebrate assemblages along the river do not differ very much, because the river do not change very much its flowing characteristics and the total length of the two rivers is not very high. The only significant differences between the macrozoobenthic invertebrate assemblages were shown by the medians of the samples revealed by the Kuskall-Wallis test which emphasize the gradual differences between the sample sites along the river continuum (Sedell *et al.,* 1989; Yates *et al.,* 2018). Also a significant difference between sample sites was revealed by Mann Whitney test between sample site S1 and the other sample sites from the two investigated rivers. This significant difference is due to the very different hydrological and habitat characteristics of this site on Șușița River as it was mentioned above, which lead to a different assemblage structure in this site.

Conclusions

Our study revealed the fact the two analysed rivers have a very good water quality except Su1 sample site mainly because natural causes determined by the hydrological characteristics of the water as we mentioned above. In Sohodol the water quality was very good in every sample site, so the tourism in nearby the river do not affect the water quality for the moment.

The macrozoobenthic invertebrate assemblages in the two rivers are very similar, due to the similar dimensions, hydrological and physico-chemical characteristics of the investigated water courses. The only one site which has a very different assemblage is Su1 where the natural disturbances are high caused especially by the waterflow differences between periods with heavy precipitation and periods with drought.

REFERENCES

Administrația Națională Apelor Române (1992). Cadastrul Apelor – București, vol I-III, București.

Alvial, I. E., Tapia, D. H., Castro, M. J., Duran, B. C., & Verdugo, C. A. (2012). Analysis of benthic macroinvertebrates and biotic indices to evaluate water quality in rivers impacted by mining activities in northern Chile. Knowledge and Management of Aquatic Ecosystems, 407, 01.

Armitage, P. D., Moss, D., Wright, J. F., & Furse, M. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Researches*, 167(3), 333-347.

Artemiadou, V., & Lazaridou, M. (2005). Evaluation score and interpretation Index of the ecological quality of running waters in central and northern Hellas. *Environmental Monitoring and Assessment*, 110, 1-40.

Aubert, J. (1959). Plecoptera. *Insecta Helvetica Fauna*, 1, 1-140.

Azrina, M. Z., Yap, C. K., Rahim Ismail, A., Ismail, A., & Tan, S. G. (2005). Anthropogenic impacts on distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. *Ecotoxicology and Environmental Safety*, 64, 337-347.

Badea, B. A., Gagyi-Palffy, A., Stoian, L. C., & Stan, G. (2010). Preliminary studies of quality assessment of aquatic environments from Cluj suburban areas, based on some invertebrates bioindicators and chemical indicators. *Aquaculture, Aquarium, Conservation & legislation International Journal of the Bioflux Society,* 3(1), 35-41.

Benedek, A. M., Sîrbu, I., Soare, M., Toma, A., & Vasile, M. (2013). Benthic macroinvertebrate communities from the western part of the Apuseni Nature Park (Crișul Negru upper river basin, Romania). *Brukenthal. Acta Musei,* VIII (3), 487-502.

Benetti, C. J., Perez-Bilbao, A., & Garrido, J. (2012). Macroinvertebrates as indicators of water quality in running waters: 10 years of research in rivers with different degrees on anthropogenic impacts. In: Ecological water quality – water treatment and reuse., *Intech, Editors: K. Voudouris*, 95-122.

Bouchard, R. W. Jr. (2004). Guide to Aquatic Invertebrates of the Upper Midwest. *University of Minnesota*, 1-203.

Buss, D. F., & Vitorino, A. S. (2010). Rapid bioassessment protocols using benthic macroinvertebrates in Brazil: evaluation od taxonomic sufficiency. Journal of North American Benthological Society, 29(2), 562-571.

Caruso, T., Pigino, G., Bernini, F., Bargagli, R., & Migliorini, M. (2007). The Berger–Parker index as an effective tool for monitoring the biodiversity of disturbed soils: a case study on Mediterranean oribatid (Acari: Oribatida) assemblages. *Biodiversity and Conservation*, 16, 3277–3285.

Challar, G., Arocena, R., Pacheco, J. P., & Fabian, D. 2011. Trophic assessment of streams in Uruguay: a trophic state index for benthic invertebrates (TSI-BI). *Ecological Indicators,* 11, 362-369.

Chang, F. H., Lawrence, J. E., Rios-Touma, B., & Resh, V. H. (2014). Tolerance values of benthic macroinvertebrates for stream biomitoring: assessment of assumptions underlying scoring systems worldwide. *Environmental Monitoring and Assessment*, 186, 2135–2149.

Cheshire, K., Boyero, L. & Pearson, R. G. (2005). Food webs in tropical Australia streams: shredders are not scarce. *Freshwater Biology*, 50, 748-769.

Cioboiu, O., & Cismașiu, C. M. (2016). Structural and functional diversity of some aquatic ecosystems in the lower sector of Jiu (south-western Romania). *Proceedings of the 33rd Congress of International Society of Limnology SIL.*, 33, 46-47.

Cioboiu, O., & Cismașiu, C. M. (2018). The influence of abiotic factors on the reconstruction of the biocoenosis areas polluted with organic and inorganic compounds from the lower sector of the Jiu River. *Journal of International Environmental Applications and Science,* 13(2), 110-115.

Cioboiu, O., Cismașiu, C. M., & Gavrilescu, E. (2017). Heavy metal influence on the evolution of the planktonic and benthic diversity of organisms present in contaminated industrial ecosystems of Oltenia Plain. *Oltenia. Studii și comunicări. Științele Naturii,* 33(1), 171-176.

Cismașiu, C. M., Cioboiu, O., Gavrilescu, E., & Tomuș, N. (2017). The effects of ecological factors on the structure of the communities of organisms present in the industrial polluted areas from Oltenia region, *Oltenia. Studii și comunicări. Științele Naturii,* 33(1), 177-182.

Cristesco, I., & Sandor, A. (1929). Les lignites de Roumanie et leurs caracteres dans la question de carburants. *Bulletin Scientifique de l'Ecole Polytechnique de Timișoara*, 1-18.

Cuffney, T. F., Brightbill, R. A., May, J. T., & Watte, I. R. (2010). Responses of benthic macroinvertebrates to environmental changes associated with urbanization in nine metropolitan areas. *Ecological Applications*, 20(5), 1384-1401.

Cummins, K. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, 10,147-172.

Cupșa D., Covaciu-Marcov, S. D., Sucea, F., & Hercuț R. (2010). Using macrozoobenthic invertebrates to asses the quality of some aquatic habitats from Jiului Gorge National Park (Gorj County, Romania). *Biharean Biologist*, 4 (2),109-119.

Czerniawska-Kusza, I. (2005). Comparing modified biological monitoring working party score system and several biological indices based on macroinvertebrates for water quality assessment. *Limnologica,* 35, 169-176.

Dijkstra, K.-D. B, & Kalkman, V. J. (2012). Phylogeny, classification and taxonomy of European dragonflies and damselflies (Odonata): a review. *Organisms Diversity & Evolution*, 12, 209–227.

Dumbravă-Dodoacă, M., & Petrovici, M. (2010). The influence of the anthropic activities on the benthonic macroinvertebrates communities existing in the Jiu and Jiul de Vest rivers, south-west of Romania, *Aquaculture, Aquarium, Conservation & legislation International Journal of the Bioflux Society*, 3(2), 133-140.

Elliott, J.M., Humpesch, U.H., & Macan, T.T. (1988). Larvae of the British Ephemeroptera: a key with ecological notes. *Freshwater Biological Association*, 145.

Faria, M. S., Re, A., Malcato, J., Silva, P. C. L. D., Pestana, J., Agra, A. R., Nogueira, A. J. A., & Soares, A. M. V. M. (2006). Biological and functional responses of in situ bioassays with *Chironomus riparius* larvae to assess river water quality and contamination. *Science of Total Environment*, 371 (1-3), 125-137.

Fishar, M. R., & Williams, W. P. (2008). The development of a biotic pollution index for the River Nile in Egypt. *Hydrobiologia*, 598, 17-34.

Gamito, S. (2010). Caution is needed when applying Margalef diversity index. *Ecological Indicators*, 10, 550–551.

Hanelt, B., & Janovy, J. J. Jr. (1999). The Life Cycle of a Horsehair Worm, Gordius robustus (Nematomorpha: Gordioidea). Papers in the Biological Sciences. *Journal of Parasitology*, 85(1), 139-141

Hargrave, B. T. (2010). Empirical relationships describing benthic impacts of salmon aquaculture. Aquaculture Environment Interactions, 1, 33-46.

Hedric, L. B., Welsh, S. A., Anderson, J. T., Lin, L.-S., & Wei, X. (2010). Response of benthic macroinvertebrate communities to highway construction in an Appalachian watershed. Hydrobiologia, 641, 115–131.

Heino, J., Mykra, H., Hamalainen, H., Aroviita, J., & Muotka, T. (2007). Responses of taxonomic distinctness and species diversity to anthropogenic impacts and natural environmental gradients in stream macroinvertebrates. *Freshwater Biology,* 52, 1846-1861.

Iamandei, S., & Diaconu, F. (2013). The coal generating Neogene forests from the Dacian Basin. *Oltenia. Studii și comunicări. Științele Naturii,* 29(1), 30-41.

Kalkman, V. J., Clausnitzer, V., Dijkstra, K.- D. B., Orr, A. G., Paulson, D. R., & van Tol, J. (2008). Global diversity of dragonflies (Odonata) in freshwater. *Hydrobiologia*, 595, 351–363.

Keylock, C. (2005). Simpson diversity and the Shannon–Wiener index as special cases of a generalized entropy. *Oikos,* 109, 203–207.

Lee, S. J., Hwang J. M., & Bae, Y. J. (2008). Life history of a lowland burrowing mayfly, *Ephemera orientalis* (Ephemeroptera: Ephemeridae), in a Korean stream. *Hydrobiologia,* 596, 279–288.

Li, F., Cai, Q., Jiang, W., Qu, X., & Jia, X. (2012), Flow related disturbances in forestes and agricultural rivers: influences on benthic macroinvertebrates. *International Review of Hydrobiology*, 97(3), 215-232.

Longing, S. D., Voshell, J. R. Jr., Dolloff, C. A., & Roghair, C. N. (2010). Relationships of sedimentation and benthic macroinvertebrate assemblages in headwater streams using systematic longitudinal sampling at the reach scale. *Environmental Monitoring Assessment*, 161, 517-530.

Macedo, D. R., Hughes, R. M., Ferreira, W. R., Firmiano, K. R., Silva, D. R. O., Ligeiro, R., Kaufmann, P. R., & Callisto M. (2016). Development of a benthic macroinvertebrate multimetric index (MMI) for Neotropical Savanna headwater streams. *Ecological Indicators*, 64, 132-141.

Marcovič, V., Tomovič, J., Atanackovič, A., Kračun, M., Ilič, M., & Paunovič, M. (2015). Macroinvertebrate communities along the Velika Morava River. *Turkish Journal of Zoology*, 39, 210-224.

Marin, A. A., Dumbravă-Dodoacă, M., Petrovici, M., & Herlo, G. (2011). The human impact on benthic community structure and dynamics of different ecosystems from Lunca Mureşului Nature Park (West of Romania). *Aquarium, Conservation & legislation International Journal of the Bioflux Society,* 4(1), 72-78.

Marin, A. A., Lixandru, B., Ciiobanu, Gh., Morariu, S., Vlad, F. M., Ferencz, M. A., Petrovici, M., Sinitean, A., & Morariu, F. (2018). Research regarding on pollution monitoring with the help of bentonic macroinvertebrates at Bega river near Timisoara. *Global and Regional in Environmental Protection – GLOREP2018, Conference Proceedings,* 149-152.

Mesa, L. M., Reynaga, M. C., del V. Correa, M., & Sirombra, M. G. (2013). Effects on anthropogenic impacts on benthic macroinvertebrates assemblages in subtropical mountain streams. *Iheringia, Serie Zoologia*, 103 (4), 342-349.

Mititelu, E. D., Petrovici, M., & Pîrvu, R. L. (2012). The study of water quality using benthic macroinvertebrates as bioindicators in the catchment areas of the rivers Jiu, Olt and Ialomiţa, *Annals of West University of Timişoara, Series Biology*, XV (1), 45-52.

Momeu, L., Battes, K., Battes, K., Stoica, I., Avram, A., Cîmpean, M., Pricope, F., & Ureche, D. (2009). Algae, macroinvertebrate and fish communities from the Arieş River catchment area Transylvania, Romania. *Transylvanian Review of Systematic and Ecological Researches*, 7, 149-180.

Moya, N., Hughes, R. M., Dominguez, E., Gibon, F. M., Goitia, E., & Oberdorff, T. (2011). Macroinvertebrate-based multimetric predictive models for evaluating the human impact on biotic condition of Bolivian streams. *Ecological Indicators*, 11, 840-847.

Mustow, S. E. (2002). Biological monitoring of rivers in Thailand: use and adaptation of the BMWP score. *Hydrobiologia*, 479, 191-229.

Nguyen, H. H., Everaert, G., Gabriels, W., & Hoang, T. H. (2014). A multimetric macroinvertebrate index for assessing the water quality of the Cau river basin in Vietnam. *Limnologica*, 45, 16-23.

Ofenböck, T., Moog, O., & Sharma, S. (2008). Development and application of the NKH Biotic Score to assess the river quality in the Hindu Kush-Himalaya. In: Moog, O., Hering, D., Sharma, S., Strubauer, I., Korte, T. (Eds.) ASSESS-NKH: *Proceeding of the Scientific Conference "Rivers in the Hindu Kush-Himalaya-Ecology & Environmental Assessment*", 25-32.

Oliveira A. L. H., & Nessimian, J. L. (2010). Spatial distribution and functional feeding groups of aquatic insect communities in Serra da Bocaina streams, southeastern Brazil. *Acta Limnologica Brasiliensia*, 22(4), 424-441.

Olszewski, T. (2007). PBDB Intensive Summer Course 2007.Paleoecology Section – Measurement of Diversity (http://paleodb.org/public/summercourse07/Olszewski_Diversity.pdf) (downloaded in 14.04.2020).

Onciu, T. M., Cogălniceanu, D., Dunca, E., Sava, D., Traistă, E., Samargiu, M. D., Ionică, M., Csaba, L., Samoilă, C., Ciurea, A., & Radu, A. (2007). Aquatic ecosystems formed between sterile dumps. *International Conference Environment Natural Sciences Food Industry Conference Proceedings,* 275-280.

Opreanu, P. A. (2010). Changes in the structure of benthic biocoenoses on the lower course of the Danube from 1996 to 2004. *Geo-Eco-Marina* 16, 93-99.

Ortiz, M., Levins, R., Campos, L., Berrios, F., Campos, F., Jordan, F., Hermosillo, B., Gonzalez, J., & Rodriguez, F. (2013). Identifying trophic groups in benthic ecosystems: Implications for fisheries management. *Ecological Indicators*, 25, 133-140.

Pîrvu, M., & Pacioglu O. (2012). The ecological requirements of caddisflies larvae (Insecta: Trichoptera) and their usefulness in water quality assessment of a river in south-west Romania. *Knowledge and Management of Aquatic Ecosystems*, 407, 03.

Popescu, A., Ibănescu, D., Vasilean, I., & Nica, A. (2016). Preliminary aspects concerning macroinvertebrates qualitative structure in the Danube River. *Scientific Papers – Animal Science Series*, 65, 130-133.

Răescu, C. S., Dumbravă-Dodoacă, M., & Petrovici, M. 2011. Macrozoobenthic community structure and dynamics in Cerna River (western Romania). *Aquaculture, Aquarium, Conservation & legislation International Journal of the Bioflux Society,* 4(1), 79-87.

Ruiz-Picos, R. A., Sedeño-Díaz, J. E., & López-López, E. (2017). Calibrating and Validating the Biomonitoring Working Party (BMWP) Index for the Bioassessment of Water Quality. Neotropical Streams. Water Quality, Ed: 1rst, INTECH OPEN, Editors: Hlanganani Tutu, 39 – 58.

Ruuskanen, M. O., Pierre, K. A. St., Louis, V. L. St., Aris-Brosou, S., & Poulain, A. J. (2018). Physicochemical drivers of microbial community structure in sediments of Lake Hazen, Nunavut, Canada. *Frontiers in Microbiology*, 9, 1-16.

Sedell, J. R., Richey, J. E., & Swanson, F.J. (1989). The river continuum, concept: A basis for the expected ecosystem behavior of very large rivers?, *Proceedings of the International Large River Symposium*, 106, 49-55.

Sharifinia, M., Imanpour Namin, J., & Bozorgi Makrani, A. (2012). Benthic macroinvertebrate distribution in Tajan River using canonical correspondence analysis*. Caspian Journal of Environmental Sciences*, 10(2), 181-194.

Šidagytė, E., Višinskienė, G., & Arbačiauskas, K. (2013). Macroinvertebrate metrics and their integration for assessing the ecological status and biocontamination of Lithuanian lakes. *Limnologica*, 43, 308-318.

Silveira, M. P., Buss, D. F., Nessimian, J. L., Egler, M., & Baptista, D. F. (2005). Application of biological measuring for stream integrity assessment in South-East Brazil. *Environmental Monitoring and Assessment*, 101, 117-128.

Sinitean, A., & Petrovici, M. (2012). Usages of biotic indices in evaluating the impact of the urban centers on the quality of the water in rivers. *Aquaculture, Aquarium, Conservation & legislation International Journal of the Bioflux Society*, 5(2), 60-63.

Sedeño-Díaz, J. E., Kohlmnan, B., & López-López, E. (2012). Benthic macroinvertebrates as indicators of water quality in streams of Costa Rica: using an adaptation of the BMWP score. *Transylvanian Review of Systematic and Ecological Researches.* 14, 177-188.

Solem, J. O., & Gullefors, B. (1996). Trichoptera, Caddisflies. Aquatic Insects of North Europe – A taxonomic handbook. Ed. Anders N. Nilson, 223-255.

Solimini, A. G., Gulia, P., Monfrinotti, M., & Carchini, G. (2000). Performance of different biotic indices and sampling methods in assessing water quality in the lowland stretch of the Tibet River. *Hydrobiologia*, 422/423, 197-208.

Steinmann, H. (1968). Alkereszek Plecoptera. *Magyarorszag Allatvilaga, Fauna Hungariae* 92 (8), 129-186.

Stoica, C., Gheorghe, Șt., Petre, J., Lucaciu, I., & Niță-Lazăr, M. (2014). Tools for assessing Danube Delta systems with maco invertebrates, *Environmental Engineering and Management Journal*, 13(9), 2243-2252.

Șerban, S. A., & Ionuș O. (2011). Ecological status assessment of the water bodies located on the lower sector of the Jiu and the Motru Rivers (Oltenia, Romania). *Forum geographic. Studii și cercetări de geografie și protecția mediului.* 10(1), 195-206.

Tchakonté, S., Ajeagah, G., Nyamsi, N. L., Tchatcho, N., Adama, C., Diomandé, D., & Ngassam, P. (2015). Stream's water quality and description of some aquatic species of Coleoptera and Hemiptera (Insecta) in Littoral Region of Cameroon. *Biodiversity Journal*, 6, 27-40.

Truță, A. M. (2014). Research on the environmental quality in the Olt river, Drăgășani city. *Current Trends in Natural Sciences*, 3(6), 88-95.

Ujhelyi, S. (1959). Kereszek Ephemeroptera. *Magyarorszag Allatvilaga, Fauna Hungariae,* 49(5), 71-95.

Varnosfaderany, M. N., Ebrahimi, E., Mirghaffary N., & Safyanian, A. (2010). Biological assessment of the Zayandeh Rud River, Iran, using benthic macroinvertebrates. *Limnologica,* 40, 226-232.

Williams, C. 1964. Patterns in the Balance of Nature. *Academic Press*, London, 324.

Yates, A. G., Brua, R. B., Culp, J. M., Young, R. G., & Chambers, P. A. (2018). Variation in stream metabolism and benthic invertebrate composition along longitudinal profiles of two contrasting river systems. *Canadian Journal of Fisheries and Aquatic Sciences,* 75(4), 549-559.

Young, S. S., Yang, H. N., Huang, D. J., Liu, S. M., Huang, Y. H., Chiang, C. T., & Liu, J. W. (2014). Using benthic macroinvertebrate and fish communitites as bioindicators of the Tanshui River Basin around the greater Taipei area – multivariate analysis of spatial variation related to levels of water pollution. *International Journal of Environmental Research and Public Health*, 11, 7116-7143.

Zamora-Munoz, C., Sainz-Cantero, C., Sanchez-Ortega, A., & Alba-Tercedor, J. (1995). Are biological indices BMWP and ASPT and their significance regarding water quality seasonally dependent? Factors explaining their variations. *Water Researches*, 29, 285-290.

Zanfir, C. G., Mititelu-Ionuș O., & Cioboiu O. (2019). Inventory of pressures on surface water bodies and the ecological status along the lower Jiu River*. Oltenia. Studii și comunicări. Științele Naturii*, 35(2), 175-184.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 65, 1, 2020 (p. 83-92)
doi:10.24193/subbbiol.2020.1.07

Colonial wintering of Long-eared owl (*Asio otus*) in Botoșani County (N-E Romania)

Medeea C. Halici[1]✉ and Alexandru N. Stermin[1]

[1] Babeș - Bolyai University, Faculty of Biology and Geology, Department of Taxonomy and Ecology Cluj-Napoca, Romania;
✉ ***Corresponding author, E-mail: medeea.halici@yahoo.com.***

Abstract. This study explores the phenological aspects and distribution of long-eared owl (*Asio otus*) colonies in Botoșani county, N-E Romania. The anthropogenic impact was also evaluated and analyzed and the microhabitat where the colonies are located. Data was collected between November 2018 and February 2019. 9 settlements in Botoșani county, including the municipality of Botoșani, were investigated. Data about temperature, the size of the colony, the tree species where the colony is located and anthropogenic impact, were collected. 9 colonies were identified with a total of 340 individuals. Two short-eared owl (*Asio flammeus*) were identified in one of the colonies. The maximum number (51) of specimens is reached in the months of January and February, and the minimum number (30) in the months of November and December. It has been observed that when the temperatures tend to fall, the size of the colonies register significant growth. The preferred species of trees are gymnosperms (69%), the owls were found on angiosperms as well (31%). The preference for the coniferous species increases along with the drop in temperatures and the increase in rainfall or snowfall, as these types of trees offer better protection against the weather conditions. We noticed that the anthropogenic factor has a significant influence on the colonies of *Asio otus*, so we wanted to grade it with the help of a scale from 1 to 7. Grade 1 was given in only one locality (Știubieni), grade 2 was registered in 4 points (Dorohoi, Avrămeni, Botoșani, and Roma), grade 3 is found only in Bucecea, Corlăteni registers a grade of 4, while in Dobârceni the registered value is 5, grade 6 is met only in Săveni. Grade 7 represented the stage in which there is a decrease in the population of *Asio otus*, or the death of individuals for various reasons, a situation that has not been encountered.

Keywords: phenology, tree selection, anthropogenic impact.

Introduction

As a response to diverse changes in the ecosystem, each taxonomical group adopt different strategies to keep the individuals alive and the group integrity. In this context some bird species adopt a grouped distribution with the goal of creating a unitary population, which helps them be better protected against predators, weather conditions and the finding of food, all objectives being made easier this way. A relevant example of such an advantage that grouping gives is found in some strigiform species, which, in the cold season, change their solitary and territorial behavior that express during the reproduction period, and opt to form colonies in different places (Gryz and Gryz, 2015).

Such a significant example of this behavior is found in the long-eared owl, *Asio otus* (Strigidae, Strigiformes L.) spread throughout: Europe, North-West Africa, Asia and North America (Marks *et al*., 1994; Moga *et al*., 2005). The populations from the northern parts of its range are migratory, and those from the southern and western are sedentary (Cramp and Simmons, 1985; Kucherenko and Kalinovsky, 2018).

Long-eared owl, constitutes one of the most wide-spread strigiform species in Romania in particular, and in Central Europe in general (König and Weick, 2008; Gryz and Gryz, 2015). Typical of the long-eared owl is the way they form large colonies during winters, in diverse areas, where they can find a stable source of food, protecting themselves more easily against weather conditions and potential predators (Moga *et al.,* 2005). Among their favorite places there are the urban and suburban areas, an aspect which facilitates the observation of the species (Cramp, 1985; Galli *et al.,* 2015; Lövy and Riegert, 2013).

Throughout the cold season, in the long-eared owl, colonies, there can be spotted also individuals of short-eared owl, *Asio flammeus*, which appear as visitors due to the fact that this species' presence in Romania is rare in contrast to that of the long-eared owl (Papp and Sándor 2007; Ionescu *et al*., 2017).

It is known that the climate change which affects the whole planet, and the excessive urbanization, influence the behavior and phenology of birds (Møller *et al*., 2010). The majority of studies done on long-eared owl colonies (Laiu *et al*, 2003; Nistreanu and Postolachi, 2011; Chiba *et al*., 2005) are focused on the regime and trophic preference of the owls and very few studies explore aspects related with dynamics of individuals in the colonies (Sharikov *et al*., 2014). In this context, the aims of our study are to (i) evaluate the phenological aspects of the colonial wintering, (ii) the dynamics of populations and (iii) the anthropogenic impact over colonies. Our investigation represents the first study of its type in the county of Botoșani, which besides all the phenological and populations dynamics information, brings also data about the distribution of the species in the area.

Study area

Our study was conducted in the north of the region of Moldova, more specifically in Botoșani county (47 ° 50′N 26 ° 49′E) (Fig. 1). Located on the old Platform of Moldova, Botoșani county constitutes 2.1% of the surface of Romania, being spread over 4965 km^2 (Mititelu and Chifu, 1994; Varvara and Apostol, 2008). As regards this relief, it is not extremely varied, the predominant forms being: plains, plateaus and hills. Of the total area, agricultural lands account for the most part 82%, followed by natural grasslands 14% and forested lands that account for only 11% (Mititelu and Chifu, 1994).

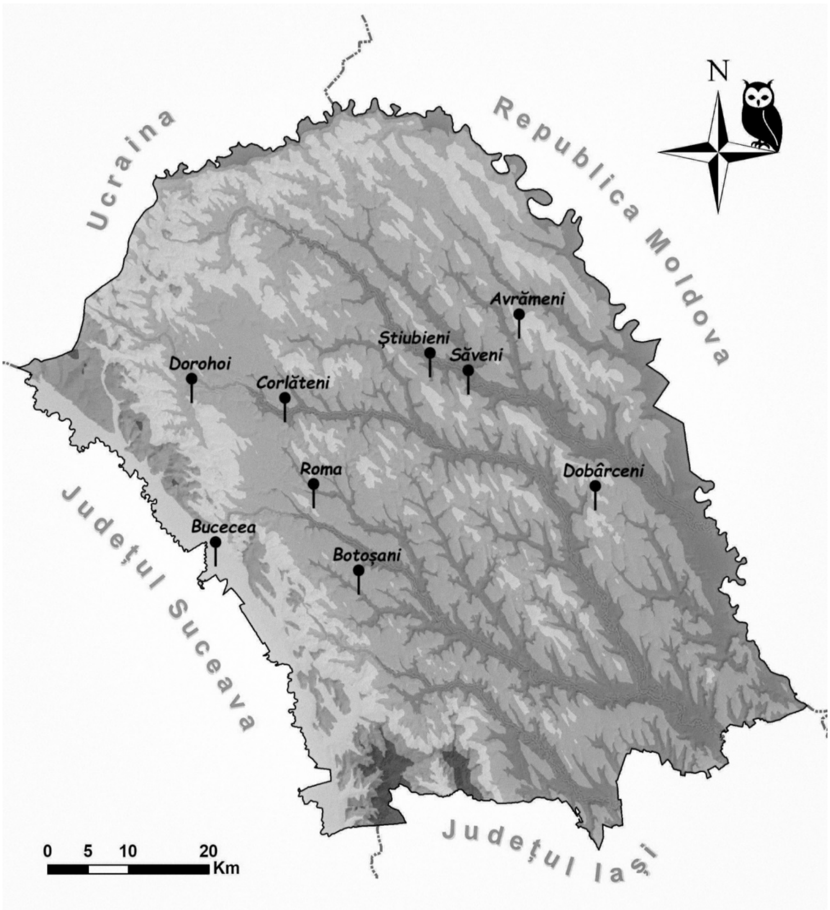


Figure 1. Study area and the points where the *Asio otus* colonies were identified

Materials and methods

Data were collected throughout the period of November – December 2018 and January – February 2019, when we conducted observation in 8 settlements from Botoșani county, as well as in the municipality of Botoșani (Fig. 1). For correctly identifying the points of interest and for an orderly gathering of data, the exploited methods were: fixed-point iteration, trail method, the counting of all individuals and taking picture to each one of them (Rang, 2002).

The identification of the colonies was accomplished by talking with locals (by showing them pictures), as well as the surveying of the areas which seemed favorable for the long-eared owl colonies (e.g.: areas with old buildings, spaces surrounded with gymnosperms). After pin pointing the colonies, we collected information about the number of individuals, the influence of the anthropic factor, tree species they occupied, as well as the number of individuals and the weather conditions. At the same time for each point of interest we registered the temperature to establish if there's a correlation between weather conditions and the type of tree chosen by the individuals. The intensity of the wind was also recorded with the help of the Beaufort scale, in which correlations were made between the visual effects produced by the wind and its type.

The antropogenic impact was evaluated on a scale from 1 to 7 as follows: Grade 1: describes the area where people are present but do not disturb the colony directly. Grade 2: here exist passing cars, but there are no street lights. Grade 3: There are people, cars and street lights at night. Grade 4: There are people who directly disturb the colony. Grade 5: The anthropic impact is big, caused by people, fireworks, noises, car lights. Grade 6: activities during the winter holidays, songs, shows. Grade 7: Death of the individuals in the colonies.

Results and discussions

Table 1. Frequency of the number of individuals, depending on the period in which the observations took place and on average temperature fluctuation

Date of observation	Settlement	Number of individuals	Temperature (^{0}C)
21.11.2018	Săveni	30	-1
21.11.2018	Avrămeni	32	-2
27.12.2018	Dobârceni	30	1
1.01.2019	Dorohoi	28	-1
1.01.2019	Săveni	51	1
12.02.2019	Dorohoi	49	-2
13.02.2019	Bucecea	30	-1
13.02.2019	Botoșani	48	0
13.02.2019	Știubieni	30	0
16.02.2019	Corlăteni	40	-1
16.02.2019	Roma	30	0

There were identified 9 colonies with a total of 340 specimens. The maximum number of a colony was of 51 individuals and the minimum of 28, with an approximated average of 38 specimens (Tab. 1). Concerning the dynamics, the highest number of individuals was observed in the months of January and February 2019 (Fig. 2).

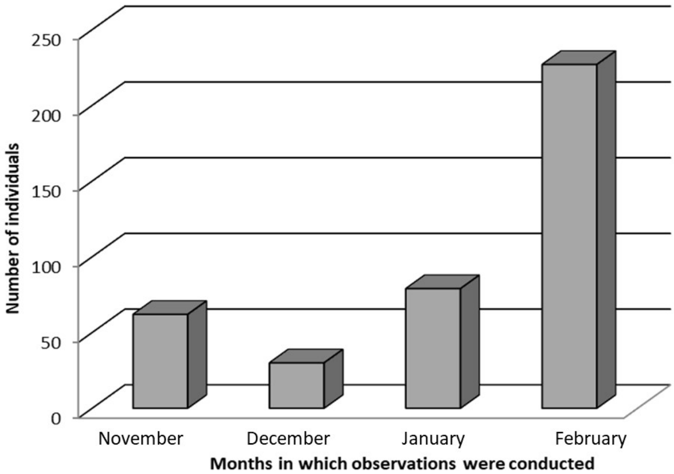


Figure 2. Frequency of the number of individuals depending on the month of the observations

It was noted that, once the average temperatures fell (from 0^0 to -2^0), the number of individuals in colonies registered an increase (from 28 to 49), fact which proves that this species' organization during the cold season is influenced by the weather conditions. According to specialty literature, in the interval of 2014-2015, the maximum number of individuals in the southern part of Romania was reached in the months of February and November (Mestecăneanu and Gava, 2015).

In other countries, the fluctuations are more different, as in Italy (Pirovano *et al*., 2000) the peak is registered in the month of December, in Russia (Sharikov *et al*., 2013) the peak is reached in the months of December and January and in Ukraine, more specifically in the Crimea (Kucherenko and Kalinovsky, 2018) the maximum number is reached in November and December. Confronting all this data, the dynamics of the populations we observed is closest to that of Russia. It is observed that, at the same time, the dynamic of long-eared owl colonies, which can differ even at the level of this country, the main reason is being represented especially by the different weather conditions.

The agricultural lands and the natural grasslands are important areas for the owl colonies, they provide a valuable food source for them. At the same time, forested areas and parks are important nesting areas for the colonies of long-eared owl. The aspects mentioned are also found in the distribution of the studied owl colonies, these being identified in the localities surrounded by meadows and agricultural lands.

The places in which the long-eared owl colonies were identified are surrounded by different old buildings, blocks, among their favorite places being: public parks (10%), block gardens (20%), private areas (20%), areas in front of public institutions buildings (30%) and areas next to the road (20%) (Fig. 3).

The species occupied by long-eared owl individuals are: *Betula pendula* (Silver birch), *Picea abies* (Norway spruce), *Fagus sylvatica* (European beech), *Thuja occidentalis* (Arborvitae), *Fraximus excelsior* (European ash), *Salix alba* (White willow) (Fig. 4), the highest preference being for the coniferous species (Pirovano *et al*., 2000) (Tab. 2, Fig. 4).

Therewith, the individuals' preference for a certain type of tree was also observed, data being collected referring to the number of specimens of long-eared owl occupying each tree species (Table 2). Most individuals were observed on the species of norway spruce, though they were present on other species too. It was observed that in the long-eared owl colonies the tree species they choose is influenced by the weather conditions as well. This fact was observed in Săveni and Dorohoi, places in which we conducted trips in different periods of time. Thus, once with the decrease in temperatures or the increase in intensity of rainfall and snowfall the long-eared owl individuals opted to group in larger numbers on species of gymnosperms, these offering a better protection against the weather. In days in which showers didn't occur, the individuals had random groupings, them being found on angiosperms as well as gymnosperms. The direction of the air currents was another parameter recorded during the study. According to Beaufort's scale, 5 types of wind were recorded, respectively: noticeable, light, weak, moderate and significant. It has been observed that the wind does not change the dynamics or degree of grouping of individuals in a colony. Moreover, in most cases, the colonies of long-eared owl were located in such a way that the buildings around them provided protection against the climatic conditions (Fig. 4) (Kucherenko and Kalinovsky, 2018).

In one of the colonies of long-eared owl, two specimens of short-eared owl (Științibieni) were identified, a species with few reports in the region of Moldova. At the same time, they were also observed among the colonies and individuals of *Streptopelia decaocto* (Eurasian collared dove), *Corvus monedula* (Western jackdaw) and *Corvus frugilegus* (Rook).

Table 2. Distribution of *Asio otus* individuals depending on the tree species for each observation point

Settlement	Tree species selected and their number	Number of *Asio otus* individuals
Dorohoi	*Salix alba (2)*	18
	Fraxinus excelsior (1)	31
Săveni	*Picea abies (4)*	32
	Thuja occidentalis (3)	12
	Fagus sylvatica (1)	4
	Betula pendula (2)	3
Avrămeni	*Picea abies (3)*	32
Dobârceni	*Picea abies (4)*	30
Bucecea	*Picea abies (2)*	30
Botoșani	*Picea abies (4)*	48
Știubieni	*Picea abies (4)*	30
Corlăteni	*Picea abies (7)*	40
Roma	*Picea abies (6)*	30

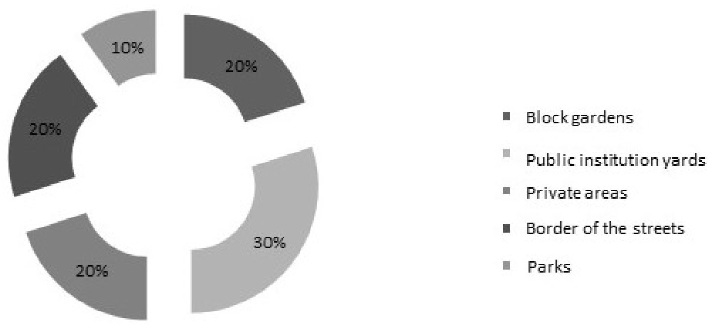


Figure 3. Areas where *Asio otus* colonies were located

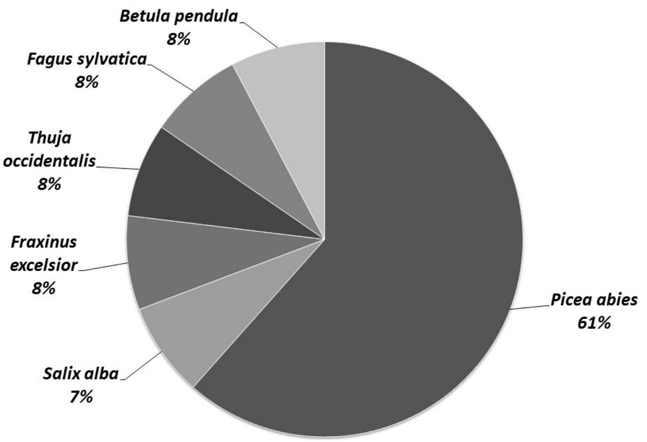


Figure 4. Distribution of *Asio otus* individuals on different tree species in the colonies.

Throughout the study, no injured or dead owls were identified, even though the anthropogenic impact exerts significant pressure on some colonies (Kucherenko and Kalinovsky, 2018). We point out that habitat destruction and disturbance of this species can have a major impact on its future. Thus, for each point we recorded the following situations: Grade 1 describes the area in which the presence of man does not influence at all the colonies of long eared owl, these being located in secluded areas (e.g: Știubieni). Grade 2 represents a space in which the long-eared owl individuals are phonically disturbed by the passing cars in their vicinity (e.g: Dorohoi, Avrămeni, Botoșani and Roma). Bucecea is the settlement registered with grade 3, due to the fact that the long-eared owl colony is disturbed phonically from the cars, shops and pubs nearby, as well as by the light emitted from the surrounding buildings. In the settlement of Corlăteni the colony of long eared owl was disturbed and cast off by the locals because of the owl pellets that piled up in their yards, thus grading the impact in this case with a 4. In Dobârceni, the grade given was 5, due to the fact that to the disturbance of the long-eared owls, fireworks used on hildays contributed as well. Grade 6 illustrates activities during the winter holidays, such as: placing of a stage near the colonies, installing of Christmas lights near the owls, the carols which were broadcast during the day in from of the Săveni town hall, the place around which the long-eared owls were located. The 7th grade indicates a decline in the population of owls, the death of some individuals from diverse reasons, a situation which thankfully was not registered in any of these locations.

Conclusions

In the period of November – December 2018 and January – February 2019, there were 9 long-eared owl colonies identified, with a total of 340 specimens in the county of Botoșani.

In Botoșani county the maximum number of long-eared owl individuals is reached in the months of January and February, and the minimum number is observed in the months of November and December.

The dynamic of long-eared owl colonies is influenced by the climate conditions, more precisely by temperatures and rainfall/ snowfall, these being a cause of the grouping degree of individuals.

The preferred tree species by them are represented by gymnosperms, these offering an enhanced protection against weather conditions.

The pursuance and the effectuation of the census on the species represents an important element due to the fact that the anthropic impact affects the long-eared owl colonies more and more.

Global warming is getting stronger, this being a factor which destroys habitats and limits food resources for more and more species, including the species of long-eared owl and short-eared owl.

REFERENCES

Chiba, A., Onojima, M., & Tohru, K. (2005). Prey of the Long-eared Owl *Asio otus* in the suburbs of Niigata City, central Japan, as revealed by pellet analysis. *Ornithol. Sci.,* 4, 169–172.

Cramp, S. (1985). The Birds of the Western Palearctic. Vol. 4. Ed. *Oxford University Press* Oxford.

Cramp, S., & Simmons, K. (1985). The Birds of the Western Palearctic. Vol. 4. Ed. *Oxford University Press* Oxford, pp. 970.

Galli, L., Baroni, D., Gelli, I., Launo, S., Puppo, C., & Rossi, R. (2015). Data about Long-eared owl *Asio otus* diet in a winter roost in Imperia (Western Liguria, North Italy) and notes on their daily activity cycle. *Boll. Mus. Ist. Biol. Univ. Genova,* 77, 72-83.

Gryz, J., & Gryz, D.K. (2015). Seasonal variability in the diet of the long-eared owl *Asio otus* in a mosaic of field and forest habitats in central Poland. *Acta Zool. Cracov.*, 58(2), 173-180.

Ionescu, D.T., Hodor, C., & Sándor, A.D. (2017). Diet of Wintering Short-eared Owl *Asio flammeus* (Pontoppidan, 1763) (Strigiformes: Strigidae) in South-Eastern Romania. *Acta Zool. Bulg.*, 69(2), 295-297.

König, C., & Weick, F. (2008). Owls of the World. Ed. *Christopher Helm* London, pp. 528.

Kucherenko, V., & Kalinovsky, P. (2018). Winter Roost Tree Selection and Phenology of the Long-Eared Owl (*Asio otus*) in Crimea. *Diversity*, 10(4), 105.

Laiu, L., Paşol, P., & Murariu, D. (2003). Winter food of the Long eared Owl (*Asio otus otus* L.) in the rural environment (Romania). *Travaux du Muséum National d'Histoire naturelle "Grigore Antipa"*, 45, 365 - 372.

Lövy, M., & Riegert, J. (2013). Home Range and Land Use of Urban Long-eared Owls. *The Condor*, 115(3), 551-557.

Marks, J.S., Evans, D.L., & Holt, D.W. (1994). Long-eared Owl (*Asio otus*), In: *The Birds of North America*, Poole, A., & Gill, F. (eds.), The Academy of Natural Sciences, Philadelphia and American Ornithologists' Union, Washington D.C.

Mestecaneanu, A., & Gava, R. (2015). The evaluation of the strength of Long-eared Owl (*Asio otus*) that wintered in the Arges country (2014–2015)*. Curr. Trends Nat. Sci.*, 4: 56–65.

Mititelu, D., & Chifu, T. (1994). Flora și vegetația județului Botoșani. *Stud. Comunic. Muz. Bacău*, 13, 109-126.

Moga, C.I., David, A., Harteli, T., & Coroiu, I. (2005). Trophic spectrum of long-eared owl *Asio otus* during a winter lacking significant snow cover. *Biota,* 6: 43-48.

Møller, A.P., Fiedler, W., & Berthold P. (2010). Effects of Climate Change on Birds. Ed. *Oxford University Press* Oxford.

Nistreanu, V., & Postolachi, V. (2011). Trophic spectrum of long-eared owl (*ASIO OTUS OTUS* L.) in nesting period*. Studii și comunicări*, *Compl. Muz. Şt. Nat. Ion Borcea, Bacău*, 24, 76 – 79.

Papp, T., & Sándor A.D. (2007). Arii de Importanţă Avifaunistică din România/ Important Bird Areas in Romania. *Ed. Societatea Ornitologică Română & Asociaţia pentru Protecţia Păsărilor şi a Naturii „Grupul Milvus"* Târgu Mureş, pp. 252.

Pirovano, A., Rubolini, D., & Michelis, S. (2000). Winter roost occupancy and behavior at evening departure of urban Long-eared Owls. *Ital. J. Zool.*, 67(1), 63–66.

Rang, P.C. (2002). Studiul dinamicii unor comunități de păsări din bazinul mijlociu al râului Siret incluzând zonele lacurilor de acumulare. Ed. *Societatea Ornitologică Română* Cluj-Napoca, pp. 33-38.

Sharikov, A.V., Makarova, T.V., & Ganova, E.V. (2014). Long-term dynamics of Long-eared Owls *Asio otus* at a northern winter roost in European Russia. *Ardea,* 101(2), 171–176.

Varvara, M., & Apostol, E. (2008). Diversity and the main ecological requirements of the epigeic species of carabidae (COLEOPTERA, CARABIDAE) in the sun flower ecosystem, Broscăuţi (BOTOŞANI COUNTY). *Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, s. Biologie Animală,* 54, 81-89.