
***TRANSYLVANIAN REVIEW OF
SYSTEMATICAL AND ECOLOGICAL
RESEARCH***

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The Wetlands Diversity

Editors

Angela Curtean-Bănăduc, David Serrano & Doru Bănăduc

**Sibiu – Romania
2023**

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Applied Ecology Research Center,
“*Lucian Blaga*” University of Sibiu

				
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**Sibiu – Romania
2023**

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IN MEMORIAM
Angela Curtean-Bănăduc
(1971 – 2023)

Tragically, the period of the preparation of the *Transylvanian Review of Systematical and Ecological Research* 2023 issues was the last period of life for one of its main founders and leaders since 1999, namely **Angela Curtean-Bănăduc**, who passed away in Clermont-Ferrand, France, on 1 November 2023. The others all over the world editors, reviewers, authors and friends of this journal wish to pay here an “in memoriam” loving tribute to someone who was both their very good friend and colleague and the beloved life partner and wife of **Doru**.



She was born in Orăștie (Transylvania, Romania) on 27 January 1971, into a caring family of intellectuals whose main educational moral rule, “everything you do, do it right!”, deeply influenced her contented golden childhood and teenage years. This happy family and the location of their picturesque, multicultural native city, lying near the Carpathians where the legendary Sarmizegetusa Regia, capital of the ancient Dacians, over the last 2000 years beneficially influenced Romanian dreams and realities – if only to recall here a temple of the Romanian language, “Palia de la Orăștie”, the monumental translation of the Old Testament into the Romanian language carried out in the 16th century – created the necessary milieu which inspired **Angela** to achieve something important with her life.

The vicinity of one of the largest Danube River tributaries, the scenic Mureș River, gave her the impulse to love the blue like in her gorgeous eyes, rivers, streams, and lakes. The chance to dream, imagine, organize, support and shape directly the lives of people of goodwill involved in the continental waters scientific understanding and protection, and the forces which uphold them, came through the professional opportunities afforded by her studies in chemistry at the “Nicolaus Olahus” Highschool of Orăștie, in ecology at the “Lucian Blaga” University of Sibiu, for her PhD in aquatic ecology at “Ovidius” University of Constanța, and her post-doctoral research in water resources management at “Costin C. Kirițescu” Romanian Academy National Institute of Economic Research in Bucharest, etc.

The water was symbolic for her beautiful blue life on this Blue Planet! Nature was her temple, home and support for many projects in ecology and biology. Macro-invertebrates and fish populate her written output, scientific episodes which are on permanent record in over 200 publications. To give just some examples of what **Angela** founded and/or led over the years with fabulous positive and honest energy, efforts and results: *Transylvanian Review of Systematical and Ecological Research* since 1999 including the *Wetlands Diversity* series; *Aquatic Biodiversity International Conference* since 2007; *Acta Oecologica Carpatica* since 2008; environmental *Ecotur Sibiu Association* since the end of the XX century; *Applied Ecology Research Center* in XXI century; “Lucian Blaga” University of Sibiu Faculty of Sciences where she worked in teaching and research since 1996, finally for 10 years as a Dean; etc.

Many devoted friends accompanied this genuinely extraordinary beautiful personal and professional life, the hundreds of editors, reviewers and authors all over the world of *Transylvanian Review of Systematical and Ecological Research* being among them.

A unique magic personal and professional match was achieved without a break from 1998 between the beloved **Angela** and her husband **Doru**, who not by chance pays his deep loving respect for an unbelievable fabulous shared lifetime experience.

A cruel illness took her too soon from this world, but till the end she encouraging smiled to us with her characteristic kindness and care.

All of us, who had the good fortune to meet and love **Angela** in this all too short life, and to benefit from her angelic heart and mind, will keep her in the sunny part of our memories forever!

Family, friends, colleagues and students wish her eternal blue waters on the other side, where sooner or later we shall all meet to again be happy together!

Transylvanian Review of Systematical and Ecological Research
all over the world Editors, Reviewers, Authors and Friends

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Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand already the presence of the medium and long-term significant change in the “average weather” all over the world, the most common key words which reflect the general concern are: heating, desertification, rationalisation and surviving.

The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of approaches and efforts.

With the fact in mind that these approaches and efforts should be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch three annual volumes dedicated to the wetlands, volumes resulted mainly as a result of the *Aquatic Biodiversity International Conference, Sibiu/Romania, 2007-2017*.

The term wetland is used here in the acceptance of the Convention on Wetlands, signed in Ramsar, in 1971, for the conservation and wise use of wetlands and their resources.

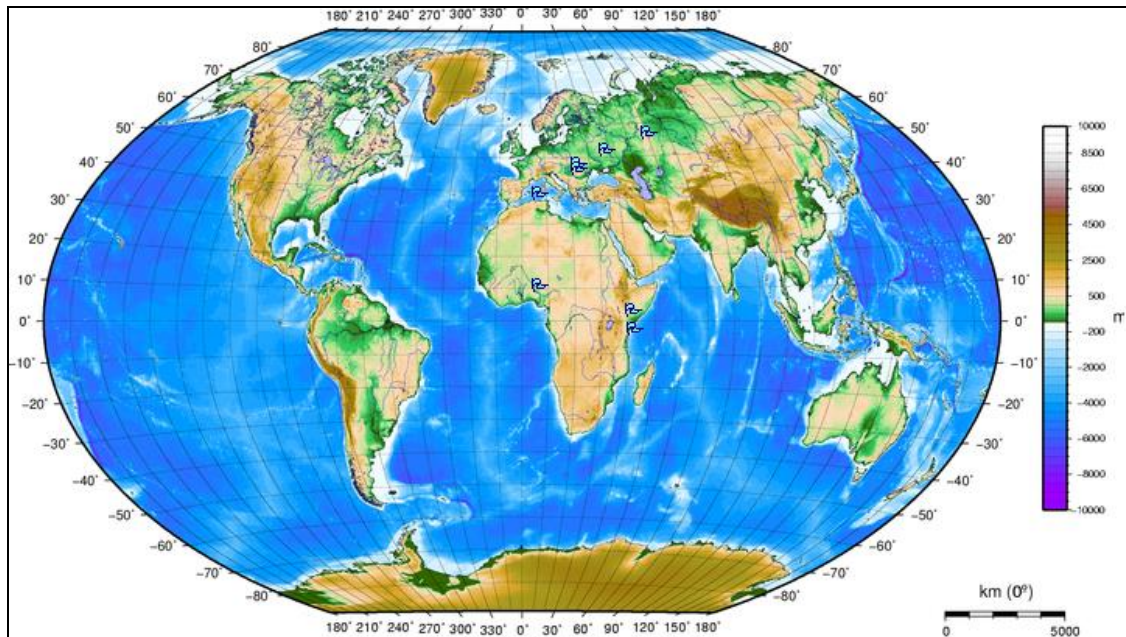
Marine/Coastal Wetlands – Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, sea cliffs; Sand, shingle or pebble shores, includes sand bars, spits and sandy islets, includes dune systems and humid dune slacks; Estuarine waters, permanent water of estuaries and estuarine systems of deltas; Intertidal mud, sand or salt flats; Intertidal marshes, includes salt marshes, salt meadows, saltings, raised salt marshes, includes tidal brackish and freshwater marshes; Intertidal forested wetlands, includes mangrove swamps, nipah swamps and tidal freshwater swamp forests; Coastal brackish/saline lagoons, brackish to saline lagoons with at least one relatively narrow connection to the sea; Coastal freshwater lagoons, includes freshwater delta lagoons; Karst and other subterranean hydrological systems, marine/coastal.

Inland Wetlands – Permanent inland deltas; Permanent rivers/streams/creeks, includes waterfalls; Seasonal/intermittent/irregular rivers/streams/creeks; Permanent freshwater lakes (over eight ha), includes large oxbow lakes; Seasonal/intermittent freshwater lakes (over eight ha), includes floodplain lakes; Permanent saline/brackish/alkaline lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peat-swamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland.

Human-made wetlands – Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue the annual sub-series (*Wetlands Diversity*) as an international scientific debate platform for the wetlands conservation, and not to take in the last moment, some last heavenly “images” of a perishing world ...

This volume included varied original researches from diverse wetlands around the world.



The subject areas (→) for the published studies in this volume.

No doubt that this new data will develop knowledge and understanding of the ecological status of the wetlands and will continue to evolve.

Acknowledgements

The editors would like to express their sincere gratitude to the authors and the scientific reviewers whose work made the appearance of this volume possible.

The Editors

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MAPPING WETLANDS OF KENYA USING GEOGRAPHIC RESOURCES ANALYSIS SUPPORT SYSTEM (GRASS GIS) WITH REMOTE SENSING DATA

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KEYWORDS: Africa, Kenya, wetlands, remote sensing, image processing, cartography, GRASS GIS.

ABSTRACT

Monitoring wetlands of Kenya is critical for analysis of environmental changes since they present unique ecosystems with special hydrological balance and biodiversity. In this study, the Landsat 8-9 OLI/TIRS satellite images for 2015-2022 were classified using GRASS GIS scripts to evaluate changes in the Lorian Swamp wetland, north-eastern Kenya. The results of the image analysis presented maps of land cover changes including wetlands. The study demonstrated technical effectiveness of the GRASS GIS for image analysis, and contributed to the environmental monitoring of African wetlands.

RÉSUMÉ: Cartographie des zones humides du Kenya à l'aide de données de télédétection et de scripts de GRASS GIS.

La surveillance des zones humides du Kenya est essentielle pour l'analyse des changements environnementaux car elles présentent des écosystèmes uniques avec un équilibre hydrologique et une biodiversité particuliers. Dans cette étude, les images satellite Landsat 8-9 OLI/TIRS pour 2015-2022 ont été classées à l'aide de scripts SIG GRASS pour évaluer les changements dans la zone humide du marais de Lorian, au nord-est du Kenya. Les résultats de l'analyse d'images ont présenté des cartes des changements d'occupation du sol, y compris les zones humides. L'étude a démontré l'efficacité technique du SIG GRASS pour l'analyse d'images et a contribué à la surveillance environnementale en Afrique.

REZUMAT: Cartografierea zonelor umede din Kenya folosind date de teledetectie și scripturi GRASS GIS.

Monitorizarea zonelor umede din Kenya este esențială pentru analiza schimbărilor de mediu, deoarece acestea prezintă ecosisteme unice cu echilibru hidrologic și biodiversitate deosebite. În acest studiu, imaginile satelitului Landsat 8-9 OLI/TIRS pentru 2015-2022 au fost clasificate folosind scripturile GRASS GIS pentru a evalua schimbările din zona umedă Lorian Swamp, din nord-estul Keniei. Rezultatele analizei imaginii au prezentat hărți ale modificărilor acoperirii solului, inclusiv zonele umede. Studiul a demonstrat eficacitatea GIS GRASS pentru monitorizarea mediului în zonele umede africane.

INTRODUCTION

Water, a key resource and generator of secondary resources in the 21st century, is under high threats and risks of a number of stressors (Bănăduc et al. 2022). In Kenya, recent environmental changes led to negative processes which include land degradation, vegetation decline, fragmentation of landscape patterns, changed functionality (Gomes et al., 2023) and land cover changes (Balaka Opiyo et al., 2022). Recent studies on land cover change assessment in Kenya noticed the conversions of grassland and forestland to cropland, increase of cropland and built-up area and decrease of forest, grassland, and bare lands (Rotich et al., 2022). The overexploitation and land degradation in Lake Victoria basin of Kenya resulted in decline in wetlands, vegetated landscapes, and farm lands (Onyango and Opiy, 2022). Climate effects on the environmental sustainability arise from the increasing temperature and decreased precipitation which led to the increase in aridity and scarcity of water resources (Böhme et al., 2013; Goman et al., 2020; Lemenkova, 2022a,b). This results in changed vegetation patterns such as expansion of shrubs in areas earlier occupied by pastures, the distribution of gullies due to the erosional processes (Maua et al., 2022; Lemenkova, 2022b).

Wetland fishery potential depends on water level in lakes, owing to the effects of a decline of water depth during the dry season (Kipkemboi et al., 2007). Degraded examples of biodiversity in Kenya include alien species that contribute to the decline of endemics and increase in water and food insecurity. The integrated effects of all these climate-environmental factors increase and accelerate land degradation processes and environmental unsustainability. Rehabilitation and restoration of land and water resources is a complex process which takes time, resources, and efforts. Therefore, preventive mapping of land cover changes may contribute to the operative environmental monitoring in easter Africa.

Mapping land cover types as reliable identifiers of environmental changes presents an effective baseline for assessing land degradation and environmental sustainability (Lemenkova, 2023; Steinbach et al., 2023). Wetlands in Kenya present unique ecosystems with specific features of hydrology, soils, and vegetation patterns (Böhme et al., 2016). Wetlands play a key role in hydrological balance of water resources, maintain biodiversity as habitats for rare species, and have high potential in agricultural production (Leauthaud et al., 2013). As important transition zones between land and water areas, wetlands support cycling of nutrients and energy flow. Highly sensitive to changes in hydrology, wetland complexes also present valuable data for paleoenvironmental and paleoclimate reconstructions and climate modelling, since they represent the interrelation between terrestrial and lacustrine environments in the past (Kiage and Kam-biu Liu, 2099; Goman et al., 2017; Githumbi et al., 2021).

The study region focuses on the area of wetlands in the north-eastern Kenya (Fig. 1). The origin and formation of Kenyan wetlands has a deep connection with topographic, geomorphic, and geologic setting which in turn affect the climatic conditions and hydrologic regulations including the level of drainage (Job and Sieben, 2022). With this regard, the geology of the Kenyan Rift Valley has the most prominent impact on the distribution of lacustrine and wetland environments through the formation of small shallow lakes located in gently sloping depressions in the rift floor.

Wetland ecosystems of Kenya combine the characteristics of terrestrial and aquatic habitats with special features on water, soil, and vegetation types. These wetlands play a main hydro-ecological buffer role through protecting lake shallows from excessive sedimentation and eutrophication, controlling the growth of aquatic plant and algae. In this way, wetlands maintain the livelihoods of the riparian communities and ecosystems (Morrison et al., 2013).

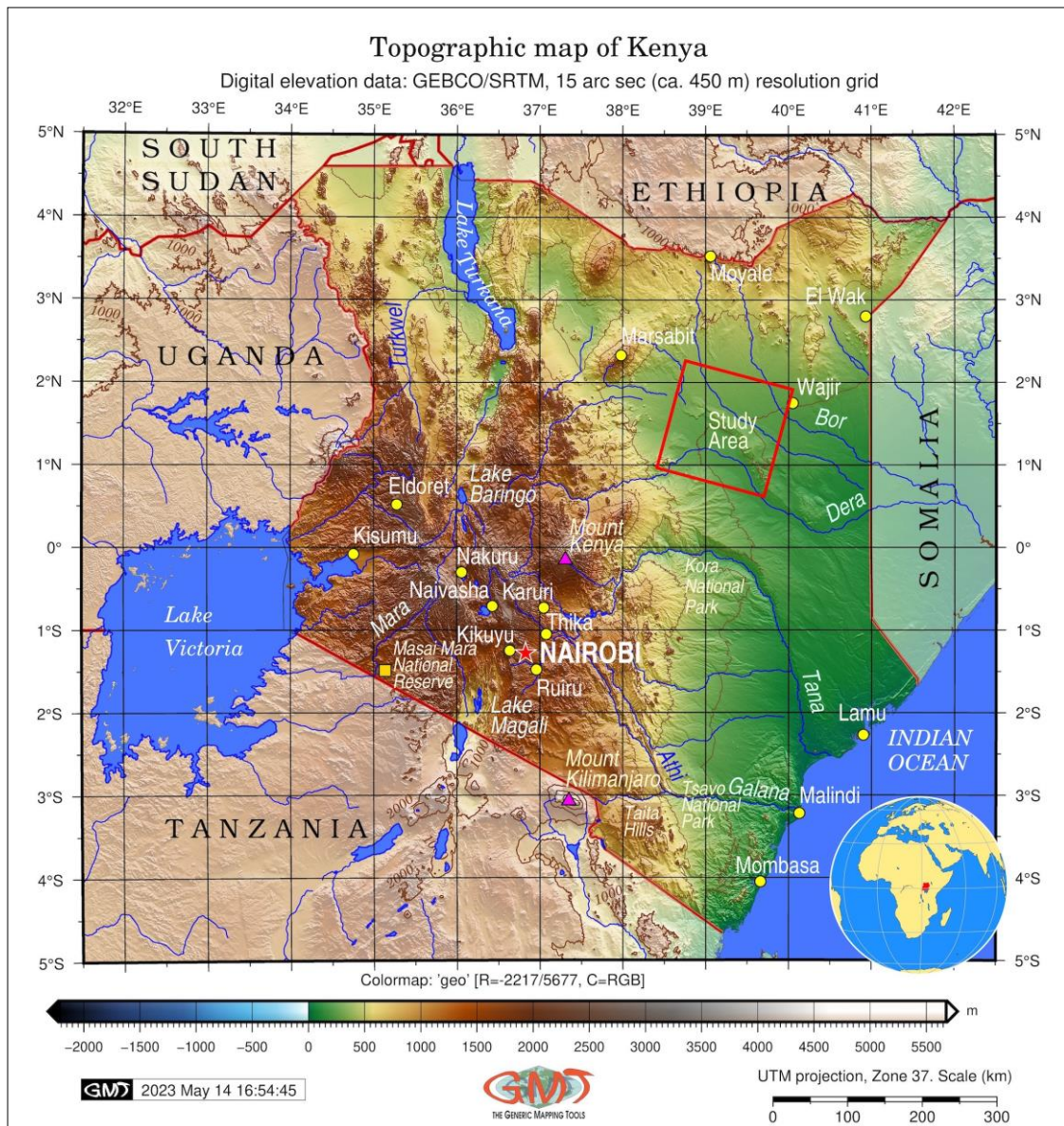


Figure 1: Topographic map of Kenya with study area (red rotated square).

Data source: GEBCO. Mapping: GMT scripting toolset.

Furthermore, wetlands play a critical role in biodiversity and ecosystem services in rural areas of Kenya, which are essential to health and welfare. Thus, disturbed patterns of water supply, stagnant water and storage may affect sanitation and hygiene (Anthonj et al., 2016, 2017, 2019). At the same time, due to the associated climate threats and anthropogenic challenges, wetlands in Kenya degrade and wetland landscapes become partially or completely lost at an increasing rate. Changes in wetland landscapes are triggered by several factors including human activity, changing river hydrology and climate-change-related coastal processes (Gitau et al., 2023). The wetland habitats loss will necessarily affect the distribution of wildlife species, and will have negative effects on livelihoods of the selected communities.

The hydrogeological parameters of soil such as permeability and plasticity, content of organic mass, granularity and viscosity affect water drainage in lakes and wetlands and regulate water circulation (stagnant waters in swamps versus currents in lakes). Nowadays, the region of the Kenyan Rift Valley forms a part of the large complex of the East African Rift System (Garcin et al., 2012; Michon et al., 2022; Lemenkova, 2022e), is presented by the Quaternary extrusive and intrusive rocks (Qv), outcrops of Tertiary (Ti) sediments (Fig. 2).

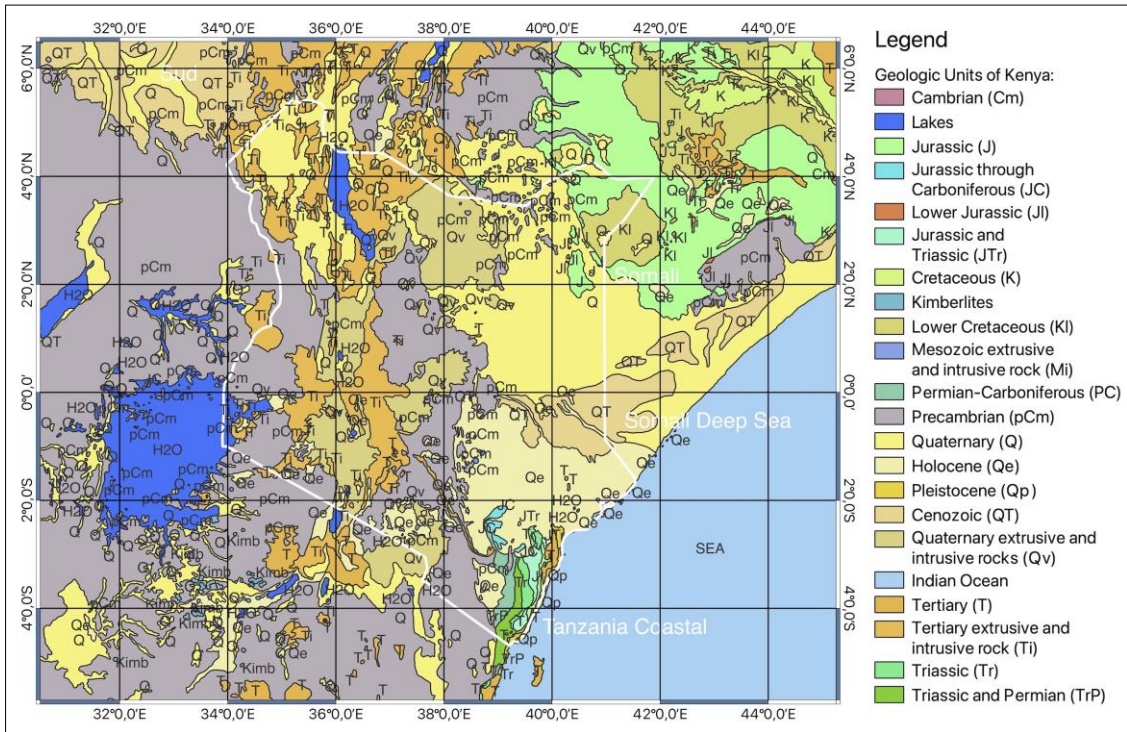


Figure 2: Geologic units, lithology and provinces in Kenya.

Data source: geologic vector layers obtained from USGS.

Other units include Quaternary sediments, and occasional Jurassic outcrops. Such formation includes fine-grained caoline, montmorillonite, kaolinite, and illite as the principal clay minerals (Yurevich, 1979). The subsequent Cenozoic extension is recorded in northern Kenya in the Turkana-Lokichar rift zone (Torres Acosta et al., 2015). Active geologic development presented conditions for formation of volcanic and tectonic lakes of the eastern branch of the African Great Rift Valley which are notable by hydrological connectivity. Besides, volcanic activity generated endorheic basins (Fazi et al., 2018).

The Olorgesailie Formation in southern Kenya Rift Valley contains lacustrine, wetland and terrestrial facies formed during the last 1.2 M years. The remaining aquatic indicators, such as diatoms, fossils and rhizolith, evidence the presence of shallow fresh to mildly saline waters in Pleistocene in this region (Owen et al., 2009; Scott et al., 2008). Such geological setting creates favourable conditions for the formation of lakes and wetlands in Kenya. Here the depth of the valley affects the velocity of streams, river discharge and ground water storage with shallow basins that better correspond to the formation of wetlands and swamps. In turn, the topographic shape of valley reflects the geomorphic parameters and soil types.

The present paper aims at mapping changes in wetlands of Kenya over the recent decade. To this end, a series of the satellite images was used to reveal changes in land cover types occasioned by the intensive human activities which requires enhancing the protection of wetlands in Kenya. Depicting the dynamics in Kenya's wetlands using remote sensing data and GIS has been documented in existing papers (Kiage et al., 2007; Olang et al., 2011; Mwitia et al., 2013; Mwaniki et al., 2017; Okotto-Okotto et al., 2018; Wanjala et al., 2020). However, these papers mostly use traditional methods of mapping such as GIS. In contrast, this study presents an advanced script-based approach by the GRASS GIS scripts. Scripts used in cartographic tasks significantly improve the mapping workflow through automation and programming (Lemenkova, 2019, 2021).

A specific focus of this study is placed on the Lorian Swamp. One of the important wetlands ecosystems of north-eastern Kenya, Lorian Swamp is situated on a vast floodplain (Fig. 3). The swamp is located in the arid zone with high mean annual temperatures and excessive evaporation. The swamp is fed by occasional rainfall which have a highly irregular pattern of occurrence due to the recurrent floods and drought (Mati et al., 2005). The effects from arid and semi-arid regions result in seasonal variations of the Lorian Swamp which is a subject to occasional droughts affecting its size, the extent and level of water. Thus, the swamp varies in area from almost zero to about 5.8 km² (Crafter et al., 1992).

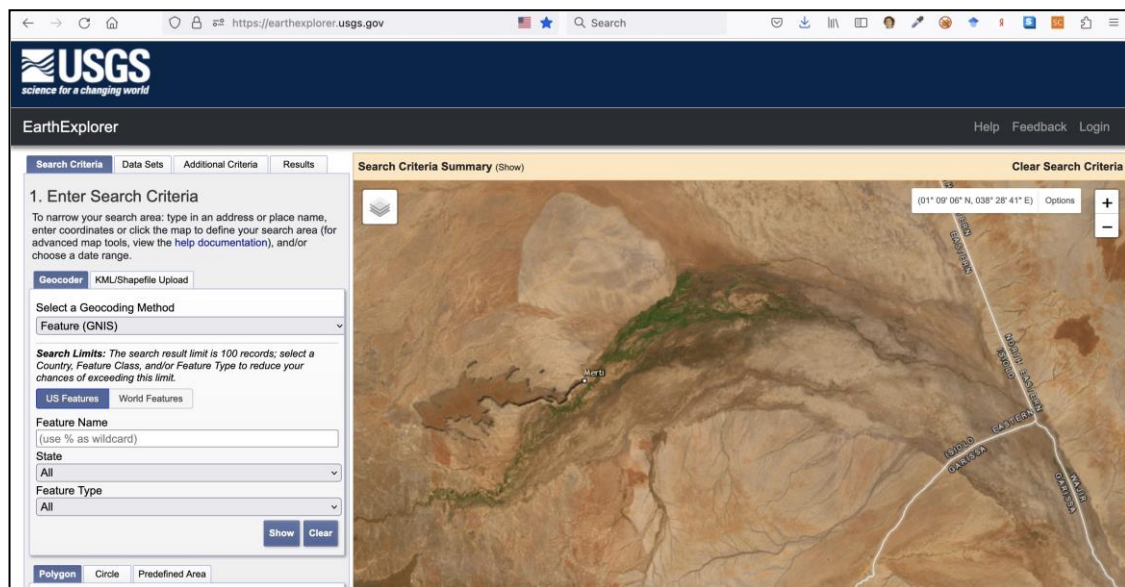


Figure 3: Enlarged view of the Lorian swamp, Kenya, on the aerial image: USGS.

The wetland area of the Lorian Swamp presents a vital resource for dry-season grazing as well as a sanctuary for the nomadic herds. Such climate setting creates unfavourable conditions for swamp ecosystem and affect its coverage and extent. Major water sources that fed the Lorian swamp include Ewaso Ng'iro North River with its tributaries originating in the slopes of Mountain Kenya and forming a river basin. Minor sources include seasonal wadis (Ministry of Environment and Mineral Resources, Kenya, 2012).

MATERIAL AND METHODS

Data

The data include two multispectral Landsat 8-9 OLI/TIRS satellite images covering target area of the Lorian swamp on 14 January 2015 and 28 January 2023 (Fig. 4). Technical characteristics common for both images are the following. The images are acquired from the USGS EarthExplorer repository with Landsat Collection Category T1 and Collection Number 2. The Landsat Worldwide Reference System (WRS) Path of the images is 167, the WRS Row is 59 which coincide with the target path and row of the satellite's orbit. The Station Identifier is LGN; the images were taken during day period with Nadir on. The Data Type L2 is OLI_TIRS_L2SP for both of the scenes and Sensor Identifier is OLI_TIRS and Ground Control Points Version 5. The Product Map Projection L1 is Universal Transverse Mercator (UTM), Zone 37, Datum and Ellipsoid WGS84. The rest of the metadata is summarised in the table 1.

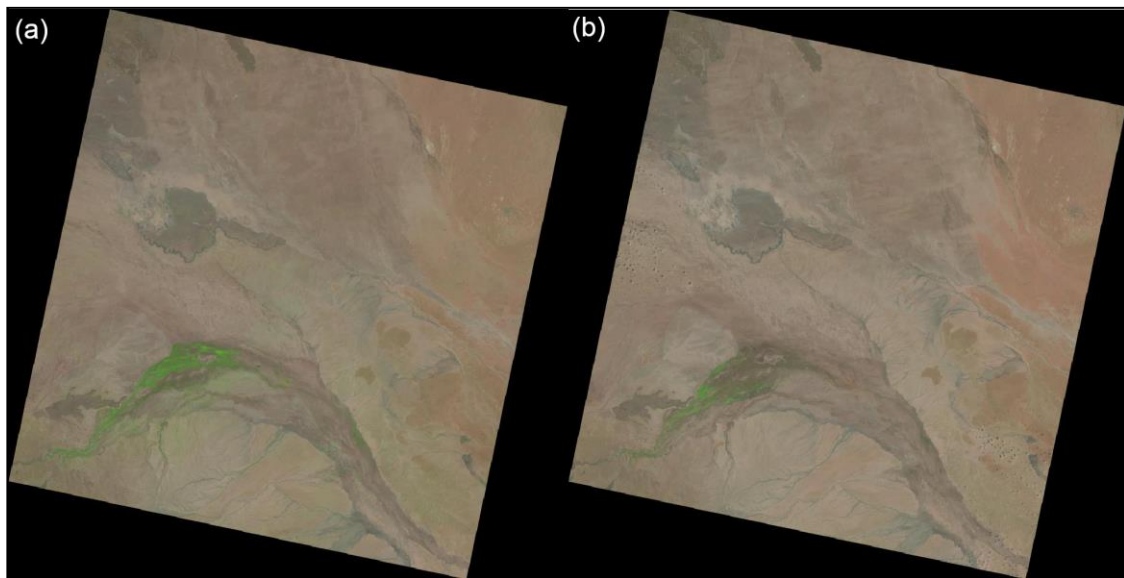


Figure 4: Remote sensing data (RGB) captured from the USGS EarthExplorer repository: Landsat 8-9 OLI/TIRS C2 L12 images. (a): 14 January 2015; (b): 28 January 2023.

Satellite images contain repetitions of pixels arranged along the matrix structure of the raster with different brightness. This is illustrated in figures 5 and 6, which show the original Landsat OLI/TIRS scenes and the segments of the study area. Therefore, the analysis of the satellite images relies on the information containing spectral reflectance of the pixels corresponding to the land cover types on the Earth's surface including wetlands.

The identification of the land cover types in general and wetlands in particular enables to collect information regarding the extent of these landscapes, while changes of contours over time enabled to assess the variations of the land cover types caused by the climate effects.

Table 1: Metadata of the two images Landsat 8-9 OLI/TIRS used in this study.

Data Set Attribute	Attribute Value (2015)	Attribute Value (2023)
Landsat Product Identifier L2	LC08_L2SP_167059_20150114_20200910_02_T1	LC09_L2SP_167059_20230128_20230309_02_T1
Landsat Product Identifier L1	LC08_L1TP_167059_20150114_20200910_02_T1	LC09_L1TP_167059_20230128_20230309_02_T1
Landsat Scene Identifier	LC81670592015014LGN01	LC91670592023028LGN02
Date Acquired	2015/01/14	2023/01/28
Roll Angle	0.000	-0.001
Date Product Generated L2	2020/09/10	2023/03/09
Date Product Generated L1	2020/09/10	2023/03/09
Start Time	2015-01-14 07:35:58.666005	2023-01-28 07:36:21
Stop Time	2015-01-14 07:36:30.436001	2023-01-28 07:36:53
Land Cloud Cover	0.00	0.01
Scene Cloud Cover L1	0.00	0.01
GCP Model	866	791
Geometric RMSE Model	2.482	4.544
Geometric RMSE Model X	1.460	3.172
Geometric RMSE Model Y	2.008	3.254
Processing Software	LPGS_15.3.1c	LPGS_16.2.0
Sun Elevation LORA	53.70218735	54.66787672
Sun Azimuth LORA	130.48808928	125.24360389
TIRS SSM Model	FINAL	N/A
Satellite	8	9
Scene Center Lat DMS	1°26'47"N	1°26'46.90"N
Scene Center Long DMS	39°15'05.98"E	39°13'35.08"E
Corner Upper Left Lat DMS	2°29'29"N	2°29'28.93"N
Corner Upper Left Long DMS	38°13'44.90"E	38°12'07.74"E
Corner Upper Right Lat DMS	2°29'27.56"N	2°29'27.67"N
Corner Upper Right Long DMS	40°16'28.24"E	40°15'00.83"E
Corner Lower Left Lat DMS	0°23'36.67"N	0°23'36.67"N
Corner Lower Left Long DMS	38°13'47.42"E	38°12'10.37"E
Corner Lower Right Lat DMS	0°23'36.46"N	0°23'36.46"N
Corner Lower Right Long DMS	40°16'24.06"E	40°14'56.72"E
Scene Center Latitude	1.44639	1.44636
Scene Center Longitude	39.25166	39.22641
Corner Upper Left Latitude	2.49139	2.49137
Corner Upper Left Longitude	38.22914	38.20215
Corner Upper Right Latitude	2.49099	2.49102
Corner Upper Right Longitude	40.27451	40.25023
Corner Lower Left Latitude	0.39352	0.39352
Corner Lower Left Longitude	38.22984	38.20288
Corner Lower Right Latitude	0.39346	0.39346
Corner Lower Right Longitude	40.27335	40.24909

The colour composites were made using the GRASS GIS module “r.composite”. The following code used for the false color composite: “r.composite blue = L8_2023_03 green = L8_2023_04 red = L8_2023_05 output = L8_2023_rgb_FCC” (here, the example is given for the image of 2023, repeated likewise for 2015). The following code was used for generating the true color composite: “r.composite blue = L8_2023_02 green = L8_2023_03 red = L8_2023_04 output = L8_2023_rgb_TCC”.

The Landsat 8 true (or natural) color composite uses visible spectral bands where red corresponds for Band 4, green for Band 3 and blue for Band 2 in the respecting red, green, and blue spectral channels as color composites. This results in the image composed in a natural colored product, which is a representation of the Earth’s landscapes on the photo image as naturally visible by human’s eyes.

Methods

The study utilizes the Geographic Resources Analysis Support System Geographic Information System (GRASS GIS) (Neteler and Mitsova, 2008; Neteler et al., 2008) as a major tool for cartographic data processing. The existing techniques and scripts of the GRASS GIS for mapping tasks were applied (Lemenkova, 2020). Image processing started from creating the color composites of the images which were generated for natural (true) and false colour composites (Figs. 5 and 6).

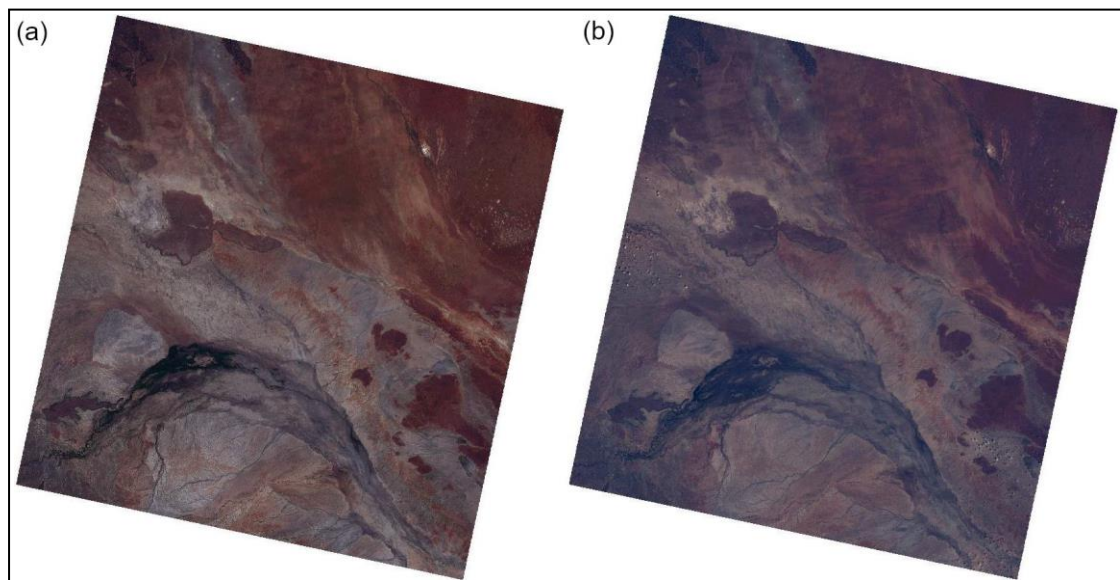


Figure 5: True color composites of the Landsat 8-9 OLI/TIRS images. (a) Bands composed from the image on 14.01.2015; (b) Bands composed from the image on 14.01.2015.

To this end, the images were processed, visualised and saved as bitmap graphics using the following sequence of GRASS GIS commands and modules (here, the example for the false color composite): “d.mon wx0 g.region raster = L8_2023_rgb_FCC -p d.rast L8_2023_rgb_FCC d.out.file output = L8_2023_rgb_FCC”.

The false color composite with 5-4-3 band combination of the Landsat OLI/TIRS images is useful for monitoring plant density in wetlands and health monitoring of vegetation, since the chlorophyll contained in leaves of the plants strongly reflects NIR light while absorbing red, therefore the areas covered by vegetation are colored by bright red. In this way, the settlements and the areas of sparsely populated villages in the surroundings, as well as

exposed ground are coloured grey or tan/middle brown colors, while water appears blueish or black. In contrast, area covered by dense vegetation near the Lorian swamp is represented by bright red with differences visible for 2015 and 2023 (Fig. 6).

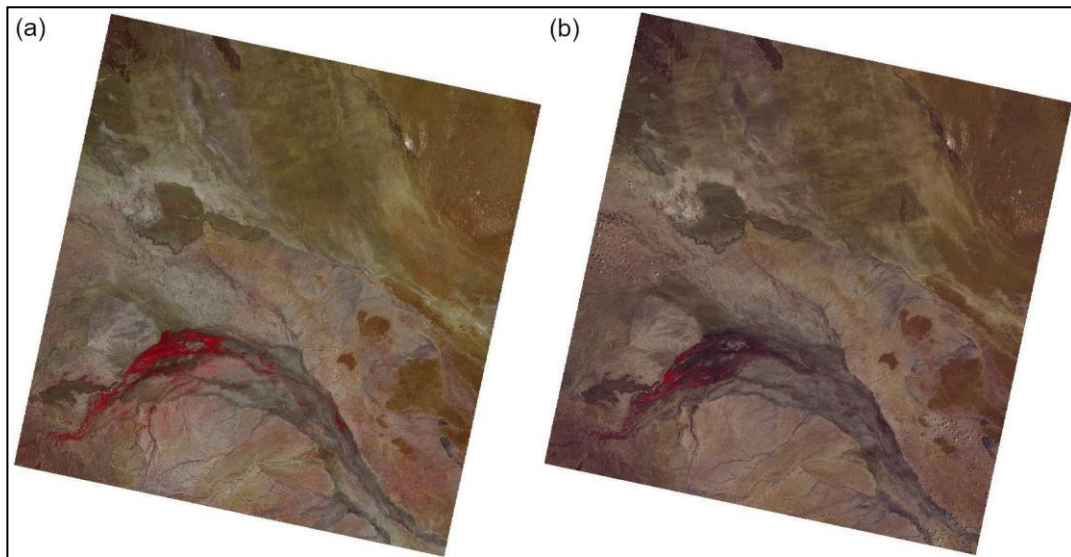


Figure 6: False color composites of the Landsat 8-9 OLI/TIRS images: Band B05 as the Red channel, Band B04 in the Green channel, and Band B03 in the blue channel. (a) Bands composed of the image on 28.01.2023; (b) Bands composed of the image on 28.01.2023.

Spectral reflectance of the pixels that differ for each case visually breaks the satellite image as multi-color scene depending on colour composites of bands (e.g., false colour composites of true colour composites). The description and interpretation of the objects identified as various land cover types was based on the information on land cover types of Kenya obtained from the FAO. The annotations, descriptions, and locations of the land cover classes were performed for images of each target year. Creating the classes was possible using the “maximum-likelihood discriminant analysis classifier” due to the functionality of this algorithm which includes image discrimination techniques by clustering and the preceding procedure of k-means clustering. After the classification, the classes were annotated as objects and features including the extent of wetlands in Kenya for comparison in multiple years.

For a GRASS GIS-based environment, the commands are called using the following sequence of commands implemented by a sequence of modules. First, the module “r.import” calls a raster TIFF files and imports it to the working folder with bilinear resampling: “r.import input=/Users/polinalemenkova/grassdata/Kenya/LC09_L2SP_167059_20230128_20230309_02_T1_SR_B1.TIF output=L8_2023_01 resample=bilinear extent=region resolution=region --overwrite”. The imported data were checked by the “g.list rast” command. As explained earlier, before using the algorithm, according to established GRASS GIS techniques using a sequence of modules “i.group”, “i.cluster”, “i.maxlik” (Lemenkova, 2022d), the snippets of the Landsat OLI/TIRS data were first visualized in the USGS to evaluate data quality and to define the cloudiness of the scenes. Both the scenes were selected with the cloudiness lesser than 2% to ensure correct classification.

Afterwards, the metadata of the raster images were checked by the “r.info” module (e.g., `r.info -r L8_2015_07`), and “g.list rast” which was used for listing the imported raster files in the next step. Thereafter, the “i.group” modules was used to create the groups and subgroups of the Landsat bands to include visible spectral bands: “i.group group=L8_2023 subgroup=res_30m input=L8_2023_01,<...>,L8_2023_07”. The following module “r.support” command was used to define semantic labels for all Landsat OLI/TIRS bands by considering the number of bands in the image: “r.support map=L8_2015_01 semantic_label=OLI_1”.

The MaxLike algorithm embedded in the GRASS GIS classifies pixels into those below the threshold as the target class objects using the centroids of the clusters generated by the “i.cluster” module in previous step: “i.cluster group=L8_2023 subgroup=res_30m signaturefile=cluster_L8_2023 classes=10 reportfile=rep_clust_L8_2023.txt --overwrite”. The rest of the image is classified automatically into the selected 10 target classes, based on the identified colour intensity of pixels. This is essentially done based on the discriminating of the breaks between the levels of the spectral reflectance of the pixels identified on the images and colour of the background of the Landsat OLI/TIRS scenes. The algorithm recognises spectral reflectance of the pixels and identifies those that do not reach the threshold level as not belonging to the target class and vice versa.

The procedure was performed using the “i.maxlike” algorithm, a commonly used robust method in the unsupervised classification of image processing to classify the cell spectral reflectances in imagery data as follows: “i.maxlik group=L8_2023 subgroup=res_30m signaturefile=cluster_L8_2023 output=L8_2023_cl_classes reject=L8_2023_cl_reject”. The aim is to select the correct inlier correspondences of the pixels to the target classes given a set of the one-to-one matches. In this model, the algorithm uses the clusters generated previously as a signature file to fit the pixels into the target groups. Using the defined parameters, the pixels were discriminated against the groups of the centroids of clusters using the objects parameters defined in a threshold. The boolean array of cells was defined as a class in each case. Thus, pixels with values exceeded the threshold indicated another land cover class different from the given one, while pixels within the given class were used for data processing. Similar to object recognition, the maximal classifier is used to define the threshold of objects parameters for identification of the land cover classes and wetlands as a target class.

The maps were plotted based on the implemented algorithm and the comparison of the actual land cover classes was performed within the several years. The image was partitioned using a threshold by assigning/rejecting pixels to/from the classed of land cover types. The object tracking was done iteratively until the Landsat image is classified and classes detected for the target region of Kenya. The procedure was repeated for each image for all the relevant years. Here, the commands used in the GRASS GIS workflow have the following meaning:

1. “g.region” – Lists the region of the images and sets region to match the scene;
2. “i.group” – Lists the necessary Landsat bands from visible spectrum available on Landsat band collection (the panchromatic and TIR are excluded);
3. “i.cluster” – Creates a new group of the classes using k-means clustering algorithm for a given image. It partitions the image and finds optimal parameters for pixels for a given target number of classes. The input signature file is generated for the following “i.maxlike” algorithm;
4. “r.support” – Creates the semantic labels for repetitive entries for automation;
5. “i.maxlike” algorithm – Classified the image into land cover classes.

The classification of the images consists of training the algorithm of classifiers that discriminates pixels forming clusters from the spectral bands of the image, detecting their spectral reflectance, assigning to clusters according to the centroids. The clusters of the pixels are defined by a series of the automatic trial tests with defined parameters, changing pixels' distance to the centroids of the clusters and closeness to the centers of the clusters to reach optimal combination. Natural clusters of the land cover classes are based on the location or attribute values using the k-means algorithm embedded in the GRASS GIS. Plotting the classified maps is implemented using the "d.mon wx0" command by the "d.rast.legend L8_2014_cluster_classes" command that defines color legends and visualizes them on the maps.

RESULTS AND DISCUSSION

Figures 7 and 8 show the images representing classes for each of the two images with the assigned land cover classes for various groups of pixels, number of pixels in a class and percentage of the correctly classified pixels represented in grey colour. The cluster groups used as empirical testing and training data during K-means algorithm for identification of optimal classes in the landscapes of north-east Kenya are groups of land cover types. The assignment was performed without the replacements and visualised as a continuous plot for both images (Figs. 7 and 8) to visually compare the outputs.

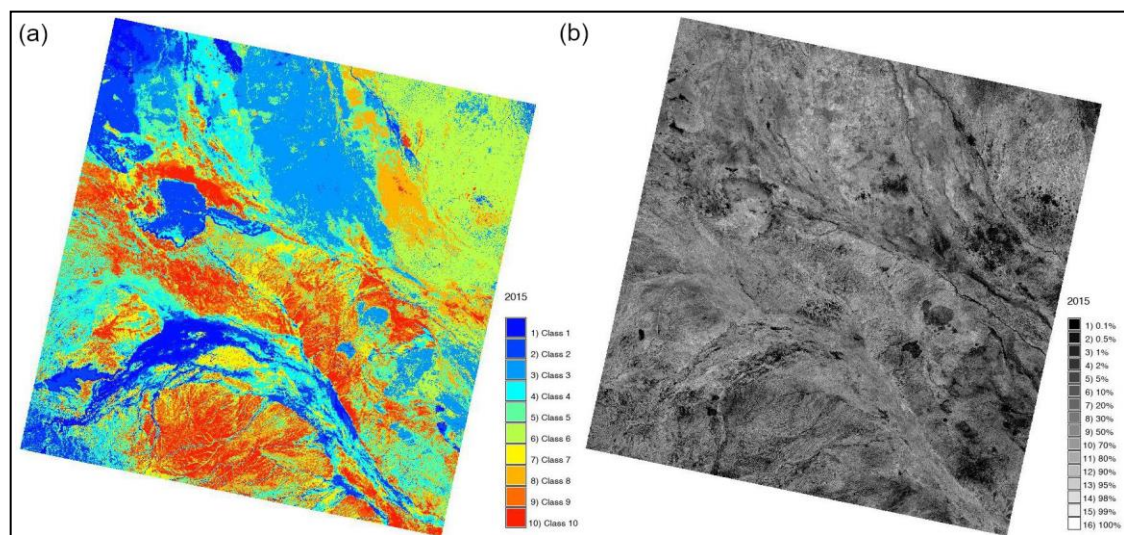


Figure 7: (a) Classification the Landsat 8-9 OLI/TIRS on 14.01.2015 of the Lorian Swamp wetland, Kenya with pixels classified into 10 classes. (b) Rejection probability values for image on 14.01.2015 with pixel classification confidence levels.

Land cover classification is applied to each image from the USGS. The clusters were optimized using the cycles of the k-means algorithm executed iteratively by the GRASS GIS until only the suitable pixels of the images are remained using the following parameters: number of clusters forming the scene, radius of pixel's neighborhood and threshold of colour intensity. Similar classes of the landscapes (tree cover: open, deciduous broadleaved, evergreen broadleaved, mixed type, unknown type, evergreen needle-leaved) defined by the k-means parameters were merged according to the landscape structure and resolution of the Landsat images (30 m) where combined classes signify the common type of the landscapes.

Each land cover class on the image was identified with dominated vegetation patterns crossing the landscapes and indicating the presence or absence of wetlands. This resulted in a series of the segments visualised assigned to the land cover classes as randomly coloured areas. The classified images were then converted to maps with added legends explaining the land cover types, computed correctness of the pixels assigned for data quality control, and compared with the original raster files of the Landsat 8-9 OLI/TIRS scenes. Quality control of the classified images was performed in pixels' level with identified examples (Figs. 7 and 8).

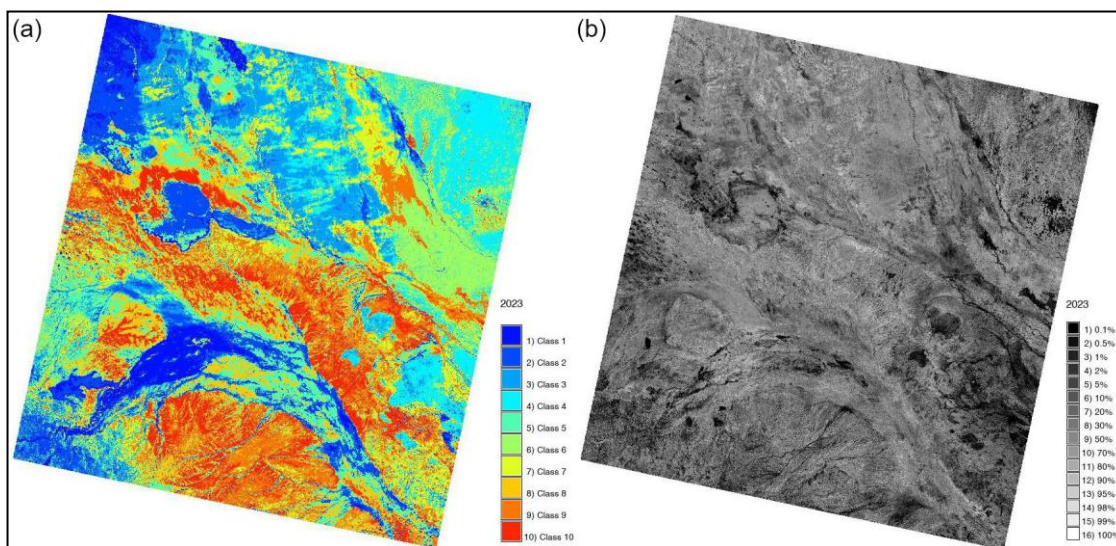


Figure 8: (a) Classification the Landsat 8-9 OLI/TIRS on 28.01.2023 of the Lorian Swamp wetland, Kenya with pixels classified into 10 classes. (b) Rejection probability values for image on 28.01.2023 with pixel classification confidence levels.

Here, the white colour signify the high rejection probability values with pixel classification confidence levels, while black to dark grey colours in the middle of the images signify the successfully classified pixels. Middle grey coloured pixels crossing the main image mean the successfully classified land cover classes, slant grey pixels mean the occasional pixels with occasional noise. The interpretation of the land cover classes and wetlands in Kenya by GRASS GIS approach performed well, while the overlapping of the neighboring classes required more attention when defining the parameters for automatic land cover class detection. The overlap cases existed between the two neighbor classes with similar spectral reflectance due to similar vegetation patters. In these cases, border classes were reclassified. The correction of such cases was done semi-automatically by checking the border regions of land cover classes and ignoring the overlapped segments in the neighbouring classes.

The final data structure of the mosaic patters in the north-eastern Kenya is presented by the areas of the 10 land cover classes. The presented maps were compared with the original raster TIFF files of the Landsat OLI/TIRS images for quality control and visual inspections in the GRASS GIS environment. The following 10 land cover classes were mapped using data adopted from ESA CCI-LC project derived from Kenya Land Degradation Neutrality Target Setting Final Report, Secretariat and the Global Mechanism of the UNCCD, based on UN Land Cover Classification System developed by FAO: 1) Cropland (rainfed and irrigated); 2) Wetlands, swamps and flooded areas; 3) Grassland; 4) Forests and tree cover (broadleaved,

evergreen); 5) Forests and tree cover (broadleaved, deciduous); 6) Mosaic natural vegetation of mixed leaf type (broad-leaved and needle-leaved); 7) Shrubland (deciduous); 8) Settlements and urban areas; 9) Bare areas; 10) Water bodies.

For classification, we applied two possible algorithm steps provided by the GRASS GIS modules "i.cluster" and "i.maxlik" for fusing pixels into the structured land cover classes which represent a complex mosaic of the wetland landscapes in Kenya. A k-means clustering method was used for partition of the images into 10 classes using estimation of distance from each pixel to the cluster centroid. A more principled approach is presented by the "i.maxlik" module which selects the 10 cluster categories as land cover classes, and performs the assignment of pixels into these classes according to their spectral reflectance and spectral signature file generated earlier by the "i.cluster". Such sequence of the GRASS GIS modules demonstrated an integrated workflow concept for the task of vegetation objects detection, showing how structured classes support image classification process for multi-spectral imagery with a case of the Landsat scenes.

CONCLUSIONS

Mapping land cover types is necessary to meet the environmental needs of the sustainable development in Africa. It is useful for monitoring land resources to support ecosystem in Kenya. Here, we presented a way to integrate remote sensing data and process them using GRASS GIS scripts for the task of image classification, analysis and monitoring landscape changes. In particular, we incorporated the clustering technique by k-means using "i.cluster" module in the training process of image partition. The module "i.maxlik" enabled to assign pixels into valid land over classes and perform image partition according to adjacent object categories. During image classification, a topological description of the land cover classes by FAO was adopted for a selected region in the Lorian swamp surroundings for exploring structured pattern of the land cover types in the north-eastern Kenya.

Scripting algorithm of GRASS GIS provides classification more accurate and faster than the GIS tools, since the processing of one image takes few seconds. Such performance is achieved due to automation of image processing through the GRASS GIS scripting approach. Image capture from the USGS format was performed using the EarthExplorer repository which enables the repeatability of the workflow in similar projects. We demonstrated the sequential use of several modules of the GRASS GIS. Selecting visual spectral Landsat bands was performed by "i.group"; assigning semantic labels was done by "r.support"; "i.cluster" was used for image partition by k-means clustering algorithm, and the unsupervised classification by the maximum likelihood discriminant analysis classifier was implemented by "i.maxlik".

To classify the Landsat imagery using GRASS GIS scripts, we used a workflow in GRASS GIS interface and several modules as described and explained in the Methodology section. The GRASS GIS modules were used as a sequence of separate tools to run the GRASS GIS scripts on the MacOS machine through a high-level scripting language of GRASS GIS, with an example for the images covering Lorian swamp, north-east Kenya. The workflow consisted of the following steps: image capture and preprocessing, grouping and sorting data by "i.group", creating semantic labels by "r.support", clustering by "i.cluster" module, "classification by i.maxlike" module, plotting, analysis and visualization of the completed images by "d.mon" and "d.legend" modules of the GRASS GIS. The cartographic workflow in the GRASS GIS scripting environment for classification of the satellite images included running the scripts of the GRASS GIS algorithm.

Two sensors were tested for image analysis with OLI-8 sensor for image on 2015 and OLI-9 sensor for image on 2023. We provided GRASS GIS scripting for both of them: clustering by k-means and maximum-likelihood classification for detecting changes in wetland and decline of vegetation. The GRASS GIS approach demonstrated superior results when compared to traditional GIS due to the high level of automation by scripts which resulted in higher speed and accuracy of image classification. We compared the results of classification based on the GRASS GIS algorithm and the existing state-of-the-art land cover maps of Kenya with an example of GIS performed through both digitizing the landscapes and remote sensing data processing, as well as technical assessment of image analysis. The performance of the GRASS GIS demonstrated effective approach to classification of the land cover classes aimed to find changes in vegetation patterns over the Lorian swamp wetlands, north-eastern Kenya. The study contributed to the environmental monitoring of wetlands by mapping Lorian Swamp area of Kenya, east Africa using GRASS GIS scripts for remote sensing data processing.

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**STRUCTURE, ECOLOGY AND PHYTHO GEOGRAPHICAL
CHARACTERISTICS OF RIPARIAN VEGETATION
ALONG THE BÂRZAVA AND CARAȘ RIVERS
(BANAT, ROMANIA)**

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KEYWORDS: environment quality indicator, thermophile riparian species, calcareous rocky thermophile species.

ABSTRACT

The riparian vegetation in the studied area have its particularities, due to the geographic position in the South-Western part of Romania with mountains and plains. These come together to give a most interesting and unique mosaic of habitats of riparian vegetation with a remarkable biodiversity. The climatic conditions and the relief with plains and mountains elements give the specific character to the studied riparian vegetation.

ZUSAMMENFASSUNG: Struktur, Ökologie und pflanzengeographische Kennzeichen der Ufervegetation entlang der Flüsse Bârzava und Caraș. (Banat, Rumänien).

Die untersuchte Auenvegetation der genannten Flüsse hat ihre Besonderheiten, die bedingt sind durch die geographische Lage im Südwesten Rumäniens, wobei das Klima, aber auch das Relief mit Bergen und Ebenen eine große Rolle spielt. Diese Elemente ergeben zusammen ein besonderes Mosaik an Auenhabitaten mit einer beachtlichen Biodiversität der Bergregion und der Ebene.

REZUMAT: Structura, ecologia și caracteristicile fitogeografice ale vegetației ripariene de-a lungul râurilor Bârzava și Caraș (Banat, România).

Vegetația ripariană studiată are un caracter deosebit datorită poziției geografice în sud-vestul României, unde atât climatul, dar și poziția geografică cu segmente muntoase și de câmpie prin care trec râurile joacă un rol deosebit fiind și baza pentru o biodiversitate remarcabilă.

INTRODUCTION

The Bârzava River has its source in the Cracul Lung hills, the western side of Semenic Mountains at an altitude of 1,023 m above sea level. Breaking through the Dognecea Mountains, the river crosses the towns of Reșița and Bocșa and then turns in a north-western direction through the large Bârzava Plain near to the localities of Gătaia and Deta. South of the town of Deta the Bârzava River is canalized on a long stretch, flowing in an Eastern direction to the canal system of Timiș (Danube Basin). The Bârzava has on its course three storage lakes, in north-eastern direction the Văliug Lake, followed by the smaller Breazova and after the turn of the river to a westerly direction – to the town of Reșița – the Secu Lake. North-east from Reșița the stream of Bîrzavița flows into the main river (Fig. 1). (Dragomir et al., 1981)

The Caraș River has its source on the eastern side of the Anina Mountains near to the watershed of the Nera River. Crossing the calcareous mountain chain, the river turns in a south-western direction, flowing as a meandering river in the plain of Caraș (Caraș Depression) and then further in the direction of the Danube River, having the mouth at Banatska Palanka (Serbia), not far off the mouth of Nera River. Due to the varied rocky relief in the upper part and a harsh climate, followed in the plain by warmer temperatures and with mild climatic conditions, numerous thermophile species occur in the area. The Caraș has eroded out on its course in a northern direction the deep gorge of Caraș (Cheile Carașului), which ends in a semicircle bed near the locality of Carașova (Fig. 1). (Dragomir et al., 1981)



Figure 1: Map of the study area realized on the base of maps of the area by Lars Gerstner KIT Institute for Geography and Geoecology, Department for Wetlands Ecology – Aueninstitut.

The Nera River collects its waters from a seeping water slope with the sources streams Coșava, Nergănița, and Nergana (Schneider-Binder 2017). Parallel to the Caraș River in a western direction flows in the Buhui Stream, near to the northern opening of the gorge. The river embrace in its northern gorge stretch the mountain of Socolova (783 m altitude). Turning in western and south-western directions, the meandering river crosses the large plain of Caraș (Caraș Gorge) and flows into the Danube River at Banatska Palanka (Serbia), not far of the mouth of Caraș River. (Dragomir et al., 1981)

The cliffy area of upper Bârzava and Caraș rivers shelter a mosaic of interlocking riparian phytocoenoses of different plant communities, identified by species of diverse phyto-geographical regions such are those of the daco-illyrian province (Borza and Boșcaiu, 1965) in the south/western part of Romania with numerous thermophile species, giving this riparian zone a particular aspect.

In general view the riparian vegetation is mostly reduced to small strips along the water course, the dominant, edifying species of the riparian forest being mostly the Common Ash (*Fraxinus excelsior*) and Black Alder (*Alnus glutinosa*) (Tab. 1).

MATERIAL AND METHODS

During the growing season of the years 2015 and 2016 in the frame of Natura 2000 sites inventory and their importance for biodiversity, field researches concerning the riparian habitats were realized on the rivers Bârzava, Caraș, and Nera, and for completion information was collected concerning the transboundary Danubian tributaries in the Banat area.

Samples were taken according to the method of Braun-Blanquet with the seven degree abundance-dominance scale (Braun-Blanquet, 1964; Borza and Boșcaiu 1965). Considered were as well aspects concerning the structure of the habitats in strong relation to the water dynamics of the tributaries, the grain size of sediments and the succession of the vegetation along ecological gradients from the river banks to the higher elevations of the river valleys. From upstream to downstream the following valleys have been studied: the Caraș River on the confluence with Buhui River, the area around the old terrace of the railway, the Comarnic Stream near the forestry residence, the Toplița Stream area and the Carașova area downstream the gorge of Caraș River (August 2015).

The samples taken are included in a phyto-coenological table and grouped according to characteristic species of the different phytocoenological units and to their layers, such as the tree, shrub, tall, and smaller herbaceous layers, the regeneration and lianas.

Species occurring only in one sample are mentioned at the end of the table.

The nomenclature of species is given according to Oberdorfer (2001), Ciocârlan (2009), and Sârbu et al. (2013).

The samples were used as well for detailed studies concerning the species composition and structure of the phytocoenoses and their ecological requirements (Schneider, 2003) All the phytocoenoses were considered in strong relation to the hydro-morphological dynamics, the grain size of sediments, the water quality and the succession of the vegetation along ecological gradients

Table 1: Place of sampling: 1. Comarnic Valley; 2. Caraş River downstream of the confluence with Buhui Stream; 3. confluence of Buhui Stream with Caraş River; 4. valley on the terrace of the former forest train; 5. old terrace of the forestry railway; 6. forester house on the Comarnic Stream; 7. on the Topliţa Stream; 8. Topliţa Stream; 9. Caraşova, downstream of the Caraş Gorge; Caraşova, 10-11 Caraşova Gorge downstream part; (August 2015).

reg. forest	<i>Ulmus procera</i>	+	+	.	.	+
reg. forest	<i>Acer campestre</i>	+	.	.	.	+	+	.	+	.	.	.
tall herb.	<i>Petasites hybridus</i>	4	.	5	.	.	4
tall herb.	<i>Telekia speciosa</i>	.	.	1	.	.	3	2	+	.	.	.
tall herb.	<i>Cirsium oleraceum</i>	+	.	+
tall herb.	<i>Cirsium waldsteinii</i>	+	+	.	.	.	+
tall herb.	<i>Eupatorium cannabinum</i>	+	.	+	.
tall herb.	<i>Salvia glutinosa</i>	+	+	.	+
herb.	<i>Phyllitis scolopendrium</i>	2	+	.	+	.	+
herb.	<i>Athyrium filix-femina</i>	+	+	.	.	.	+	.	+	.	.	.
herb.	<i>Aegopodium podagraria</i>	.	+	+
herb.	<i>Urtica dioica</i>	.	+	.	.	.	2	+	3	.	.	.
herb.	<i>Lotus corniculatus</i>	+	.	.	.	3
herb.	<i>Mercurialis perennis</i>	+	.	.	2	.	+
herb.	<i>Knautia sylvatica</i>	+	2	+
herb.	<i>Brachypodium sylvaticum</i>	+	+	+	+	.
herb.	<i>Asarum europaeum</i>	+	+	.	+	+
herb.	<i>Lathyrus sylvestris</i>	+	.	.	+
herb.	<i>Lunaria rediviva</i>	.	3	.	3	.	3
herb.	<i>Stellaria holostea</i>	.	+	+
herb.	<i>Pulmonaria maculata</i>	.	+	.	+	.	+

Table 1: Place of sampling: 1. Comarnic Valley; 2. Caraş River downstream of the confluence with Buhui Stream; 3. confluence of Buhui Stream with Caraş River; 4. valley on the terrace of the former forest train; 5. old terrace of the forestry railway; 6. forester house on the Comarnic Stream; 7. on the Topliţa Stream; 8. Topliţa Stream; 9. Caraşova, downstream of the Caraş Gorge; Caraşova, 10-11 Caraşova Gorge downstream part; (August 2015).

herb.	<i>Galium sylvaticum</i>	.	.	+	+
herb.	<i>Arum maculatum</i>	.	.	+	+	.	+
herb.	<i>Parietaria officinalis</i>	2	+
herb.	<i>Angelica sylvestris</i>	+	.	+	.	.	.
herb.	<i>Lycopus europaeus</i>	+	.	.	.	+	+
herb.	<i>Ranunculus repens</i>	+	.	+	.	+	.
herb.	<i>Impatiens parviflora</i>	+	.	+	.
herb.	<i>Rumex obtusifolius</i>	+	.	+	.

Species occurring only in one sampling place. rel 1: *Athyrium filix femina*, *Polypodium vulgare*, *Festuca sylvatica*, *Lotus corniculatus*, *Mercurialis perennis*, *Sanicula europaea*, *Melica nutans*, *Sambucus ebulus*, and *Equisetum maximum*; rel. 2: *Cornus mas*, *Oxalis acetosella* and *Polygonatum multiflorum*; rel. 3: *Galium sylvaticum*, *Euphorbia amygdaloides*, *Geranium phaeum*, and *Sambucus racemosa*; rel. 4: *Galium schultesii*, *Lilium martagon*, and *Geranium sylvaticum*; rel. 5: *Glechoma hederacea*, *Daphne laureola*, *Rosa canina*, *Burnet rose (Rosa pimpinellifolia)*, *Tussilago farfara*, *Syringa vulgaris*, and *Ceterach officinarum*; rel. 6: *Salix triandra*, *Trifolium repens*, *Polygonatum latifolium*, and *Anthriscus sylvestris*; rel. 7: *Aconitum vulparia*; *Verbena officinalis*, *Bromus inermis*, *Physocaulis nodosus*, *Knautia drymeia*, *Impatiens parviflora*, and *Ajuga reptans*; rel. 11: *Oriental hornbeam (Carpinus orientalis)*, *Crataegus pentagyna*, *Lythrum salicaria*, *Deschampsia caespitosa*, and *Galinsoga parviflora*.

Due to the geographical position and the prevailing climate characteristics a number of thermophilous balcanic, balcano-anatolian, pontic-mediterranean, and mediterranean species are present in the area. They can be found almost all in the shrub layer. These species are: Cornelian cherry (*Cornus mas*), Mountain ash (*Sorbus torminalis*), European bladder nut (*Staphylea pinnata*), Common lilac (*Syringa vulgaris*), Smoke tree (*Cotinus coggygria*), Flowering ash (*Fraxinus ornus*). Downstream of the Caraşova Gorge silver lime tree (*Tilia tomentosa*) can be observed. In the herbaceous layer near to the water occur Carpatian – Balcanic hydrophilous species, for example *Veronica bachofenii*, *Parietaria officinalis*, *Lunaria rediviva*, and *Cirsium waldsteinii*. Around the gorge of Caraova, many xerothermophilous species occurring on the rock slope, for example *Centaurea atropurpurea*, are adjacent to the riparian species.

CONCLUSIONS

The riparian vegetation of the Caraş and Bârzava rivers is special, due to the geographical position brought about by the climate and the typical relief of the area, with both plain and mountain conditions. Due to this situation, we can observe in the area large scale biodiversity.

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**GENETIC SIMILARITY AND GENE FLOW
IN FRESHWATER SNAIL, *BULINUS GLOBOSUS* POPULATIONS
FROM SELECTED NATURAL HABITATS IN KANO STATE (NIGERIA)**

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KEYWORDS: diversity, variation, gene flow, polymorphism, RAPD, Nigeria.

ABSTRACT

The population genetic structure of *Bulinus globosus*, an important intermediate host snail for *Schistosoma haematobium*, in Nigeria was studied using Random Amplified Polymorphic DNA Technique. The five primers amplified genomic DNA of *B. globosus* from three populations with one region and 999 permutations. With 79 loci, the % polymorphic bands for each primer across all population samples were 55.70% (River Karaye), 58.23% (River Bagwai) and 60.76% (River Kano). The mean heterozygosity was 0.175, 0.190 and 0.197 for snails caught in Karaye, Bagwai, and Kano respectively. The highest genetic distance (0.141) and identity (0.919) were observed between the populations of Bagwai, Kano, and Karaye and Bagwai. The variations within and among the snail populations were 76% and 4% respectively, while the UPGMA dendrogram revealed no outliers. The gene pool of *B. globosus* was microgeographically fragmented.

RÉSUMÉ: Similarité génétique et flux de gènes chez des populations d'escargot d'eau douce, *Bulinus globosus*, issues d'habitats sélectionnés dans l'État de Kano, Nigéria.

La structure génétique de *Bulinus globosus*, un escargot hôte intermédiaire important pour *Schistosoma haematobium*, a été étudiée au Nigeria à l'aide de la technique d'amplification aléatoire d'ADN polymorphe. Les cinq amorces amplifiées d'ADN génomique de *B. globosus* à partir de trois populations avec une région et 999 permutations. Avec 79 loci, le pourcentage de bandes polymorphes pour chaque amorce dans l'ensemble des échantillons de population était de 55,70% (Karaye), 58,23% (Bagwai) et 60,76% (Kano). L'hétérozygotie moyenne était de 0,175, 0,190 et 0,197 pour les escargots capturés dans la rivière Karaye, la rivière Bagwai et la rivière Kano. La plus grande distance génétique (0,141) et d'identité (0,919) ont été observées entre les populations de la Bagwai et Kano, et entre la Karaye et la Bagwai. Les variations au sein des populations d'escargots étaient de 76% et 24% respectivement, tandis que le dendrogramme UPGMA ne révélait pas de valeurs aberrantes. Le pool génique de *B. globosus* était fragmenté à l'échelle micro-géographique.

REZUMAT: Asemănarea genetică și fluxul de gene la melcul de apă dulce, populația *Bulinus globosus* din habitate naturale selectate din statul Kano, Nigeria.

Structura genetică a populației de *Bulinus globosus*, un melc gazdă intermediar important pentru *Schistosoma haematobium*, din Nigeria a fost studiată folosind Tehnica de Amplificare Aleatorie a ADN-ului polimorf. Cei cinci primeri au amplificat ADN-ul genomic al *B. globosus* de la trei populații cu o regiune și 999 permutări. Cu 79 de loci, procentajul benzilor polimorfe pentru fiecare primer din toate probele populației au fost de 55,70% (râul Karaye), 58,23% (râul Bagwai) și 60,76% (râul Kano). Heterozozitateea medie a fost de 0,175, 0,190 și 0,197 pentru melcii din râul Karaye, râul Bagwai și, râul Kano. Cea mai mare distanță genetică (0,141) și identitate (0,919) au fost observate între populațiile Bagwai și Kano și, Karaye și Bagwai. Variațiile în interiorul și între populațiile de melci au fost de 76% și, respectiv, 24%, în timp ce Dendograma UPGMA a arătat fără valori aberante. Baza genetică a *B. globosus* a fost fragmentată microgeografic.

INTRODUCTION

Current rates of species loss have triggered numerous attempts to protect and conserve biodiversity (Wäldchen et al., 2018). Species conservation, however, requires species identification tools, a competence obtained through molecular technique. Accurate species identification is the basis for all aspects of taxonomic research and is an essential component of workflows in biological research (Wäldchen and Mäder, 2018). Biologists are asking for more efficient methods to meet the identification demand. Meanwhile, information on the genetic structure of fish is useful for optimizing identification of stocks, stock enhancement, breeding programs, management of sustainable yield and preservation of genetic diversity (Sultana et al., 2018).

DNA polymorphisms have been extensively employed to assess genetic diversity in aquatic organisms (Phillips et al., 2019). Randomly amplified polymorphic DNA (RAPD) fingerprinting offers a rapid and efficient method for generating a new series of DNA markers in fish (SriHari et al., 2022). RAPD analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of an arbitrary sequence (SriHari et al., 2022). This method is simple and quick to perform; most importantly, no prior knowledge of the organism's genetic make-up is required. This technique has been used extensively to detect genetic diversity in crabs (Moruf and Adekoya, 2020), lobsters (Jeena et al., 2016) and fish (Suleiman et al., 2023). It has also been used to evaluate genetic diversity in various snail species such as *Galba schirazensis* (Lounnas et al., 2018), *Biomphalaria pfeifferi* (Tian-Bi et al., 2019; Manyangadze et al., 2021), *Oncomelania hupensis* (Qiu et al., 2019) and *Hydrobioides nassa* (Bunchom et al., 2021).

The determination of taxa is particularly important in the case of organisms involved in spreading diseases. Indeed, prophylactic strategies require a thorough knowledge of the biology and ecology of parasites and their vectors. Schistosomiasis caused by *Schistosoma haematobium* and *S. mansoni* is a parasitic disease commonly found in tropical and subtropical regions and is considered as the third most important tropical disease after malaria and intestinal helminthiasis (WHO, 2014).

The freshwater snail *B. globosus* is an important intermediate host for *S. haematobium*, the causative agent of urinary schistosomiasis in tropical and sub-tropical countries (Bórquez et al., 2020). This disease is of major health concern, especially in Africa where most cases have been reported. In 2012 alone it was reported that an estimated 42.1 million people were treated for this disease, and 249 million required preventative treatment, of which 90% lived in Africa (WHO, 2014). *Bulinus* spp. are hermaphrodites that are capable of selfing or outcrossing, with different species adopting either selfing or outcrossing as the preferential reproductive mode (Keeney and Yurco, 2021). Limited gene flow encourages inbreeding and this is probably the most significant factor shaping the genetic structure of *B. globosus*, which normally has a mixed reproductive strategy. However, it adopts only one reproductive mode at any particular time, with evidence proposing outcrossing as a way to avoid inbreeding depression (Qiu et al., 2019; Koene, 2021).

The common habitats of *B. globosus* are shallow waters near shores of lakes, ponds, streams and irrigation channels (Manyangadze et al., 2021). The important factors that shape the genetic structure and spatial distribution of snails include the distribution of habitats, which is influenced by the spatial and temporal fluctuations in water availability (Bórquez et al., 2020). Habitats also vary in space with regards to environmental factors such as predation, parasitism, and competition (Pyron and Brown, 2015). Parasites have also been found to have an effect on the genetic diversity, reproduction mode and overall structure of freshwater snail populations (Tian-Bi et al., 2019). In Nigeria, a limited number of molecular techniques have

been utilized to study the genetic structure of African freshwater snail populations. Therefore, a deeper understanding of the geographical distribution and population structure of *B. globosus* is crucial in understanding its population genetic diversity and fitness because the species plays a major role as a vector of schistosomiasis. Hence, the present study was carried out to ascertain the genetic similarity and gene flow in the freshwater snail populations from selected natural habitats in Kano State using versatile RAPD markers.

MATERIAL AND METHODS

Study area

The study was conducted in three selected rivers in Kano State, Nigeria. The river Kano is located in the South while the Rivers Karaye and Bagwai are in the western part of the state (Fig. 1). The locations were selected due to their same origin. Kano State is located in the semiarid area of Northwestern Nigeria. It has a population of 9,383,682 comprising of 4,844,128 males and 4,539,534 females (NPC, 2006). Kano State is the commercial nerve centre of Northern Nigeria. It is located between latitude 10°33' and 12°27' North of the equator and longitude 7°34' and 9°29' East of the Greenwich meridian and as such it is part of Sudano-Sahelian vegetation zone of Nigeria.

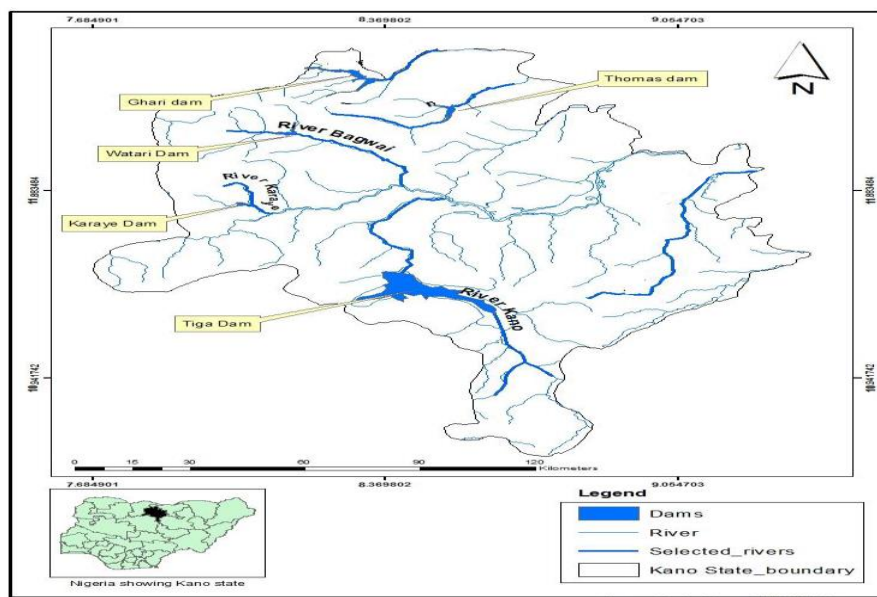


Figure 1: The map of Kano state with the sampling locations; modified from Suleiman (2017).

Sample collection and preparation

A total of 185 specimens of *B. globosus* were collected from spatially distributed water contact sites in the study area in 2023. The specimens were collected alive using a standard snail scoop, the contents washed, and the snails picked manually. The recovered snails were transported in pre-labeled plastic containers to the laboratory of the Department of Fisheries and Aquaculture, Bayero University, Kano. Each snail was identified based on its morphological characteristics using the field guide to African freshwater snails (Kristensen, 1987). The samples for molecular studies were preserved in 70% ethanol and further analyzed in conjunction with the African Bioscience Ltd in Ibadan, Oyo State.

Laboratory analysis

DNA was extracted from the head-foot tissue of each snail using the Genomic DNA Tissue MiniPrep Kit as described by Winnepeninckx et al. (1993). The concentration of the extracted DNA was spectrophotometrically estimated. DNA was stored at 4°C until needed. DNA yield was determined with a nanodrop spectrophotometer (NANO 1000, China) based on the maximum absorbance of DNA at 260 nm. One (1) µL of the DNA sample was applied on the platform of the nanodrop spectrophotometer and a reading was taken after adjustment of absorbance to zero using water as blank. The yield was measured in ng/µL. The 260 nm/280 nm ratio was obtained to give an analysis of the purity of the sample and the concentration of the extracted DNA was also found.

The amplification reaction was performed in 50 µl volume mixtures consisting of Polymerase Chain Reaction buffer (50 mM KCl, 0.1% Triton X-100, 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂), 2.5 mM dNTP (BioBasic, Canada), 5.0 µM of each RAPD primers, 50 ng of template DNA and 3U. Taq DNA polymerase with the protocol described by Simpson et al. The five RAPD primers: 1(CTGCTGGGAC), 2(AGGGAACGAG), 3(GTGAGGCGTC), 4(GTTGCCAGCC), and 5(TGCCGAGCTG), used in the PCR reaction were arbitrarily selected from laboratory stocks. Amplifications of DNA fragments were carried out by using a thermal cycler (Hamburg, Germany) with the following cycling profile: pre-denaturation at 94°C for four minutes, followed by 35 cycles of amplification (one min. denaturation at 94°C, one min. annealing at 36°C and one min. extension at 72°C). The process concluded with an extension at 72°C for 10 min. analysis of the resultant amplification products was done at 100 V for four hours with 1.8% agarose gel electrophoresis (BioRAD, USA) using TBE 1 × buffer (0.9 M Tris, 0.9 M Boric acid and 20 mM EDTA, pH 8.3). Furthermore, a DNA size criterion of 100 bp molecular weight marker was used. In order to visualize the amplified products with a digital camera, ethidium bromide was used to stain them.

Agarose gel (1.5 gm/100 ml) was prepared in pH 8.0 buffer which contained 89 mmol of Tris-borate, 2 mmol of EDTA, and 89 mmol of boric acid. After mixing the DNA samples with the loading buffer, they were electrophoresed at 50 volts for one hour. Afterward, agarose gel was stained with ethidium bromide (0.5 µg/ml) for 30 minutes and then photographed on UV light with a digital camera.

Data analysis

The RAPD Polymerase Chain Reaction (PCR) banding patterns generated with the primer were analyzed using Phyllip software (version 2.1, USA). Existence or non-existence of amplicons in each lane of Agarose Gels was premised on scores recorded in binary format. Scores were exclusively allotted only to the intense and reproducible bands that ranged between 400 and 1200 bp. Electrophoresis gels were scored into binary matrix using PyElph 1.4. Genetic analyses were conducted using GenAlEx 6.5 and UPGMA Phylogeny was constructed using MEGA 11.

RESULTS AND DISCUSSION

The five RAPD primers amplified the genomic DNA of 96 individuals of *B. globosus* from three populations (Fig. 2) with one region and 999 permutations. With 79 loci, the percentages of polymorphic bands for each primer across all population samples were 55.70% (River Karaye), 58.23% (R. Bagwai), and 60.76% (R. Kano) suggesting that inbreeding is not a major concern for this species. The study revealed a wide variation of polymorphic loci among the three populations. The highest level of polymorphism (60.76%) was exhibited by the River Kano population, whereas the lowest level of polymorphism (55.70 %) was exhibited by the River Karaye population. The percentage of polymorphic bands in *B. globosus* was

greater than that of the Indian mangrove crab, where the level of polymorphic bands was 24.60% in *Grapsus albolineatus* (Suresh and Madhuri et al., 2017). Although the sample size from each geographic site in this study was limited, specimens were collected from different geographic locations in Kano State. This should be sufficient to generate the preliminary data on genetic diversity and population differentiation of *B. globosus* in Kano State, Nigeria.

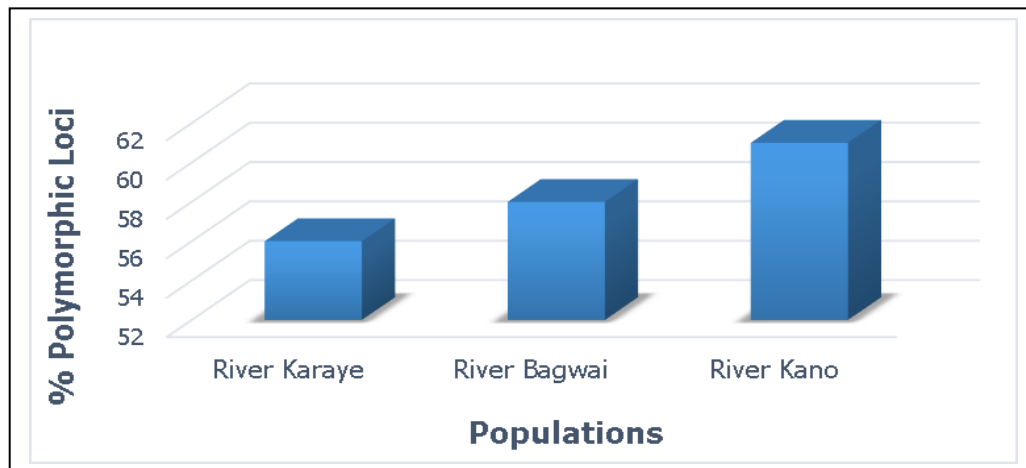


Figure 2: Percentage of Polymorphic Loci.

As indicated by the total band patterns for binary (diploid) data by populations in figure 3, the number of bands (or a number of bands freq. $\geq 5\%$) ranges between 45 (River Karaye), 46 (River Bagwai) and 53 (River Kano) while the mean heterozygosity was 0.175, 0.190 and 0.197 for snail caught in River Karaye, River Bagwai and River Kano respectively. The heterozygosity displayed by *B. globosus* is lower than the mean heterozygosity of 0.463 recorded in *Oreochromis niloticus* (Mahboob et al., 2019). These population-specific unique bands can be used to detect any possible mixing of these populations, especially during selective breeding programs (Houston et al., 2020). Hassanien and Al-Rashada (2019), Kajungiro et al. (2019), Lounnas et al. (2018), and Hobbs et al. (2021) also observed population-specific bands in *Penaeus semisulcatus*, *Oreochromis niloticus*, *Galba schirazensis*, and *Segmentina nitida* respectively.

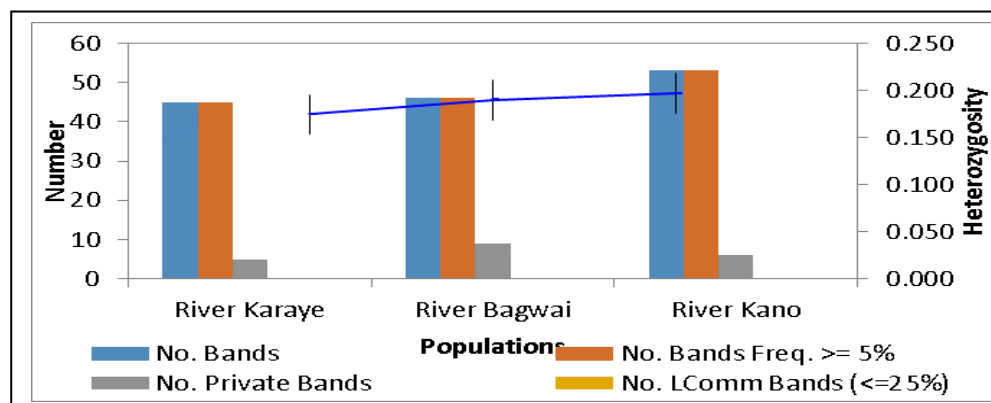


Figure 3: Total band patterns for binary (diploid) data by populations.

Estimates of Nei's genetic distance demonstrated sufficient genetic divergence to discriminate the samples of different populations of *B. globosus* (Tab. 1). The highest genetic distance (0.141) and identity (0.919) was observed between the populations of River Bagwai and River Kano, and River Karaye and River Bagwai, respectively. Generally, the levels of genetic distance between paired geographic samples did not reveal larger genetic distance with greater geographic distance (Moruf and Adekoya, 2020).

Table 1: Pairwise Population Nei Genetic Values of *B. globosus* from three water bodies in Nigeria.

Population 1	Population 2	Nei Distance	Nei Identity
River Karaye	River Bagwai	0.085	0.919
River Karaye	River Kano	0.086	0.918
River Bagwai	River Kano	0.141	0.868

The variations within and among the snail populations are 76% and 24% respectively (Fig. 4) while the UPGMA Dendrogram among *B. globosus* populations using Nei's genetic distance obtained three main clusters, River Kano, River Kano, and River Bagwai, with no outliers (Fig. 5). The configuration of the first clade revealed that the snails are more closely related. The present study indicated that the gene pool of *B. globosus* was microgeographically fragmented intraspecifically. Patterns of genetic differentiation at the fine-scale level in *B. globosus* are similar to other locations and species. For example, significant genetic homogeneity was previously reported for *Achatina achatina* (Etukudo et al., 2018), *Oreochromis niloticus* (Mahboob et al., 2019), and *Labeo ariza* (Ahammad et al., 2022), between geographic samples from different aquatic habitats.

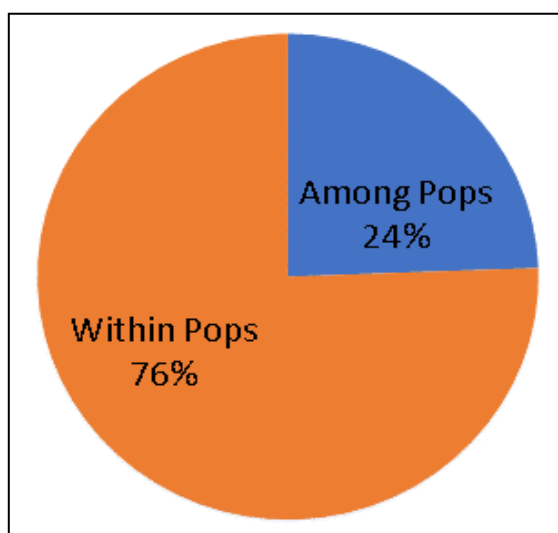


Figure 4: Percentages of molecular variance in *B. globosus* populations from three water bodies in Nigeria.

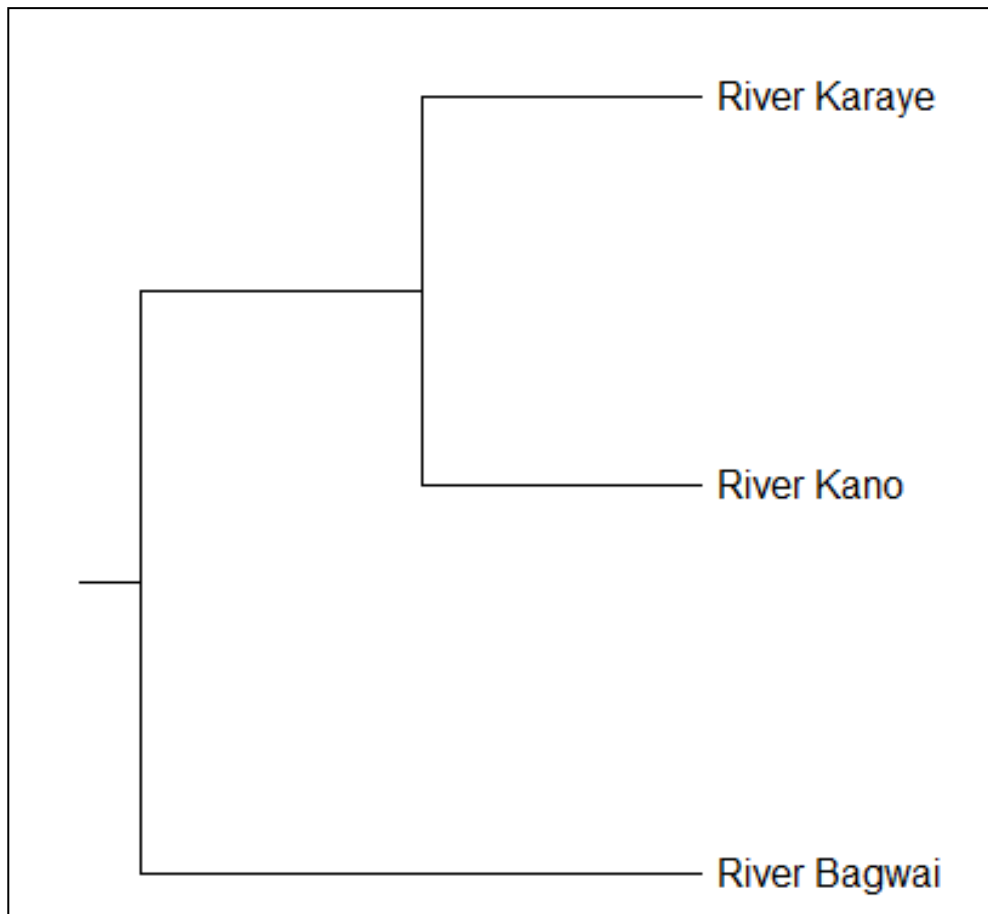


Figure 5: UPGMA Phylogeny constructed from Nei's genetic distance.

CONCLUSIONS

The present study revealed a wide variation of polymorphic loci among the three populations of *B. globosus*. The highest level of polymorphism (60.76%) was exhibited by the River Kano population, whereas the lowest level of polymorphism (55.70%) was exhibited by the River Karaye population. Restricted gene flow and high intraspecific population differentiation, but micro geographically fragmentation were observed. Moreover, RAPD confirmed the previous knowledge about its application as a quick and efficient method for generating DNA markers in aquatic organisms.

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HISTORICAL AND CURRENT DISTRIBUTION OF THE BROWN TROUT, EUROPEAN GRAYLING AND VOLGA NASE IN SMALL RIVERS IN THE REPUBLIC OF TATARSTAN

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KEYWORDS: historical and current distribution, environmental factors; probability of occurrence; brown trout, European grayling, Volga nase.

ABSTRACT

We have studied the historical and current distribution of brown trout, European grayling, and Volga nase in the Republic of Tatarstan. The influence of environmental variables on the current distribution of these fish was assessed using regression methods. The probability of occurrence in small rivers of all three fish species is currently low, ranging from 2.8 to 5.3%. Each of the recorded environmental variables had statistically significant relationships with presence/absence of at least one fish species, but only two variables (elevation and human impact) significantly affected the probability of occurrence of all species in the study.

RÉSUMÉ: Répartition historique et actuelle de la truite commune, de l'ombre commun et de la Volga nase dans les petites rivières de la République du Tatarstan.

Nous avons étudié la répartition historique et actuelle de la truite commune, de l'ombre commun et de la Volga nase dans la République du Tatarstan. L'influence des variables environnementales sur la répartition actuelle des poissons a été évaluée à l'aide de méthodes de régression. La probabilité d'occurrence dans les petites rivières des trois espèces de poissons est actuellement faible et varie de 2,8 à 5,3 %. Chacune des variables environnementales enregistrées avait des relations statistiquement significatives avec la présence/l'absence d'au moins une espèce de poisson, mais seules deux variables, l'altitude et l'impact humain, affectent de manière significative la probabilité d'occurrence de toutes les espèces dans l'étude.

REZUMAT: Distribuția istorică și actuală a păstrăvului indigen, a lipanului și a scobarului de Volga în râurile mici din Republica Tatarstan.

Am studiat distribuția istorică și actuală a păstrăvului indigen, a lipanului și a scobarului de Volga în Republica Tatarstan. Influența variabilelor de mediu asupra distribuției curente a peștilor a fost evaluată folosind metode de regresie. Probabilitatea de apariție în râurile mici a tuturor celor trei specii de pești este în prezent scăzută și variază de la 2,8 la 5,3%. Fiecare dintre variabilele de mediu înregistrate au avut relații semnificative statistic cu prezența/absența a cel puțin a unei specii de pești, dar doar două variabile, altitudinea și impactul uman, au afectat semnificativ probabilitatea de apariție a tuturor speciilor din studiu.

INTRODUCTION

The distribution, occurrence, and abundance of animals changes over time, caused by many factors. Rivers are characterized by spatial and temporal differences in environmental factors (Birzaks, 2012; Askeyev et al., 2017, 2021a). Environmental conditions, which include geomorphological, physical, and chemical factors in river systems, have a great influence on the distribution of freshwater fish species (Matthews, 1998). However, over the past two centuries it has been human activity that has been the main variable affecting the distribution of most fish species, including in the Republic of Tatarstan. The creation of large reservoirs in the middle of the 20th century put even more pressure on river ecosystems and led to a change in the hydrological balance of small and medium-sized rivers. The discovery and development of oil fields in the southeast of Tatarstan as well as the use of chemicals in oil production have had a detrimental effect on the ichthyofauna of the rivers of this region, leading to the partial or complete disappearance of fish from watercourses (Askeyev et al., 2015, 2016). Another negative aspect of human activity lies in the ecologically unjustified introduction or re-introduction of certain animal species. For example, the release of Eurasian beaver (*Castor fiber* Linnaeus, 1758) led to extremely negative consequences for small rivers in Tatarstan. A decrease in the flow rate, and eutrophication of small rivers, due to the construction of dams by beavers has had a detrimental effect on the abundance of a number of rheophilic fish species.

We focused on the following fish species: brown trout (*Salmo caspius morpha fario*, Kessler, 1877); European grayling (*Thymallus thymallus*, Linnaeus, 1758), and Volga nase (*Chondrostoma variable*, Yakovlev, 1870). All these species are native representatives of the rheophilic complex with limited distribution and low abundance (all are listed in the Red Book of the Republic of Tatarstan, 2016). Modern climatic changes (IPCC, 2019; Askeyev et al. 2020, 2022) can have an additional negative impact on the state of fish populations (Comte and Grenouillet, 2013; Buisson et al., 2013; Bănăduc et al., 2021). Therefore, their ecological parameters require especially careful monitoring, and their habitats deserve strong protection.

The main aims of the study were to compare historical and current distributions of brown trout, European grayling, and Volga nase and identify their preferences on environmental gradients in rivers in Tatarstan in current time.

MATERIAL AND METHODS

Study area

The Republic of Tatarstan is located in the eastern part of the Russian Plain, at the confluence of the largest rivers in Europe: the Volga, Kama, Belaya, and Vyatka. Traditionally, this area is considered to be within the historical and geographical provinces of European Russia – the Middle Volga and the Pre-Ural region and includes two natural zones – forest and forest-steppe. The largest rivers divide Tatarstan into three clearly separated natural and geographical parts: the Predvolga region (to the west and south of the Volga River valley), the Predkamye region (to the north of the Volga and Kama river valleys), and the Zakamye region (to the south of the Kama River valley). Predkamye, in turn, is divided by the valley of the Vyatka River into Western and Eastern Predkamye; Zakamye is divided by the valley of the Sheshma River into Western (low) and Eastern (high) Zakamye (Stupishin, 1964).

The Predvolga region, bounded to the east and north by the Volga River, is the northeastern part of the Volga Upland, with elevations of about 200-260 m above sea level. The elevated plain has a general slope to the north and is drained by the Sviyaga River. The eastern part of the interfluvium of the Volga and Sviyaga rivers is higher and abruptly breaks off to the Volga River, forming the Uslonsky Mountains. The relief surface is heavily dissected by erosion (Stupishin, 1964). In figures 1-3 this region is shaded pink.

Western Predkamye occupies a vast area of the north-west of Tatarstan, bounded in the west by the Volga River, in the south by the Kama River, and in the east by the Vyatka River. In relief, it is a ridged plain with a general slope to the southwest, towards the valley of the Volga River. The undulating surface of the interfluves of the Ashit-Kazanka, Kazanka-Mesha, and Mesha-Kama rivers in the valleys are dissected by numerous small tributaries, ravines, and gullies (Stupishin, 1964). In figures 1-3 this region is shaded green.

Eastern Predkamye, in the northeastern Tatarstan, in relief is the southern spurs of the Mozhginskaya and Sarapulskaya uplands 150-220 m heights, drained by Vyatka, Toima, and Izhem rivers. Slightly undulating interfluve areas are characterized by asymmetry: the western and northeastern slopes are gentler (Stupishin, 1964). This region is shaded blue in figures 1-3.

Western Zakamye occupies a low-lying plain with heights of 100-150 m above sea level, located east of the Volga River, south of the lower reaches of the Kama River and up to the Sheshma River in the east, the prevailing slopes are to the southwest and west, to the valley of the Volga River. The region is drained by the Bolshoi Cheremshan and Malyi Cheremshan rivers and their tributaries (Stupishin, 1964). In figures 1-3 this region is shaded red.

Eastern Zakamye contains the Bugulma-Belebeevskaya upland with the highest point in the central parts of the interfluves in the south (up to 380 m above sea level). The northern slope is rather gentle; the height of the surface of the basins of the lower reaches of the Sheshma, Zaya, and Ika rivers is 100-200 m, and only in the interfluves reaches up to 230-250 m (Stupishin, 1964). In figures 1-3 this region is shaded brown.

Fish assemblage data

Historical information

Information about the historical distribution of the studied species was taken from literary sources (Pallas 1773; Varpakhovsky 1886; Ruzsky 1887; Berg 1906, 1948-49; Meisner 1907; Pokrovsky 1909; Logashev 1933; Tikhii 1933; Zhdanov and Muratova 1935; Lukash 1940; Popov and Lukin 1949; Platonova 1952; Kirikov 1966; Kuznetsov 2005).

Current research

Fish sampling was conducted at 318 locations in the 11 years from 2012-2022. We focused on the fish assemblages of small rivers (length up to 500 km). We excluded rivers strongly impacted by large reservoirs. Detailed information on the method of catching fish and the distribution of study sites was presented in earlier work (Askeyev et al., 2015, 2021b)

For each site, we calculated the occurrence and abundance of each fish species. The following nine variables were obtained for each site: elevation above sea level (range from 53.2 to 270 m, mean 105.3 m, SD-standard deviation 39.0 m), mean width (0.5 to 55 m, mean 7.7 m, SD 10.3 m), mean depth (0.15 to 1.7 m, mean 0.71 m, SD 0.31 m), water velocity (0 to 1 m/s, mean 0.31 m/s, SD 0.16 m/s), tree/shrub cover along banks (0% to 100%, mean 48.0%, SD 27.5%), dominant bottom substrate (1 – mud, 2 – clay/peat, 3 – sand, 4 – gravel, 5 – pebbles, 6 – stones up to 150 mm, 7 – stones 150-300 mm, 8 – boulders > 300 mm), human impacts (as a 7 point scale: 0 – no agriculture or forestry; 1 – light agricultural impact – hay meadows, light grazing, and forestry within 250 m of the river bank; 2 – moderate agricultural impact – average grazing within 250 m of the river bank, the presence of a ford and a watering hole for livestock; 3 – strong agricultural impact – strong grazing with cattle driving trails visible, arable land, and shelter for animals within 250 m of the river bank; 4 – moderate agriculture impact and oil pollution – average grazing and exploitation of oil and gas within 250 m of the river bank; 5 – urban impact – river site in town or large village; 6 – strong oil and chemical pollution – chemical or oil odour is smelt or spots seen), latitude, and longitude.

Data analysis

Relationship between fish species and environmental variables

For each of the three fish species the nature and strength of relationships with the nine environmental variables was examined using binary logistic regression with the environmental variables as predictors on presence-absence data. Only statistically significant variables were retained in these regressions. In order to assess the accuracy of the final models, we used the area under the ROC curve (AUC), which indicates the predictive performance expressed as an index ranging from 0.5 to 1. The accuracy of the model was interpreted after Swets (1988) as follows: 0.90-1.00 excellent; 0.80-0.90 good; 0.70-0.80 fair; 0.60-0.70 poor; and 0.50-0.60 fail.

Calculation and visualization were done in PAST version 4.04 and XLSTAT 2021.

RESULTS AND DISCUSSION

Historical and current distribution for brown trout, European grayling, and Volga nase

Our current study estimated the probability of occurrence of brown trout as 5.3%, but all the occupied sites of this species fall in Eastern Zakamye in the southeast of Tatarstan (Fig. 1). Compared to historical data, the number of sites of brown trout has decreased by almost 80%; in particular, the populations north of the Kama River and in the Predvolga region have been completely lost (Fig. 1).

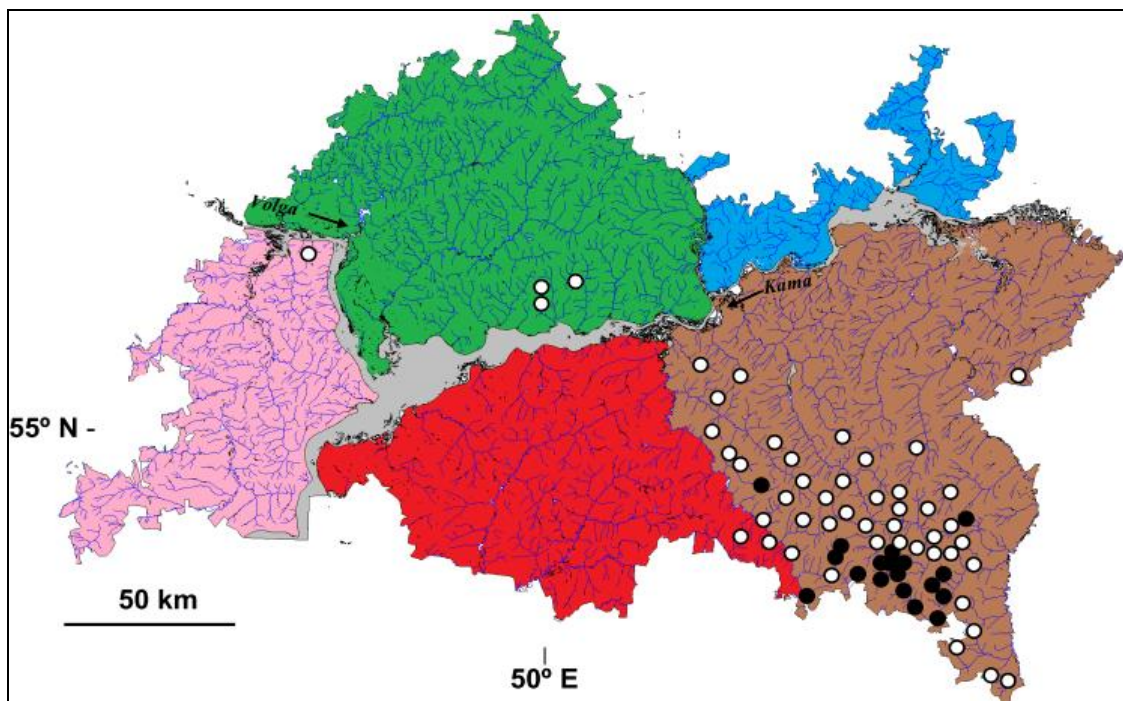


Figure 1: Historical and current distribution of brown trout in the Republic of Tatarstan. White circles show historical locations now absent, black circles historical locations which are still occupied.

In current times, the probability of occurrence of European grayling in the small rivers of Tatarstan is 2.8%. The few locations of European grayling are now very fragmented and can be divided into two clusters (Fig. 2). The first cluster is located in two sites in Western Predkamye: in tributaries of the Kama River (in the Bersut and Karmalka rivers which have been occupied by European grayling since the 19th century (Varpakhovsky 1886)), and one site in Eastern Predkamye in a tributary of the Vyatka River (the Lubyanka River – also a known historical site (Lukash, 1940)). Three others historical sites in this cluster for European grayling have been lost. Thus, compared to historical data, the number of sites for European grayling in these clusters has halved (Fig. 2). Another historical location in Western Predkamye for European grayling was the upper Kazanka River, located in the northwest of Tatarstan. A European grayling in this place was caught by Gontserovsky at the end of the nineteenth century (Meisner 1907).

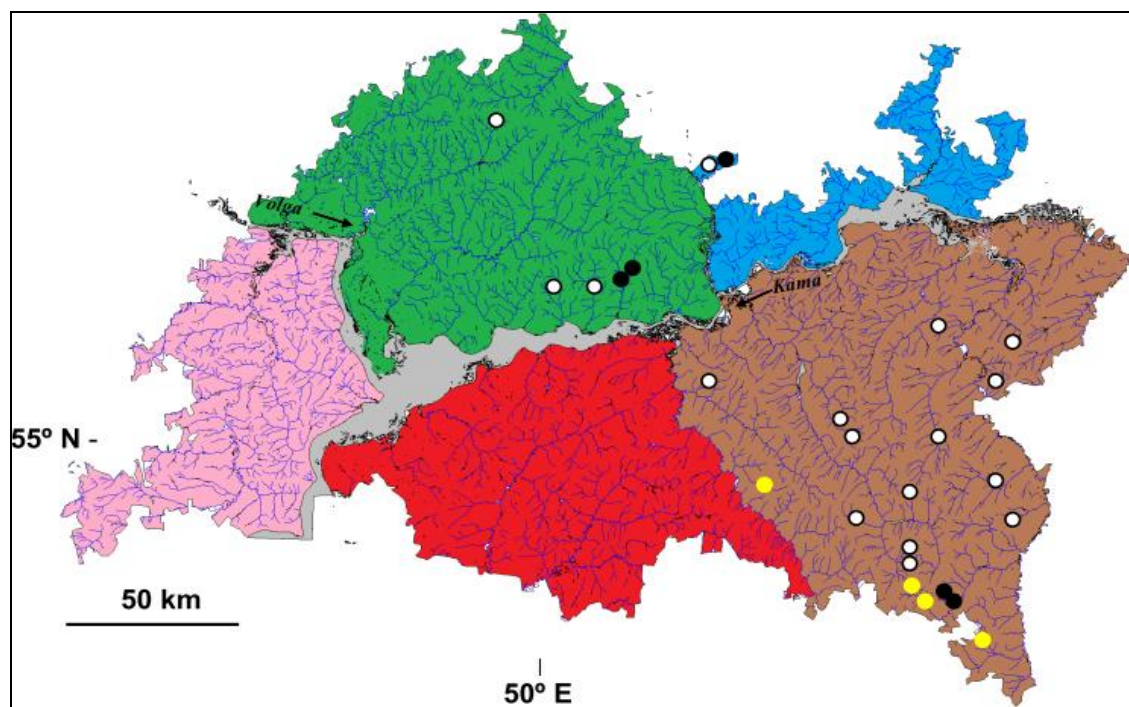


Figure 2: Historical and current distribution of European grayling in the Republic of Tatarstan. White circles show historical locations now absent, black circles – historical locations still occupied, yellow circles – new locations discovered in the current study.

The second cluster in current time is located in the southeast of Tatarstan, in Eastern Zakamye – in the basin of the Ik River – the Sula River and its tributary (the Lipovka River), the Dymka River and its tributary (the Shaitanka River), where this species was recorded in the 18th century (Pallas 1773). At present, European grayling has also been noted in the basin of the Sheshma River (the Bagryazhka River) (Fig. 2). Compared to the historical distribution, several locations have now been lost in the basins of the Zai, Ik, and Sheshma rivers. In general, despite the discovery of new sites of the European grayling, the total number of sites in Tatarstan has decreased markedly (Fig. 2).

Presently, the probability of occurrence for Volga nase is 4.7%. Populations of the Volga nase are distributed in Tatarstan in a scattered pattern, in separate river basins in all three orographic zones (Fig. 3).

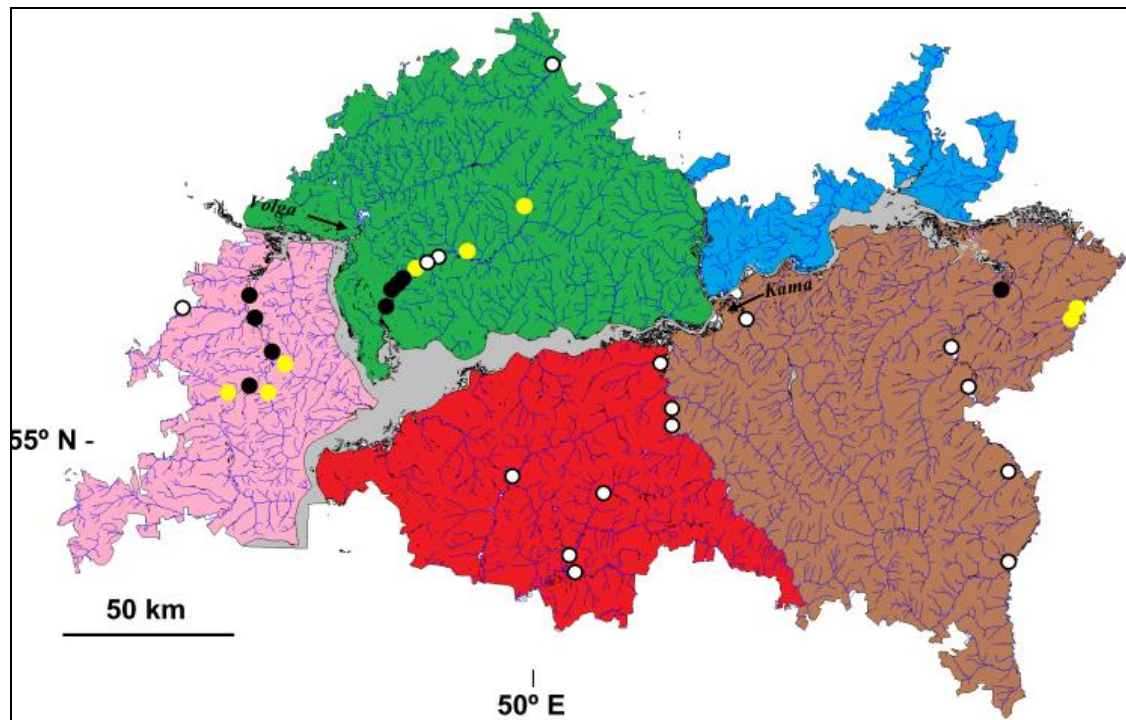


Figure 3: Historical and current distribution of Volga nase in the Republic of Tatarstan. White circles show historical locations now absent, black circles – historical locations still present, yellow circles – new locations discovered in the current study

The largest number of Volga nase locations is now in the Predvolga region, in the basin of the Sviyaga River (Sviyaga, Suhaya Ulema, Ulema, and Bula rivers). In Predkamye, Volga nase were noted in the Mesha River basin. There were only three locations in Zakamye (two in the Syun River basin and one in the Ik River basin). According to historical data, the Volga nase was a relatively common species, but mainly in large rivers (such as the Volga, Kama, and Vyatka), as well as in the lower parts of their tributaries, currently flooded by reservoirs. Compared with the modern distribution of Volga nase, the historical distribution was smaller in the Predvolga region, but significantly greater in Zakamye. In Western Zakamye, the Volga nase is now no longer observed (Fig. 3).

Environmental variables influencing the distribution of the fish species

Each of the selected environmental variables had statistically significant relationships with the presence/absence of at least one fish species (Tab. 1). All species were associated with elevation and human impact, two with width of the river, one with each of water velocity, tree and shrub cover, depth, longitude, latitude, and bottom substrate. All final models had satisfactory predictive power (AUC) varying from 0.87 to 0.98 (Tab. 1).

Table 1: Coefficients and summary for models of the relationship between presence/absence of brown trout, European grayling and Volga nase, with environmental variables. Environmental variables included in the models are abbreviated as follows: elevation above sea level (Elev); mean width (Width); mean depth (Depth); water velocity (Vel); tree/shrub cover along banks (Cover); dominant bottom substrate (Substrate); human impacts (Human); latitude (Lat); longitude (Long).

Species	Equation of the model	AUC
Brown trout	$Y = 4.41 + 2.81 * \text{Elev} - 0.32 * \text{Width} + 3.83 * \text{Vel} - 1.41 * \text{Human} - 1.62 * \text{Lat} + 0.53 * \text{Long}$	0.984
European grayling	$Y = -2.94 + 2.49 * \text{Elev} + 4.31 * \text{Cover} + 0.47 * \text{Substrate} - 1.10 * \text{Human}$	0.869
Volga nase	$Y = -1.87 - 3.87 * \text{Elev} + 3.35 * \text{Width} - 1.12 * \text{Human} + 1.71 * \text{Depth}$	0.932

In Europe, brown trout is a widespread species and inhabits various types of watercourses (Logez et al., 2012). However, in the studied region, brown trout is a rare species that prefers narrow, fast flowing rivers at high elevation, with a low anthropogenic load and with a gravel-stone substrate. A similar distribution of brown trout in the rivers of Western Europe was typical for the first half of the 20th century. At that time the occurrence of brown trout was quite low; the species predominantly occupied areas in the headwaters of rivers at high elevation (Huet, 1959). Currently, in the rivers of Europe, due to different types of human activity, brown trout populate rivers from source to mouth, and its distribution is essentially independent of elevation (Grenouillet et al., 2007; Buisson et al., 2008). In our study, there was a strong decrease in the occurrence of brown trout in rivers with a high anthropogenic impact. This species prefers "clean" rivers little touched by humans. A similar decline in numbers with increased agricultural pressure on rivers has been noted in Finland (Sutela and Vehanen, 2010). Similar trends were also noted in Tatarstan. At the end of the 19th century, Ruzksky (1887) noted a reduction of brown trout in the Sviyaga Basin, while Zhdanov and Muratova (1935) noted a strong decrease in the number of brown trout locations due to intensive human activity in the rivers. All these facts indicate that man has a serious impact on aquatic ecosystems and it is impossible to ignore this influence. For brown trout, the probability of occurrence decreased with increasing river width. Similar preferences for this variable were noted in the rivers of Latvia, where brown trout inhabits narrow sections of watercourses (Birzaks, 2012). The nature of the bottom substrate has a significant impact on the presence of brown trout. This species avoids areas with "soft" substrates, preferring gravel and areas with small and medium-sized pebbles. This is due to the ecological preferences of this species; in particular, brown trout is more conservative in terms of breeding substrate, spawning only on "hard" substrates (Greenberg et al., 1996). Brown trout are more likely to populate areas of watercourses with relatively high water velocity, preferring places with a powerful flow and high oxygen content. Similar preferences by brown trout have also been noted elsewhere in Europe (Greenberg et al., 1996; Logez et al., 2012). The absence of a significant relationship between brown trout and river depth is explained by the fact that different age groups of this species have contrasting preferences for this variable (Greenberg et al., 1996). An increase in the probability of occurrence of brown trout in the southeastern regions of Tatarstan is associated with the historical distribution of the species, as well as more suitable environmental conditions.

In Tatarstan, European grayling is a rare species and is found in rivers at higher elevations with a high percentage of tree cover, low human impact, and hard substrates. The frequency of occurrence of European grayling in European river basins varies greatly. The probable reason for these differences lies in genetic and historical differences in European grayling populations from different regions of Europe (Marić et al., 2014). In the rivers of Europe, European grayling also prefers rivers with tree cover (Logez et al., 2012; Maire et al., 2016). In this regard, a serious reason for the decrease in the number and distribution of European grayling in Tatarstan is that forest area has decreased by two thirds over the past 200 years. The deterioration of the state of European grayling populations as a result of the destruction of bank vegetation has also been noted in Romania (Curtean-Bănăduc and Bănăduc, 2016). Anthropogenic pressure has a significant negative impact on the probability of finding European grayling in rivers. Household and agricultural emissions, waste, and products of the petrochemical industry entering the rivers directly or indirectly have led to a decrease in numbers, and elimination of, European grayling from the rivers. In boreal Europe (Sutela and Vehanen, 2010), a significant relationship has been found between agricultural pollution and a decrease in the number of European grayling in rivers.

The main limiting factors for European grayling and brown trout are the marked fragmentation and geographic isolation of populations, oil and chemical pressure on the rivers, and a low percentage of riparian forests. Poaching of these species may be a further contributory factor. It is also important to note that in addition to humans, beavers also have a negative effect on the populations of these species, since their activity leads to a reduction in habitats and the number of suitable biotopes for spawning of brown trout and European grayling. In addition, for brown trout and European grayling, it should be noted that climate change will probably create additional negative pressure, reducing their numbers.

The Volga nase, in contrast to the other two species in our study, is characterized by an increase in the probability of occurrence on larger rivers at low elevation; but, as for brown trout and European grayling, it avoids sites with high anthropogenic impact. Unfortunately, it is difficult to compare the ecological preferences of the Volga nase in Tatarstan with those from other European regions due to the unclear genetic status of the species in different parts of the continent. The main limiting factors for the Volga nase are river flow regulation, and water pollution by industrial, domestic, and agricultural effluent (Red Book of Republic of Tatarstan, 2016).

CONCLUSIONS

The changing distribution of brown trout, European grayling, and Volga nase raises serious concerns for their future conservation, especially in the context of sharp and rapid climate change.

Based on the results of our work, a specially protected natural area “Rychkovskaya Forest-Steppe” has been formed, which includes a number of sites on small rivers which are characterized by a high number of brown trout and European grayling.

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**A FIRST RECORD OF
HYSTEROETHYLACIUM RELIQUENS (NORRIS AND OVERSTREET, 1975)
PARASITIZING *MULLUS BARBATUS BARBATUS* (L.)
FROM THE ALGERIAN COAST**

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KEYWORDS: Raphidascarididae, *H. reliquens*, *M. barbatus barbatus*, Algeria.

ABSTRACT

Mullus barbatus barbatus (Linnaeus, 1758) is a teleost fish with high commercial value in Algeria. 120 fish specimens of *Mullus barbatus barbatus* (L.) sampled from Algerian coasts were examined for their parasitic nematodes. Collected larvae were identified according to morphological and molecular analysis. Collected larvae belong to *Hysterothylacium reliquens*. This parasitic nematode is reported for the first time in Algerian coasts.

RÉSUMÉ: Premier signalement d'*Hysterothylacium reliquens* (Norris et Overstreet, 1975) parasitant *Mullus barbatus barbatus* (L.) sur la côte algérienne.

Mullus barbatus barbatus (Linnaeus, 1758) est un poisson téléostéen à haute valeur commerciale en Algérie. 120 poisson spécimens de *Mullus barbatus barbatus* (L.) prélevés sur les côtes algériennes ont été examinés pour leurs nématodes parasites. Les larves collectées ont été identifiées selon une analyse morphologique et moléculaire. Les larves collectées appartiennent à *Hysterothylacium reliquens*. Ce nématode parasite est signalé pour la première fois sur les côtes algériennes.

REZUMAT: O primă înregistrare a *Hysterothylacium reliquens* (Norris et Overstreet, 1975) ca parazit pe *Mullus barbatus barbatus* (L.) pe coasta algeriană.

Mullus barbatus barbatus (Linnaeus, 1758) este un pește teleostean cu o valoare comercială ridicată în Algeria. 120 de exemplare de pești *Mullus barbatus barbatus* (L.) prelevate de pe coastele algeriene au fost examinate pentru depistarea nematozilor paraziți. Larvele colectate au fost identificate în conformitate cu analiza morfologică și moleculară. Larvele colectate aparțin speciei *Hysterothylacium reliquens*.

INTRODUCTION

Fish are one of the most important group of organisms, as aquatic ecosystem's cornerstone structural and functional elements, as ecosystem service generators, as key components in building ecosystem resilience and increasing and speeding up productivity, as an important link in food chain dynamics and nutrient cycling as food, as a source of income and employment, and last but not least as diseases and parasites control control vectors (Alieva et al., 2023).

Mullus barbatus barbatus Linnaeus, 1758 is a teleost, marine, demersal fish with high commercial and gamefish value including in Algeria. This species infection by parasite nematodes, can affect its biological and ecological performances (feeding, growth, reproductive capacity, physiology, survival, etc.). Many studies were conducted on nematodes infecting marine fishes in Algerian coast: on *Trachurus trachurus* (Ramdani et al., 2022), on *Sardinella aurita* (Ramdani et al., 2020), on *Pagellus erythrinus* (Saadi et al., 2019), on *Phycis blennoides* and *Mullus surmuletus* (Hassani et al., 2015), on *Trachurus trachurus* (L.) and *Boops boops* (L.) (Ichalal et al., 2015).

The identification of parasitic nematodes larvae depending solely on their morphological features is not sufficient, thus, molecular methods are used for an accurate identification at the level species. Recent studies have proved that integrating DNA data is an essential tool in their identification (Costa et al., 2018; Simsek et al., 2018). Unfortunately, works considering these precise methods in the identification of nematodes are still rare in Algeria.

The goal of the present research is to report for the first time the presence of *H. reliquens* collected on *M. barbatus barbatus* from the eastern coast of Algeria, using morphological analysis combined with sequence data of selected of 28S of the ribosomal DNA (Zhao et al., 2017; Li et al., 2018). Moreover, the variation of infection parameters such as prevalence and mean abundance regarding season, host size and sex of this parasite was evaluated.

MATERIAL AND METHODS

Sampling method

A total of 120 specimens of *Mullus barbatus barbatus* (L.) were sampled (from local fisheries) from November 2017 to October 2018 from the Gulf of Bejaia (the eastern coast of Algeria). Sampled specimens were transported to the laboratory immediately after landing. Each fish specimen was dissected fresh and examined for its parasites and especially for parasitic nematodes (all parts of the body including organs) using both naked eye and with a binocular magnifying glass. For each fish specimen, the following parameters were recorded: total length (TL) in cm using an ichthyometer, total mass body weight (Wt), and gutted fish weight (We) in g using a digital scale (precision = 0.1 g). Subsequently, the sex was determined. Once the parasitic nematodes were collected, they were cleaned with distilled water and immediately preserved in pure ethanol (98%) for subsequent identification and for molecular analyses. The number and the location of each collected parasite were noted for each infected fish. They were photographed under a light microscopy. The parasites were identified using identification keys based on their morpho-anatomic characteristics. Prevalence (P%) and Mean intensity (MI) were calculated (Bush et al., 1997). These infection parameters (prevalence and mean intensity) were compared according to seasons, size classes, and sex using Chi square test (P%) and Anova test (MI), respectively.

Molecular analysis

All collected specimens (fifteen) of *Hysterothylacium reliquens* were selected for molecular analyses. Each specimen was prepared individually for DNA extraction according to PureLink® Genomic DNA Mini Kit extraction kit following the manufacturer's protocol. The rDNA fragment corresponding to the 28S sequence amplified using 28SrD1.2a (CCCSSGTAATTTAAGCATTA) and 28SB (TCGGAAGGAACCAGCTAC) primers (Whiting, 2002). The PCR protocol was as follows: preliminary denaturation for 13 min at 95°C, followed by 30 cycles from 40s at 95°C, 45 s at 55°C and 90 s at 72°C; and a final extension at 72°C for seven min. PCR products have been tested on 2% agarose gel. We also sequenced the gene of the cytochrome oxidase subunit "barcoding" mitochondrial 1 (COI), using the primers jgLCO1490 and jgHCO2198 (Whiting, 2002; Geller et al., 2013) following the protocol: preliminary denaturation for 13 min at 95°C, followed by five cycles of 40s at 95°C, 40s at 45°C and one min at 72°C; followed by 30 cycles of 40s at 95°C, 40s at 51°C, one min at 72°C and a final extension at 72°C for seven min. DNA sequencing was performed at Genewiz company, Germany.

Phylogenetic analysis

The sequences determined were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>). The analysis was performed on the Phylogeny.fr platform and comprised the following steps; sequences were aligned with MUSCLE (v3.7) configured for highest accuracy (MUSCLE with default settings). After alignment, ambiguous regions (i.e. containing gaps and/or poorly aligned) were removed with Gblocks (v0.91b) using the following parameters: minimum length of a block after gap cleaning: 5, positions with a gap in less than 50% of the sequences were selected in the final alignment if they were within an appropriate block, all segments with contiguous non conserved positions bigger than 8 were rejected, minimum number of sequences for a flank position: 55%. The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0). The TN93 substitution model was selected assuming an estimated proportion of invariant sites and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data. Reliability for internal branch was assessed using the aLRT test (SH-Like). Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3).

RESULTS

Fifteen larvae of *Hysterothylacium reliquens* were collected. The majority of the collected parasite specimens were encysted or free larvae dwelling in the organs of the abdominal cavity. Our collected specimens of *Hysterothylacium reliquens* (Fig. 1) present relatively small body length regarding the previous works. This difference may be related to the host species, the studied geographical area and the low number of collected species (may be large species were not sampled).

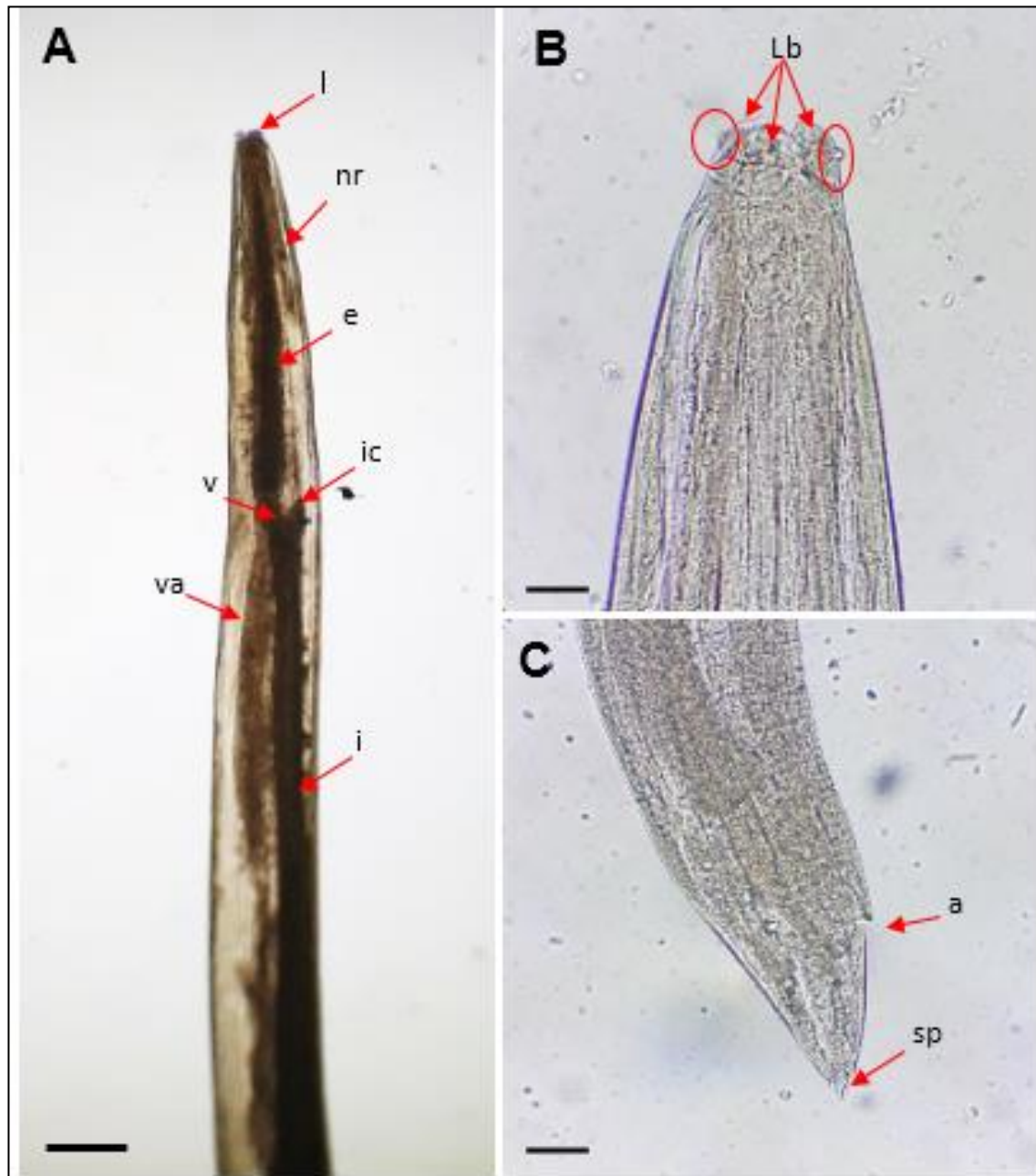


Figure 1: Morphology of *H. reliquens* collected on *M. barbatus barbatus*.

A: anterior part showing lips (l), nerve ring (nr), oesophagus (e), intestinal caecum (ic), ventriculus (v) ventricular appendix (va), and intestine (i).

B: dorsal labium showing labia, see red arrows (Lb), and papillae (see red circle).

C: posterior end showing anus (a) and spinous tail (sp).

Scale bars A: x10 = 100 μ m. B, C: x40 = 50 μ m).

Whereas, the oesophagus length of our specimens (of *H. reliquens*) is not different to the measurement reported on specimens from Iraq by Zhao et al., (2017) and Ghadam et al., (2017) (Tab. 1). Measured lengths of ventriculus, ventricular appendix and intestinal caecum are approximately similar to the measurements reported by Norris and Overstreet (1975) on specimens collected from Mississippi and Florida (Tab. 1). The tail length is close to the measurement reported on specimens from Iraq Ghadam et al. (2017) (Tab. 1).

Table 1: *Hysterothylacium reliquens* measurements and morphometric comparisons between our specimens with the previous studied specimens (measurements in mm).

Characteristics features /References	Present study	Norris and Overstreet (1975)	Deardorff and Overstreet (1980)	Petter and Sey (1997)	Zhao et al. (2017)	Ghadam et al. (2017)
Hosts	<i>Mullus barbatus barbatus</i>	<i>Archosargus probatocephalus</i> , <i>Chilomycterus schoepfi</i> , <i>Halichoeres bivittatus</i> , <i>Micropogon undulatus</i>	<i>Micropogonias undulatus</i>	<i>Acanthopagrus berda</i> , <i>Epinephelus tauvina</i> , <i>Elisha elongate</i> , <i>Polydavtylus sextarius</i> , <i>Plotosus anguillaris</i> , <i>Pseudorhombus arsius</i> , <i>Synaptura orientalis</i> , <i>Therapon puta</i> , <i>Trachinotus blochi</i>	<i>Brachirus orientalis</i>	<i>Otolithes ruber</i> <i>Brachirus orientalis</i>
Localisation	Algeria	Mississippi, Florida	Mexico, Atlantic Ocean	Kuwait City	Basrah, Iraq	Iraq
Number of specimens	15	23	30	12	11	8
Body length	15.36 ± 1.45	2–79	25.0–40.0 (21.0–44.0)	40.28 (18.00–54.75)	25.1 (12.6–38.1)	23.81 (17.23–34.78)
Oesophagus length	3.97 ± 1.78	2.2–9.7	3.30–4.50 (2.90–5.00)	4.33 (2.10–6.00)	3.37 (1.47–4.73)	2.57 (1.90–3.78)
Ventriculus length	0.07 ± 0.4	0.06–0.39 × 0.14–0.53	/	/	0.17 (0.07–0.30) × 0.21 (0.10–0.30)	0.16 (0.14–0.20)
Ventricular appendix length	0.84 ± 0.62	0.940–3.360	1.20–1.70 (1.00–2.90)	1.71 (0.89–2.70)	1.46 (0.74–2.00)	1.16 (0.98–1.40)
Intestinal caecum length	0.25 ± 0.17	0.255–1.400	0.32–0.60 (0.39–0.79)	0.675 (0.36–1.00)	0.42 (0.20–0.65)	0.30 (0.28–0.37)
Tail length	0.23 ± 0.12	0.120–0.235	0.12–0.19 (0.37–0.57)	2.07 (0.20–0.25)	/	0.19 (0.17–0.21)

Molecular characterization and phylogenetic analysis

Fifteen samples were used for genetic studies, but only thirteen sequences suitable for analysis were obtained. We choose one sequence and deposited on GenBank under the accession number (MZ320999). The sequence of *H. reliquens* obtained herein ranged from to 1089 bp in length. According to BLASTn search, the query 28S rDNA sequence shared over 98.11% identity with those isolates from Iraq (KX815307, KX815306, KX815305, KX815304, KX815303, KX815302, KX815301, KX815300), and to other sequence of various geographical area of the world, were collected from GenBank and used to build a 14 sequences alignment with *Raphidascaris acus* (KT633862) as outgroup (Tab. 2).

The phylogenetic tree showed strong statistical support for the *H. reliquens* branch with a bootstrap value of 82% with *H. reliquens* from Mexico (MK558800) (Fig. 2). The obtained results of phylogenetic analyses converge with those carried out on the morphometric analyses. The phylogenetic analyses show that our specimens are closer to specimens from Mexico (Vidal-Martinez et al., 2019 (MK558800)) and support the similitude with sequenced the 28S region of *H. reliquens* among various host from around the world.

Table 2. GenBank accession numbers, hosts, locality and references for *Hysterothylacium* species from various geographical area used for multiple alignment and phylogenetic tree construction.

GBan	Organism	Host	Locality	References
MZ320999	<i>Hysterothylacium reliquens</i>	<i>Mullus barbatus barbatus</i>	Algeria	Present study.
MK558800	<i>Hysterothylacium reliquens</i>	<i>Syacium papillosum</i>	Mexico	Vidal-Martínez et al. (2019)
KU527061	<i>Hysterothylacium reliquens</i>	<i>Rhomboplites aurorubens</i>	USA	Claxton et al. (2017)
MF094283	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	China	Li et al. (2018)
KX815307	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815306	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815305	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815304	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815303	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815302	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815301	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
KX815300	<i>Hysterothylacium reliquens</i>	<i>Brachirus orientalis</i>	Iraq	Zhao et al. (2017)
U94762	<i>Hysterothylacium reliquens</i>	<i>Micropogonias undulatus</i>	USA	Nadler and Hudspeth (1998)
KF601900.1	<i>Hysterothylacium liparis</i>	<i>Liparis tanakae</i>	China	Guo et al. (2014)

Table 2 (continued): GenBank accession numbers, hosts, locality and references for *Hysterothylacium* species from various geographical area used for multiple alignment and phylogenetic tree construction.

JX028282.1	<i>Hysterothylacium zhoushanensis</i>	<i>Pseudorhombus oligodon</i>	East China Sea	Li et al. (2012)
AF115571.1	<i>Hysterothylacium auctum</i>	<i>Zoarces viviparus</i>	Southern	Szostakowska et al. (2001)
JX845137.1	<i>Hysterothylacium aduncum</i>	<i>Zoarces viviparus</i>	Denmark	Haarder et al. (2013)
KF736944.1	<i>Hysterothylacium fabri</i>	<i>Liparis tanakae</i>	China	Guo et al. (2014)
KX084795.1	<i>Hysterothylacium sinense</i>	<i>Conger myriaster</i>	China	Li et al. (2016)
LT717077	<i>Hysterothylacium persicum</i>	<i>Marine fish</i>	Iraq	Ghadam et al. (2017)
JQ520159.1	<i>Hysterothylacium longilabrum</i>	<i>Marine fish</i>	South China Sea	Li et al. (2012)
JX982129.1	<i>Hysterothylacium thalassini</i>	<i>Marine fish</i>	South China Sea	Liu et al. (2012)
MH211527.1	<i>Hysterothylacium amoyense</i>	<i>Lophius litulon</i>	China	Zhang et al. (2018)
JN005769.1	<i>Hysterothylacium</i> sp. PB-2010	<i>Pagellus bogaraveo</i>	Portugal	Hermida et al. (2012)
HF680324.1	<i>Hysterothylacium rigidum</i>	<i>Lophius piscatorius</i>	Ireland	Canas et al. (2015)
KP419719.1	<i>Hysterothylacium</i> sp. Type IV-A LL-2015	<i>Polydactylus sextarius</i>	China	Zhao et al. (2015)
MF276917.1	<i>Hysterothylacium deardorffoverstreetorum</i>	<i>Genypterus brasiliensis</i>	Brazil	Di azevedo and Iniguez (2015)
HE862225.1	<i>Hysterothylacium australe</i>	<i>Marine fish</i>	Australia	Shamsi (2016)
HE862229.1	<i>Hysterothylacium kajikiae</i>	<i>Marine fish</i>	Australia	Shamsi (2016)
HE862230.1	<i>Hysterothylacium brucei</i>	<i>Marine fish</i>	Australia	Shamsi (2016)
MW456073.1	<i>Hysterothylacium incurvum</i>	<i>Xiphias gladius</i>	Mediterranean Sea	Mattiucci et al. (2005)
MW456072.1	<i>Hysterothylacium corrugatum</i>	<i>Xiphias gladius</i>	Mediterranean Sea	Mattiucci et al. (2005)
AY603539.1	<i>Hysterothylacium bidentatum</i>	<i>Marine fish</i>	Poland	Kijewska et al. (2004)
KT633862	<i>Raphidascaris Acus</i>	<i>Anguilla anguilla</i>	Turkey	Simsek et al. (2018)

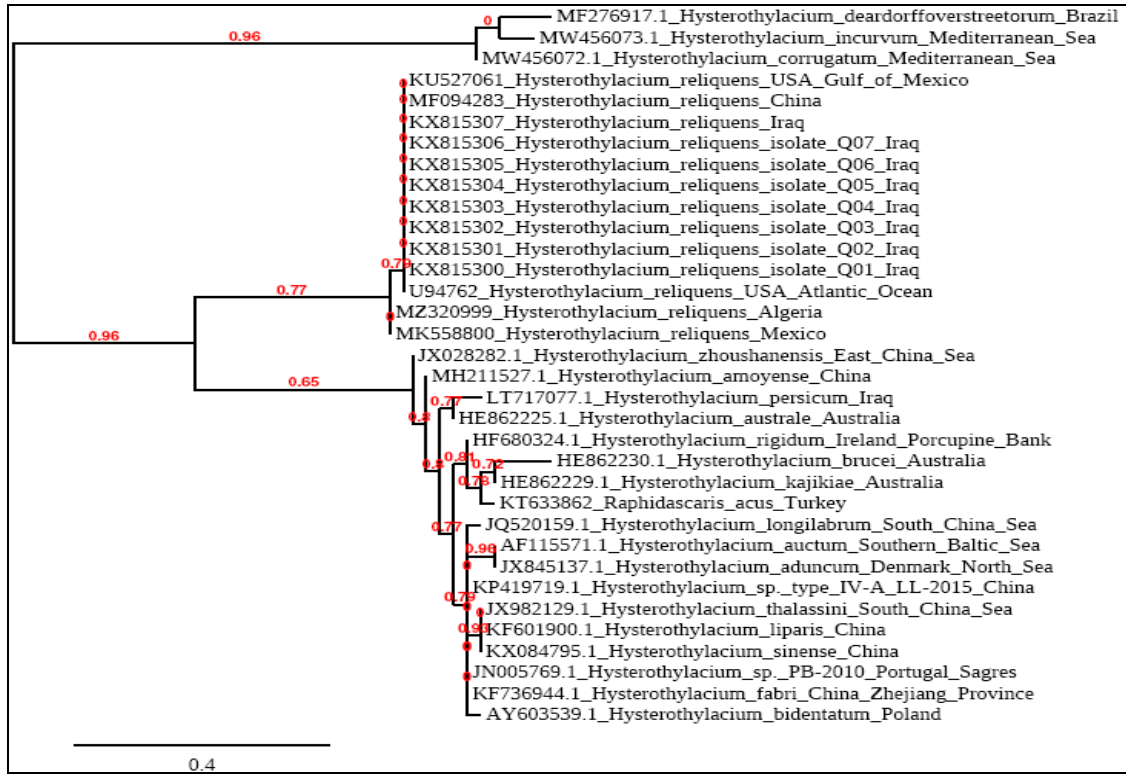


Figure 2: Phylogenetic relationships between *H. reliquens* from Algerian coast and other species recorded from the same genus (*Hysterothylacium*) as inferred by maximum likelihood tree obtained from the sequenced the 28S region. The scale bar indicates the distance in substitutions per nucleotide. *Raphidascaris acus* was used as outgroup taxa.

Infection parameter: prevalence and mean intensity infection

Our results show that *M. barbatus barbatus* presents a high prevalence infection ($P = 92\%$, 110 fish specimens were infected) and mean intensity infection ($MI = 4.02$ parasites/infected fish). All size classes of *M. barbatus barbatus* were parasitized by nematodes. Specimens over 15 cm present a high prevalence ($P = 100\%$) (Fig. 3). The infection parameters of *M. barbatus barbatus* are higher in autumn with 97.92% of prevalence infection and 5.88 parasite/infected fish of mean intensity infection. The lowest prevalence ($P = 82.75\%$) and mean intensity infection ($MI = 3.2$ parasite/infected) were recorded in winter.

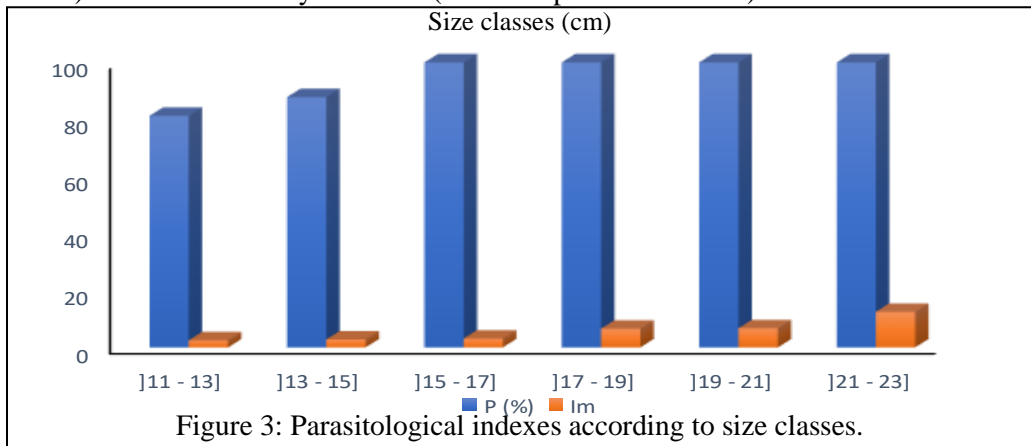


Figure 3: Parasitological indexes according to size classes.

No significant differences were observed in the prevalence infection between seasons, size classes and both sexes ($P > 0.05$) (Tab. 3). Significant differences were recorded between the mean intensity infection of size classes (Tab. 3). However, no significant differences were observed in the mean intensities between seasons and sex ($P = 0.9112 > 0.05$; $P = 0.86 > 0.05$) respectively (Tab. 3).

Table 3: Parasitological indexes according to seasons, size classes, and sex; n: number of examined fish; P: prevalence (%), Im: Mean intensity and $p = p\text{-value} < 0.05$.

Seasons	Autumn	Winter	Spring	Summer	Sizes classes	(11-13)	(13-15)	(15-17)	(17-20)	Sex	Females	Males
n	44	29	26	21	n	32	41	25	14	n	63	57
P	97.9 2	82.7 5	96.1 5	95.2 3	P	81.2	87.8	100	100	P	93.6	89.5
P	0.0708				p	0.31				p	0.62	
Im	5.88	3.2	1.44	3.35	Im	2.5	2.8	3.08	6.57	Im	3.86	4.2
P	0.9112				p	2.372*10 ⁻¹⁴				p	0.86	

DISCUSSION

In the present study, we used morphometric and molecular methods, for an accurate identification of specimens (larvae) of *Hysterothylacium reliquens* collected for the first time on *Mullus barbatus barbatus* from Algerian marine coasts.

The morphological analysis of the collected specimens of *H. reliquens* is similar (especially for internal features) to the original description of the collected specimens by Norris and Overstreet (1975) and the description performed later by Deardorff and Overstreet (1980) and Petter and Cabaret (1995) on specimens from the Gulf of Mexico and Morocco, respectively. The main characteristic features including the morphology of the oesophagus, lips, the number and arrangement of the labial papillae on the lips, the shape of tail were similar to the specimens collected from the Persian Gulf off Kuwait and Iraq by Petter and Sey (1997), Al-Salim and Ali (2010), and Ghadam et al., (2017) respectively. The present study confirms the existence of morphological similarities in comparison with specimens described by El-Sayed et al., (2004) and Simsek et al., (2018) from the Mediterranean Sea (Egypt and Turkey, respectively). It seems that this parasite species has a wide geographical distribution across continents. Morphological features of the studied specimens of *H. reliquens* are similar to those reported in the previous studies as suggested by Zhao et al., (2017).

Our collected specimens of *Hysterothylacium reliquens* present relatively small body length regarding the previous works. Whereas, the oesophagus length of our specimens (of *H. reliquens*) is not different to the measurement reported on specimens from in Iraq by Zhao et al., (2017) and Ghadam et al., (2017) (Tab. 1). The variability in ventriculus length of the examined specimens (*H. reliquens*) may be related to the host size on which they were collected (small or large host specimens), fish specimens maybe sampled in different period or different sites, and finally, the low number of examined specimens. The diagnostic characters of *H. reliquens* (Williams and Bunkley-Williams, 1996) allow us more accurate morphological identification taking into the count other specific characters: the minute alae begin just posterior to the lips; the lips are longer than wide, constricted at their midpart, and lack interlabial grooves; the tip of the tail is covered with numerous minute spines. However, this

variability in the morphometric characters need particularly in larvae parasitic nematode, more precise method (molecular method) in order to confirm if they are enclosed to the same species. The tail length is close to the measurement reported on specimens from Iraq Ghadam et al. (2017) (Tab. 2).

All collected specimens of *H. reliquens* were sequenced taking into the count the general similarities in their morphological features although the variability noticed in ventriculus. Given the range in sizes of *H. reliquens* between the present study and previous studies (Tab. 2). We hypothesis the existence of different species. Regarding the difficulties of an accurate identifications of nematode larvae on the basis of their morphological and morphometric characters (low differentiation of their morphology, geographical variability of specimens and low number of collected specimens in different areas), many researchers suggest molecular methods for the taxonomic study of parasitic nematodes (Ferris, 1994; De Ley, 2006; Ichallal, 2015). Using this method, we can easily verify if there are low or high genetic differences. Costa et al. (2018) reported that specimens from close areas present low genetic differences; however, specimens from distant areas present high genetic differences.

The obtained results of phylogenetic analyses converge with those carried out on the morphometric analyses. The phylogenetic analyses show that our specimens are closer to specimens from Mexico (Vidal-Martinez et al., 2019 (MK558800)) and support the similitude with sequenced the 28S region of *H. reliquens* among various host from around the world.

Our results revealed that *M. barbatus barbatus* is highly infected by parasitic nematodes. Total prevalence is higher than that reported by Saadi et al. (2020) in the same host fish *M. barbatus barbatus* from Algerian coast and also higher than that reported by Ichalal et al. (2015) in *Boops boops* and *Trachurus trachurus*. The high infection by parasitic nematodes is may be attributed to intermediate hosts (like benthic crustaceans) that could enhance infection. The feeding behavior of marine fishes is the mean way enhancing parasitism (Ichalal et al., 2015). Fluctuation in the infection levels may be related to many environmental factors (temperature, prey availability, feeding behaviour of the host, etc.).

H. reliquens is a common parasite of a wide range of fish species. This parasitic nematode was collected on various fish species (Euryxenic specificity) from different geographical areas worldwide (especially in Kuwait and Mexico) (Tab. 4).

Table 4: Check-list of *H. reliquens*'s hosts from various geographical areas.

Parasite	Host species	Study area	References
<i>Hysterothylacium reliquens</i> (Norris and Overstreet, 1975)	<i>Archosargus probatocephalus</i> (Walbaum, 1792)	Northern Gulf of Mexico and eastern USA (north western Atlantic Ocean)	Norris and Overstreet (1975)
	<i>Chilomycterus schoepfii</i> (Walbaum, 1792)		
	<i>Halichoeres bivittatus</i> (Bloch 1791)		
	<i>Micropogonias undulatus</i> (Linnaeus, 1766)	Mexico (Atlantic Ocean)	Deardorff and Overstreet (1980)
	<i>Opsanus beta</i> (Goode and Bean, 1880)		
	<i>Micropogonias undulatus</i> (Linnaeus, 1766)		
	<i>Pagellus acarne</i> (Risso, 1827)	Morocco (eastern Atlantic Ocean)	Petter and Cabaret (1995)
	<i>Microchirus azevia</i> (Capello, 1867)		

Table 4 (continued): Check-list of *H. reliquens*'s hosts from various geographical areas.

Parasite	Host species	Study area	References
<i>Hysterothylacium reliquens</i> (Norris and Overstreet, 1975)	<i>Acanthopagrus berda</i> (Forsskål, 1775) <i>Epinephelus tauvina</i> (Forsskål, 1775) <i>Ilisha elongata</i> (Bennett, 1830) <i>Plotosus anguillaris</i> (Bloch, 1794) <i>Polydactylus sextarius</i> (Bloch and Schneider, 1801) <i>Pseudorhombus arsius</i> (Hamilton 1822) <i>Synaptura orientalis</i> (Bloch and Schneider, 1801) <i>Terapon puta</i> Cuvier 1829 <i>Trachinotus blochii</i> (Lacepède, 1801)	Kuwait (northern Indian Ocean)	Petter and Sey (1997)
	<i>Micropogonias undulatus</i> (Linnaeus, 1766)	USA (Atlantic Ocean)	Nadler and Hudspeth (1998)
	<i>Mullus barbatus barbatus</i> (Linnaeus, 1758)	Egypt (port said province) Mediterranean Sea	El-Sayed et al. (2004)
	<i>Trichiurus lepturus</i> (Linnaeus, 1758) <i>Cynoglossus arel</i> (Bloch and Schneider, 1801) <i>Lethrinus nebulosus</i> (Forsskål, 1775)	Iraq	Al-Salim and Ali (2010)
	<i>Brachirus orientalis</i> (Bloch and Schneider, 1801)	Iraq	Zhao et al. (2017)
	<i>Otolithes ruber</i> (Bloch and Schneider, 1801) <i>Brachirus orientalis</i> (Bloch and Schneider, 1801)	Iraq (Indian Ocean)	Ghadam et al. (2017)
	<i>Boops boops</i> (Linnaeus, 1758)	Turkey (Mediterranean Sea)	Simsek et al. (2018)
	<i>Mullus barbatus barbatus</i> (Linnaeus, 1758)	Algeria	Present study

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BIOLOGICAL DIVERSITY OF SULA RIVER (UKRAINE) UNDER DIFFERENT HYDROLOGICAL CONDITIONS

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ABSTRACT

Hydrobiological research of the main components of the biota of the lower reaches of the Sula River was conducted. The river flows into the Kremenchuk Reservoir. Zoobenthos, zooperiphyton, vascular water plants, and ichthyofauna in lotic and lentic microbiotopes were studied. Species and communities of protected status were stated. It was shown, that a significant share (about 30%) consists of rheophilic species. 28 species of fish were registered in the studied area, of which eight are invasive species and 10 are under protection. The biotopic distribution of different fish species, and the intensity of the downstream migration of juvenile fishes in spatial, temporal, and species aspects were estimated. It was identified, that about 12 million young fish migrate from the researched part of the Sula River to the Kremenchuk reservoir during the season.

RÉSUMÉ: Diversité biologique de la rivière Sula (Ukraine) dans différentes conditions hydrologiques.

Des études hydrobiologiques des principales composantes du biote du cours inférieur de la rivière Sula, où la rivière se jette dans le réservoir de Kremenchuk, ont été menées. La végétation aquatique supérieure, les groupes zoobenthos et périphyton, les plantes vasculaires aquatiques et les roches, l'ichtyofaune, des microbiotopes lotiques et lenticques ont été étudiés. Les espèces et les groupes d'hydrobiontes à statut protecteur ont été déterminés. Il est montré qu'une partie importante (environ 30%) est constituée d'espèces rhéophiles. La composition des espèces et la structure des groupes végétaux, ainsi que les groupes de zoobenthos, d'épiphyton et d'épilithon ont été déterminées. 28 espèces de poissons ont été recensées dans la zone étudiée, dont huit invasives et 10 sous protection. La distribution des biotopes de diverses espèces de poissons a été déterminée, l'intensité de la migration descendante des jeunes poissons a été calculée dans les aspects spatiaux, temporels et spécifiques. Il a été établi qu'au cours de la saison, environ 12 millions de spécimens de jeunes poissons migrent de la rivière Sula dans le réservoir de Kremenchuk, au niveau de la zone d'étude.

REZUMAT: Diversitatea biologică a râului Sula (Ucraina) în diverse condiții hidrologice.

Au fost efectuate cercetări hidrobiologice ale principalelor componente ale biotei din cursurile inferioare ale râului Sula. Râul se varsă în lacul de acumulare Kremenchuk. Au fost studiate zoobentosul, zooperifitonul, plantele de apă vasculare și ihtiofauna în microbiotopii lotici și lentici. Au fost precizate speciile și comunitățile cu statut protejat. S-a demonstrat că o pondere semnificativă (aproximativ 30%) este formată din specii reofile. În zona studiată au fost înregistrate 28 de specii de pești, dintre care opt specii invazive și 10 aflate sub protecție. Au fost estimate distribuția biotopică a diferitelor specii de pești și intensitatea migrației în aval a peștilor juvenili din punct de vedere spațial, temporal și a speciei. S-a identificat că aproximativ 12 milioane de pești tineri migrează din partea cercetată a râului Sula către lacul de acumulare Kremenchuk în timpul sezonului.

INTRODUCTION

The vast majority of rivers in Ukraine are regulated by dams at a considerable length. There are very few areas with an unmodified, natural lotic regime, and they are under threat of further regulation or anthropogenic transformation.

Hydrobiological researches of the sections with a natural flow are very important for the investigation of river biota, its biodiversity, which is one of the main characteristics of a “good ecological status”. And one of the important factors in the formation of biodiversity is the hydrological regime.

The Sula River in the research area is characterized by an almost natural state, with the clearly expressed riverbed, additional floodplain waterbodies, and well-developed floodplain, although polluted by discharges from the upstream located settlements.

Due to its structure and location, the lower reaches of the Sula River serves as an area of mass spawning and feeding of fishes, which migrate here from the Kremenchuk reservoir.

Recently, work on the engineering and building of hydraulic structures on small rivers has noticeably intensified in Ukraine. This makes it necessary to study the current ecological state of river ecosystems prior to the start of such construction. In particular, there was a project for the building of a hydraulic construction in the lower reaches of the Sula River.

This research was carried out to assess the possibility of building sluice-regulator construction in the considered river section, which will significantly change its hydrological regime.

MATERIAL AND METHODS

The whole length of the Sula River is 363 km, and the river basin is 19,600 km² (State Water Cadastre, 2012). A 10 km long section of the lower Sula River was studied (10-20 km from the confluence of the river into the Sula Bay of the Kremenchuk Reservoir).

The research was carried out in June-July 2018 in the lower reaches of Sula River (from estuary of the Orzhytsia River to the Gorikhove village), on the riverbed, oxbows, and bays (Figs. 1a, b). The riverbed is considered a lotic biotope, and the oxbow, and bays – as a lentic biotope. The research covered vascular aquatic plant communities, invertebrates (benthos and periphyton), and ichthyofauna.



Figure 1a: View of the Sula River in the research area, sampling hydrobiological materials.



Figure 1b: View of the Sula River in the research area, collected fish fry.

We investigated vascular aquatic plants (VAP), identified the species composition, overgrowing features of the VAP in different parts of the waterbodies and its further mapping of the plant cover (Arsan et al., 2006).

Benthos samples were taken by the Petersens bottom grab (capture area = 0.025 m²). Zooperiphyton samples from submerged VAP (with weights of 0.5 kg) and stones, epiphyton (on VAP) and epilyton (on stones) were distinguished. The samples of invertebrates were preserved in 4% formaldehyde.

Ichthyological material was collected using the ichthyological net into the coastal aquatic plant thickets and by the fishing rod in the open parts of the river. Fish larvae were collected in the spawning sites. A drift of the commercial fishes' juveniles was studied by setting daily experiments using the drift trap (inlet orifice area – 0.24 m², 1.5 m long cone from the mill sieve N 12). The exposure lasted for 30 min, the material was taken every two hours.

Concentration of fish juveniles per volume unit (C_{vol} , ind/m³) was calculated by the formula:

$$C_{vol} = Q/Svt$$

where Q – number of fish juveniles in the trap (ind.), S – the inlet orifice area (m²), v – flow velocity (m/s), t – exposition (s).

Total drift of fish juveniles was calculated by multiplying the found value by the river water yield. Water yield was set according to the water-meter post located upstream (in the town of Lubny).

Totally 32 samples of juveniles and adult fish were processed. The species of young fish and the development stage of larvae were determined by Koblitskaya (1981). Rare species were released into the river alive. In addition, the local fisherman were interviewed, results were considered reliable if the fish name was repeated more than five times.

RESULTS AND DISCUSSION

The formation of aquatic plant communities is mostly determined by the hydrological regime (presence and speed of flow), and the type of biotope. The formation of **vascular aquatic plants** in the researched area took place under the influence of the construction of the Kremenchuk Reservoir in 1961, which caused significant degradation of natural phytophilous complexes, and in some cases their complete disappearance. Subsequently, their gradual restoration took place, and today there is a complete inventory of flora and fauna of this section of the river with justification for the creation of protected areas here.

At the same time, the lower reaches of the Sula River have undergone a relatively small anthropogenic transformation, there are preserved natural floral complexes, typical of such habitats. In modern conditions, the main riverbed is surrounded by a thick strip of floodplain reed and cattail thickets, the width of which in some places reaches 150-500 m, and in some places even more. Dominant was *Phragmites australis* (Cav.) Trin. Ex. Staud, as well as *Typha angustifolia* L. and *T. latifolia* L. These thickets also included *Glyceria maxima* (Hartm.) Holmb., *Acorus calamus* L., *Alisma plantago-aquatica* L., *Oenanthe aquatica* (L.) Poir., *Iris pseudacorus* L., *Carex riparia* Curt., *Carex acuta* L. and *C. acutiformis* Ehrh., *Equisetum palustre* L., *Mentha aquatica* L. and *Cicuta virosa* L.

Aquatic plants in the riverbed are situated in belts depending on the depth. The first belt is formed by helophytes, the composition of which is almost similar to the composition of the coastal strip. The second belt is formed by plants with floating leaves on the water – *Lemna trisulca* L., *Lemna minor* L., and *Spirodela polyrrhiza* L. At depths of 0.9-1.2 m on muddy-sandy bottom sediments in this zone, there are also *Nuphar lutea* (L.) Smith., *Nymphaea alba* L., *Hydrocharis morsus-ranae* L. At a depth of 1.2-1.5 m on silty soils, thickets of submerged plants are formed, consisting of *Ceratophyllum demersum* L., *Potamogeton perfoliatus* L., *P. crispus* L., *P. lucens* L. and *Myriophyllum spicatum* L. Areas of the riverbed with a depth of more than three m are free of thickets. All bays and oxbows within the research area are completely overgrown. As a rule, there are also three ecological groups of plants: helophytes, plants with floating leaves and submerged. In general, the flora of channel areas is richer than the flora of lentic biotopes, combining both species typical of non-flowing areas and rheophilic plant species, the share of which is about 8%.

The rare part of flora confirmed during the research includes one species of vascular plant – *Salvinia natans* L. – a holarctic plurizonal relict (tertiary) species that occur in fragments throughout the study area, sometimes forming communities. This species is protected by the Berne Convention and listed in the Red Book of Ukraine.

Three identified formations are listed in the Green Book of Ukraine: 1) communities of the *Nuphareta luteae*; 2) communities of the *Nymphaeeta albae*; 3) communities of the *Salvinieta natantis*, all occurred in small areas.

The communities are protected by the Berne Convention and the Habitats Conservation Directive in the researched area:

- Fen-sedge beds;
- Water soldier rafts;
- Frogbit rafts;
- Magnopotamion or Hydrocharition type vegetation of natural eutrophic lakes.

The analysis of our data in comparison with the data of previous studies shows that the plant communities of the researched area of the river are in a relatively stable state, which has developed here for a long time after the creation of the reservoir (Klestov et al., 2016).

In communities of **aquatic invertebrates** were identified 67 LIT (lower identified taxa), in the epiphyton – 33 LIT, in the epilithon – 39 LIT, in the zoobenthos – 34 LIT (Fig. 2). The number of taxonomic groups in the communities was similar – 10-12. The zoobenthos was distinguished by a large number of species of Oligochaeta and Chironomidae, epiphyton – larvae of Insecta and Gastropoda, three species of Spongia were noted only in the epilithon. The diversity number of taxa in the taxonomic groups was quite high: in epiphyton – 2.92, epilithon – 3.06, and zoobenthos – 2.33 LIT. One species (medicinal leech *Hirudo medicinalis* (L.)), which was found in epiphyton, was included in the Red Book of Ukraine (Red Book, 2009).

The applying of cluster analysis (Bray-Curtis Cluster Analysis) confirmed the low similarity of the zoobenthos taxonomic composition with other communities (the similarity was 32%), which stood out in a separate cluster. At the same time, the taxonomic composition of the epiphyton and epilithon were similar (the similarity was 80%).

It is necessary to remark, that in the list of aquatic invertebrates is a number of rheophilic and oxyphilic species, including larvae of Ephemeroptera, Trichoptera, *Simulium* sp., *Rheotanytarsus exiguus* Johannsen (Chironomidae), *Tubifex newaensis* (Michaelsen) (Tubificidae). Gastropods *Theodoxus fluviatilis* L., which prefer stony substrates, are also indicators of rheophilic conditions. The relatively large taxonomic richness of Trichoptera takes our attention – six species were found there: *Hydroptila tineoides* Dalman, *Leptocerus tineiformis* Curtis, *Neureclipsis bimaculata* (L.), *Hydropsyche angustipennis* (Curtis), *Oecetis lacustris* (Pictet), *O. furva* (Rambur).

In general, about 24% of the registered taxa of aquatic invertebrates are characteristic of rheophilic living conditions. Changing the lotic conditions to limnic ones can lead to a reduction in the part of these species or their complete loss from the hydrobiocenoses.

The taxonomic composition of aquatic invertebrates allows us to assess the water quality in the lower Sula River. According to the Trent biotic index (TBI) (Woodiwiss, 1964) (recommended by the EU Water Framework Directive), water in this area can be classified as Class II – “Pure” (TBI = 7).

Ichthyofauna of the lower reaches of the Sula River comprised 28 fish species of the eight fish families: Cyprinidae – 17, Gobiidae – three, Percidae, and Cobitidae – two species each, and families of Siluridae, Esocidae, Gasterosteidae, and Syngnathidae comprised one species each. Juvenile fishes of 15 species were found.

The major part of caught fishes belong to the river type (dace *Leuciscus leuciscus* (Linnaeus, 1758), chub *Squalius cephalus* (Linnaeus, 1758), ide *Idus idus* (Linnaeus, 1758), asp *Aspius aspius* (Linnaeus, 1758), and spiny loach *Cobitis taenia* Linnaeus, 1758); and river-lake type (bleak *Alburnus alburnus* (Linnaeus, 1758), bream *Abramis brama* (Linnaeus, 1758), blue bream *Ballerus ballerus* (Linnaeus, 1758), silver bream *Blicca bjoerkna* (Linnaeus, 1758), Chinese chebachok *Pseudorasbora parva* (Temminck and Schlegel, 1846), silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), carp *Cyprinus carpio* Linnaeus, 1758, catfish *Silurus glanis* Linnaeus, 1758, and pike perch *Sander lucioperca* (Linnaeus, 1758)).

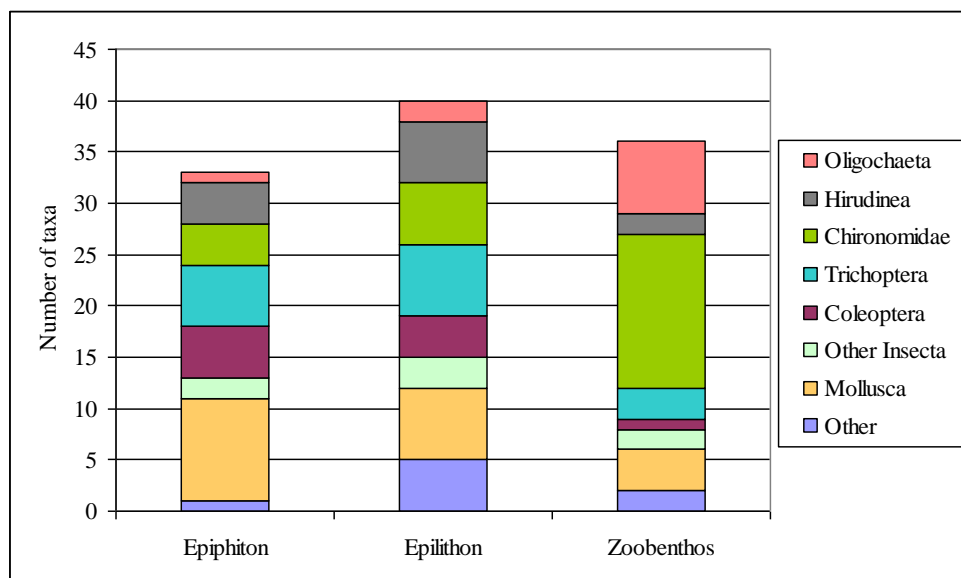


Figure 2: Taxonomic structure of aquatic invertebrate communities in the the lower reaches Sula River, July 2018. The “Other Insecta” included – Ephemeroptera, Simuliidae, Culicidae, Odonata, “Others” – Spongia, Turbellaria sp., Nematoda sp., Crustacea (*Asellus aquaticus* L.).

Fish of river type and river-lake type were respectively 17.9% and 32.1% of the total fish species number, adult specimens were found mainly in the free of plants and flowing sections of the river, of depth up to 4-5 m, with the flow velocity 0.5-0.7 m/s and sand bottom.

The Ponto-Caspian species round goby *Neogobius melanostomus* Pallas, 1814 and monkey goby *Neogobius fluviatilis* (Pallas, 1814) also favored lotic conditions (7.1%) and were found in free coastal areas of the river with overgrown with VAP (up to 35% projective water surface cover in the researched area) with clean sand bottom and stream up to 0.5 m/s.

In coastal biotopes, areas covered by VAP, up to 1.5 m deep with sandy and silty bottom, with the flow velocity of 0.2-0.5 m/s there were mainly fish species representatives of lake type (pearl roach *Scardinius erythrophthalmus* (Linnaeus, 1758), amur bitterlink *Rhodeus amarus* (Bloch, 1782)) lake-river type (roaches *Rutilus rutilus* (Linnaeus, 1758), crucian carp *Carassius carassius* (Linnaeus, 1758) and gibel carp *C. gibelio* (Linnaeus, 1758), tench *Tinca tinca* (Linnaeus, 1758), loach *Misgurnus fossilis* (Linnaeus, 1758), pike *Esox lucius* Linnaeus, 1758 and perch *Perca fluviatilis* Linnaeus, 1758). This type of species was 7.1 and 25.0% of the total number of species. Such Ponto-Caspian species, as western tubenose goby *Proterorhinus semilunaris* (Heckel, 1837), three-spined stickleback *Gasterosteus aculeatus* Linnaeus, 1758 and pipefish *Syngnathus nigrolineatus* Eichwald, 1831) preferred lentic conditions (10.7%) and were found in the same habitats as lake and lake-river species.

15 young fish species were registered in the samples. Nine species of fish were observed in the samples in June (Tab. 1).

Table 1: The ratio of young fish species from the lower stream of the Sula River in different biotopes, %.

Species	June		July	
	lotic biotope	lentic biotope	lotic biotope	lentic biotope
<i>Rutilus rutilus</i> (L.)	10.9	41.2	6.2	2.5
<i>Scardinius erythrophthalmus</i> (L.)	1.0	1.2	12.5	25.0
<i>Alburnus alburnus</i> (L.)	79.2	27.1	54.3	23.7
<i>Leucaspius delineatus</i> (Heckel)	–	–	–	2.5
<i>Blicca bjoerkna</i> (L.)	–	18.8	4.9	20.1
<i>Ballerus ballerus</i> (L.)	1.0	–	–	–
<i>Abramis brama</i> (L.)	2.1	2.4	–	0.5
<i>Rhodeus amarus</i> (Bloch)	3.7	4.7	22.2	21.2
<i>Cobitis taenia</i> L.	–	4.7	–	1.8
<i>Esox lucius</i> L.	–	–	–	0.9
<i>Pungitius platygaster</i> (Kessler)	–	–	–	0.9
<i>Perca fluviatilis</i> (L.)	2.1	–	–	–
<i>Proterorhinus semilunaris</i> (Heckel)	–	–	–	0.9

Seven young fish species were registered in the samples in the clean areas, among which the larvae of *A. alburnus* were predominant – 79.2%, on average 95 fish/m² (lim 5-210). The youth of the species differed in the widest range of development stages: from C1 to E (Tab. 2). Part of the youth of roach *R. rutilus* was much smaller and was 10.9%, which was 47 fish/m² (lim 0-60). Larvae of bream *A. brama*, blue bream *B. ballerus*, pearl roach *S. erythrophthalmus*, roach *R. amarus* and perch *P. fluviatilis* were represented by the separate fish, their part was from 1.0 to 3.7%, and quantity – 5-23 fish/m². Late larvae of bream, roach, and pearl roach were in stages of development E, F, G. Youth of the bitterlink was represented as early larvae (stage D₁) and late larvae (stages D₂, E). Perch youth was 0+ age.

The stocks of young fish in the thickets of submerged aquatic plants at the beginning of the summer were identical to the clear water for the number of species, but differed slightly in species composition. The largest part of the young fish in the samples was roach – 41.2%, on average 55 fish/m² (lim 42-71). Late larvae and fry of bleak were – 27.1% and 35 fish/m² (lim 10-67), bleak *B. bjoerkna* 18.8% and 13 fish/m² (lim 6-20). Frys of bream, roach, bitterlink, and spiny loach *C. taenia* were found in much smaller quantities – from 1.2 to 4.7% of the composition of young fish in the thickets of VAP.

In the flooded areas of meadow vegetation were found crucian carp *C. gibelio* – 78.6%, carp fry *C. carpio* was – 21.1%, as single fish were found loon *M. fossilis* – 0.3%.

In July 11 species were found in the samples of young fish: five of them (roach larvae *R. rutilus*, silver bream *B. bjoerkna*, rudd *S. erythrophthalmus*, bleak *A. alburnus* and bitterling *R. amarus*) took place in the lotic biotope. In the inflows and abandoned river channels among the water vascular plants the abundance of juvenile ribs was larger (Tab. 1), as there were bleak *L. delineatus*, bream *A. brama*, pike *E. lucius*, spiny loach *C. taenia*, Southern nine-spined stickleback *P. platygaster* and blunt-nosed scourge of late *P. semilunaris*.

Table 2: Stages of development of young fish in the lower reaches of the Sula River, summer 2018; stages of development of young fish (Koblitskaya, 1981). C₁, C₂, D₁ – youthful larva; D₂, E, F, G – last stage larva.

Species	June	July
<i>Rutilus rutilus</i> (L.)	E, F, G	G, fry
<i>Scardinius erythrophthalmus</i> (L.)	E	E, G, fry
<i>Alburnus alburnus</i> (L.)	C ₂ , D ₁ , D ₂ , E	D ₂ , E, F, G
<i>Leucaspis delineatus</i> (Heckel)	–	G
<i>Blicca bjoerkna</i> (L.)	E, F, G	E, G
<i>Ballerus ballerus</i> (L.)	G	–
<i>Abramis brama</i> (L.)	E, F	Fry
<i>Rhodeus amarus</i> (Bloch)	D ₁ , D ₂ , E	D ₂ , G, fry
<i>Cyprinus carpio</i> L.	fry	–
<i>Carassius gibelio</i> (Bloch)	fry	–
<i>Cobitis taenia</i> L.	–	E, fry
<i>Esox lucius</i> L.	–	Fry
<i>Pungitius platygaster</i> (Kessler)	–	Fry
<i>Perca fluviatilis</i> (L.)	fry	–
<i>Proterorhinus semilunaris</i> (Heckel)	–	Fry

In July, 11 fish species were found in the samples of young fish: five of them (roach larvae *R. rutilus*, silver bream *B. bjoerkna*, rudd *S. erythrophthalmus*, bleak *A. alburnus*, and bitterling *R. amarus*) took place in the lotic biotope. In the bays and oxbows among the VAP thickets the abundance of juvenile ribs was larger (Tab. 1), as there were bleak *L. delineatus*, bream *A. brama*, pike *E. lucius*, spiny loach *C. taenia*, Southern nine-spined stickleback *P. platygaster* and blunt-nosed scourge of late *P. semilunaris*. The juveniles of almost all of the above fish species were in the fry stage, except for portion spawning species (Tab. 2).

The main part in the samples of fish youth from the riverbed part of the river consists of the larvae of the bleak *A. alburnus* – 54.2%, on average 73 fish/m² (lim 44-103), larvae and fry of such limnophilous species, as blitterlink *R. amarus* and common rudd *S. erythrophthalmus* were represented singly – 22.2 and 12.5%, 45 and 16 fish/m² respectively. Part of young fish *R. rutilus* and *B. bjoerkna* was non significant – 6.2 and 4.9%. Their quantity did not exceed 25 fish/m², and the frequency of occurrence was 32.0%.

From juvenile fish in lentic biotope among the VAP thickets besides the *A. alburnus*, *B. bjoerkna*, *S. erythrophthalmus*, and *R. amarus*, which were mainly in equal parts (20-25% with the quantity 9-21 fish/m²) and the separate representatives were met of gossip *R. rutilus*, *L. delineatus*, *A. brama*, *E. lucius*, *C. taenia*, *P. platygaster*, and *P. semilunaris*.

One of the key of fish species' abundance support in rivers is downstream migrations of the fish youth. A drift of the fish youth in June was mainly in the nighttime. The biggest intensity of fish youth downstream migration was registered from the first to the fourth hour at nighttime. Taking into consideration the confidence interval, the size of the drift value calculated for the 10 hours in the dark part of the day, including twilight. The flow velocity during the study period was 0.2 m/s. The biggest part of the samples was the larvae of *A.*

brama and *B. bjoerkna*, 51.7 and 36.2%. The larvae of *R. rutilus* and *A. alburnus* were less common and had a much smaller share in drift, namely 6.9 and 5.2%. The youth of all the above species were in the late stages of larvae development (E, F, G). The drift samples were dominated by larvae of *B. bjoerkna* and *A. alburnus*. The number of larvae of *B. bjoerkna* was on average 0.0617 fish/m³ (lim 0.0116-0.1620). *A. alburnus* youth was 0.0173 fish/m³. The larvae of *A. brama* drifted in the night and pre-morning hours, their number was highest at 4-5 A.M., averaging 0.0839 specimens/m³ (lim 0.0752-0.0926) (Tab. 3). Young of *R. rutilus* drifted downstream day and night, the larvae were 0.0231 fish/m³ (lim 0.0115-0.0347). In July drift samples were empty. The water flow in the lower stream of the Sula River within the study period was 30 m³/s (equivalent to 108,000 m³/h), we can calculate the total quantity of young fish carried down by flow per day (Tab. 3).

Table 3: Quantitative meanings of fish drift at the night.

Species	Part in drift, %	Concentration of fish larvae in the sample, org/m ³	Drift of young fish at the studied area, org/year	Quantity of fish larvae for 10 hours of night, org.
<i>Abramis brama</i> (L.)	51.7	0.0839	9061	90610
<i>Blicca bjoerkna</i> (L.)	36.2	0.0617	6663	66630
<i>Rutilus rutilus</i> (L.)	6.9	0.0231	2495	24950
<i>Alburnus alburnus</i> (L.)	5.2	0.0173	1868	18680

Thus, during the young fish migration, in the Sula River estuary, about 200 thousand fish (182.2 thousand) of young fish are carried out to the Kremenchuk Reservoir per day.

A drift of young fish in the stages of late larval development (E, F, G) occurred at night (21-6 A.M.) because the larvae of fish were enough developed to navigate and are able to actively avoid the rapid flow during the day, but at night they endure on the core of the river. During the day fish larvae which rolled downstream also appeared in the traps but in much smaller quantities. It is known that fish youth downstream rolling begins from the stages B-C₁ (Abramiuk et al., 2018). Taking into account that every stage of larvae development takes 3-5 days depending on water temperature, we can assume that the drift of larvae of each species of fish in early summer continues for at least 24 days and the total period of downstream migration continues from the middle of April to the middle of June (near 60 days). This means, that for the entire period, at least 12 mln different species of fish fry are rolled down.

Mostly all species of young fish that we met during the summer, relating to the current belong to the river-lake type (Movchan, 2011). Only roach, perch, and pike belong to the lake-river type, but redfin and rudd, and bitterling – to the lake type, they prefer lentic conditions.

Despite the fact that in adulthood fish species of river and river-lake types in relation to the current often keep to the river areas with a pronounced current, the method of reproduction most of them are phytophiles, and therefore the young of these fish species develop in thickets of VAP, along with the young limnophilous fish species. In the first half of the summer, almost the same number of fish and species composition were observed for groups of young fish in both the lotic and tape biotopes. However, the distribution by the number of juveniles of separate fish species is noteworthy. Thus, in the channel areas, the quantity of bleak youth was into three times larger than in the backwaters (79.2%). The youth of blue bream *Balerus balerus* were found mostly in the armbeds. In the lentic conditions, the quantity of youth roach was mostly four times larger (41.2%), and the quantity of the youth of silver bream was 18.8%.

The change in the structure of communities of young fish in the second half of the summer is explained by the increase from the spawning portion-spawning fish species and is quite natural. The ratio of the number of roach in the riverbed and in the bays has changed in the direction of the predominance of roach fry in areas with currents. The general quantity of the fry of bream, silver bream, and roach decrease in the samples is explained by their move to the bigger depth connected with their growth and feeding style change.

One of the most important hydrological factors which have a great influence on taxonomic composition is the speed of the stream. Downstream migration of the fish fry from the spawning places to the mouth of the river is the important mechanism of population quantity support and spreading of fish species and can be realized only in lotic conditions.

Among all fish species represented, eight are alien with varying degrees of naturalization: *H. molitrix*, *C. gibelio*, *P. parva*, *N. melanostomus*, *N. fluviatilis*, *P. semilunaris*, *P. platygaster* and *S. nigrolineatus*. Part of alien species was 32.0% from the general fish species.

According to the results of the poll among the fishermen and fish caught by their own methods in the lower reaches of the Sula River were registered adult representatives of *L. leuciscus*, *C. carassius*, *R. amarus*, *A. aspius*, *S. glanis*, *C. taenia*, *S. nigrolineatus*, *N. fluviatilis*, and *P. semilunaris*, which are listed in the different lists of endangered species.

As was mentioned above the spatial distribution of young fish depends on the presence of VAP, but correlates more with the flow velocity than with the species composition of VAP. Biotopic meanings of VAP associations for the fish fry, therefore, are to provide shelter and create conditions for the development of a forage base consisting of aquatic invertebrates.

The research demonstrates that the lower reaches of Sula River has a well-developed biota with high biodiversity, which mainly depends on the diversity of habitats. It is important to provide the existence of the flow regime in this section of the river, which supports the existence of lotic habitats. Due to this, the river biota is enriched by rheophilic species of aquatic organisms, which in general make up about a third of the researched area. 57% of the river fish species were found and were met in lotic habitats. The smallest part of rheophilic species (8%) is registered among higher aquatic vegetation. The highest percentage of rheophilic species is observed in the riverbed, but they are also found in the lentic habitats of bays and backwaters. At the same time, the basis of taxonomic abundance (about 70%) is species with wide ecological diapasons, which can exist in non-flowing conditions. This ratio coincides with the distribution of biotopes within the area because, in the research area, lotic habitats of the channel also occupy about a third of the water surface.

The river with a developed system of floodplain waterbodies has a high biotope diversity and, a high biodiversity, much of which is provided by the presence of lotic habitats. Changes in hydrological conditions due to dehydration or dam construction have little effect on belt biotopes and effectively eliminate lotic habitats, with corresponding losses of biodiversity. Therefore, for the researched river we should expect a decrease in biodiversity with decreasing flow. This pattern should be true for rivers with well-developed biotope diversity, which largely depends on the size of the river. Thus, for small rivers and streams with low biological and biotope diversity, the opposite picture can be observed: increasing biodiversity during dam construction. However, the general increase in species abundance does not preclude the extinction of rheophilic and migratory species, many of which are vulnerable and protected, so each case requires separate research and decision-making.

CONCLUSIONS

The lower reaches of the Sula River are an example of lotic ecosystem with high biodiversity. The biotic complexes of the lower reaches of Sula River were historically formed under the conditions of a rheophilic river regime and contain a third of the rheophilic and oxyphilic species of hydrobionts, some of which are included in international and state protection lists of various levels.

The floristic complexes of the lower Sula River are typical for such habitats and contain six plant communities with conservation status. The structure of phytocomplexes remains stable for many years. The maximum number of vascular aquatic plants is typical for the riverbed sections of the river, rheophilic species are about 8% of the total number of species.

The fauna of aquatic invertebrates in the studied area contains 67 taxa, 15 of which are characterized by rheophilic biotopes. The composition and structure of zoobenthos, epiphiton, and epilithon indicate the presence of anthropogenic impact and pollution of the river ecosystem.

Research has registered 28 species of fish in the lower reaches of the Sula River, the most common of which were bleak, roach, and white bream, 10 of the species found have conservation status, eight are alien.

Downward migration of young industrial fish species occurs in late spring and early summer in the Sula River, during which the Kremenchuk Reservoir receives about 12 million young fish.

Construction of a dam will cause significant changes in the hydrological regime of the river, fundamentally changing the entire structure of the river and near-river biocenoses. The rise of the water level above the lock will lead to a decrease in flow, eutrophication, the creation of limnic conditions, siltation, and overgrowth with VAP. Flooding of large areas of shallow water will lead to a deterioration of the oxygen regime due to the decomposition of a large mass of organic substances, an increase in water loss through evaporation, and, accordingly, a decrease in the water content of the river below the lock. Water depleted in oxygen and saturated with decomposition products entering the river, as well as Sulyansk Bay, can contribute to fish's mass death from suffocation not only in winter but also in summer. Thus, the building of hydraulic structures is undesirable and will lead to a breach of integrity of the river continuum, restructuring, and impoverishment of the ecological communities of the river biota.

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THE ROLE OF INTEGRATED COASTAL MANAGEMENT APPROACH IN THE PROTECTION OF COASTAL AND MARINE RESOURCES IN THE EASTERN COAST OF TANZANIA

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ABSTRACT

This scientific paper examines the role of Integrated Coastal Management (ICM) approach in the protection of coastal and marine resources in Tanzania. It engages coastal resource users and practitioners in focus group discussions and interviews, and complements the data obtained with documented sources. A modified version of the Benefit Analysis Framework is adopted from Wenger et al. (2011) to analyze the extent to which ICM yields a wide range of benefits that may potentially promote the protection of coastal ecosystem and enhance the commitment of communities that live adjacent to the coastal strip to manage mangroves, fisheries, coral reefs, and coastal land in a sustainable manner.

RÉSUMÉ: Le rôle de la gestion intégrée des zones côtières dans la protection des ressources côtières et marines de la côte orientale de la Tanzanie.

Cet article examine le rôle de l'approche de gestion intégrée des zones côtières (GIZC) dans la protection des ressources côtières et marines en Tanzanie. Il fait participer les utilisateurs (des ressources côtières) et les praticiens à des discussions de groupe et à des entretiens, et complète les données obtenues par des sources documentées. Une version modifiée du cadre d'analyse des avantages est tirée de Wenger et al. (2011) pour analyser dans quelle mesure la GIZC offre un large éventail d'avantages susceptibles de promouvoir la protection de l'écosystème des côtes et de renforcer l'engagement des communautés vivant à proximité de la bande côtière pour gérer les mangroves, les pêcheries, les récifs coralliens et les terres côtières de façon durable.

REZUMAT: Rolul abordării managementului integrat de coastă pentru protecția resurselor de coastă și marine pe coasta de est a Tanzaniei.

Această lucrare examinează rolul abordării managementului integrat de coastă (MIC) în protecția resurselor de coastă și marine din Tanzania. Acesta implică utilizatorii și practicienii resurselor de coastă în discuții și interviuri focusate pe grup și completează datele obținute cu surse documentate. O versiune modificată a cadrului de analiză a beneficiilor este adoptată după Wenger et al. (2011) pentru a analiza măsura în care MIC oferă o gamă largă de beneficii care ar putea promova protecția ecosistemului de coastă și pot spori angajamentul comunităților care trăiesc adiacent de fâșia de coastă pentru a gestiona mangrovele, pescuitul, recifele de corali și terenurile de coastă într-o manieră durabilă.

INTRODUCTION

Aquatic ecosystems and resources are under a high natural and anthropogenic stress around the world (Niinemets et al., 2017; Bănăduc et al., 2022, 2023a, b). The Tanzanian coastal ecosystems are not at all an exception in this respect (Sabai and Sisitka, 2013; Sabai, 2017; Schuijt et al., 2021).

The world has witnessed the adoption of integrated approaches across continents for a wide range of reasons. The approaches are either adopted or developed in terrestrial and coastal contexts in order to harmonise prevailing natural resource use conflicts, discourage overlapping institutional mandates and promote sustainable use of natural resources (Campuzano et al., 2013; Stori et al., 2023). Some of the common integrated approaches preferred in terrestrial ecosystems include Integrated Forest Management (Kulshreshtha, 2014), Integrated Natural Resource Management (Wang et al., 2021), Integrated Land Use Planning (FAO, 2020), and Integrated Water Resource Management (Nagata et al., 2022).

The Integrated Coastal Management (ICM) approach is widely preferred and applied in coastal areas due to its benefits in terms of attracting active community participation and harmonizing local resource use challenges (Sabai, 2021). In Asia, particularly in the coastal states, the specified approach is called Integrated Coastal Zone Management (ICZM). This approach is also common among countries that are located on the Indian Ocean, including Madagascar, Seychelles, Mozambique, Kenya, Tanzania, and South Africa (WIOMSA, 2020).

In the Black Sea region, a very dynamic area affected by the human impact along its history (Bănăduc et al., 2016, 2020, 2023), ICM was adopted as a means for mobilising and promoting coherent use of coastal resources (Bat et al., 2012). The specified approach has also been adopted in other contexts on sustainability grounds (Saha, 2019). Some researchers have applied the approach in climate change issues, arguing that it has the potential of drawing lessons and offering significant contributions that may deduce experiences for addressing climate change concerns in coastal contexts (Ojwang et al., 2017). It is quite clear that some countries have adopted the approach due to its potential to resolve emerging and prevailing challenges in coastal areas (Khelil et al., 2019).

In Tanzania, the ICM approach was adopted and applied in the mid-90s as an initiative for promoting sustainable management of coastal and marine resources, particularly mangroves, fisheries, and coral reefs and discouraging mismanagement practices such as overfishing, mangrove clearance, and dynamite fishing (TCMP, 1999a, NICMS, 2003). Despite the challenges experienced in the initial stages of adopting the ICM approach (Sabai, 2021), a wide range of benefits have been identified as a result of its adoption in the coastal regions of Tanzania such as Dar es Salaam, Tanga, Pwani, Mtwara, and Lindi (KICAMP, 2004, 2005; TCMP, 1999b, TCZCD, 2004).

This journal article seeks to describe the extent to which the ICM approach has contributed to the protection of coastal and marine resources in the eastern coast of Tanzania. It creates a platform for describing what the ICM approach may potentially offer when adopted in coastal and marine contexts. The article also serves as an eye opener to coastal practitioners and local communities that there are many benefits that may be accrued from ICM than some negative challenges that are reported in a body of coastal literature.

MATERIAL AND METHODS

Context of the study

Data for this article were captured from two coastal sites namely Kijiru and Moa in Tanga region (Fig. 3), where 26 coastal resource users (selected intentionally on the grounds of their long term experience in coastal resource use) were engaged in face-to-face interviews. The same target group was involved in three Focus Group Discussion sessions as a means of verifying data that had been previously generated from interview sessions. Captured data were later analyzed qualitatively and complemented with documented coastal and marine literature sources to yield insights that depicted the potential role of the ICM approach.

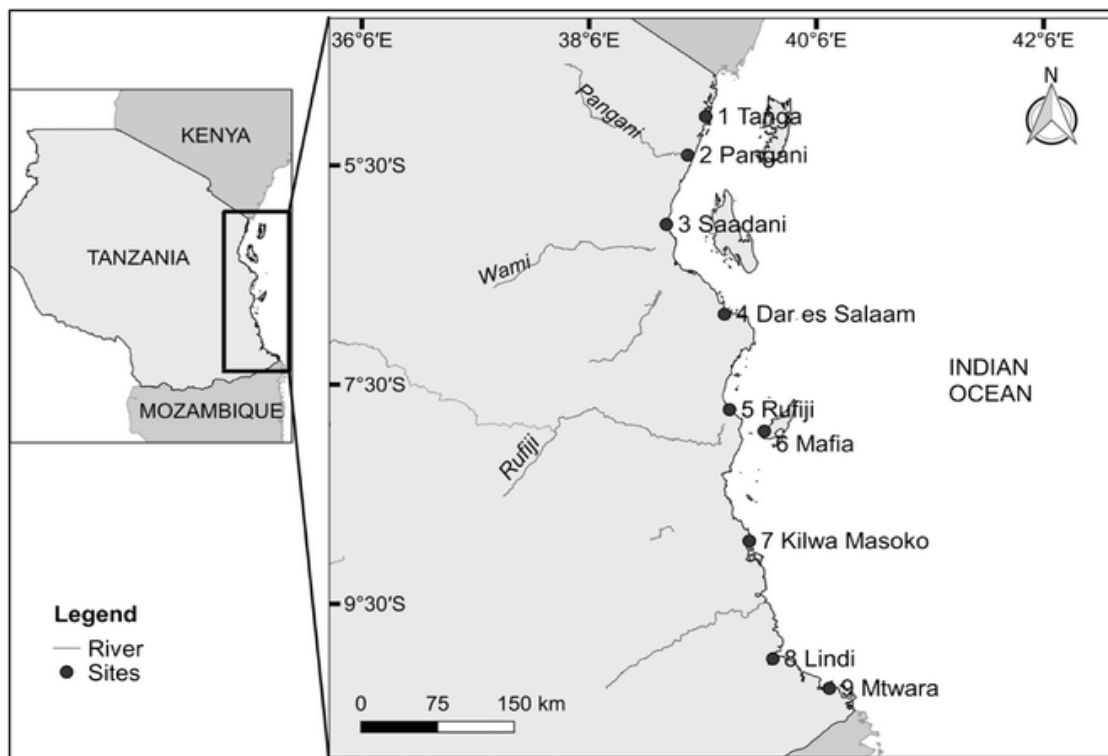


Figure 3: ICM sites (1-9) in the Eastern Coast of Tanzania;
Researchgate.net.

Theoretical framework

This article adopts the Benefits Analysis Framework (Wenger et al., 2011; Fig. 4) in setting the theoretical background. The framework offers analytical, explanatory, and methodological support for uncovering potential benefits that may be realised in contexts where ICM approach is adopted. It also offers the language of description and serves as a content descriptive tool.

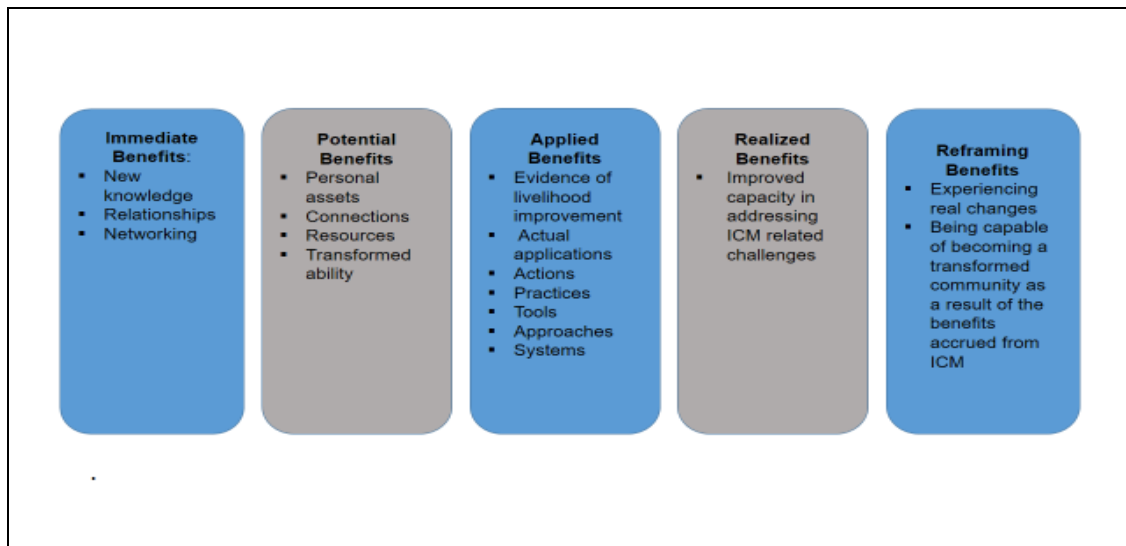


Figure 4: Benefit Analysis Framework;
Wenger et al., 2011-modified.

According to the framework, *immediate benefits* are realized when target coastal communities gain new knowledge such as preparation of nursery plots, planting of mangrove seeds in the plots, transplanting and installation of eco-friendly facilities for instance beekeeping. They are also realized when relationships and networks are established.

Potential benefits are realized when local communities engage in income generation activities as an alternative to overreliance on coastal and marine resources. Such benefits also extend to creation of connections between one community of practice and adjacent coastal communities and it is facilitated by activities such as study tours, experience sharing and resource sharing (TCZCD, 2004). Under *potential benefits*, people may benefit from sustainable practices and eventually develop transformed capabilities.

Applied benefits are accrued when there is evidence on ground that the livelihoods of coastal communities have improved. Under such benefits, target local communities also start involving themselves in actual application of gained knowledge and skills such monitoring of coastal and marine resources, mangrove transplanting and community patrols. It is under this level that various tools and systems are created to aid practices such as implementation of conservation and restorative initiatives.

Realized benefits are revealed when there is improved community capacity in addressing emerging and prevailing social, ecological, and economic challenges that occur in the coastal and marine ecosystem.

Reframing benefits are realized when target coastal communities experience real changes, being capable of becoming a transformed community as a result of the benefits accrued from ICM.

RESULTS AND DISCUSSION

The results suggest that ICM may potentially attract sustainable fishing in the study sites, build capacity for transplanting mangrove species, expose coastal communities to monitoring of coastal and marine resources, yield knowledge of the creation of nurseries for mangrove species, promote learning by doing, create opportunities for income generation, protect mangrove resources and avail opportunities for women to participate in mangrove restorative activities. Moreover, the approach has the potential to protect coastal land by encouraging land use planning. These results are analyzed hereafter, and discussed with the aid of the Benefits Analysis Framework presented in figure 4, in five categories of benefits namely immediate, potential, applied, realized, and reframing benefits.

Attraction of sustainable fishing practices

The Integrated Coastal Management (ICM) approach provides a space for promoting the protection of fish breeding grounds such as coral reefs and mangrove sites. It also encourages the use of acceptable fishing gear such as large-meshed nets and discourages the use of small-meshed nets, illegal fishing, dynamite fishing, and the use of different poisons. There is also evidence that some fishers pluck off live corals and place them on top of traditional fishing gears commonly known as *madema* to prevent them from being pushed away by turbulence and thus keep them in their original position. Captured insights from the study sites describe various attempts that have been previously made by the Tanga Coastal Zone Conservation and Development (TCZCD) Program to promote sustainable fishing practices in the target sites (TCZCDP, 2004). When local fishers refrain from destructive activities and adopt the use of sustainable fishing practices, they illustrate a form of transformation which results from experiencing real changes. In other words, when such a situation is realized, they have obtained *reframing benefits* (Fig. 4; Tab. 1, no. 1).

Builds capacity in mangrove transplanting

It is also evident that ICM exposes coastal communities to mangrove transplanting activities, including training on how to prepare nurseries for raising mangrove seeds. This suggests that they are being enabled to engage directly in practices that will attract *applied benefits* (Fig. 4; Tab. 1, no. 2). Applied benefits are realised when target communities apply gained knowledge. This category of benefits is revealed in a form of actions, practices or actual application of what had been introduced or delivered by facilitators in hands-on training sessions. Analysed documented sources reveal that mangrove restorers in Kijiru and Boma sites were initially trained by coastal and marine experts on the manner of preparing nursery plots and transplanting mangrove seedlings and acquired transplanting techniques that were later shared in other coastal localities (Sabai, 2014). The new knowledge that is acquired by coastal communities through participating in transplanting activities falls under *immediate benefits* (Fig. 4; Tab. 1, no. 4). Mangrove transplanting may potentially improve coastal forests, hydrological cycle and carbon sinks (Hu et al., 2018).

Builds the capacity to monitor coastal resources

In contexts where ICM had been adopted as the main approach, community monitoring of coastal and marine resources had been encouraged. This practice has many ecological benefits. It aids in the identification of changes, threats, trends, and condition of resources and thus helps community groups to understand well the status of the target ecology and make proper intervention for the identified gaps (KIMP, 2005). The practice also allows local resource users to share monitoring experiences with facilitating teams. Gained knowledge

is later spread to other parts of the coastal strip and attracts the protection of coastal and marine resources. Ecological monitoring also falls under *applied benefits* (Fig. 4; Tab. 1, no. 3).

Table 1: Perceived role of the ICM in the study sites.

SN	ICM role	Context	Beneficiaries	Benefit category
1.	Attracts sustainable fishing	Fishing	Fishers	Reframing benefits
2.	Generates knowledge on tree nursery creation and mangrove transplanting	Mangroves	Mangrove restorers and mangrove-based fishers	Immediate and applied benefits
3.	Builds capacity to monitor coastal resources	Mangroves, fisheries, coral reefs, seagrass and coastal land	Coastal resource users	Applied benefits
4.	Promotes learning by doing	Coastal area	Coastal resource users	Realised benefits
5.	Protects mangrove resources	Mangroves	Coastal resource users	Applied benefits
6.	Attracts participation of women in conservation	Coastal area	Coastal women	Reframing benefits
7.	Creates a space for income generation	Coastal area	Coastal resource users	Potential benefits
8.	Promotes greening in schools	Coastal schools	Schools in the coastal area	Applied benefits
9.	Protects coastal land	Coastal strip	Coastal communities	Applied benefits
10.	Promotes knowledge sharing	Coastal area	Coastal communities	Immediate benefits

Promotes learning by doing

ICM encourages coastal communities to participate fully in a wide range of activities under the facilitation of coastal experts. This causes mangrove restorers, fishers and other social groups to learn as they participate actively in the management of coastal resources and eventually attain *realised benefits* (Fig. 4; Tab. 1, no. 4)

Protects mangrove resources

The implementation of ICM-oriented activities provides a space for village councils to formulate and approve by-laws that protect mangrove forests and other coastal and marine resources from being mismanaged. Programmes that are being implemented under the ICM approach also encourage local communities to initiate patrols in the mangrove forests. Patrol activities are evident in the study area and other ICM sites in the eastern coast of Tanzania. These kinds of practices cause them to obtain *applied benefits* (Fig. 4; Tab. 1, no. 5).

Creates opportunities for women to participate fully in the management of coastal and marine resources

Analyzed data suggest that women were initially being excluded in the management of coastal and marine resources (KICAMP, 2000, 2001). Their main role was to collect sea cucumber during low sea tides and assume minor roles in fish selling. The adoption of the ICM approach opened up opportunities for them to play various roles in the management of all coastal resources. Currently, women assume a leading role in guiding ecological restoration activities that aim at protecting the coastal ecosystem. A study carried out by Sabai (2019) indicated that they are also assuming leadership positions in groups, CBOs and NGOs that are directly involved in the management of coastal and marine resources. These kinds of transformation indicate that they have attained *reframing benefits* (Fig. 4; Tab. 1, no. 6).

Creates alternative sources of income generation

Implementation of the ICM strategy encouraged the development and initiation of alternative sources of income in the coastal area to reduce pressure on the use of coastal fisheries, mangroves, and other coastal and marine resources. It was envisaged that if local communities who are involved in the mismanagement practices will be exposed to alternatives sources of income, they will reduce their total reliance on coastal and marine resources and the affected ecosystem will thus rejuvenate and regenerate. In Pangani (Tanga), a crab fattening project was introduced to encourage mariculture. In other coastal areas such as Dar es Salaam, beehives were installed in the mangrove forests to attract double benefits. Mangroves that are located near beehives are normally not disturbed by people for fear of being attacked by swarms of bees (Sabai, 2014). Bees also contribute to the pollination of mangrove species.

In Dar es Salaam, revolving fund schemes were introduced between 2002 and 2005. These accommodated different income generating activities such as poultry farming, urban vegetable growing, petty trade businesses and food selling. Seed money came from the Swedish Development Agency (Sida) in collaboration with the Government of Tanzania. Under this scheme, borrowers were supposed to recover their loans on weekly bases at agreed affordable rate (KICAMP, 2004). This suggest that they are continuing to realize *potential benefits* (Fig. 4; Tab. 1, no. 7).

Promotes greening programs in schools

The ICM promoted and encouraged the introduction of greening programmes in local schools particularly primary and secondary schools in the coastal regions. These witnessed the integration of environmental aspects in the school curriculum. The first initiatives were carried out by the Tanga Coastal Development Programme in the late 1990s. School greening programmes have since then been initiated in other parts of the coastal area to orient students in the protection of environment. Under the Benefit Analysis Framework (Fig. 4), greening of schools may be regarded as falling under *realised benefits* since it is being practiced to improve schools capacity in addressing environmental challenges (Fig. 4; Tab. 1, no. 8).

Helps in the development of land use plans which prioritise the protection of coastal and marine resources

The adoption of the ICM strategy and policy has also necessitated the development of coastal land use plans. In 2005, Kinondoni Integrated Coastal Area Management (KICAMP) facilitated the development of a draft land use plan for Mbweni, Kunduchi, and Ununio localities (Fig. 4, no. 4) in collaboration with Kinondoni Municipal Council. The plan generally protected the coastal land and forests from human induced encroachment. It was later approved by the relevant organs and became operative (KICAMP, 2007).

Promotes knowledge sharing in the coastal area

It was found that in places where ICM was adopted, most of the initiatives prioritized study tours to other ICM sites prior to commencement of planned activities. Tanga Coastal Zone Conservation and Development (TCZCD) programme facilitated a study tour for selected representatives to visit Mombasa (Kenya) to learn from communities that had experience in coastal and marine management. In Dar es Salaam, representatives from the newly established ICM programme KICAMP visited the TCZCD to learn before preparing its coastal management plans. This suggests that knowledge sharing is a key aspect in effective implementation of the ICM approach. It is evident that knowledge exchange continued in the life time of the specified ICM programmes (KICAMP, 2004).

Contributes to development of ICM policy and strategy

The adoption of the ICM approach influenced the development of the policy in Tanzania in 1999 (TCMP, 1999a, 1999b; NICMS, 2003). Four years later (2003), the strategy was developed and implemented. The ICM policy provided general guidance on how coastal and marine resources should be managed and governed, and specified the actors and strategies for implementation of ICM-oriented activities in the country.

CONCLUSIONS

The Integrated Coastal Management approach (ICM) may potentially contribute to the protection of coastal and marine resources by providing a space for yielding ecological, economic, and socio-economic benefits; a situation which causes coastal communities to reduce pressure on coastal and marine resources. The adoption of ICM in similar coastal contexts is likely to be successful, if necessary procedures and conditions are considered prior to their implementation. Knowledge exchange and experience sharing emerge to be key aspects that ignite successful implementation of ICM.

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ROMANOGOBIO BANATICUS (BĂNĂRESCU, 1960) IN THE NERA RIVER (DANUBE BASIN)

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ABSTRACT

Romanogobio banaticus it is a species of conservation interest with a small distribution range in the south-west of the Romanian Carpathians basin. In spite of the presence of some moderate anthropogenic threats and risks, the Nera River is a sanctuary for this fish. The lower latitude of Nera in comparison with other rivers where this fish is living, explain its presence in an atypical ichthyological zone. This species presence is permanent, the abundance is relatively high, the age structure is well balanced, more than that, in some sectors is a co-dominant species, all of these revealing a favourable conservation status.

RÉSUMÉ: *Romanogobio banaticus* (Bănărescu, 1960) dans la rivière Nera (bassin du Danube).

Romanogobio banaticus est une espèce d'intérêt pour la conservation avec une petite aire de répartition dans le sud-ouest du bassin des Carpates roumaines. Malgré la présence de quelques menaces et risques anthropiques modérés, la rivière Nera est un sanctuaire pour ce poisson. La latitude plus basse de Nera par rapport aux autres rivières où vit ce poisson explique sa présence dans une zone ichtyologique atypique. Cette présence d'espèce est permanente, l'abondance est relativement élevée, la structure d'âge est bien équilibrée, plus que cela dans certains secteurs est une espèce co-dominante, tout cela révélant un état de conservation favorable.

REZUMAT: *Romanogobio banaticus* (Bănărescu, 1960) în râul Nera (bazinul Dunării).

Romanogobio banaticus este o specie de interes conservativ cu o arie de distribuție mică în partea de sud-vest a bazinului Carpaților Românești. În ciuda prezenței unor amenințări și riscuri antropogene, râul Nera este un sanctuar pentru acest pește. Latitudinea mai scăzută a Nerei în comparație cu alte râuri unde acest pește trăiește, explică prezența acestuia într-o zonă ihtiologică atipică. Prezența acestei specii este permanentă, abundența este relativ ridicată, structura pe vârste este echilibrată, și mai mult decât atât în unele sectoare este specie co-dominantă, toate acestea relevând o stare favorabilă de conservare.

INTRODUCTION

The talent, inspiration and dedication of pioneering scientific personalities like Grigore Antipa or Petru Mihai Bănărescu, to mention only the most representative Romanian ichthyologists, as well as the next generations of fish biology and ecology experts, led to the initiation and consolidation in this country at an international scientific level of an ichthyology school with exceptional results. The almost three centuries of ichthyological studies have led to the accumulation of some special qualitative and quantitative data, and new studies can benefit in principle from the previous results obtained in the field, for comparison reasons.

Why in principle? Although the number of existent fish taxa is relatively small and the national territory has been relatively well covered by studies, the increase of direct or indirect human impact on them, create a necessity for more screenings of them.

Following the bibliographical analysis regarding genus *Gobio* representatives since 1726 on the Romanian territory (Curtean-Bănăduc et al., 2014, 2019; Bănăduc, 2001, 2003, 2004a-d, 2007a-e, 2008a-d, 2009, 2017; Bacalu et al., 1995; Bănărescu, 1947, 1952, 1953, 1954, 1956, 1962, 1964, 1965a,b, 1970, 1992a,b, 1994; Bănărescu and Nalbant, 1973; Antonescu 1934, 1957; Băcescu, 1947; Simionescu, 1923; Antipa, 1909), some elements still require extensive or intensive studies in the Romanian freshwaters. Consequently such a bibliographical analysis reveal the need for update field work based studies related to the *Gobio* genus representatives in terms of distribution and ecological status.

Such a case is addressed in the present study, the authors proposing the identification of the current distribution, threats and risks, and the evaluation of the general ecological status of the species *Romanogobio banaticus* in the Nera River (Danube Basin).

Romanogobio banaticus/*Gobio kessleri banaticus* (Bănărescu, 1960) (Fig. 1) (Teleostei, Cypriniformes, Gobionidae) it is a freshwater, benthopelagic fish, with a maximum length of 8.5 cm living in the temperate zone, in Europe, Romania, namely with important populations in two medium sized watersheds: Nera and Timiș. This species was found also in Crișul Alb, Crișul Negru, Bega, and Caraș rivers (Bănărescu, 1964; Bănăduc, 2004a).

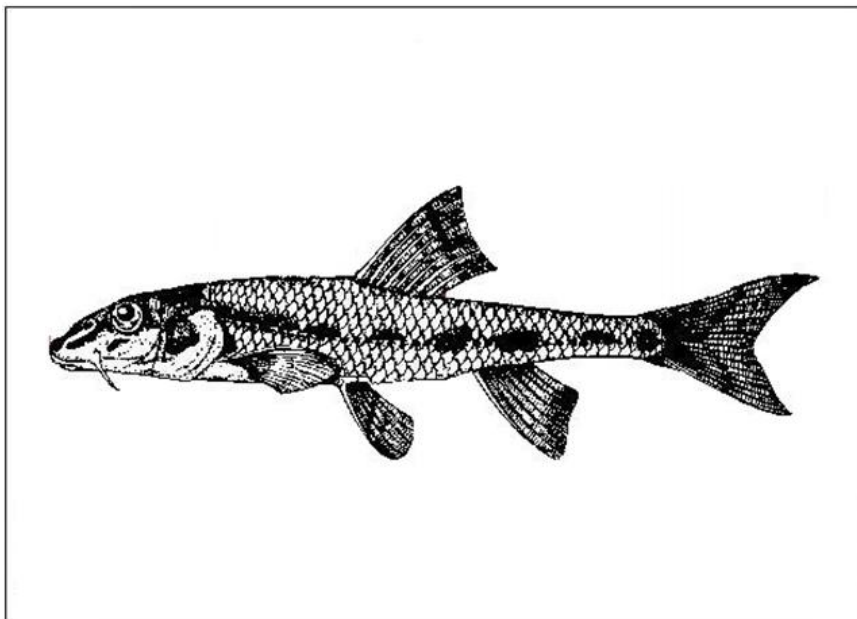


Figure 1: *Romanogobio banaticus*/*Gobio kessleri banaticus* (Bănărescu, 1964).

Nera and Timiș rivers springs both from the Semenic Mountains and cross a very complex and relatively isolated geographical areas (Diaconu, 1971; Ujvari, 1972; Oancea and Velcea, 1987; Badea and Bugă, 1992).

At least at a first glance the specific local/regional environmental conditions push the evolutionary processes in forming and conserving this fish in the area. With a different general flow direction, another two relatively similar rivers as dimensions of the area, Bârzava and Caraș rivers suffered a more accentuated human impact in the last centuries and this fish was not signalled in them, may be correlated with this significant human impact acting on them. In comparison with the majority of other species of this genus the distribution range of *Romanogobio banaticus* is relatively limited. This area defined from the relief point of view by the Banatului Mountains and its surrounding hills and plains landscape with some interrelated environmental characteristics, probably offer the conditions for this species appearance and survival. Still the relatively small distribution area under a direct and indirect human impact on the characteristic habitats and associated biota including fish, can be a risk factor for this species existence and survival. This induces the need for periodical updates regarding information about distribution range and ecological status of *Romanogobio banaticus*. This was the goal of this study due to the old and incomplete data regarding this species.

The Nera River is located in the south-west part of Romania and has an east-west orientation, with a length of 131 km, springs from the Semenic Mountains, below the Piatra Goznei Peak (1,447 m), and flows into the Danube River; before its discharge, along a length of 15 km, the Nera constitutes the border between Romania and Serbia. The narrow valley carved into the Jurassic rocks constitutes the Nera Gorges. It collects 36 tributaries, the length of the hydrographic network is 574 km, the density is 0.42 km/km². The multiannual average runoff has values between 20 l/s x km², in the highland area with altitudes of 800-900 m, and below 8 l/s x km², in the lower areas, below 400 m altitude. The average flow in the Sasca section is around 1,700 l/s. The area of the basin is 1,240 km². The Nera River runs 23 km through the Nera National Park, where it separates the Anina Mountains from the Locvei Mountains. (*; Diaconu, 1971; Ujvari, 1972; Oancea and Velcea, 1987; Badea and Bugă, 1992)

This hydrological basin includes a lotic systems network formed of Nera River and its tributaries: left side tributaries – Cremenita, Ogașul Bogozului, Ogașul Babei, Ogașul Mare, Valea Haimeliug, Ogașul Porcului, Ogașul Ulmu Mic, Ogașul Ulmu Mare, Ogașul Porcarului, Ogașul Rintu, Ogașul Cracu Lung, Șușara, Valea Fântâna Seacă; and right side tributaries – Valea Miniș, Ogașul Lighidia, Ogașul Agrișul, Ogașul Lăpușnic, Ogașul Mocerîș, Valea Ducin, Ogașul Bresnic, Ogașul Țârcovița, Ogașul Alunilor, Ogașul Radovanului, Valea Rea, Valea Padina Seacă, Valea Lindina, Valea Beiului, Pârâul Vicinic, Valea Ilidia, Valea Baca, Valea Ghicin, Valea Ciclova, Valea Oraviței, etc. (*; Diaconu, 1971; Ujvari, 1972; Oancea and Velcea, 1987; Badea and Bugă, 1992; Posea, 2006)

The diversity of habitats and ecosystems in a relatively small geographical area is induced by the complexity of the regional relief. The Semenic Mountains are part of the Banat Mountains. This geographical area has a very complex geology and varied relief, a fact which created the characteristic, extremely variable habitats, biocoenosis, and ecosystems. The main features of the relief include interfluvial ridges, interfluves, and deep river valleys and gorges. (*; Diaconu, 1971; Ujvari, 1972; Oancea and Velcea, 1987; Badea and Bugă, 1992; Posea, 2006)

MATERIAL AND METHODS

The presence/absence of *Romanogobio banaticus* (Figs. 2 and 3) in Nera River was signaled based on electrofishing with a Hans Grassl IG 600TL device, in time (45 minutes)/effort unit, from every two-three km length river sectors, from the river springs to its outflow in the Danube, in a total of 48 sampling sectors of 100 m long. After fast visual identification, the fish were released in the habitat of origin. In this paper is presented only the river sector where the target species was found, between Pătaș and Zlatița.



Figure 2: Sampled *Romanogobio banaticus*.



Figure 3: Sampled *Romanogobio banaticus*.

RESULTS

The studied species was found in 25 sampling stations on all the Nera River (Tab. 1).

Table 1: Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.. In the table is presented only the river sector where the target species was found, between Pătaș and Zlatița localities.

S.S.	L.	GIS	D.	N.	H.D.	P.F.S.
N1	Pătaș Village, downstream of Borlovenii Vechi	N 44°57.125' E 22°06.140'	07.07.2022	1	Substrate dominated by rocks, gravel and isolated patches of sand and mud. Water width between 10-15 m. Riparian vegetation in favorable condition, consisting of tall trees (<i>Alnus glutinosa</i> , <i>Salix alba</i>). High degree of water shading. Submersed aquatic vegetation. Stable banks, without signs of erosion.	<i>Alburnoides bipunctatus</i>
N2	Upstream of Pârlipeț Village	N 44°55.866' E 22°04.401'	08.07.2022	3	Gravel and boulder substrate, with isolated rocky sections. Water width between 10-15 m. Steep right bank, small slope on the left bank. Moderate water turbidity. Riparian vegetation in favourable condition consisting of tall trees, shrubs and herbaceous vegetation. High degree of water shading. Stable banks, without signs of erosion.	<i>Alburnoides bipunctatus</i>
N3	Downstream of Pârlipeț Village	N 44°55.726' E 22°02.887'	08.07.2022	1	Gravel and boulder substrate, with isolated rocky sections. Water width between 10-15 m. Steep right bank, smoother left bank. Moderate water turbidity. Riparian vegetation in favourable condition consisting of tall trees, shrubs and herbaceous vegetation. High degree of water shading. Stable banks, without signs of erosion.	<i>Alburnoides bipunctatus</i>
N4	Upstream of Bozovici Commune – at the upstream bridge	N 44°55.04' E 22°01.02'	08.07.2022	1	Substrate consisting of gravel, coarse sand, fine sand, and mud. Water width between 10-15 m. Moderate water turbidity. Riparian vegetation in favourable condition consisting of tall trees, shrubs and herbaceous vegetation. High degree of water shading. Stable banks, without signs of erosion.	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N5	Bârz – upstream of Bârz, downstream of Moceriş	N 44.85013° E 21.91517°	19.10.2022	21	Substrate consisting of rocks, gravel, boulders, with patches of sand, and silt. Water width between 10-15 m. Riparian vegetation in favourable condition consisting of tall trees (<i>Salix alba</i> predominantly), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i>
N6	Bârz – between Bârz and Boinița	N 44.84145° E 21.89474°	19.10.2022	6	Substrate consisting predominantly of mud and sand, with isolated patches of gravel. Water width between 14-15 m. Riparian vegetation in favourable condition consisting of tall trees (<i>Salix alba</i> predominantly), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Cobitis elongata</i>
N7	Upstream of the Șopotul Nou Commune	N 44.82892° E 21.88104°	19.10.2022	3	Substrate predominantly consisting of gravel, sand with isolated patches of silt. Water width between 14-17 m. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> and in lower number <i>Fagus sylvatica</i> and <i>Carpinus betulus</i>), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>
N8	At the bridge in Șopotul Nou Commune, next to the confluence with the Buceaua River.	N 44.82127° E 21.86562°	20.10.2022	6	Substrate consisting predominantly of sand, gravel and in some areas mud. Water width between 10-20 m. Riparian vegetation in favourable condition consisting of tall trees (<i>Salix alba</i> predominantly), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i>
N9	Breșnic-Corniș	N 44.83781° E 21.84897°	20.10.2022	10	Substrate consisting predominantly of sand, gravel and in some areas mud. Water width between 10-20 m. Riparian vegetation in favourable condition consisting of tall trees (<i>Salix alba</i> predominantly), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N10	Downstream of Bozovici Commune	N 44.89908° E 21.98630°	18.10.2022	1	Substrate consisting of gravel, coarse sand, fine sand and mud, isolated patches with boulders. Riparian vegetation in favourable condition, consisting of tall trees (<i>Salix alba</i> , <i>Alnus glutinosa</i>), shrubs and herbaceous vegetation. Steep banks, with areas of erosion.	<i>Alburnoides bipunctatus</i>
N11	Dabloșeț – under the bridge located upstream from Dalboșeț	N 44.87735° E 21.95813°	18.10.2022	2	Substrate consisting of gravel, boulders and sand covered with organic matter. Water width between 12-15 m. Riparian vegetation in favourable condition consisting of tall trees (<i>Salix alba</i> predominantly), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> , <i>Rhodeus amarus</i> , <i>Squalius cephalus</i> and <i>Barbus meridionalis</i>
N12	Mocerîș – at the Mocerîș bridge	N 44.86355° E 21.92986°	19.10.2022	5	Substrate consisting of gravel, boulders and sand covered with organic matter. Water width between 12-15 m. Riparian vegetation in favourable condition consisting of tall trees (<i>Salix alba</i> predominantly), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i>
N13	3 km upstream of Dracului Lake	N 44.84746° E 21.83561°	20.10.2022	2	Substrate consisting predominantly of sand, with isolated patches of mud. Water width between 10-20 m. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , and <i>Carpinus betulus</i>), shrubs and herbaceous vegetation. Adequate degree of water shading	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>
N14	At the entrance of the river into the Nera Gorges	N 44.8594° E 21.81687°	20.10.2022	14	Substrate consisting predominantly of sand, with isolated patches of mud. Water width between 15-20 m. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Quercus petraea</i> and <i>Carpinus betulus</i>), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Cobitis elongata</i> , <i>Romanogobio kessleri</i> <i>banaticus</i> and <i>Squalius cephalus</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N15	In the vicinity of the Dracului Lake	N 44.863432° E 21.813235°	28.10.2022	10	Substrate consisting predominantly of sand, with isolated patches of mud. Water width between 15-20 m. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Quercus petraea</i> and <i>Carpinus betulus</i>), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Squalius cephalus</i> , <i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>
N16	In Nera Gorges, Poiana Alunilor area	N 44.873248° E 21.807432°	28.10.2022	5	Substrate consisting predominantly of boulders, gravel and coarse sand. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Carpinus betulus</i> , <i>Quercus robur</i> , <i>Quercus petraea</i> and <i>Populus alba</i>), shrubs and herbaceous vegetation. Adequate degree of water shading.	<i>Barbus meridionalis</i> and <i>Alburnoides bipunctatus</i>
N17	2 km downstream of Poiana Alunilor	N 44.88885° E 21.80057°	29.10.2022	8	Substrate consisting predominantly of boulders, gravel, coarse sand and isolated patches with mud. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Carpinus betulus</i> , <i>Quercus robur</i> , <i>Quercus petraea</i> and <i>Populus alba</i>), shrubs (<i>Sambucus nigra</i> and <i>Cornus sanguinea</i>) and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> and <i>Cobitis elongata</i>
N18	In Nera Gorges, at La Cârlige area	N 44.89057° E 21.78882°	29.10.2022	–	Substrate consisting predominantly of boulders, gravel, coarse sand and isolated sections with mud. Stones covered with silt and organic matter. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Carpinus betulus</i> , <i>Quercus robur</i> and <i>Quercus petraea</i>), shrubs and herbaceous vegetation (<i>Sambucus nigra</i> and <i>Cornus sanguinea</i>). Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> and <i>Cottus gobio</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N19	In Nera Gorges, Cantonul lui Damian area	N 44.89965° E 21.77771°	04.11.2022	4	Substrate consisting predominantly of boulders, gravel, coarse sand and isolated patches with mud. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Carpinus betulus</i> , <i>Quercus robur</i> , <i>Quercus petraea</i> and <i>Populus alba</i>), shrubs (<i>Sambucus nigra</i> and <i>Cornus sanguinea</i>) and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> , <i>Chondrostoma nassus</i> and <i>Cobitis elongata</i>
N20	In Nera Gorges, at Gura Lindinii meadow	N 44.89987° E 21.76553°	05.11.2022	–	Substrate consisting predominantly of gravel, coarse and fine sand, and isolated sections with boulders and mud. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Carpinus betulus</i> , <i>Quercus robur</i> and <i>Quercus petraea</i>), shrubs and herbaceous vegetation (<i>Sambucus nigra</i> , <i>Cornus sanguinea</i> and <i>Rubus fruticosus</i>). Adequate degree of water shading.	<i>Cobitis elongata</i> , <i>Squalius cephalus</i> and <i>Sabanejewia balcanica</i>
N21	In Nera Gorges – Bei Bridge – at the confluence of Nera River with Bei River	N 44.90284° E 21.74546°	05.11.2022	16	Substrate consisting predominantly of boulders, gravel, coarse sand and isolated patches with mud. Riparian vegetation in favourable condition consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Fagus sylvatica</i> , <i>Carpinus betulus</i> , <i>Quercus robur</i> , <i>Quercus petraea</i> and <i>Populus alba</i>), shrubs (<i>Sambucus nigra</i> and <i>Cornus sanguinea</i>) and herbaceous vegetation. Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> and <i>Romanogobio banaticus</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N22	At the entrance of Nera River in Sasca Română Village	N 44.90055° E 21.72236°	05.11.2022	3	Substrate consisting predominantly of gravel, coarse sand, boulders, and areas with fine sand and mud. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> , <i>Populus alba</i>), shrubs and herbaceous vegetation (predominantly <i>Rubus fruticosus</i>). Adequate degree of water shading. Submerged vegetation present.	<i>Alburnoides bipunctatus</i>
N23	At the bridge in Sasca Montană, near the Cheile Nerei Beușnița Park Administration	N 44.88572° E 21.70699°	06.11.2022	2	Substrate consisting predominantly of gravel, coarse sand, boulders and areas with fine sand and mud. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> , <i>Alnus glutinosa</i> and <i>Populus alba</i>), shrubs and herbaceous vegetation (predominantly <i>Rubus fruticosus</i>). Adequate degree of water shading. Submerged vegetation present.	<i>Squalius cephalus</i>
N24	2 km downstream of Sasca Montană Village	N 44.90039° E 21.68874°	06.11.2022	2	Substrate consisting predominantly of gravel, coarse sand, and boulders. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> and <i>Alnus glutinosa</i>), shrubs and herbaceous vegetation (predominantly <i>Rubus fruticosus</i>). Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>
N25	Upstream from Bogodint Village	N 44.90724° E 21.67430°	06.11.2022	8	Substrate consisting predominantly of gravel and boulders, coarse sand and isolated areas with mud. The rocky substrate covered with vegetation. Presence of submerged vegetation. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> and <i>Alnus glutinosa</i>), shrubs and herbaceous vegetation (predominantly <i>Rubus fruticosus</i> and <i>Sambucus nigra</i>). Adequate degree of water shading.	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N26	Downstream from Bogodiņ Village	N 44°53.8486' E 021°39.0468'	24.03.2023	13	Meadow landscape with pasture. Steep left bank, smooth right bank – meadow. Substrate consisting predominantly of gravel and boulders, coarse sand, and isolated areas with mud. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> and <i>Populus alba</i>), shrubs and herbaceous vegetation. Presence of the rapids with strong water current.	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>
N27	Between Bogodiņ and Naidāš villages	N 44.88525333 E 21.63783833	24.03.2023	1	Slightly steep banks with riparian vegetation in favourable condition. Left bank – forest, right bank - meadow surrounded by forest. Substrate consisting predominantly of gravel, boulders, coarse sand, and isolated areas with mud. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> and <i>Populus alba</i>), shrubs and herbaceous vegetation. Presence of the rapids, strong water current.	<i>Alburnoides bipunctatus</i>
N28	Between Bogodiņ and Petrilova villages	N 44°53.3944' E 021°37.4752'	24.03.2023	3	Left bank – forest, right bank - meadow surrounded by forest. Gravel covered with organic matter. Presence of submerged vegetation. Riparian vegetation in favourable condition, consisting of tall trees (predominantly <i>Salix alba</i> , <i>Quercus</i> , <i>Fagus</i> sp., <i>Acacia</i> sp., <i>Acer</i> sp., <i>Alnus</i> sp.) with shrubs (predominantly <i>Sambucus nigra</i>). Smooth flow of water.	<i>Rhodeus amarus</i>

Table 1 (continued): Sampling stations in which *Romanogobio banaticus* was sampled and the habitats characteristics: Sampling station – S.S., Location – L., GIS coordinates – GIS, Date of sampling – D., Number of *Romanogobio banticus* individuals – N., Habitat description – H.D., Predominant fish species – P.F.S.

N29	Upstream of Naidăș Village	N 44°52.9534' E 021°36.3409'	25.03.2023	8	The right bank – slightly steep with riparian vegetation in a strip, followed by pasture. Steep, eroded left bank, poorly represented riparian vegetation, pasture area. Substrate consisting predominantly of gravel, boulders, sand, and fine sand with isolated areas of mud. Submerged vegetation present. Presence of rapids, followed by smooth flow of water.	<i>Romanogobio kessleri</i> <i>banaticus</i> and <i>Cobitis elongata</i>
N30	At Naidăș Village bridge	N 44°53.0731' E 021°35.2273'	25.03.2023	4	Smooth banks, riparian vegetation in inadequate condition. Substrate consisting predominantly of gravel, boulders, sand, and fine sand with isolated areas of mud. Submerged vegetation present.	<i>Alburnoides bipunctatus</i> and <i>Barbus meridionalis</i>
N31	Downstream of Naidăș – upstream of Lescovița villages	N 44.8768133 E 21.5643683	25.03.2023	-	Low banks, left bank – meadow, right bank – meadow and agricultural land. Substrate consisting predominantly of gravel, boulders, silt, and isolated areas with boulders. Strong water flow. Moderate riparian vegetation, dominated by <i>Salix alba</i> .	<i>Neogobius melanostomus</i> and <i>Cobitis elongata</i>
N32	At Lescovița Village bridge	N 44°52.1892' E 021°32.3322'	25.03.2023	2	Left Bank – slightly steep. Right Bank characterized by meadow and pasture. Substrate consisting predominantly of gravel, coarse sand, fine sand, and isolated patches with mud. Strong water flow. Moderate riparian vegetation, dominated by <i>Salix alba</i> .	<i>Cobitis elongata</i>
N33	Between Lescovița and Zlatița villages	N 44°52.5772' E 021°31.4735'	26.03.2023	-	Slightly steep right bank with signs of erosion, smooth left bank characterized by a meadow landscape. The predominant substrate consists of gravel, boulders, with submerged vegetation. Portions of sand and silt. Strong water flow. Moderate riparian vegetation, dominated by <i>Salix alba</i> .	<i>Rhodeus amarus</i>

DISCUSSION

Natural and anthropogenic induced environmental conditions and fish fauna vary both qualitatively and quantitatively in the Carpathian Basin streams and rivers (Simalcsik and Bates, 1973; Curtean-Bănăduc and Bănăduc, 2002, 2008; Bănăduc, 2010; Popa et al., 2013, 2019; Bănăduc et al., 2012, 2013, 2017, 2020a,b, 2021; Didenko, et al, 2014; Curtean-Bănăduc et al., 2014, 2015, 2019; Popescu et al., 2015; Afanasyev et al., 2023) the habitat characteristic variation led to the establishment of fish zonation based on the characteristic indicator fish species. Large and medium size Carpathian rivers which spring in mountain areas, like the Nera, have five such specific fish zones: brown trout zone, grayling and Mediterranean barbel zone, nase zone, barbel zone, and carp zone. (Bănărescu, 1964)

Due to the habitats general characteristics (high river flow, less moderate-accentuated water current, generally devoid of waterfalls, permanently rocky bottom made up by boulders, water saturated in oxygen, oscillations of temperature 12-14°C) and dominance of *Alburnoides bipunctatus* in the majority of the sampling stations where *Romanogobio banaticus* was identified, with the co-dominance of *Barbus balcanicus* and *Cobitis elongata*, it can be stated that the target fish species of this study is located slightly atypically a little upstream on the river in the grayling and Mediterranean barbel zone, in comparison with its presence in the nase zone or even carp zone in other more northern Carpathian rivers (Bănărescu, 1964).

The relatively lower latitude of Nera River with its environmental characteristics in comparison with other rivers where this fish is living can be an explanation for this species presence upstream on the river than in other northern rivers and missing in the lower sectors of the river and their associated ichthyological zones.

It is worth highlighting the fact that this species presence is permanent, the abundance of this species is relatively high, around 5,000 individuals are estimated to be present in the area, the age structure is well balanced. More than that, in three of the sampling stations it is a co-dominant species, all of these revealing a good ecological status of this species populations, and last but not least that the conservation status is favourable.

In spite of the fact that this species populations in the Nera Basin are relatively protected by its natural characteristics and status, and the relatively low human impact presence, there are still some threats and risks from this perspective as following: riverbed mineral exploitation and overexploitation; bridges and viaducts; localities; household wastes; fishing; off-road vehicle driving; pollution; etc.

The riverbed and riverine areas mineral exploitation of the Nera River influence the fish populations through the increasing of the noise and vibrations level, the modification of the downstream sedimentation rate, and the modification of the specific habitat substrate.

The negative impact of bridges and viaducts manifests itself especially during their construction phase, rehabilitation of the bridge legs or when unclogging the riverbed in the near areas. The pressure is represented by the intervention that is carried out with machines in the minor riverbed, affecting the ichthyofauna during construction/rehabilitation by changing the physico-chemical properties of the water (increasing the turbidity of the water, high vibrations, various substances that can reach the water during the pouring of concrete, etc.).

All the localities through which Nera flows exert an impact on the respective lotic sectors. In this sense, household wastes were most often identified thrown into the riverbed or stored on the banks of the water, part of it being carried away by the floods or wind affecting the downstream sectors as well. The micro-plastic released by these wastes represents a significant impact for the ichthyofauna. Ad hoc deposits of construction materials have been identified on the banks, which can affect by releasing various substances and changing the physio-chemical water properties, through runoff during rains or floods. Also, loading with organic matter facilitates the eutrophication process, changing the microbiology of river water,

changing the physio-chemical properties of water, pollution with toxic and corrosive substances from detergents are among the most significant pressures identified, especially in the sectors where the sewage system mouths pours household waste water directly into the river without any filtration or purification system. These pressures have a significant negative impact on both fish and benthic invertebrates' species, which are their main food source. Often in these areas, the number of species is small and the fish can present a danger to the local human population if they are consumed, the toxic substances eliminated in the water being stored in the muscle mass of the fish through bioaccumulation. (Gokul et al., 2023; Curtean-Bănăduc et al., 2023)

The household wastes illegal deposition is another frequent problem. Very often there were identified household wastes in the riverbed or on the river banks, a part of them present there due the wind and water transport action. The intensity of this pressure is related with the presence of the multitude of localities and the abundance of the wastes in some sectors.

Inappropriate and even illegal fishing it is also a problem which create a pressure on fish. This pressure is manifested through the capturing and retaining of the fish of conservation interest, as food for human also as baits for fishing. The majority of the people have no idea about what fish species are protected, they can not identify them, they do not understand their role in nature and for humans, or consider that breaking the laws it is an option.

Off-road driving common practice induces vibrations and noise in water, the water turbidity changings, pollution with oil and gas. The intensity of this pressure is related with the presence of the multitude of localities and by the level of their traffic.

The pollution is present in the rivers sectors where the lotic system pass through or near the localities. The main pollution sources are: household water and wastes, building materials, organic matters, etc.

All these threats and risks should be managed in an integrated way not only in the protected areas but in the whole basin, to create optimum conditions for *Romanogobio banaticus* species of conservation interest protection, the Nera River basin is and still can remain in the future a sanctuary area for *Romanogobio banaticus*.

CONCLUSIONS

Romanogobio banaticus it is a species of conservation interest with a small distribution range in the south-west of the Romanian Carpathians basin. In spite of the presence of some moderate anthropogenic threats and risks, the Nera River is a sanctuary for this fish. The lower latitude of Nera in comparison with other rivers where this fish is living, explain its presence in a rather atypical ichthyological zone. This species presence is permanent, the abundance is relatively high, the age structure is well balanced, more than that, in some sectors it is a co-dominant species, all of these revealing a favourable conservation status. An integrated management plan for the entire Nera River basin should be enforced to keep this area safe, a response to the extending anthropogenic impact.

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