TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

19.3

The Wetlands Diversity

Editors

Angela Curtean-Bănăduc & Doru Bănăduc

Sibiu – Romania
2017

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IN MEMORIAM

Dan Munteanu
(1937 – 2017)

The Romanian biologist *Dan Munteanu* was born in Cluj, in Transylvania, on the 2nd of June 1937.

After graduating from the Natural Sciences Faculty at Babeş-Bolyai University of Cluj, in 1969 he obtained his Doctorate in Biology at the University of Bucharest by defending his doctoral thesis on the Bird fauna of the Mountain Areas of the Moldavian Bistrița River.

A complex personality, *Dan Munteanu* was known in scientific circles as one of the most competent and active ornithologists of Romania, with a solid and wide-ranging background in classical biology.

He chaired the Romanian Ornithological Society; he was member of the Executive Board of the International Waterfowl and Wetlands Research Bureau of Slimbridge (UK); and was representative of the International Council for Bird Preservation of Cambridge (UK). In this last quality he took part in a variety of European nature conservation and preservation programmes.

His bird fauna studies represent a comprehensive list of works, not only in terms of numbers but also of quality, the most important being the following: Provisional Atlas of Romanian breeding birds, a work carried out for the international committee responsible of the European Atlas; the Birds Chapter in the Romanian Red Book of Vertebrates, in the Editions of the Romanian Academy, 2005; Romanian Bird Areas – Documentations, ALMA MATER Editions, Cluj-Napoca, 2004; Rare, vulnerable and endangered birds in Romania, ALMA MATER Editions, Cluj-Napoca, 2009; Romanian Fauna, Aves, Volume XV, Fascicule 2, Editions of the Romanian Academy, 2015.

Dan Munteanu, as a member of the Romanian Academy, was also very effective in advising on broader environmental issues faced by Romania.

Starting with 2000, he chaired the Commission for the Protection of Nature Monuments of Romania, and solved very competently all problems related to its organisation and functioning. In the same quality, he coordinated the activity of the Romanian Scientific Councils of Nature Parks and National Parks. He contributed substantially to the establishment of new protected areas and proposed viable solutions for the conservation of biodiversity and the protection of natural heritage.

The death of *Dan Munteanu*, after a long and painful illness, is a great loss for Romanian biology in the many important areas to which he dedicated his tireless lifelong activity.

A generous and modest man, who dedicated his life to the protection of nature, has left us and will be greatly missed.

The Editors

Transylvanian Review of Systematical and Ecological Research

19.3 The Wetlands Diversity ***2017***

CONTENTS

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 allready the presence of the medium and long-term significant change in the “average weather” all over the world, the most comon key words which reflect the general concern are: heating, desertification, rationalisation and surviwing.

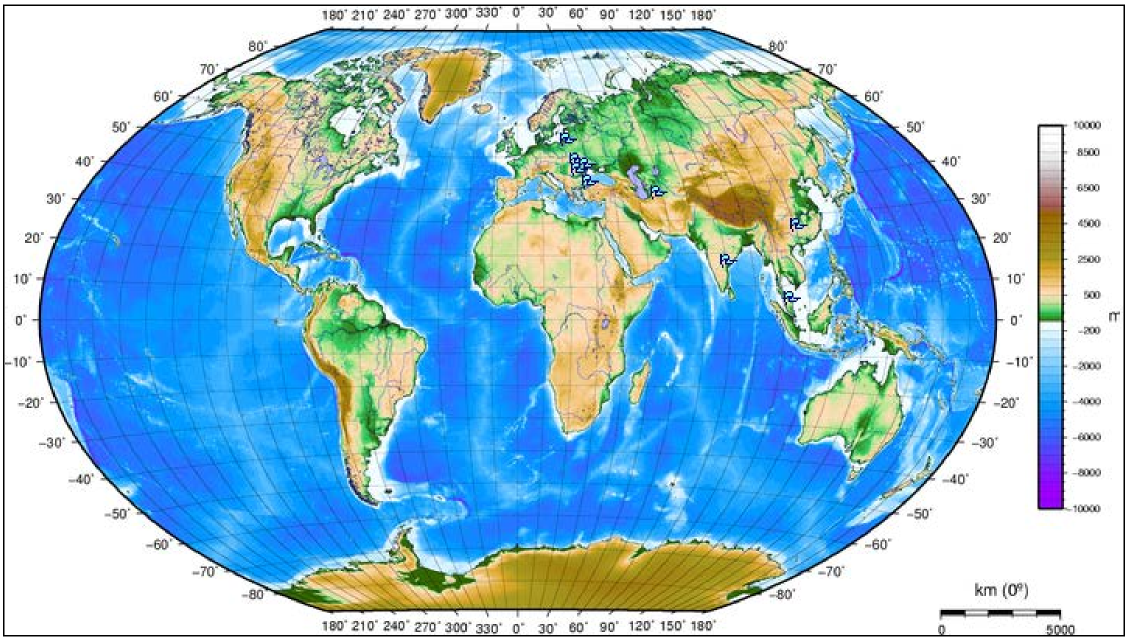
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch a second annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2011.

The therm 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; peatswamp 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 this new 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 sixth volume included variated 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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FLORISTIC DIVERSITY OF LAKES SUBJECTED TO LONG TERM CHANGES IN THE WATER NETWORK OF THE WEST POLESIE (EASTERN POLAND)

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KEYWORDS: amelioration, canal, lake, water reservoir, macrophytes, changes.

ABSTRACT

The Wieprz-Krzna Canal, built in 1961, is one of the longest in Poland (142 km). Although the drainage construction was intended to revitalize the region of wetlands and peat-bogs of the West Polesie, it caused large hydrological changes. Research on catchments of three natural lakes and three retention reservoirs involved cartographic analysis using photointerpretation, as well as the Braun-Blanquet method. In the studied area, between 1939 and 2016 the length of rivers and ditches increased more than three times. Macrophytes covered about 20-27% of the natural lakes water surface, whereas in retention reservoirs the coverage was 12-15.5%. Also a greater diversity of macrophytes occurred in natural lakes. In retention reservoirs it was restricted to only emerged macrophytes.

RESUMEN: Diversidad floristica de lagos sujetos a cambios a largo plazo en la red de aguas del Polesia Occidental (Polonia Oriental).

El Canal Wieprz-Krzna, construido en 1961, es uno de los más largos de Polonia (142 km). Aunque la construcción del drenaje tenía por objeto revitalizar la región de humedales y turberas de la Polesia Occidental, causó grandes cambios hidrológicos. Las investigaciones sobre captación de tres lagos naturales y tres reservorios de retención involucraron análisis cartográficos con el método de fotointerpretación, así como el método de Braun-Blanquet. En el área estudiada, entre 1939 y 2016 la longitud de ríos y zanjas aumentó más de tres veces. Los macrófitos cubrían alrededor del 20-27% de la superficie del agua de los lagos naturales, mientras que en los reservorios de retención era desde 12 al 15,5%. También se observó una mayor diversidad de macrófitas en lagos naturales. En tanques de retención sólo se restringió a las macrófitas emergidas.

REZUMAT: Diversitatea floristică a lacurilor supuse modificărilor pe termen lung în reţeaua hidrografică a Polesiei de vest (estul Poloniei).

Canalul Wieprz-Krzna, construit în 1961, este unul dintre cele mai mari din Polonia (142 km lungime). De altfel, realizarea drenării a fost destinată revitalizării regiunii zonelor umede şi a turbăriilor din Polesia de Vest, a provocat schimbări hidrologice mari. Cercetările privind captarea a trei lacuri naturale şi a trei rezervoare de retenţie au implicat analize cartografice utilizând metoda de interpretare a imaginilor – metoda fotointerpretării, precum şi metoda Braun-Blanquet. În aria studiată, între anii 1939 şi 2016 lungimea râurilor şi a canalelor a crescut de mai mult de trei ori. Macrofitele acoperă aproximativ 20-27% din lacurile naturale de suprafaţă, în timp ce în rezervoarele de retenţie între 12 şi 15,5%. De asemenea, o mare diversitate de macrofite este în lacurile naturale. În tancurile de reţinere acestea sunt limitate doar la macrofitele emerse.

INTRODUCTION

It is well known, that each melioration action changes ecosystem and natural balance, e.g. in diversity of flora species. One of the most important factors determining the occurrence and diversity of aquatic and riverine plants in aquatic ecosystems is the water level fluctuations. (Soszka et al., 2012; Curtean-Bănăduc et al., 2014)

Since the 50s, in the researched Łęczna-Włodawa Lake District have been significant changes in the hydrological network. Almost in all lakes it caused the changes in the management of reservoirs, as well as in water supplying of lakes.

The creation of the Wieprz-Krzna Canal was proposed in 1953. The construction was intended to revitalize the region of the West Polesie by building a drainage infrastructure on wetlands and peat-bogs (Radwan, 1994).

Building of the Canal began in 1954 and was completed and opened in 1961. The Wieprz-Krzna Canal is the longest of its type in Lubelskie Region and one of the longest in Poland at 142 km in length. It draws water from the Wieprz River in the Borowica Village, where it begins, ending in the area of the Międzyrzec Podlaski City, where flows into the Krzna River (Dawidek et al., 2004).

The total area under the Wieprz-Krzna Canal's influence is almost 528,000 ha. More than 290,000 ha of meadows and about 280,000 ha of agriculture lands were under melioration works between 1956 and 1960 (Grzyb et al., 1982; Pichla and Jakimiuk, 2008). In the studied mesoregion of Łęczna-Włodawa Lake District most of the water reservoirs are included in the melioration system. Some of them are used for fishery management (Harasimiuk et al., 1998).

Melioration works caused large hydrological changes in the studied area and consequently changes in the land use, as well in the reservoirs. The aim of the study was to assess the degree of these changes and to identify trends in further changes related with phytocenoses of the examined lakes. Lakes with different land cover forms and management of catchment were analyzed.

The mesoregion of Łęczna-Włodawa Lake District is included in the area of the East European Plain (Harasimiuk et al., 1998; Kondracki, 2013), as well as the macroregion of Polesie Zachodnie (Kondracki, 1995, 2013).

A very small slope of terrain results in a specific slow surface runoff (Kowalczyk, 1974).

The researched area is characterized by a high variety of hydrogenic landscapes (peat-bogs, wetlands, rivers, ponds) and the occurrence of more than 60 natural reservoirs (Fig. 1). That's why a mesoregion of the Łęczna-Włodawa Lake District has high natural values (Harasimiuk et al., 1998) and constitutes one of the biggest touristic regions in the Lublin Region (Krukowska, 2009).

The whole research area is included inside the "West Polesie" Transboundary Biosphere Reserve. The study area is also included in many other types of protection areas, e.g. Polesie National Park, landscape parks, nature reserves, areas of landscape protection, as well as NATURA 2000 sites: OSO and SOO.

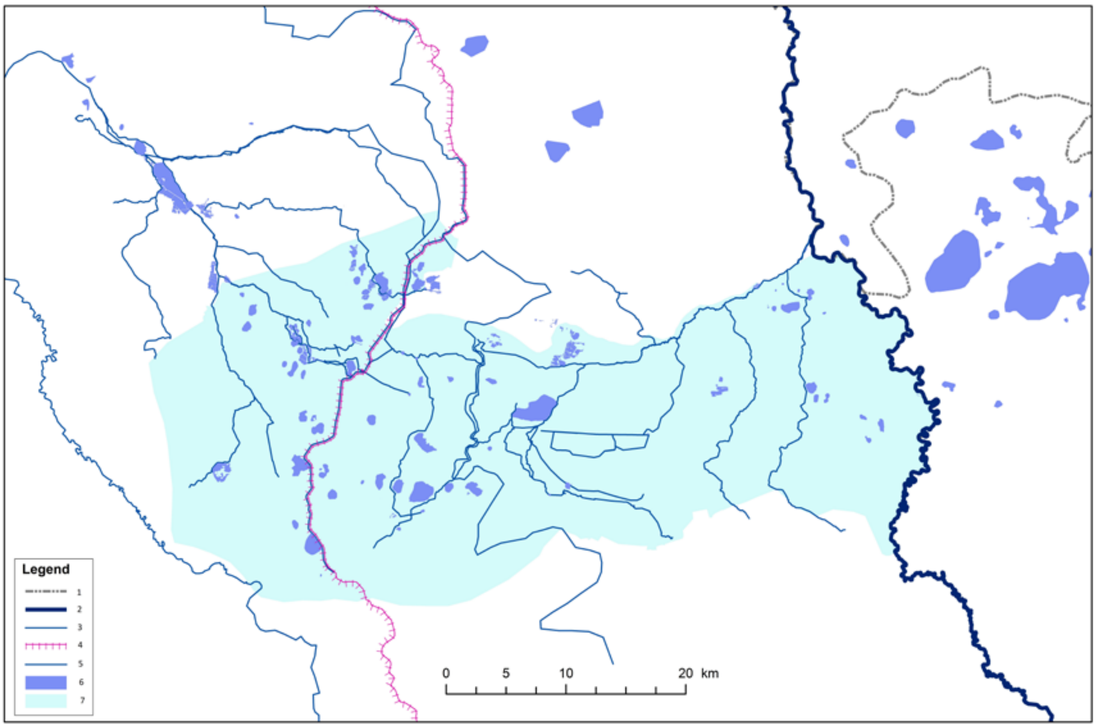

Figure 1: Studied area of Łęczna-Włodawa Lake District: 1 – national border, 2 – cross-border Bug River, 3 – rivers of studied area, 4 – Wieprz-Krzna Canal, 5 – other rivers, 6 – lakes and reservoirs, 7 – Łęczna-Włodawa Lake District.

Study area included lakes located in the central part of the Łęczna-Włodawa Lake District (Fig. 1). Three natural lakes, as well as three lakes converted into retention reservoirs after 1954, were chose under the study. For the group of natural lakes the following were selected:

1. Lake Łukcze (51°23'48.8" N, 22°57'57.8" E) is the smallest and the shallowest of the studied lakes, with surface of 56.5 ha, and maximum depth of nine m. Length of its shoreline is 3,876 km, and capacity of the lake is 2,091,000 m^2 (Wilgat et al., 1991).
2. Lake Rogóźno (51°22'36" N, 22°58'21" E) with a surface area of 57.1 ha, is relatively deep (25.4 m). The shoreline, in a shape similar to a circus, is poorly developed. The capacity of the lake is 4,209,000 m^2 (Michalczyk and Wilgat, 1998).
3. Lake Krasne (51°25'35" N, 22°57'31" E) is the deepest (33 m) and the largest (75.9 ha) of the studied lakes. Length of the shoreline is about 3.6 km, whereas the capacity is 8,180,000 m^2 (Michalczyk and Wilgat, 1998).

While the group of retention reservoirs included:

1. Tomaszne Reservoir (51°28'11.9" N, 23°00'09.5" E), before building the Wieprz-Krzna Canal it was a natural lake with a surface of 67 ha and maximum depth of 3.5 m. Nowadays its surface is 85.5 ha. Length of the shoreline is 3.65 km, whereas water capacity is 2,208,000 m^3.

2. Krzczeń Reservoir (51°23'59.64" N, 22°56'5.03" E), after building the Wieprz-Krzna Canal its surface increased eight times – from 20 ha to 160 ha. Length of the shoreline is about 6.7 km, length is about 1.84 km, width is 1.67 km, whereas maximum depth is 5.2 m.
3. Dratów Reservoir (51°20'26" N, 22°56'45" E), after building the Wieprz-Krzna Canal its surface increased almost two times – from 87 to 168 ha. Length of the shoreline is about five km, length is about 1.83 km, width is 1.5 km, whereas maximum depth is two m.

MATERIAL AND METHODS

Cartographic analysis was made using raster maps, orthophotomaps and field studies. Maps were downloaded from the Geoportal service (www.geoportal.gov.pl). Lakes' catchments were designated on the base of the topographic map in a scale 1:25,000, in ArcGIS 10.4. Maps of land cover and terrain formation, as well as analysis of hydrographical network of the studied Wieprz-Krzna Canal's impact area were prepared. An analysis of the land cover structure of the catchment area was made using photointerpretation, a method often used in landscape ecology research. It was based on transformation of digital image data (so-called quantitative continuous data) into vector thematic data (so-called discrete qualitative data) in the form of land cover classes (Chmielewski et al., 1996; Lu and Weng, 2007; Chmielewski and Chmielewski, 2009; Lechowski, 2013; Kozak et al., 2014). A definition of land cover was adopted by the physical properties of the Earth's surface (Fisher et al., 2005). A photointerpretation method was performed by visual – manual procedure, which involved a detailed analysis of the direct and indirect distinctive characteristics (size, shape, or color tone, structure and texture of the image, shadow, interconnected objects, etc.) of teledetection materials detected on the monitor screen and then manually outlining all elements of land cover forms by the interpreter (Pyka and Mularz, 1998; Longley et al., 2010). Outlining of all elements was made with the principle of mutual complementation of polygons and the correctness and topological consistency of the entire coverage.

Field studies were carried out in August 2015 and August 2016 and included a phytolittoral of all studied lakes. The studies were conducted by the Braun-Blanquet method (Braun-Blanquet, 2013). Phytosociological units were determined by the analysis of dominant species using Matuszkiewicz's nomenclature (2008). The plant communities analysis of lakes were carried out in transects from the shore to the maximum depth of the plant occurrences (from four transects in lakes to eight in reservoirs). In addition to the surface, the range (depth) of the occurrence of particular groups of macrophytes was analyzed.

In order to distinguish a group of similar lakes due to the researched features (like height above sea level, management of catchment and the qualitative and quantitative structure of macrophytes), cluster analysis was used. The Euclidean distance and the Ward method was used to estimate distance between clusters. Then a non-hierarchical method was applied – grouping by three-means, to indicate which variables play the main role in the division into clusters (that differentiate the examined lakes).

Spearman's rank correlation coefficient was used to investigate a relationship between different forms of land development around lakes and the height above sea level.

Due to the fact that the Shapiro-Wilk test rejected the normality of some tested features, the Wilcoxon's nonparametric test was investigated to determine whether the average surface area of particular land cover forms had changed since 1939.

RESULTS

The conducted analysis, covering 29,039 ha, proved large changes in the hydrographic network (Fig. 2). After the construction of the Wieprz-Krzna Canal, the length of the rivers and ditches nearly tripled. The surface of the water reservoirs have also changed considerably. Some of them completely changed the shape of the shoreline (Dratów, Tomaszne, and Krzczeń) leading to the surface of standing waters increasing about 300 ha (Tab. 1).

Table 1: Length of rivers and ditches and surface of reservoirs in 1939 and 2016 in studied area.

Length of rivers and ditches (m)		Surface of reservoirs (ha)	
1939	2016	1939	2016
149,573	482,189	1,061	1,324

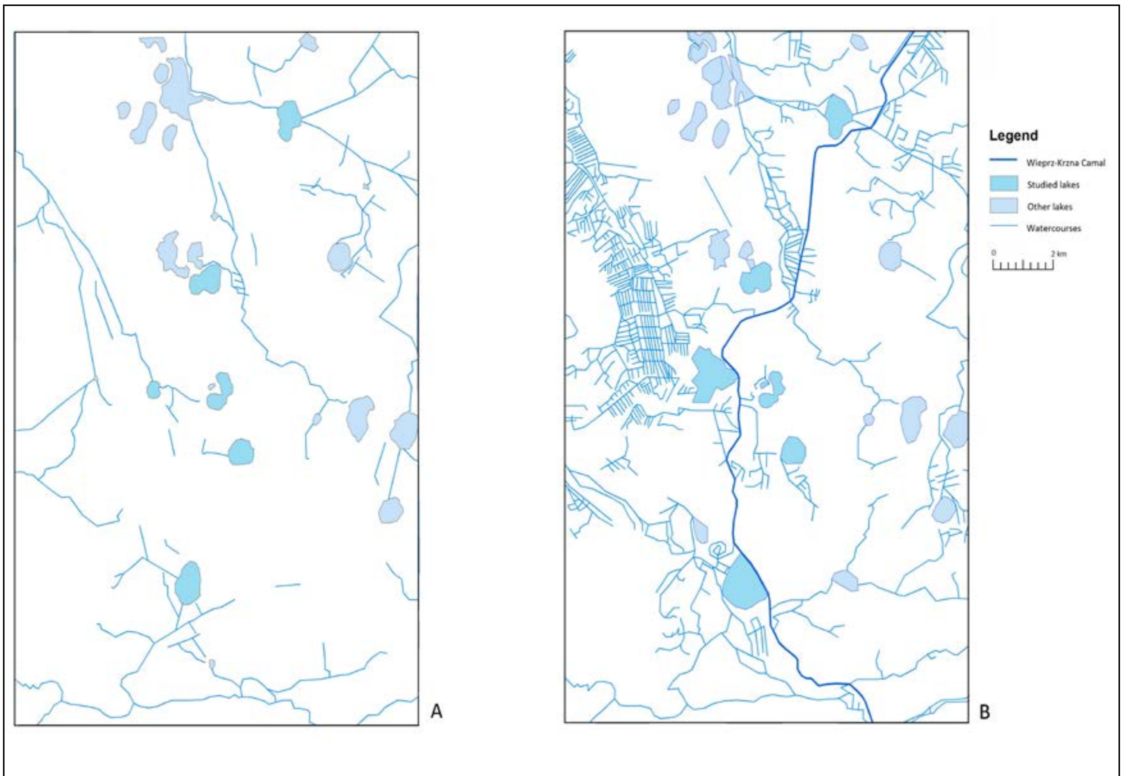

Figure 2: Water network changes in investigated area: A – 1939, B – 2016.

The largest catchment among examined lakes surrounded lakes Rogóźno (796 ha) and Łukcze – 437 ha, while lake Krasne was characterized by the smallest catchment – only 261 ha. Currently, the dominant land cover forms in the lakes' catchment area was agriculture fields, as well as forests in the Rogóźno and Łukcze lakes catchments. The smallest surface was covered by marshes and peat-bogs (Tab. 2). Since 1939, the dominant type of land cover has not changed substantially. Since the 1930's in the Łukcze Lake catchment the meadows dominated. It is the only lake area in which agriculture lands increased. Forests' surface increased in all studied lakes (Fig. 3).

Table 2: Land cover forms in studied natural lakes' catchment (ha).

Land cover forms	Rogóźno (ha)			Krasne (ha)			Łukcze (ha)		
	1939	2016	changes	1939	2016	changes	1939	2016	changes
Buildings	100	63	– 37	22	28	+ 6	64	93	+ 29
Wetlands	70	40	– 30	0	0	0	6	23	+ 17
Meadows	46	55	+ 9	28	34	+ 6	183	48	– 135
Forests	190	276	+ 86	0	34	+ 34	31	113	+ 82
Water lakes surface	55	52	– 3	71	74	+ 3	58	54	– 4
Agriculture lands	333	308	– 25	97	54	– 43	95	106	+ 11
Ponds	0	0	0	43	37	– 6	0	0	0

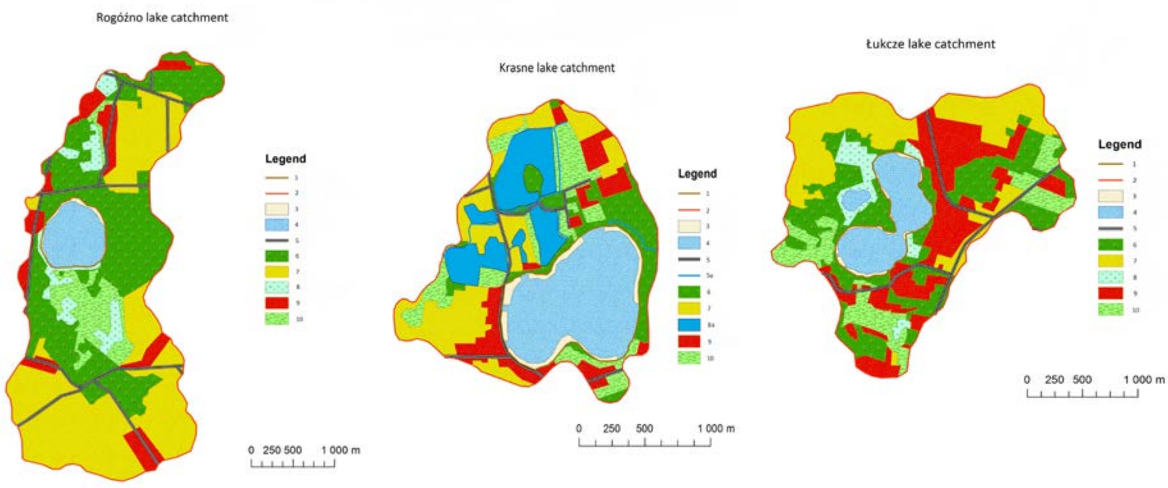

Figure 3: Land cover forms in studied natural lakes' catchments in 2016:
1 – shoreline, 2 – catchment border, 3 – rushes community, 4 – lake, 5 – road,
5a – river, 6 – forests, 7 – agriculture lands, 8 – wetlands, 8a – ponds, 9 – buildings,
10 – meadows.

Among the examined retention lakes (reservoirs) the largest catchment area surrounded reservoirs Dratów (1,283 ha) and Krzczeń (623 ha), while Tomaszne Reservoir was only 355 ha. These catchments covered mainly forest and meadow. Whereas Krzczeń Reservoir catchment covered mainly farmlands and meadows (Tab. 3). In the period of more than 70 years the biggest changes took place in the Krzczeń Reservoir' catchment: surface of the reservoir increased by 140 hectares, while the area of meadows and buildings decreased significantly (Fig. 4).

Table 3: Land cover forms in studied retention lakes' catchments (ha).

Land cover forms	Dratów (ha)			Tomaszne (ha)			Krzczeń (ha)		
	1939	2016	changes	1939	2016	changes	1939	2016	changes
Wieprz-Krzna Canal	0	26	+ 26	0	7	+ 7	0	9	+ 9
Forests	0	42	+ 42	7	87	+ 80	33	83	+ 50
Meadows	604	421	– 183	175	73	– 102	294	141	– 153
Agriculture lands	342	373	+ 31	79	58	– 21	158	134	– 24
Wetlands	176	179	+ 3	0	0	0	34	58	+ 24
Buildings	74	74	0	25	37	+12	84	38	– 46
Water lakes surface	87	168	+ 81	69	89	+ 20	20	160	+ 140
Ponds	0	0	0	0	4	– 4	0	0	0

In other catchments of retention reservoirs, surface of meadows was significantly reduced, while the area of forests increased (Fig. 4).

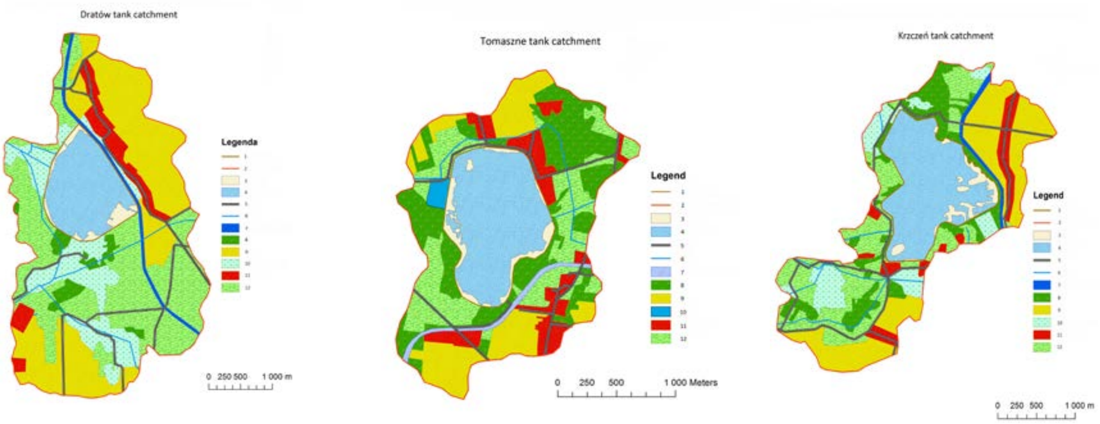

Figure 4: Land cover forms in studied retention lakes' catchments in 2016:
1 – shoreline, 2 – catchment border, 3 – rushes community, 4 – water lake,
5 – road, 6 – river, 7 – Wieprz-Krzna Canal, 8 – forests, 9 – agriculture lands, 10 – wetlands,
11 – buildings, 12 – meadows.

The largest area in the catchments of all examined lakes was located between 166 and 169 m a.s.l. The area around lakes Rogóźno and Łukcze was relatively the highest, respectively covering 652 ha and 197 ha (Fig. 5). The lowest terrain, between 162 and 165 m a.s.l., occurred in the most extensive areas in catchments of reservoirs Krzczeń (229 ha) and Tomaszne (160 ha) (Fig. 6).

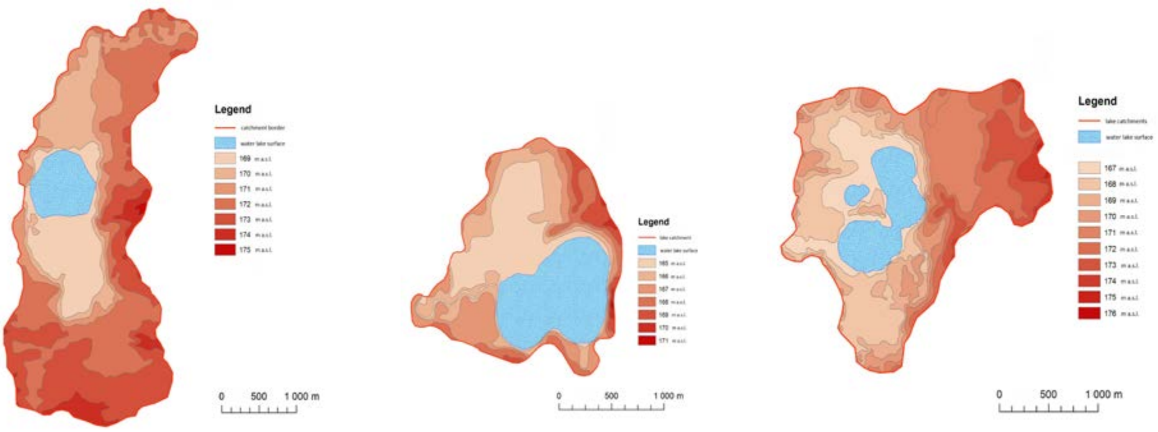

Figure 5: Terrain formation of studied natural lakes' catchments; from the left: Rogóźno, Krasne, and Łukcze.

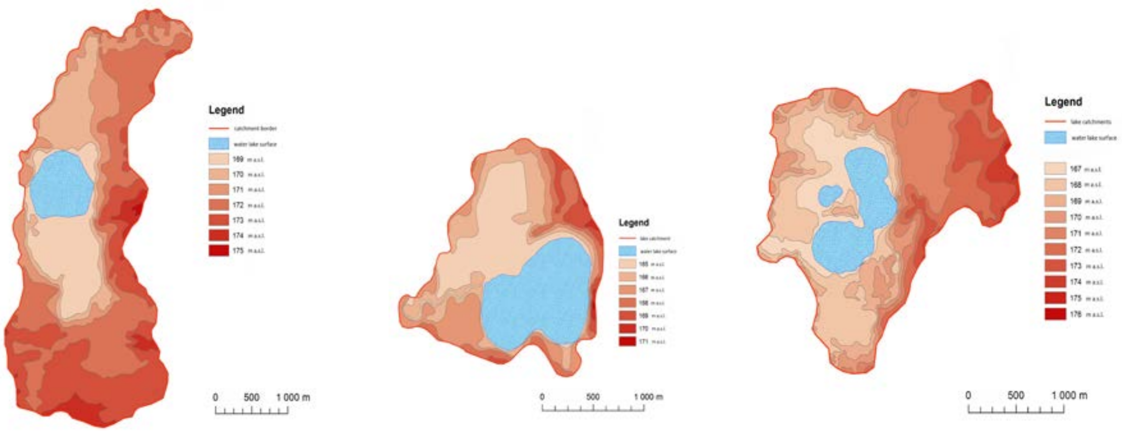

Figure 6: Terrain formation of studied reservoirs' catchments; from the left: Dratów, Tomaszne, and Krzczeń.

Differences of height around the lakes were noticeable. Usually the fastest height increase occurred from the north-east side of the lakes. The western and southern sides of the reservoirs usually constituted flat areas and were situated the lowest (Fig. 5).

On the base of agglomeration course made for catchment development and its terrain three groups of lakes were distinguished: Ist: Dratów, IInd: Krasne, Tomaszne, Łukcze, Krzczeń and IIIrd: Rogóźno (Fig. 7).

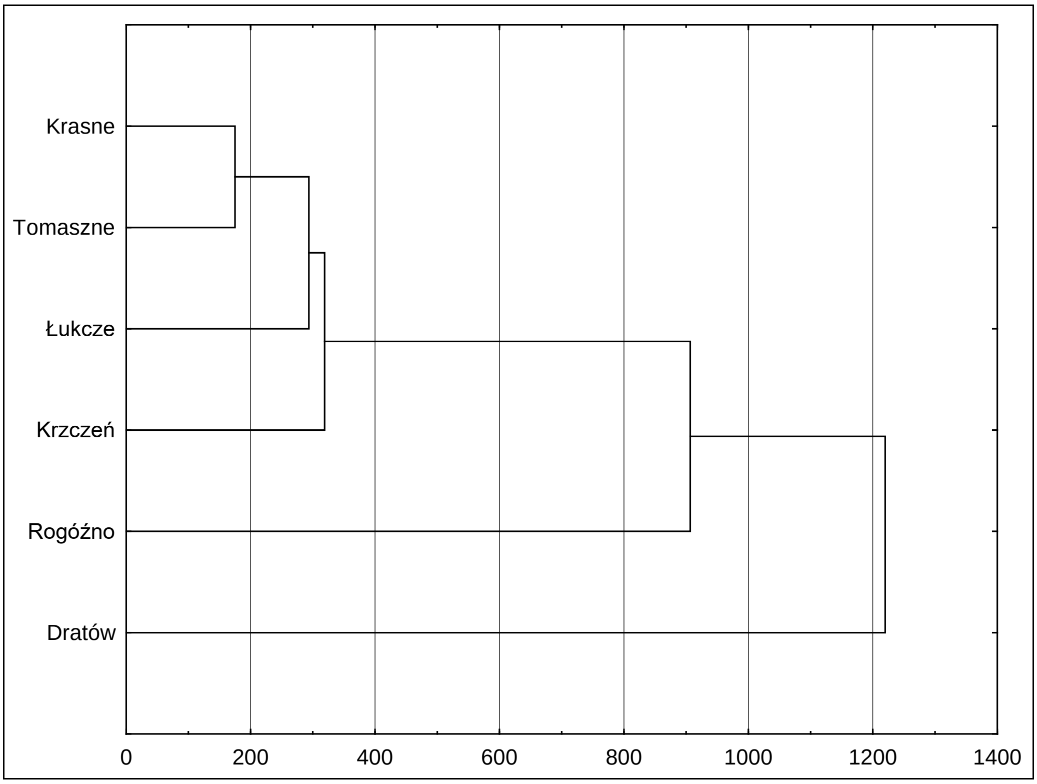

Figure 7: Similarity of studied lakes
due to catchments' land cover forms and terrain formation.

On the base of non-hierarchical method of grouping by the three-median, a size of the area over 165 m a.s.l., the area of marshes, peatlands, forests and fields ($p < 0.05$) were indicated as variables which decisively differentiated the examined lakes (Tab. 4).

Table 4: Variance analysis.

Variables	df	F	p
162-165 m a.s.l.	3	0.857	0.507
166-169 m a.s.l.	3	60.840	0.003
170-173 m a.s.l.	3	16.563	0.023
174-177 m a.s.l.	3	35.453	0.008
Buildings	3	0.312	0.744
Wetlands	3	13.645	0.031
Meadows	3	20.799	0.015
Forests	3	16.495	0.024
Water lakes surface	3	1.659	0.327
Agriculture lands	3	19.066	0.010
Wieprz-Krzna Canal	3	7.213	0.045

Compared to the lakes Krasne, Tomaszne, Łukcze, and Krzczeń, the Dratów Reservoir was surrounded by much lower area above sea level, had less wetlands and agricultural fields in its catchment area, on the other hand it was surrounded by similar surface of buildings and forests. However, lake Rogóźno was surrounded by the area of 170-173 m a.s.l. with a dominant share of forests.

Spearman's rank correlation coefficient indicates a very strong and positive relationship between the area of agriculture fields and the area at 170-173 m a.s.l. ($r = 0.8697$).

Research has shown that since 1939 significant changes have occurred only in forested area ($p = 0.0277$). More than half of the surveyed lakes were characterized by forest areas exceeding 80 hectares. In other cases, there were no statistically significant differences.

Floristic diversity

Usually macrophytes covered about 20-27% of the natural lakes water surface, while in retention reservoirs they covered 12-15.5%. Share of particular groups of macrophytes was also distinctly different. In the lakes, the share in the phytolittoral of emerged macrophytes ranged from 8% (Łukcze, Rogóźno) to 12% (Krasne), in retention lakes this value was similar and amounted to 13%. However, submerged macrophytes covered from 0.06% in Dratów Reservoir to 3.6% in Tomaszne, while in lakes these values were much higher: from 9% in Łukcze Lake to 19% in Rogóźno. In the lakes emerged macrophytes occurred from 1.4 m (Rogóźno) to two m (Krasne), while submerged from 2.1 m (Rogóźno Lake), even to 3.5 m in Łukcze Lake. In retention lakes any group of macrophytes didn't exceed one m. A width of rushes was significantly higher in the retention lakes and ranged from 160 m (Tomaszne) to 411 m (Krzczeń). The rushes in the lakes formed a belt reaching a maximum width of 76 m (Krasne) (Tab. 5; Fig. 8).

Table 5: Phytolittoral characteristic in studied lakes (E – emergent; S – submerged macrophytes).

Lakes	Surface (ha)	Phytolittoral of emergent macrophytes (ha)	Phytolittoral of submerged macrophytes (ha)	Total phyto-littoral (ha)	Width of rushes (m)			Max. depth of macro-phytes occu-rrence		Number of plant commu-nities	
					max.	min.	av.	E	S	E	S
Dratów	168	21.7	0.1	21.8	303	12	156	0.6	0.7	5	1
Krzczeń	160	20	2.6	22.6	411	12	212	0.5	0.3	4	1
Tomaszne	89	11	3.2	14.2	160	17	89	0.7	0.9	5	2
Krasne	71	9.2	7.3	16.5	76	10	43	2	2.7	5	6
Łukcze	54	4.4	4.8	9.2	41	8	25	1.9	2.1	5	6
Rogóźno	52	4.2	9.8	14	61	11	36	1.4	3.5	6	6

The phytolittoral of studied lakes consisted of a different number of plant communities. In the lakes this number ranged from 11 (Łukcze, Krasne) to 12 (Rogóźno), whereas in reservoirs the range was from five (Krzczeń) to seven (Tomaszne). In retention reservoirs communities of emergent macrophytes dominated, whereas in natural lakes submerged macrophytes were dominant (Tab. 5; Figs. 8 and 9).

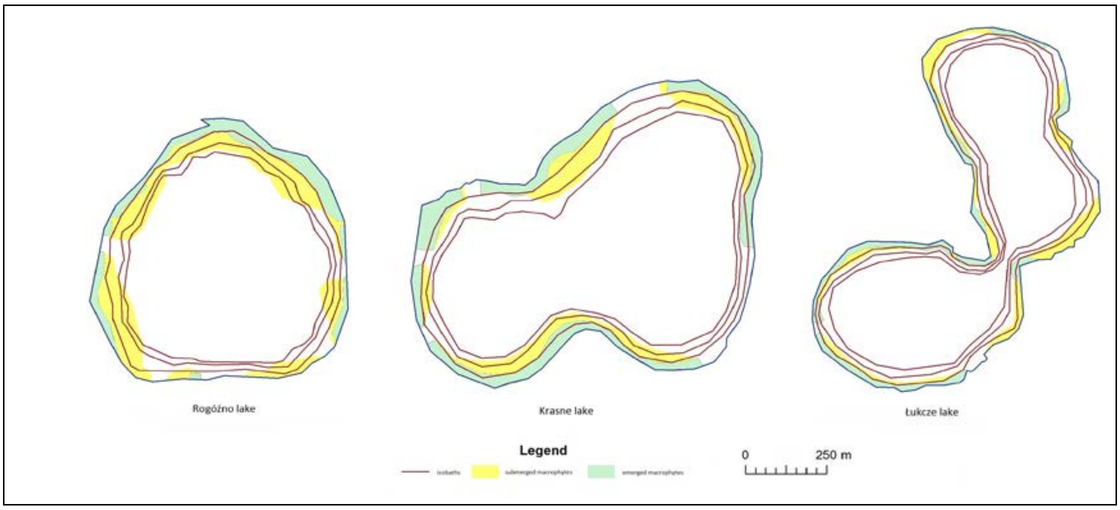

Figure 8: Distribution of macrophytes communities in natural lakes.

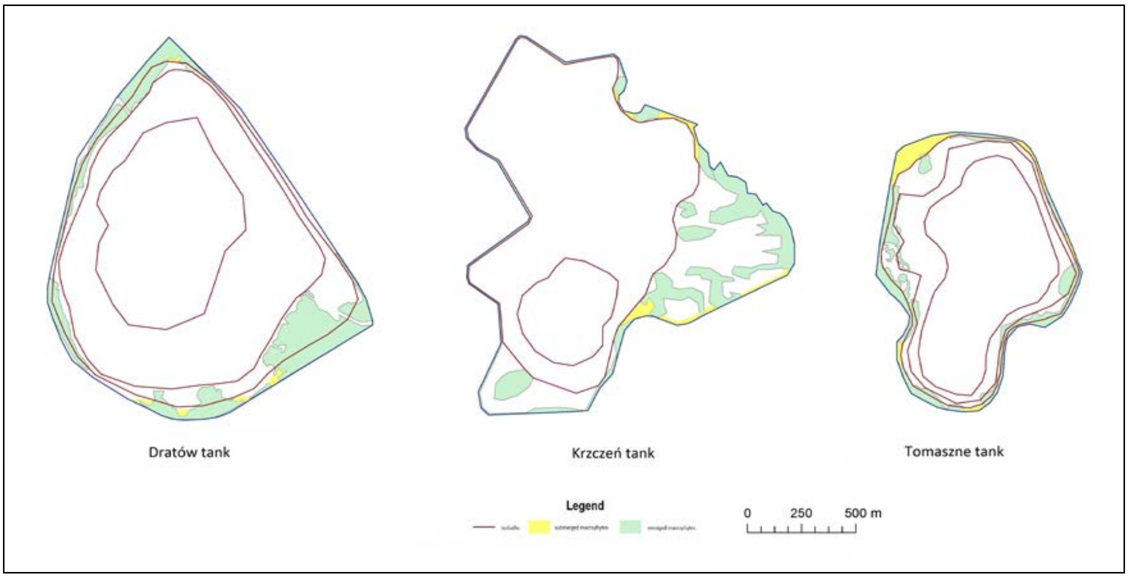

Figure 9: Distribution of macrophytes communities in retention reservoirs.

Macrophytes' cluster analysis of six aquatic ecosystems, based on qualitative and quantitative composition indicated a significant similarity of macrophytes' groups (Fig. 10). Retention reservoirs formed a floristically similar group. The greatest similarity in this group was shown by macrophytes of Krzczeń and Dratów lakes (89%). High similarity was also observed between macrophytes of studied lakes (80-85%). By far the smallest similarity of only 32-37% occurred between retention reservoirs and natural lakes (Fig. 10).

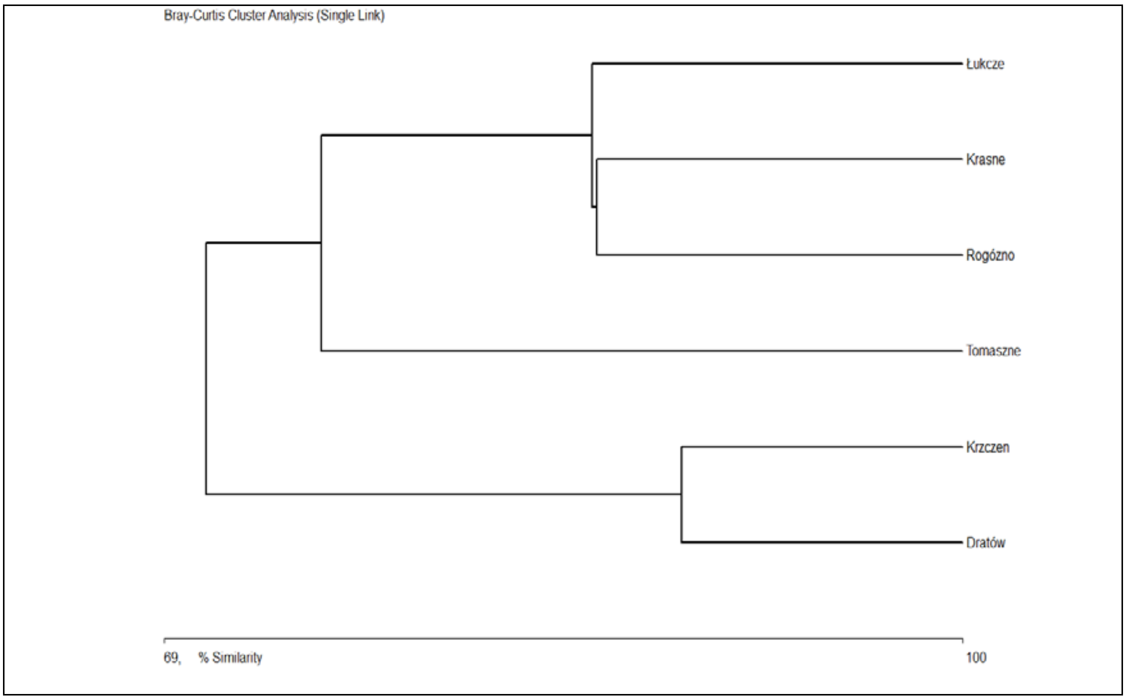

Figure 10: Floristic similarity of studied lakes.

DISCUSSION

The previously planned target of economic recovery of the region did not produce the expected results. It was rather degraded to nature, which is the pearl of that part of Poland. Certainly in the present day another direction of development, identical with regional possibilities, would allow for dynamic development, especially in the direction of tourism. At present, however, many lake ecosystems in the Łęczna-Włodawa Lake District are subjected to continuous eutrophication, at various rates. The construction of the Wieprz-Krzna Canal contributed to changes in water relations, primarily resulting in a decrease of the water level in lakes (Radwan and Chmielewski, 1997). The appropriate amount of water was provided by a network of retention reservoirs (six with embankments and five newly built retention tanks) (Solis, 2012).

By the construction of a drainage system in the Łęczna-Włodawa Lake District, the surface of wetlands and peat lands has decreased significantly (Pichla, 2011). In the analyzed area it resulted in almost three times increase of the length of watercourses and ditches. Human activity has contributed to the multiplication of an occurrence of a large number of waterways in the Lake District (Wojciechowski, 1976; Wilgat et al., 1997). The surface of the lakes also increased by as much as 300 hectares. The changes that occurred during the period 1939-2016 in the management of the catchment area were noticeable. In the catchments of natural lakes forested area increased, whereas area of meadows was reduced. The surface area of the natural lakes has changed only slightly.

Changes in water relations in the Łęczna-Włodawa Lake District led not only to morphometric changes, but also affected their trophy. Currently, apart from mesotrophic Rogóźno Lake, all of the studied lakes belonged to eutrophic type (Wojciechowska and Solis, 2009). Retention tanks were characterized by very high trophic levels, primarily caused by direct impact of waters of the Wieprz-Krzna Canal (Solis, 2012).

Apart from differences in trophy between tanks and lakes, there was a clear distinction among macrophytes. Retention reservoirs, exposed to greater influences, both connected with their origin and impact of Wieprz-Krzna Canal waters were significantly poorer in qualitative and quantitative vegetation than natural lakes.

In the past the studied retention reservoirs were natural lakes. By transformation into retention tanks and connection to the Wieprz-Krzna Canal, their surface area increased. Because of their shallowness they are characterized by fewer predispositions for self-cleaning (Traczewska, 2012). Water relations in retention reservoirs have undergone a great change, and the factor that fostered a trophic growth was touristic and recreational use of the catchment areas, mainly by development of summer buildings, the creation of beaches or fishing (Michalczyk and Wilgat, 1998; Krukowska and Krukowski, 2012). In the retention tanks there was also an intensive fishery. Inadequate fishery management can lead to negative impacts, resulting in decreased biodiversity, reduces of water transparency or disappearance of underwater vegetation (Opuszyński, 1997), and vice versa the high trophy reduces the quality of habitat conditions for fish (Jezierska-Madziar and Pińskwar, 2008).

The visible difference between lakes and reservoirs, their trophy and vegetation is primarily a result of the way of management and changes occurring inside. The method of the tanks catchments' management was dominated by natural forms of land cover. Lake catchments were more transformed by human activity.

CONCLUSIONS

Based on the analysis of the catchment characteristics, the studied lakes formed three groups without a clear division into natural lakes and retention reservoirs, whereas botanical analysis clearly distinguished them.

After construction of the Wieprz-Krzna Canal the number of watercourses and lakes' surface increased in the researched area.

Despite lakes catchments being subject to greater anthropogenic pressure, they are characterized by far greater natural values.

Catchments of examined lakes and retention reservoirs have changed the land management towards increased forest cover and reduction of agricultural fields.

Greater diversity of macrophytes occurred in natural lakes. In retention tanks it was only restricted to emerge macrophytes.

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REPRODUCTIVE ECOLOGY OF *ACANTHUS ILICIFOLIUS* L., A NON-VIVIPAROUS MANGROVE ASSOCIATE IN CORINGA MANGROVE FOREST, ANDHRA PRADESH (INDIA)

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KEYWORDS: Facultative xenogamy, temporal dioecy, melittophily, explosive fruit dehiscence.

ABSTRACT

Acanthus ilicifolius L. (Acanthaceae) is an evergreen non-viviparous mangrove associate. It is hermaphroditic, strongly protandrous, self-compatible, facultative xenogamous, temporally dioecious and melittophilous. The floral mechanism is highly specialized and adapted for pollination by large-bodied bees. The natural fruit set is below 30%. The fruits mature within a month and usually contain four seeds. The fruit is a capsule and splits explosively in the dorsi-ventral plane ejecting the seeds away. This makes it anemochorous. The gregarious occurrence of the plant at the study site is attributed to propagation by seed and vegetative modes.

ZUSAMMENGASSUNG: Die Vermehrungsökologie von *Acanthus ilicifolius* L., eine nicht-vivipare Mangrove im Gefüge des Coringa-Mangrovenwaldes von Andhra Pradesh (Indien).

Acanthus ilicifolius L. (Acanthaceae) ist ein immergrüne, nicht-vivipare Art im Bestand der Mangrovenwälder Sie ist zweigeschlechtlich, stark protandrisch, selbstkombatible, fakultativ xenogam sowie vorübergehend zweihäusig und melittophil. Der florale Mechanismus ist hoch spezialisiert und für die Bestäubung durch große Bienen angepasst. Die natürliche Fruchtmenge liegt unter 30%. Die Früchte reifen innerhalb eines Monats und enthalten meist vier Samen. Die Frucht ist eine Kapsel, die explosiv in der dorsi-ventralen Richtung aufreißt, die Samen hinauschleudert und sich daher anemochor d.h. durch den Wind verbreitet. Das gesellige Auftreten der Pflanze am Untersuchungsort wird der Ausbreitung durch Samen und auf vegetative Weise zugeschrieben.

REZUMAT: Ecologia reproductivă la *Acanthus ilicifolius* L., o mangrovă non-vivipară asociată din pădurea de mangrove Coringa, Andhra Pradesh (India).

Acanthus ilicifolius L. (Acanthaceae) este o mangrovă non-vivipară asociată veşnic verde. Este hermafrodită, cu protandrie accentuată, auto-compatibilă, cu xenogamie facultativă, temporar dioică şi melitofilă. Mecanismul floral este foarte specializat şi adaptat pentru polenizare de către albinele mari muncitoare. Setul natural de fructe este sub 30%. Fructele se coc în termen de o lună şi conţin în majoritatea cazurilor patru seminţe. Fructul este o capsulă şi se sparge în mod exploziv în plan dorso-ventral expulzând seminţele şi prin urmare este o plantă anemocoră. Apariţia gregară în zona studiată este atribuită propagării seminţelor cât şi a modului vegetativ de înmulţire.

INTRODUCTION

Mangrove biodiversity is important for human well-being through climate regulation, food security and poverty reduction, and also a bio-shield against natural calamities. (Alang et al., 2010; Aziz and Hashim, 2011; Aluri, 2013; Sabai D. and Sisitka H., 2013) Acanthaceae, with some 200 genera and 3,000 species (Souza and Lorenzi, 2005) predominantly have a pan tropical distribution and the American continent harbours the greatest richness and morphological diversity of species (Ezcurra, 1989). Mariette (2000) provided an elaborate account on the function and evolution of stamina filament complex in Acanthaceae with reference to its role in the evolution of pollination syndromes. *Acanthus* is a genus of about 30 species in the family Acanthaceae. It is native to tropical and warm temperate regions, with the highest species diversity in the Mediterranean Basin and Asia (Barker, 1986; Tomlinson, 1986). Three species *A. ilicifolius, A. ebracteatus* and *A. volubilis* are characteristic associates of mangroves and range from India to the Western Pacific (New Caledonia), tropical Australia, and the Philippines (Tomlinson, 1986). *Acanthus* is poorly known with reference to its reproductive ecology. Fragmentary information on the pollination ecology of *A. ilicifolius* is available based on brief field studies. Primack and Tomlinson (1980) noted that *Acanthus ilicifolius* is pollinated by the sunbird, *Nectarinia jugularis* in Queensland, Australia. Tomlinson (1986) mentioned that *A. ilicifolius* offers nectar as the main floral reward; it is pollinated by insects, especially bees. Solomon Raju (1990) reported that *A. ilicifolius* is pollinated by sunbirds, *Nectarinia asiatica* and *N. zeylanica*, carpenter bees, *Xylocopa latipes* and *X. pubescens*, and the wasp, *Rhynchium* sp. in India. Keeping this state of information in view, the present study was conducted to provide detailed information on the reproductive ecology of *Acanthus ilicifolius* L. growing in Coringa Mangrove Forest in Andhra Pradesh.

MATERIAL AND METHODS

Acanthus ilicifolius L. is a landward plant and commonly occurs in oligohaline zone in Coringa Mangrove Forest (16°30’-17°00’ N and 82°10’-80°23’ E) in Andhra Pradesh State, India. But, it also grows occasionally in mesohaline zone where true mangrove plants are removed and kept open. Field investigations and experiments were conducted during the period from February 2013 to October 2016. The inflorescence type and the number of flowers per inflorescence were noted. Ten inflorescences prior to commencement of their flowering were tagged and followed daily to record the flowering duration. Twenty five fresh flowers were used to record the flower type, sex, shape, color, odor, symmetry, calyx, corolla, stamens and style. The floral configuration and rewards presentation aspects were examined in relation to the forage collection activity of insects. Anthesis was initially recorded by observing the marked inflorescences in the field. Later, the observations were made three to four times on different days in order to record accurate anthesis schedule. Similarly, the mature buds were followed to record the time of anther dehiscence. The pollen presentation pattern was also investigated by recording how anthers dehisced and the same was confirmed by observing the anthers under a 10 x hand lens. The presence of nectar was determined by gently pulling a flower from its calyx and firmly pressing its base against a hard surface. The protocols provided by Dafni et al. (2005) were used for measuring the nectar volume, sugar concentration and sugar types. The micropipette was inserted into the flower base to extract nectar for measurement. The average of ten flowers was taken as the total volume of nectar/flower and expressed in µl. Similarly, a sample of nectar was used for measuring nectar sugar concentration at selected intervals of time; the Hand Sugar Refractometer (Erma, Japan) was used for this purpose. Nectar was spotted on Whatman no. 1 filter paper along with the standard samples of glucose, fructose and sucrose. The paper was run ascendingly in chromatography chamber for 24 hours with a solvent system of n-butanol-acetone-water (4:5:1), sprayed with aniline oxalate spray reagent and dried at 120°C in an electric oven for 20 minutes for the development of spots from the nectar and the standard sugars. The developed spots were compared with the spots of the standard sugars to record the sugar types present. Ten mature but un-dehisced anthers were collected from different individuals and placed in a Petri dish. Later, each time a single anther was taken out and placed on a clean microscope slide (75 x 25 mm) and dabbed with a needle in a drop of lactophenol-aniline blue. The anther tissue was then observed under the microscope for pollen. The pollen mass was drawn into a band, and the total number of pollen grains was counted under a compound microscope (40 x objective, 10 x eye piece). This procedure was followed for counting the number of pollen grains in each anther collected. Based on these counts, the mean number of pollen produced per anther was determined. The mean pollen output per anther was multiplied by the number of anthers in the flower for obtaining the mean number of pollen grains per flower. Five dehisced anthers were collected in a Petri dish and the pollen removed from these anthers was examined under microscope for recording the pollen grain features. The pollen-ovule ratio was determined by dividing the average of the number of pollen grains per flower by the number of ovules per flower. The value thus obtained was taken as pollen-ovule ratio (Cruden, 1977). The stigma receptivity was observed by H_2O_2 test. In visual method, the stigma physical state (wet or dry) and the unfolding of its lobes were considered to record the commencement of receptivity; withering of the lobes was taken as loss of receptivity. H_2O_2 test as given in Dafni et al. (2005) was followed for noting the stigma receptivity period.

The insect species were observed visually and with binoculars; the species that could not be identified on spot were captured and later identified with the help of the specimens available in Andhra University, Visakhapatnam. Butterflies were identified to species level by consulting the books of Kunte (2007) and Gunathilagaraj et al. (1998). The foraging activities of insects were recorded for 10 minutes per hour for the entire day on three or four occasions and the data was or further analysis, especially to understand the foraging activity rate at different times of the day. Fifty inflorescences were used to record the foraging visits of insects. The data thus obtained was used to calculate the percentage of foraging visits made by each category of insects per day to evaluate their association and pollination role. The insects feeding on nectar and/or pollen were carefully observed to assess their role in effecting pollination. They were observed on a number of occasions for their foraging behavior such as mode of approach, landing, probing behavior, contact with essential organs to result in pollination, and inter-plant foraging activity in terms of cross-pollination. Ten individuals of each insect species were captured while collecting pollen and/or nectar on the flowers; the collection was done during their peak foraging activity period. The captured specimens of insects were brought to the laboratory, washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under a microscope to count the number of pollen grains present and evaluate their relative pollen carryover efficiency and pollination role.

Mature flower buds of different individual inflorescences were tagged and enclosed in paper bags to test different modes of pollination. The stigmas of flowers were pollinated with the pollen of the same flower manually by using a brush and bagged to test manipulated autogamy. The flowers were fine-mesh bagged as such without hand pollination to test spontaneous autogamy. The emasculated flowers were hand-pollinated with the pollen of a different flower on the same plant and bagged to test geitonogamy. The emasculated flowers were pollinated with the pollen of a different individual and bagged to test xenogamy. All these types of pollinations were kept under regular observation until fruit set. Then, the percentage of fruit set and seed set was calculated. The flowers/inflorescences on ten individuals were tagged prior to anthesis and followed for fruit and seed set in open-pollinations. The resulting fruit and seed output were pooled up for calculating fruit and seed set rates. During the fruit maturation period, the fruit and seed characteristics were recorded. Regularly field notes record fruit and seed dispersal modes. Casual observations were also made to record whether the seeds germinated immediately after they were dispersed or not.

RESULTS

Phenology. *Acanthus ilicifolius* L. inhabits soft muddy soils of brackish water areas but extends to mangrove zonations and becomes dominant after clearing of mangroves (Fig. 1a). The plant is a gregarious bushy shrub due to its ability for vegetative spread to its reclining stems as a result of which it forms large patches by vegetative means. It is erect and grows up to two m tall. The flowering occurs during April-November with peak phase during September-October (Fig. 1b). Inflorescence is terminal or pseudo-axillary bracteate spikes producing 18.21 ± 6.3 (Range nine-26) flowers, eight-20 cm long, the spike extends with age, peduncle terete and glabrous (Fig. 1c). The flowers are produced acropetally (Fig. 3a). The chronological events of sexual reproduction in this species are detailed in table 1.

The flower. Flowers are sessile, very large, 35-40 mm long 35 mm across, bisexual and zygomorphic (Figs. 1d, e and Figs. 2a-i). The calyx is green, sepals four, lobes glabrous, 12-15 mm long and one mm broad, shortly connate in two opposite pairs, the outer pair larger, the upper lobe conspicuous enclosing the flower in bud, the lower lobe somewhat smaller, lateral calyx lobes narrow, wholly enclosed by the upper and lower sepal. The corolla is bluish-violet, 30 mm long with a short tube closed by basal hairs, upper lip obsolete, lower lip broadly three-lobed and recurved by the middle portion. Four stamens, epipetalous, attached to the throat of corolla tube, didynamous, filaments with thick hairy connectives, 13-16 mm long, stout, curved, more or less flat. The anthers are bilobed (one sterile and one fertile), aggregated around the style, 12 mm long with thick hairy connectives and medifixed. The pistil is glabrous, ovary superior, two-loculed each with two ovules on axile placenta. The style is 27 mm long, slender, terete, protruded beyond the stamens while the stigma is semi-wet, bifid.

Table 1: Chronological events of sexual reproduction in *Acanthus ilicifolius*.

Floral event	*Acanthus ilicifolius*
Anthesis	06.00-08.00 h
Anther dehiscence	Mature bud stage
Sepals	Persistent
Petals	Bi-lipped, bluish-violet
Stamens	Four, fall off after two-four days
Stigma receptivity	2nd day of anthesis
Nectar volume/flower (µl)	4.12 ± 0.89
Nectar sugar concentration (%)	41.06 ± 2.86
Pollination system	Entomophily
Pollinators	Insects, primarily carpenter bees
Breeding system	Self-compatible
Fruit set in open pollinations (%)	28
Fruit maturation time (days)	One month
Seed set per fruit	Four

Floral biology. The mature buds enlarge and open during 06.00-08.00 h. Since the upper lip is obsolete, the stamens and stigma are exposed without any shelter. The cartilaginous corolla tube has a three-lobed lower lip that acts as landing base for the forager. The staminal filaments hold the sterile and fertile anther locules together with fringed hairs. The development and orientation of stamens are such that the fertile anther lobes are locked in the sterile locule of the facing anther, the pollen receptacle is thus kept firmly closed and can only be separated by the large-bodied probing insect or animal. The open flowers present this state of stamens. Anthers dehisce by longitudinal slits in the mature bud stage (Fig. 3d). The pollen output per anther is 71,837 ± 186.14 (Range 71,651-72,023) and the per flower average is 2,87,350. The pollen grains are monosiphonous, dispersed as single grains, aperturate, tri-colporate, yellow, powdery, and 49.8 µm in size. Pollen-ovule ratio is 71,837: 1. The stigma rests on the top of the anthers in bud stage and after anthesis (Fig. 2j; Fig. 3b). It becomes receptive on the morning of the second day of anthesis by growing beyond the height of the stamens, curving down the anthers and slightly diverging its two lobes; the receptivity is lost by the evening of the same day (Figs. 3c, e). The configuration of sex organs and the differential maturation of stamens and stigma were found to prevent self-pollination within the flower but not within the plant and it is further supplemented by strong protandry. The ring of dense hairs present at the base of the stamens where the floral tube narrows, points upward and outward; this arrangement prevents insects from crawling into the floral tube. A flower secretes 4.12 ± 0.89 µl of nectar at the corolla base. The nectar sugar concentration is 41.06 ± 2.86% and the common sugars include sucrose, glucose and fructose with the first as dominant. The flowers usually last two days while some fall off after three to four days.

Figure 1: *Acanthus ilicifolius*: a. habitat – vegetative phase,
b. flowering phase, c. inflorescence, d. and e. flowers.

Breeding systems. Flower bud abortion is absent. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. Apomixis is absent. The fruit set is absent in spontaneous and manipulated autogamy; but fruit set is 58% in geitonogamy, 100% in xenogamy and 28% in open-pollination (Tab. 2).

Table 2: Results of breeding experiments on *Acanthus ilicifolius*.

Breeding system	Number of flowers pollinated	Number of flowers set fruit	Fruit set (%)
Apomixis	50	0	0
Autogamy (bagged)	50	0	0
Autogamy (hand-pollinated and bagged)	50	0	0
Geitonogamy	50	29	58
Xenogamy	50	50	100
Open pollinations	150	42	28

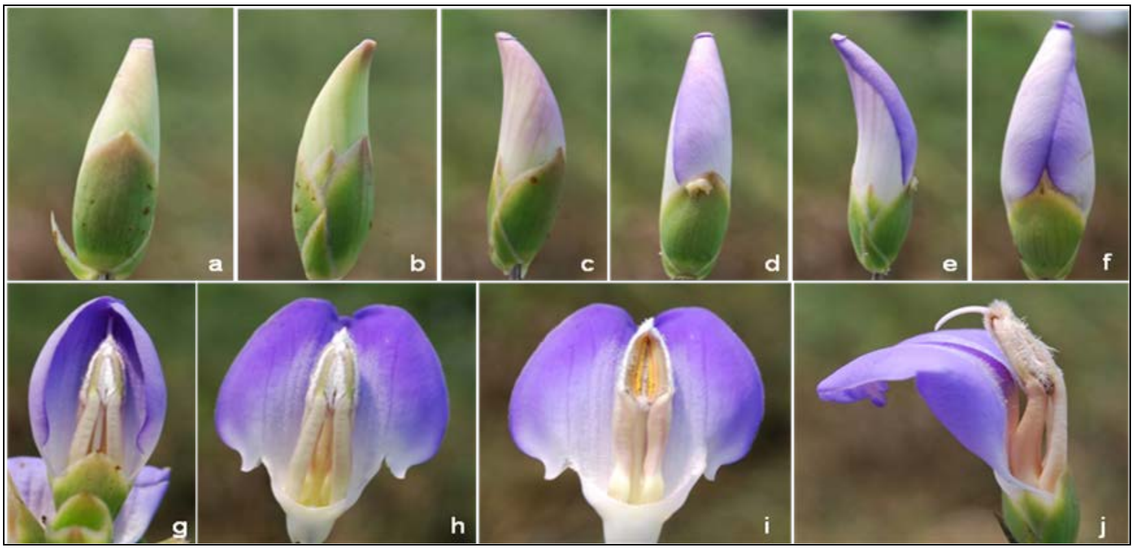

Figure 2: *Acanthus ilicifolius*: a-i different stages of anthesis, j. unreceptive stigma.

Figure 3: *Acanthus ilicifolius*: an acropetal anthesis of inflorescences, b. un-receptive stigma, c. receptive stigma by divergent lobes, d. anther, e. flower with curved receptive stigma extended beyond the length of anthers.

Table 3: List of insect foragers on *Acanthus ilicifolius*.

Family	Genus	Species	Common name	Forage sought
Hymenoptera				
Apidae	*Xylocopa*	*pubescens* Spinola	Large Carpenter Bee	Nectar
	Xylocopa	*latipes* Drury	Large Carpenter Bee	Nectar
Anthoporidae	*Anthophora*	*cingulata* F.	Blue Banded Bee	Pollen + Nectar
Vespidae	*Odynerus*	sp.	Black-headed Mason Wasp	Pollen
Lepidoptera				
Hesperiidae	*Borbo*	*cinnara* Wallace	Rice Swift	Nectar

Figure 4: *Acanthus ilicifolius*: a.-c. different postures of nectar collecting *Xylocopa pubescens*, d. *Anthophora cingulata* collecting pollen, e. *Odynerus* sp. Collecting pollen, f. Hesperiid butterfly, *Borbo cinnara* collecting nectar.

Pollination

The flowers are unspecialized for pollination by large-bodied animals. Fresh flowers are available from morning and remain attractive for two-days for day-active foragers. They were foraged by three bee species, *Xylocopa pubescens* (Figs. 4a-c), *X. latipes* and *Anthophora cingulate* (Fig. 4d), one wasp species, *Odynerus* sp. (Fig. 4e) and one Hesperiid butterfly, *Borbo cinnara* (Fig. 4f; Tab. 3). *Xylocopa* species and *Borbo* foraged nectar only, *Anthophora* for both pollen and nectar, *Odynerus* for pollen only. Both bees and the wasp were the regular and consistent foragers throughout the flowering season while the butterfly was an occasional forager. All these insects except the butterfly foraged from 07^{00} to 17^{00} h with more foraging activity during 10^{00}-12^{00} h (Fig. 6). The butterfly foraged from 07^{00} to 13^{00} h only (Fig. 6). *Xylocopa* bees probed the flower channel formed between the stamens and the large corolla lobe below for nectar collection. The bees touched the bases of the stamens due to which they readily diverged in pairs while the style and stigma descended. Following the release of pressure on the stamen bases, the stigma lifted up and the stamens returned back to their original position. In effect, the stigma contacted the back of these bees first and picked up pollen resulting in cross-pollination. The bees captured pollen while departing from the flower and simultaneously the stamens returned back to their original position. The functionality of this floral mechanism rarely led the stigma and the dehisced anthers to contact one another to result in self-pollination. *Anthophora* bee was medium-sized and its weight was not effective to place pressure on the stamen bases to release anthers from their locked-up position. But, the pollen was placed on the ventral and dorsal side of the bee by the flower when this bee probed for pollen collection. During pollen collection from the frontal portion of the anthers, it contacted the stigma and hence was considered to be important for effecting self- and cross-pollination. *Odynerus* wasp was small-bodied and it also exhibited the same behavior that was exhibited by *Anthophora* bee during pollen collection and hence was considered to be important in effecting both self- and cross-pollination. *Borbo* butterfly while collecting nectar had contact with stamens and stigma facilitating the occurrence of self or cross-pollination but its role in pollination was considered to be negligible due to its occasional foraging activity. The bees and wasps were found to visit the same flowers several times in order to gather the forager multiplying the chances for the occurrence of pollination. Such a foraging behavior exhibited by them on the same plant or different plants in the same patch or different patches scattered in the entire width and breadth of the mangrove forest in quest of the forage was found to be promoting the occurrence of cross-pollination. Of the total foraging visits made by the insects, bee visits constituted 83%, wasp visits 9% and butterfly visits 8% (Fig. 7). Body washings of these insects revealed the presence of pollen grains; the mean number varied from 244 to 308 for bees, a mean of 235 pollen grains in the wasp and a mean of 66.4 pollen grains in butterflies (Tab. 4). The results indicated that each species is a pollen carrier and the pollen carry-over capacity is related to the body size and probing behavior within or at the flower.

Table 4: Pollen pick up efficiency of foraging insects on *Acanthus ilicifolius*.

Insect species	Sample size	Range	Mean ± S.D.
Xylocopa latipes	10	218-356	308.2 ± 53.12
Xylocopa pubescens	10	134-376	238.2 ± 95.73
Anthophora cingulata	10	173-367	244 ± 79.54
Odynerus sp.	10	173-314	235.8 ± 59.18
Borbo cinnara	10	49-89	66.4 ± 15.12

Fruiting behaviour. Fruits mature in about a month time. An inflorescence produces 8 ± 3.2 (Range four – 12) fruits. Fruit is a loculicidal compressed capsule, nut-like, ellipsoid or ovoid-oblong, glabrous, 30 mm long and 10 mm wide and apiculate. It is initially green and light and dark brown when ripe and dry (Fig. 5a). There are four seeds in each fruit, 10 mm long, reniform, supported on short-hooked retinacula, testa delicate, wrinkled and whitish (Fig. 5c). The dry fruit capsule splits violently in the dorsiventral plane; the seed stalk is modified into a hook-shaped jaculator that flings out the seeds in an explosive way during fruit dehiscence (Figs. 5b, d). The seeds disperse up to a distance of two m. Such a seed dispersal in its muddy habitats contribute to the formation of monotypic stands and the spread of population if there is no disturbance to these populations from locals. Seeds germinate and produce new plants during the rainy season.

Figure 5: *A. ilicifolius*: a. maturing fruits, b. dehisced fruit seeds, c. seeds, d. dehisced fruits.

DISCUSSION

Acanthus ilicifolius L. is a landward gregarious bushy shrub that naturally inhabits muddy soils in the estuarine region. But, it is showing up recently as an invasive non-viviparous species in cleared areas of meso- to poly-haline zones of the study region. It is a very prominent species along the brackish water canals and is involved in assisting the accumulation of soil sediments and stabilization of the floor of the brackish water areas. Different authors have reported the flowering and fruiting seasons differently. Mulik and Bhosale (1989) noted that it flowers during January-May and fruits during April to July. Solomon Raju (1990) reported that it flowers during April-June. Ramasubramanian et al. (2003) noted that it flowers and fruits during May-August in Krishna and Godavari mangrove forests, Andhra Pradesh. Anupama and Sivadasan (2004) recorded that the flowering and fruiting occurs during March-August in the Kerala mangrove forest. Upadhyay and Mishra (2010) recorded that the flowering occurs during January-March in Bhitarkanika and the Andaman and Nicobar Islands. The present study records that *A. ilicifolius* L. flowers during April-November while fruiting occurs during May-December. These various reports suggest that the flowering and fruiting season in this species is not fixed and the occurrence of these annual recurring events appears to be a function of salinity levels which change annually depending on the rainfall levels as a consequence of climate change.

Tomlinson (1986) noted that *Acanthus ilicifolius* L. exhibits weak protandry that is likely to restrict self-pollination. In the present study, this species has been found to be strongly protandrous and exhibits temporal dioecy by being staminate on the day of anthesis and pistillate by showing stigma receptivity on the second day. Within the flower, there is no possibility of autogamy or self-pollination in Day one flowers due to un-receptive stigma but geitonogamy is possible due to the simultaneous display of staminate and pistillate phase flowers at plant level. In this context, the hand-pollination tests have been conducted and the results indicated that the plant does not fruit through autogamy but fruits through geitonogamy and xenogamy. The results therefore indicate that pollination and subsequent fertilization is essentially a function of pollinators. The plant has mixed breeding system with out-crossing as the principal mode of pollination. This is further substantiated by the high pollen output per flower and pollen-ovule ratio (Cruden, 1977). Primack and Tomlinson (1980) mentioned that *A. ilicifolius* L. produces fruits from most of the flowers produced. Upadhyay and Mishra (2010) reported that pollination is normally very effective and there is abundance of fruit set in the populations of *A. ilicifolius* L. studied by them in Bhitarkanika and in Andaman and Nicobar Islands. In the present study, the natural fruit set rate did not exceed 30% and the fruit set rate is relatable to the intensity of pollinator activity and the state of the nutrient environment of the plant population.

In *Acanthus* genus, the floral mechanism is very complex and it is evolved with reference to pollination syndrome. Mariette (2000) described four independent floral characters that are involved in the structure of the filament curtain: a fusion of the filaments, decurrent filaments along the corolla wall, a slanting border between the synstapetal and apostapetal corolla regions, and geniculate lower, lateral corolla lobe traces at this border. The evolutionary origin of the filament curtain is considered to be connected with its functions in pollination biology, which are proposed to be those of restricted nectar access, prevention of nectar evaporation, lever arm function facilitating dorsal pollen deposition, and stabilizing of posticous position of anthers and style. Variation in the filament curtain structure appears to be related to different pollination syndromes of the flowers. But, in the total absence of studies on the floral mechanism of individual species of *Acanthus*, the functioning of such a complex structure of staminal filaments in relation to flower foragers can not be evaluated. Primack and Tomlinson (1980) stated that in *A. ilicifolius*, the size of the flower and its mechanics require a large pollinator for effective pollination. These authors also mentioned that the sunbird, *Nectarinia jugularis* is the pollinator in Queensland, Australia. Tomlinson (1986) described that *A. ilicifolius* flowers offer nectar as the main floral reward and are pollinated by insects, especially bees and birds. Solomon Raju (1990) reported that this plant species is pollinated by sunbirds, *Nectarinia asiatica* and *N. zeylanica*, carpenter bees, *Xylocopa latipes* and *X. pubescens*, and the wasp, *Rhynchium* sp. in the present study area. After 24 years, the present study was taken up and it is found that *A. ilicifolius* is pollinated by bees consisting of *Xylocopa*, *Anthophora* and the wasp *Odynerus* sp. at the same site. Of these, *Xylocopa* is the most appropriate forager to work out the floral mechanism to access nectar and pollinate the flowers effectively and this observation refutes the report by Primack and Tomlinson (1980) that *Xylocopa* bees do not visit the flowers of *A. ilicifolius*. During the study period, the sunbirds never visited the flowers and their presence was also not sighted. Similarly, the wasp, *Rhynchium* sp. has never visited the flowers of *A. ilicifolius* but it is present in the area and concentrated on the flowers of *Lumnitzera racemosa* for nectar. The absence of sunbird activity seems to be related to land use changes and conversion of certain pockets of the mangrove forest.

In *A. ilicifolius* L., the flowers are nectariferous and available during day time. The complex floral mechanism functional in this species appears to have evolved to conceal and protect the nectar from the flower foragers that do not bring about pollination during nectar collection. This floral mechanism is highly specialized and only those foragers that forcefully touch the bases of stamens can access the nectar. When such foragers touch the stamens, the latter readily diverge in pairs and style and stigma descends; following the departure of the foragers, the pressure on the stamen bases is released, the stigma lifts up and the stamens return back to their original position. Then, the stigma sweeps against the dorsal side of the foragers first and in effect picks up the pollen (if it is already there on the bee which it carried from the previously visited flowers) and the stamens also deposit the pollen on the bee simultaneously. This is how pollination occurs. The Day one flowers serve as pollen donors only since the stigma is not receptive while the Day two flowers as pollen receivers due to the receptivity of stigma. The effective pollinators of *A. ilicifolius* L. in this study are *Xylocopa* and then *Anthophora* bees. Therefore, the success of sexual reproduction in *A. ilicifolius* under the current state of mangrove forest almost exclusively depends on *Xylocopa* bees. These bees are abundant in the forest due to availability of their nesting sites. The wood of *Excoecaria agallocha* and *Brownlowia tersa* are used as nesting sites by these bees. Other bees and wasps also nest in mangroves and are therefore more dependent on mangrove plants for their existence (Tomlinson, 1986). The study shows that *A. ilicifolius* with a highly specialized pollination mechanism is pollinated in principle by carpenter bees of the genus *Xylocopa*. The plant thrives as long as the carpenter bees are available in the mangrove forest.

Upadhyay and Mishra (2010) stated that *Acanthus ilicifolius* L. takes thirty four days to complete the phases of floral bud to mature propagules in Bhitarkanika and the Andamans. Tomlinson (1986) noted that this species ejects seeds from the fruits violently. Similarly, Das and Ghose (2003) also mentioned the same. In the present study also, it is found that *A. ilicifolius* L. takes about a month time to produce mature fruits each containing mostly four seeds. The fruit is a capsule and splits explosively in the dorsiventral plane ejecting the seeds away and hence it is anemochorous. Anemochory is effective only for those fruits that mature and split during dry season. The fruits that mature during rainy season for dispersal do not split violently due to high ambient humidity and rainfall. These fruits remain in place with the mother plant and gradually dehisce releasing seeds passively. Therefore, the expansion of the population of *A. ilicifolius* is largely dependent on effective anemochory which is functional during dry season, especially in May.

Tomlinson (1986) reported that *A. ilicifolius* has the ability for vegetative spread due to its reclining stems and as a result it forms large patches by vegetative means. This characteristic is important for the sprawling habit exhibited by this species. Perhaps the gregarious occurrence of the plant can be linked to propagation by both seed and vegetative means. The ability to have both sexual and asexual means of propagation is a “fail-safe mode” with which it is able to survive and build-up its population even in isolated areas and hence expands its distribution range.

CONCLUSIONS

Acanthus ilicifolius L. (Acanthaceae) with hermaphroditic flowers exhibits protandry, self-compatibility, facultative xenogamy and temporal dioecy. The floral mechanism is highly specialized and adapted for tripping by large bees. Fruits disperse by explosion and are anemochorous. The plant reproduces by seed as well as vegetative mode and hence has the ability to extend and expand its distribution in mangrove habitats. Cleared mangrove forests are soon occupied by this plant and its populations are an invasive species.

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THE SEA OF MARMARA: NEW LOCALITY FOR *LEPEOPHTHEIRUS EUROPAENSIS* ZEDAM, BERREBI, RENAUD, RAIBAUT AND GABRION, 1988 (COPEPODA, SIPHONOSTOMATOIDA, CALIGIDAE) FROM TURKEY

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KEYWORDS: *Lepeophtheirus, Platichthys*, the European flounder, Sea of Marmara, morphology, Turkey.

ABSTRACT

Lepeophtheirus europaensis Zeddam, Berrebi, Renaud, Raibaut and Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae) an ectoparasite of flatfishes, was reported for the first time in the Sea of Marmara Coasts.

Some morphological characters of this parasitic copepod are given using original photographs and drawings. The general morphology, the mouth parts (antenna, mandible, maxillule, maxilla, and maxilliped), the outgrowth developed between the post-antennary process and the antenna, the setal and spinal formula from first leg to fourth leg in this study are compatible according to the specific literature.

RESUMEN: El Mar de Mármara: nueva localidad de *Lepeophtheirus europaensis* Zedam, Berrebi, Renaud, Raibaut, Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae) de Turquia.

Lepeophtheirus europaensis Zeddam, Berrebi, Renaud, Raibaut y Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae), un ectoparásito de peces planos, fue encontrado por primera vez en las costas del Mar de Mármara.

Algunos caracteres morfológicos de este copépodo parásito se dan utilizando fotografías y dibujos originales. La morfología general, las partes de la boca (antena, mandibula, maxila, maxilar y maxilipedio), la extensión desarrollada entre el proceso post-antenario y la antena, la fórmula setal y espinal del primero al cuarto pie en este estudio son compatibles de acuerdo con la literatura específica.

REZUMAT: Marea Marmara: o nouă semnalare pentru *Lepeophtheirus europaensis* Zedam, Berrebi, Renaud, Raibaut, Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae) din Turcia.

Lepeophtheirus europaensis Zedam, Berrebi, Renaud, Raibaut, Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae), un ectoparazit la speciile de calcan, a fost raportat pentru prima oară pe coastele Mării Marmara.

Anumite caractere morfologice ale acestui copepod parazit sunt indicate cu ajutorul unor fotografii și desene originale. Morfologia generală, piesele bucale (antenă, mandibulă, maxilulă, maxilă și maxiliped), excrescența apărută între procesul post-antenar și antenă, formula setală și spinală de la piciorul unu la piciorul patru prezentate în acest articol sunt compatibile cu cele din literatura de specialitate.

INTRODUCTION

Copepods of the family Caligidae (Siphonostomatoida) are commonly known as sea lice among fish culturists. It is the largest family of marine copepods comprising over 450 species (Ho, 2004).

Bailly (2008) listed the occurrence of 14 parasitic copepod species that occur on the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pisces, Pleuronectidae) globally, which include: *Acanthochondria cornuta, Acanthochondria depressa, Acanthochondria limandae, Acanthochondria soleae, Caligus diaphanus, Caligus elongatus, Caligus musaicus, Chondracanthus depressus, Ergasilus sieboldi, Holobomolochus confusus, Lepeophtheirus europaensis, Lepeophtheirus pectoralis, Lernaeocera branchialis, Lernaeocera lusci.*

Platichthys flesus is a widely distributed species in coastal and brackish waters, naturally occurring in the Black Sea, the Mediterranean Sea, the European Atlantic Coast (including the British Isles and Ireland), the North, the Baltic, the Barents and the White Sea (Nielsen, 1986; Cabral et al., 2007). There are a few studies about the copepod parasites of the European flounder in Turkey (Oğuz, 1991; Aydoğdu and Öztürk, 2003; Öztürk, 2005; Oğuz and Öktener, 2007).

In this study we present the morphological characters of the parasitic copepod *Lepeophtheirus europaensis* found on European flounder from Turkey. We aimed to confirm the occurrence of *Lepeophtheirus europaensis* (Zeddam et al., 1988) from the previously unstudied location of Bandirma Bay in the Sea of Marmara, Turkey, with drawings and photos including morphological characters. The morphological characters given in the study highlight the possibility of comparing our findings with those of other countries in the future. Thus, those who would like to use these methods, can obtain these samples from the Museum National d'Histoire Naturelle (MNHN), Paris, France.

MATERIAL AND METHODS

14 of the European flounder, *Platichthys flesus* (Linnaeus, 1758) (Pisces, Pleuronectidae) were collected by local fishermen from Bandırma Bay (Fig. 1) of Turkey in 2015. The parasites collected from these fish were fixed in 70% ethanol. Mouthparts and pleopods of the parasites were dissected using a Wild M5 stereo microscope. Some of the copepod specimens were later cleared in lactic acid before dissection of the appendages. Appendages were drawn with the aid of a lucida (Olympus BH-DA) camera. Photographs were taken with a Canon EOS 1100D camera connected to a microscope. Measurements were taken in millimetres (mm), with a micrometric programme (Pro-way). The scientific names, synonyms of parasite and host were checked with WoRMS (2016), Froese and Pauly (2016). The parasite (MNHN-IU-2013-18735) was deposited in the collections of the Museum National d'Histoire Naturelle (MNHN), Paris, France.

RESULTS AND DISCUSSION

In this study, parasites of *Platichthys flesus* was examined from the Sea of Marmara (Fig. 1c). *Lepeophtheirus europaensis* Zeddam, Berrebi, Renaud, Raibaut and Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae) was found as ectoparasite. All parasites were firmly attached to the inner wall of operculum of the the european flounder. The prevalence of parasite was 21.4%. Total and dissected parasite number were found as 10, three respectively.

Lepeophtheirus europaensis Zeddam, Berrebi, Renaud, Raibaut and Gabrion, 1988 (Copepoda, Siphonostomatoida, Caligidae) (Figs. 2-6; Tab. 1).

Figure 1a: Turkey satellite image (Google Earth).

Figure 1b: Bandırma Bay (Google Earth).

Figure 1c: Image of sampling area, Bandırma Bay (Sea of Marmara).

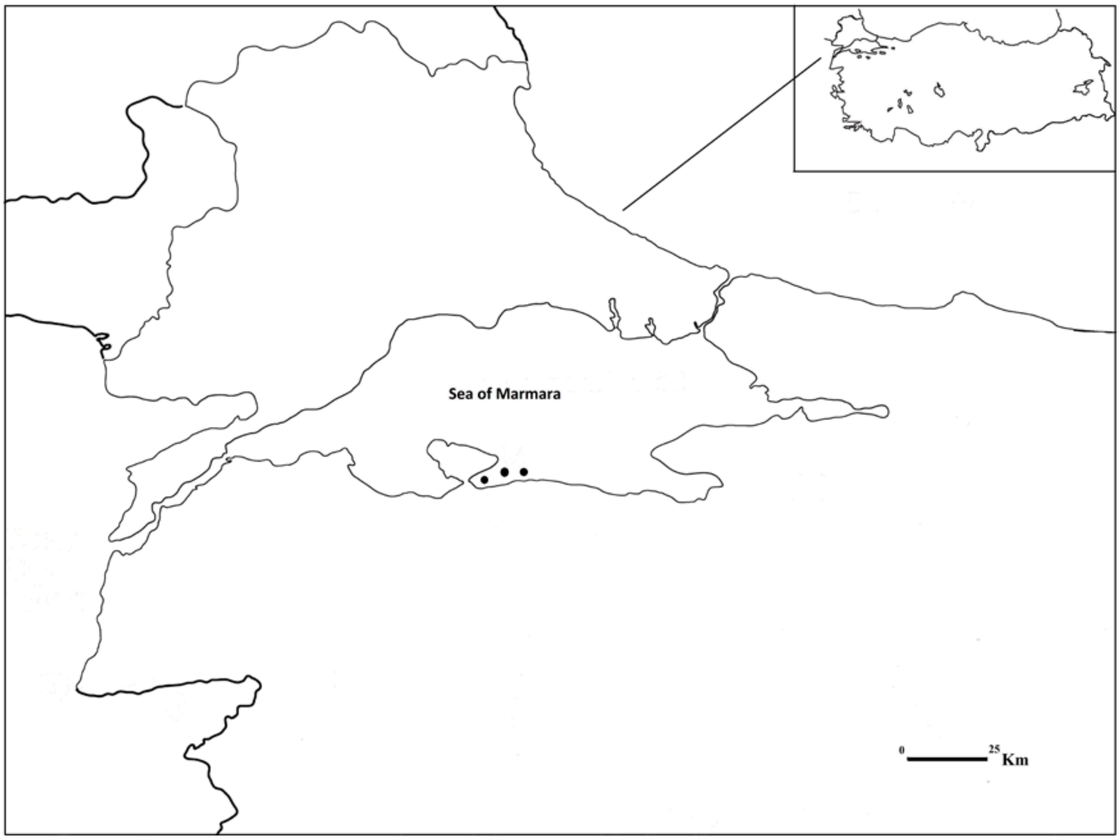

Figure 1d: Sampling area, Bandırma Bay (Sea of Marmara).

Figure 2: *Lepeophtheirus europaensis* ♀
(scale two mm).

Host: *Platichthys flesus*; Total parasite: 10 females; Dissected material: three.
All parasites were firmly attached to the inner wall of operculum of the host.
The prevalence of parasite was 21.4%.

Female morphology: Body length varies from 4.5 to 5.5 mm. Antennule two-segmented; distal segment shorter than proximal, distal segment with 15 setae and one subterminal seta on distal margin, proximal segment carrying on anterodistal surface 20-24 plumose setae. Antenna three-segmented; proximal segment smallest; second segment nearly quadrangular and unarmed; distal segment long, curved claw bearing one seta. Postantennal process a small, bent claw bearing three papillae in basal region, each tipped with three setules. The outgrowth developed between the post-antennary process and the antenna. Distal of maxillule bifurcated and basal papilla with three unequal setae. Maxilla two-segmented and brachiform; proximal segment (lacertus) thick and unarmed; slender distal segment (brachium) with subterminal hyaline membrane on outer margin and terminal calamus distinctly longer than subterminal canna. Distal segment longer than proximal segment. Maxilliped two-segmented; proximal segment (corpus) largest and unarmed; middle segment (shaft) unarmed and distal segment (claw) fused to form a claw with small medial seta. Sternal furca with subrectangular boxbearing large, parallel and sharpened tines. Caudal rami consisted with three unequal long setae and three unequal short setae. Mandible bearing 12 teeth on medial margin of distal blade. Fifth leg represented by four short plumose setae.

Table 1: The setal and spinal formula of from first leg to fourth leg are follows.

Legs	Endopod	Exopod
First leg		I-0; III-4
Second leg	1-0; 2-0; 6-0	I-1; I-1; 6-II
Third leg	1-0; 6-0	0-I; 1-I; 4-III
Fourth leg		I-0; I, III

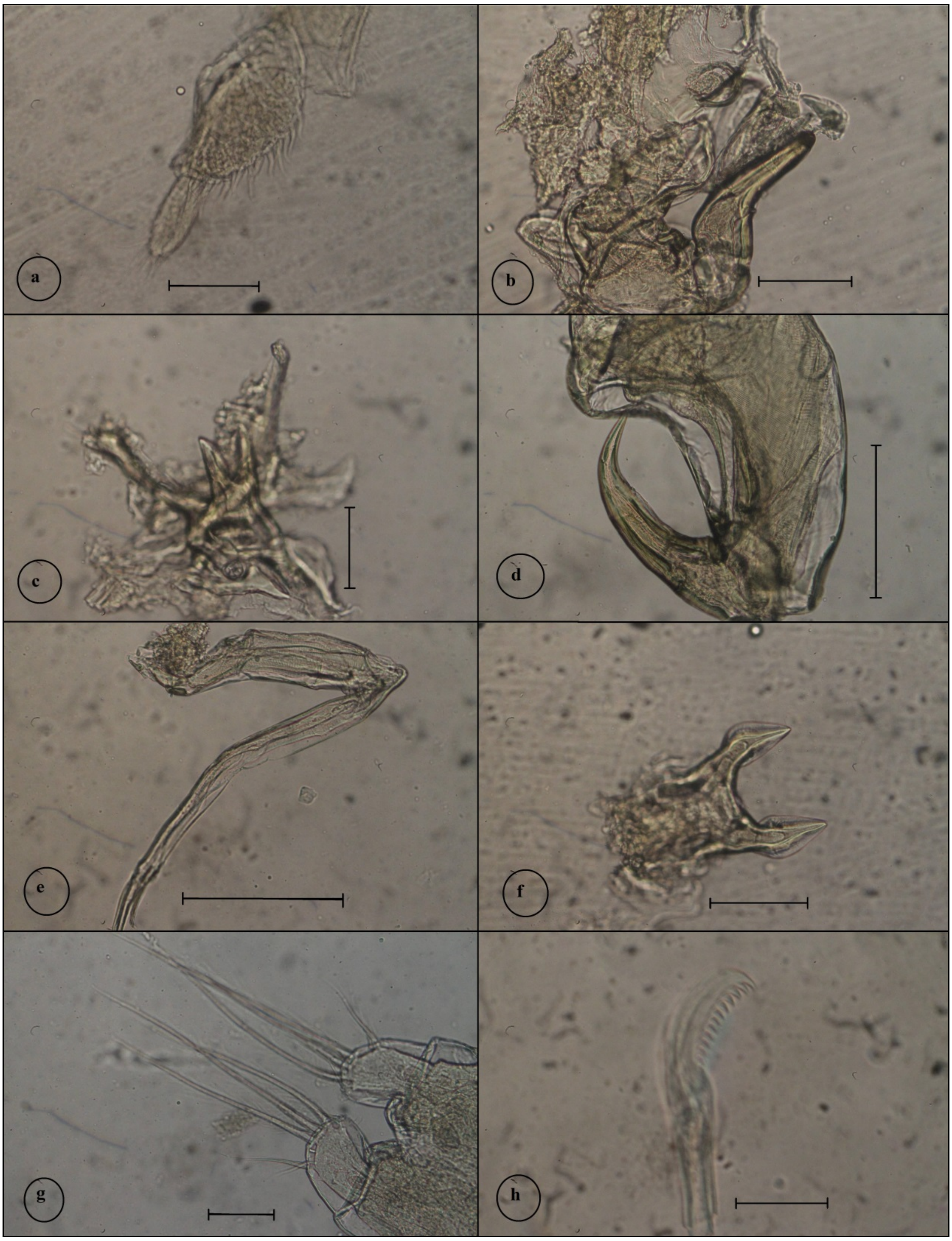

Figure 3: *Lepeophtheirus europaensis* ♀, a) Antennule (0.16 mm), b) Antenna (0.17 mm), c) Maxillule (0.16 mm), d) Maxilliped (0.28 mm), e) Maxilla (0.33 mm), f) Sternal furca (0.18 mm), g) Caudal rami (0.13 mm), h) Mandible (0.05 mm).

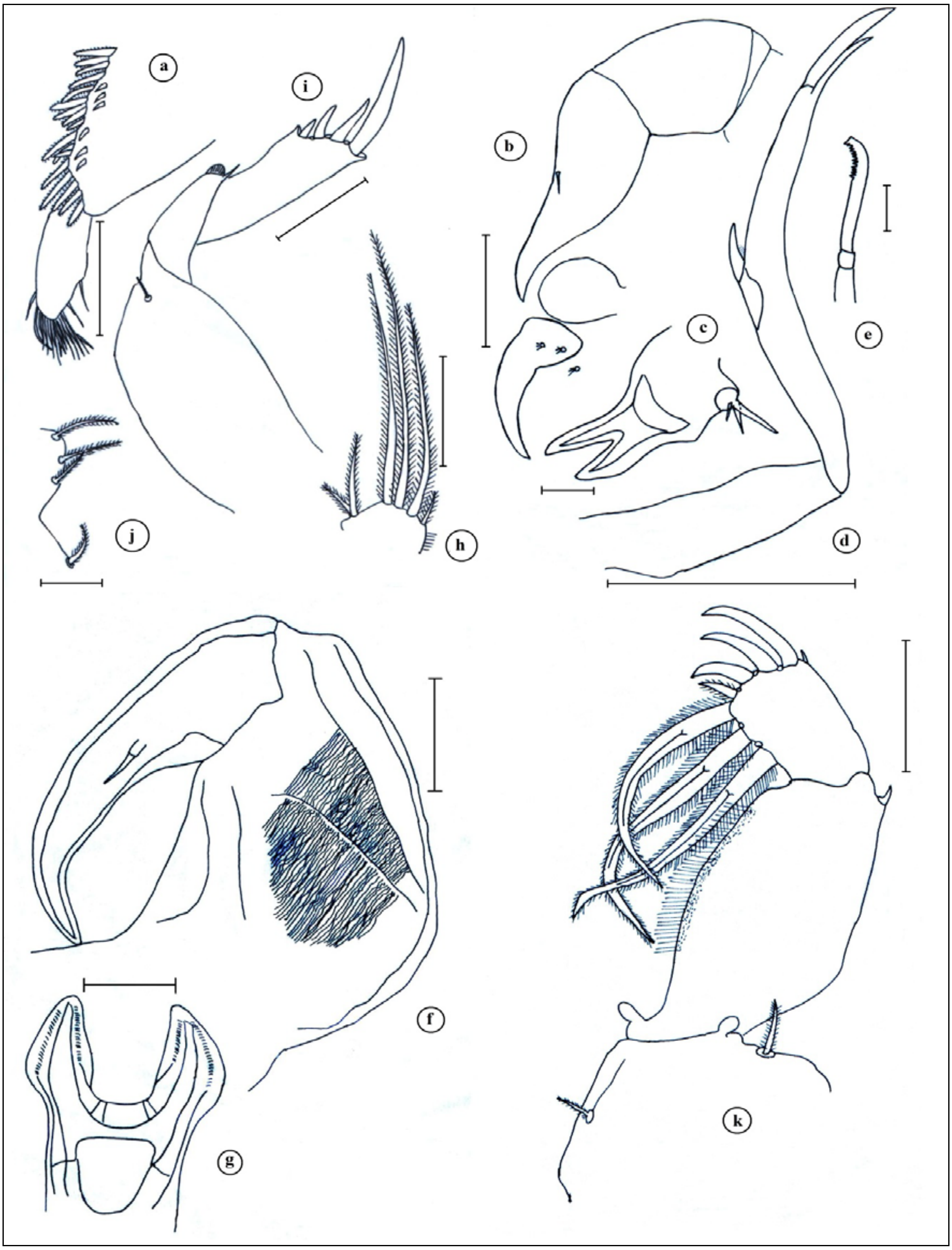

Figure 4: *Lepeophtheirus europaensis* ♀, a) Antennule (0.16 mm), b) Antenna and postantennal process (0.17 mm), c) Maxillule (0.07 mm), d) Maxilla (0.33 mm), e) Mandible (0.05 mm), f) Maxilliped (0.14 mm), g) Sternal furca (0.18 mm), h) Caudal ramus (0.20 mm), i) Fourth leg (0.15 mm), j) Fifth leg (0.11 mm), k) First leg (0.19 mm).

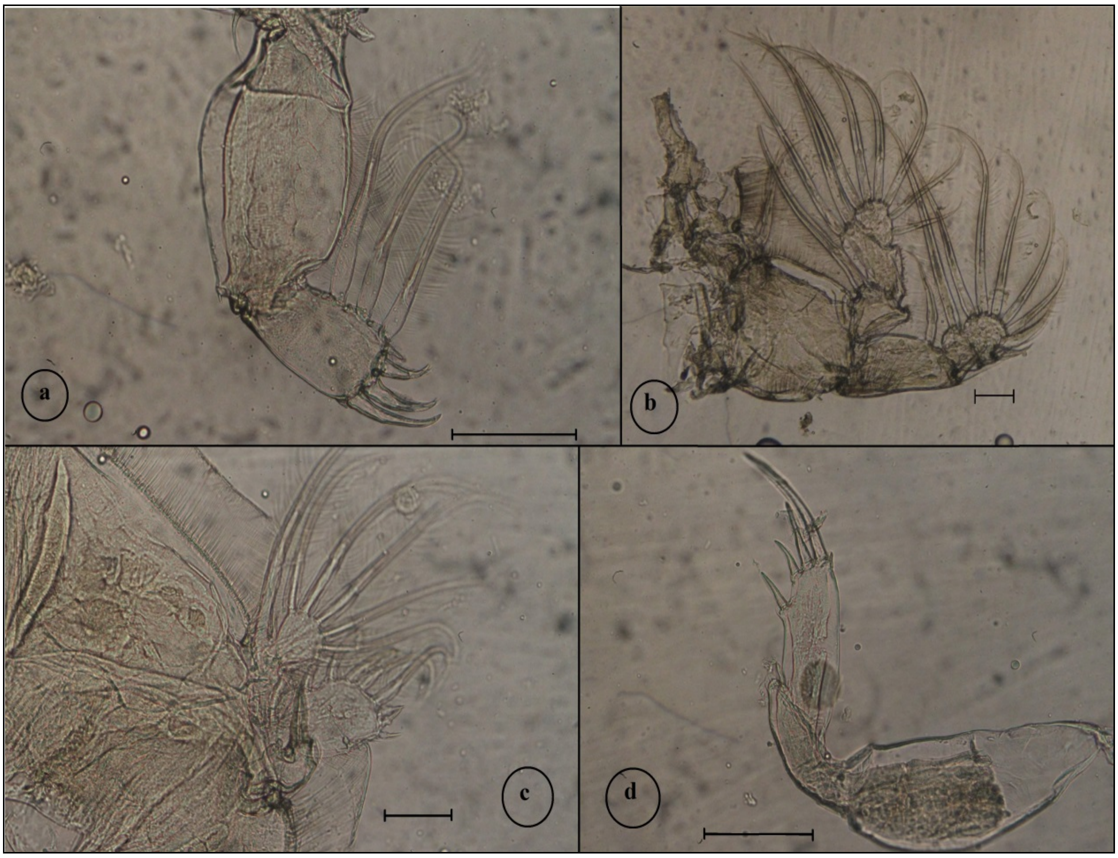

Figure 5: *Lepeophtheirus europaensis* ♀, a) First leg (0.19 mm), b) Second leg (0.10 mm), c) Third leg (0.15 mm), d) Fourth leg (0.15 mm).

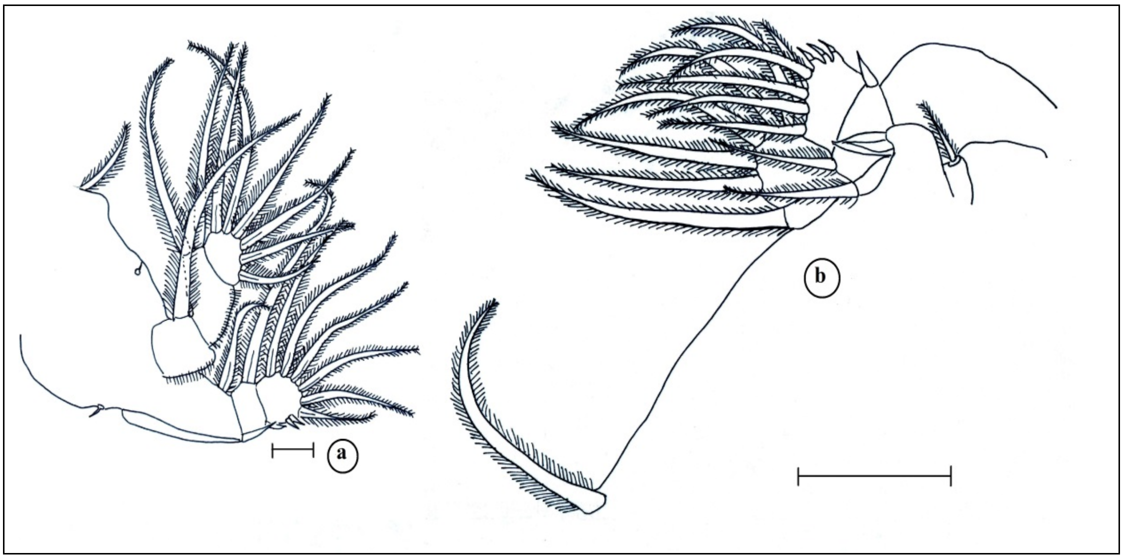

Figure 6: *Lepeophtheirus europaensis* ♀, a) Second leg (0.10 mm), b) Third leg (0.15 mm).

Lepeophtheirus europaensis has been reported in the North Atlantic Ocean (Boxshall, 2015), with the most common hosts being flatfishes *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae) and *Scophthalmus rhombus* (Linnaeus, 1758) (Scophthalmidae) (Zeddam, 1988). From these reports, It can be said that this parasite selects carnivorous and demersal fishes as hosts for habitat and feeding habits. In this study, we examined *Platichthys flesus* which is a carnivorus and a demersal fish and therefore fit to be a host preferring of *Lepeophtheirus europaensis*.

The morphological characters of *Lepeophtheirus europaensis* found in this study are compared with Zeddam et al. (1988). The general morphology, the mouth parts (antenna, mandible, maxillule, maxilla, maxilliped), the outgrowth developed between the post-antennary process and the antenna, the setal and spinal formula of from first leg to fourth leg in this study are compatible according to this literature.

There are limited studies concerning the geographical distribution and hosts of *Lepeophtheirus europaensis* (Zeddam et al., 1988). It was reported from Ekinli Lagoon, Turkey, on *Platichthys flesus* by Oğuz and Öktener (2007). In our study, the presence of this species elsewhere in Turkey was also confirmed with dissection of parasitic copepod on *Platichthys flesus* living in the Sea of Marmara (Bandirma Bay). We have presented the morphological characters of the first recorded specimiens of *Lepeophtheirus europaensis* located within the Sea of Marmara.

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FISH COMPOSITION AND DIVERSITY IN PERAK, GALAS AND KELANTAN RIVERS (MALAYSIA) AFTER THE MAJOR FLOOD OF 2014

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KEYWORDS: fish diversity, diversity index, Kelantan, Galas and Perak rivers.

ABSTRACT

Fish from three major rivers, namely the Kelantan River (KR) and the Galas River (GR) in Kelantan, and the Perak River (PR) in Perak, Malaysia, were caught using gill nets with different mesh sizes, cast nets, and the electroshock method. There were 14 fishes representing five families and five fish species were collected from the Kelantan systems in February 2015. While the Galas system holds more fish, 48 individual fishes comprising of four families and 10 fish species were found there. A total of 213 fish specimens representing 10 families and 22 species were captured in PR in May 2015. For diversity index, PR had the highest value due to the catchment area and the environmental condition: Shannon-Weiner index (H’) (2.54), Species Evenness (J’) (0.73) and Simpson’s Dominance (D’) (8.93), compared to GR (H’) (2.09) (J’) (0.603) (D’) (6.52) and KR (H’) (1.62) (J’) (0.47) (D’) (5.62).

RESUME: Composition et diversité piscicole dans les rivières de Perak, Galas et Kelantan à la suite des inondations majeures de 2014.

Les poissons des trois rivières importantes notamment la rivière de Kelantan (KR) et la rivière de Galas (GR) dans l’état de Kelantan, et la rivière de Perak (PR) dans l’état de Perak, Malaisie, ont été capturés avec des filets maillants de différentes dimensions, des filets à lancer et par la méthode d’électronarcose. Dans le système de Kelantan, 14 poissons ont été captures en février 2015, représentant cinq familles et cinq espèces. Le bassin de Galas a été plus riche avec 48 individus de quatre familles et 10 espèces de poissons. En PR, en mai 2015 ont été captures un total de 213 spécimens représentant 10 familles et 22 espèces. Concernant l’indice de diversité, PR a la valeur la plus importante due à la configuration du bassin et due aux conditions d’environnement: Indice Shannon-Weiner (H’) (2,54), distribution (J’) (0,73) et indice de dominance de Simpson (D’) (8,93), comparé avec GR (H’) (2,09) (J’) (0,603) (D’) (6,52) et KR (H’) (1,62) (J’) (0,47) (D’) (5,62).

REZUMAT: Speciile de pești și distribuția lor în râurile Perak, Galas și Kelantan după inundațiile majore din 2014.

În trei râuri mari, și anume Kelantan (KR) și Galas (GR) în statul Kelantan, și Perak (PR) în statul Perak, din Malaezia, au fost capturați pești utilizând plase de dimensiuni diferite ale ochiurilor, năvoade și prin metoda electronarcozei. În stațiile de pe Kelantan, în februarie 2015 au fost capturați 14 indivizi aparținând la cinci specii și cinci familii, pe când bazinul hidrografic Galas a prezentat o mai mare abundență, fiind capturați 48 de indivizi din zece specii și patru familii de pești. În total în PR au fost capturați 213 indivizi aparținând la 10 familii și 22 specii, în mai 2015. În ceea ce privește indicele de distribuție, PR a avut cea mai mare valoare datorită condițiilor de mediu și conformației bazinului hidrografic: indicele Shannon-Weiner (H’) (2,54), distribuția (J’) (0,73) și indicele de dominanță Simpson (D’) (8,93), față de GR (H’) (2,09) (J’) (0,603) (D’) (6,52) și KR (H’) (1,62) (J’) (0,47) (D’) (5,62).

INTRODUCTION

Rivers support a significant proportion of aquatic biodiversity including fish (Briones et al., 2004; Clausen and York, 2008; Bănăduc et al., 2011). Species' richness within some tropical systems surpasses that of marine ecosystems, including coral reefs (Giller and Malmqvist, 2002; Rohasliney and Jackson, 2009). Additionally, associated semi-aquatic/terrestrial habitats, such as seasonally-flooded forests, are an integral part of river ecosystems, and sustaining the water resources is a pre-requisite for their viability. Biologists have identified about 1.7 million species, the majority of which are insects followed by others including freshwater fishes. Worldwide, freshwater fishes are the most diverse of all vertebrate groups, but are also the most highly threatened (Mcdowall and Taylor, 2000). Chong et al. (2010) reported that a total of 1,951 species of freshwater and marine fish belonging to 704 genera, and 186 families have been recorded in Malaysia. In Peninsular Malaysia alone, about 278 native species were recorded. Thirty two fish species are currently categorized as highly threatened species (Chong et al., 2010). Cyprinidae (150 species) are the most dominant in most Malaysian aquatic ecosystems (Chong et al., 2010). To date, more than 35 freshwater species have been recorded in Kelantan (Department of Fisheries, 2005; Mohd Rezza and Rohasliney, 2009; Rohasliney et al., 2009; Ambak and Mohd Zaidi, 2010). However, previous studies have shown that there has been a substantial decline in fish densities during the last two decades, particularly affecting species such as *Barbonymus schwanenfeldii*, *Hampala macrolepidota*, *Hysibarbus cf. pierrei*, *Poropuntius* sp. undet., *Scleropages formosus*, and Tor spp. (Kvernevik, 1997). Ambak and Mohd Zaidi (2010) also reported seven species found in Kelantan River system, namely *Ompok bimaculatus, Oxygaster anomalura, Neolissochilus hexagonolepis, Labiobarbus sumatranus, Hemibagrus nemurus, Osphronemus goramy, Acrossocheilus deuratus*, are now considered to be rare species.

Most local people living along the Perak, Galas, and Kelantan rivers are part-time fishermen, especially those who live along the upper reaches of these rivers. This group of fishermen usually have other important sources of income such as agriculture, urban labour or transport, and many more with which they alternate their fishing activities. These fishermen are called subsistence fishermen, whereby they fish to supplement the family diet during slack periods in their daily schedule or seasonal calendar. Rarely do we see full-time fishermen (also known as food fishermen) in these rivers, and they comprise only 5-6% of the total population (Fatin Athirah, 2015). There are also recreational fishermen who do not depend directly on the fishery for employment, instead treating fishing as a temporary pastime. In recreational fishing, once the fish is captured, it is returned to the water for the enjoyment of others. However, there is evidence that fish caught by recreational fishermen are sometimes taken home for food (Fatin Athirah, 2015). Living near the river is an advantage for fishermen. For food fishermen, the resources are readily available for them to fish daily. Fish are high in protein and minerals such as calcium and selenium. Fish are eaten whole or in fillets and sometimes fish is preserved as “ikan pekasam” or “ikan kering”.

Floods are the most significant natural hazard in Malaysia in terms of population affected, frequency, area extent, flood duration, and socio-economic damage (Che Su et al., 2014). Normal floods are expected and generally welcomed in Malaysia as they provide rich soil, water, and a means of transport. The floods are directly caused by the weather in Malaysia, which is characterized by two monsoon regimes, i.e. the Southwest monsoon (May to September) and the Northeast monsoon (November to March). The Northeast monsoon is the major rainy season in the country. During flooding season, there is little fishing activity on most areas of the river as fishes are dispersed over the floodplain. If the river is flooding at an unexpected scale and with excessive frequency, the flood waters cause damage to life, livelihoods, and the environment. Fishing during heavy flooding is also rather risky. For example, in the year of 2014, the flooding in areas surrounding Perak, Galas and Kelantan rivers was disastrous. Thousands of people became homeless as their homes were washed away by aggressive currents, crops in the fields were destroyed, and infrastructure such as schools, hospitals, and markets were severely damaged. The three worst affected areas were Kuala Krai, Tanah Merah, and Gua Musang. Other than those districts, Kota Bharu (the state capital of Kelantan State) also was paralyzed for several days as the water level increased tremendously, almost submerging the entire town. The same tragic conditions could also be seen along Perak River, Perak, although the numbers of victims were fewer than in Kelantan. As the rising waters from Perak River and its tributaries inundated four areas statewide, many devastating tragedies followed suit and houses quickly became engulfed. Kampung Gajah, Lenggong, Kerian, Bota, Parit, Kuala Kangsar, and Sungai Siput were among the areas affected. The people who were most affected by the flood disaster were those living on the floodplain, especially the food and subsistence fishermen. They lost their fishing equipment, some of which was expensive. However, regardless of its monetary value, much equipment was lost or damaged and many months were spent repairing the damaged equipment.

Although floods inflict certain catastrophes on human beings, they play important ecological roles for other biotic components of a river. The biology and ecology of fish in large rivers are strongly linked to the annual hydrological regime in the main channel and the regular flooding of the associated floodplains (Welcomme, 1985a). A general pattern for reproductive migrations in floodplain rivers is an upstream spawning movement followed by a downstream dispersion of eggs, larvae, and spent adults into floodplain habitats (Carolsfeld et al., 2003; Borowsky, 2008).

The factors that influence the impact of floods on lotic fish communities include flood magnitude, timing, and frequency of disturbance (Kano et al., 2011; George et al., 2015). These factors determine flood strength and direction of abiotic and biotic responses. Responses of aquatic organisms to floods are also governed by the degree of environmental adaptation (Bischoff and Wolter, 2001). Living aquatic organisms can be severely affected by both abnormally high and low discharges. High discharges, for example, can wash away adult and juvenile fish (Welcomme and Halls, 2003). According to Baran (2006), diversity in catches is higher at lower discharge levels (between 2,000 and 8,000 cumecs) and decreases progressively as discharge increases. The movements of migratory movements are more diverse; they can be long, short, or at times absent; they can be upriver, downriver, or lateral into tributaries and the diversity of movements varies within and among species depending on water level (Makrakis et al., 2012).

Therefore, this study aimed to determine the composition and diversity of fishes after the major floods that occurred in 2014.

MATERIAL AND METHODS

Site description

Perak River, Perak and its creek, Rui River, are freshwater or oligohaline. Perak River is dammed for hydro electrical purposes. There are four dams allocated along Perak River: Kenering Dam, Bersia Dam, Temenggor Dam and Chenderoh Dam. Rui River is located at Kenering Reservoir, which is between Bersia and Kenering dams (Fig. 1). The study was conducted in Rui River (Fig. 1), which is the only tributary in Kenering system that flows directly into the main stream of Perak River. The river is located at the latitude and longitude coordinates of N 5˚27’27.1”, E 101˚10’38.8”. Several villages and small industries are located along Rui River. The upper stream of Rui River, Kampung Pong, is inhabited by several indigenous people and Siamese people. A small dam, named Pong Dam, was built there for power supply to transport tin mined in Klian Intan to the processing area at Gerik previously (Gerik District Council, 2011). The physical condition of the environment is still in a good state. Significant riparian zones are found there with numerous rubber plantations. Tin mining activity is still operational at the upper stream region. While downstream of Rui River, common Malay villages can be found such as Kampung Pahit Luar, Kampung Alai, Kampung Plang and Kampung Kerunai. Near the confluence of Rui River, Kuala Rui is commonly inhabited by Chinese. Plantations and housing are more prevalent in the downstream area, with acceptable riparian zones. However, some areas, such as Dataran Loma, have been altered permanently for fishing activity. Fishing is secondary work and a recreational activity for villagers. This activity becomes more active during wet season as larger numbers of fish spawn in the upper stream of Rui River. The wet season occurs in Gerik District from March to May and in September and October, while dry season is during January and February and from June to August (Zarul, 2013). Most studies conducted in the Perak River have focused on Temengor Reservoir, the most upstream reservoir in the series. More recent studies include analyses of physico-chemical or water quality and fish assemblages (Hashim et al., 2004; Hashim, 2006; Khalik and Abdullah, 2012) in Temengor Reservoir and its water catchment areas. Only Zarul (2013) reported that water quality in Kenering system (temperature 29.0˚C, conductivity 48 µs/cm, total dissolved solid 27 mg/l) was Class I during his study from July 2009 to December 2010.

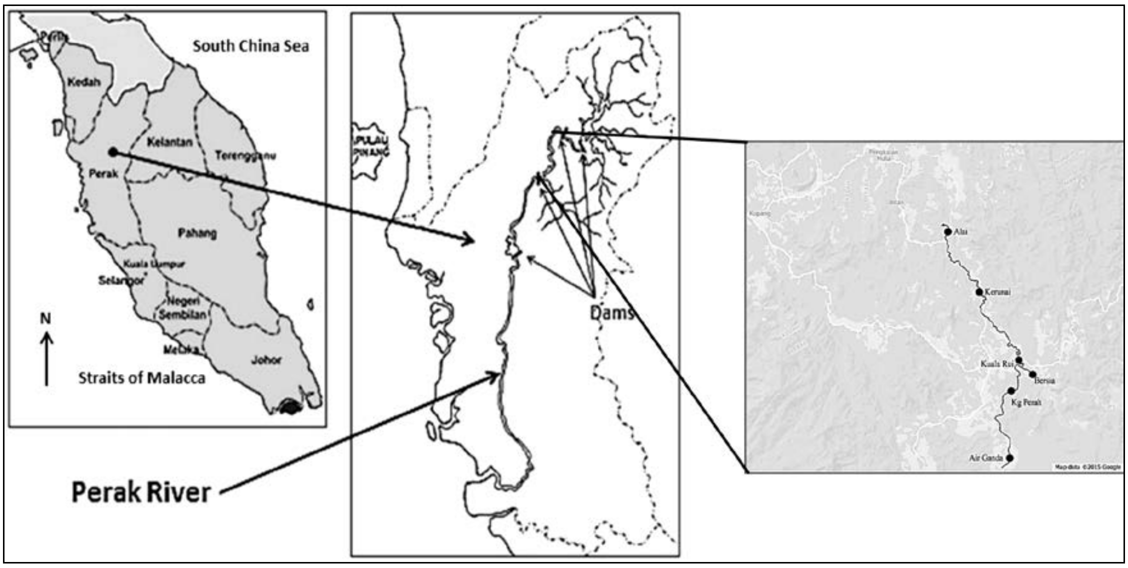


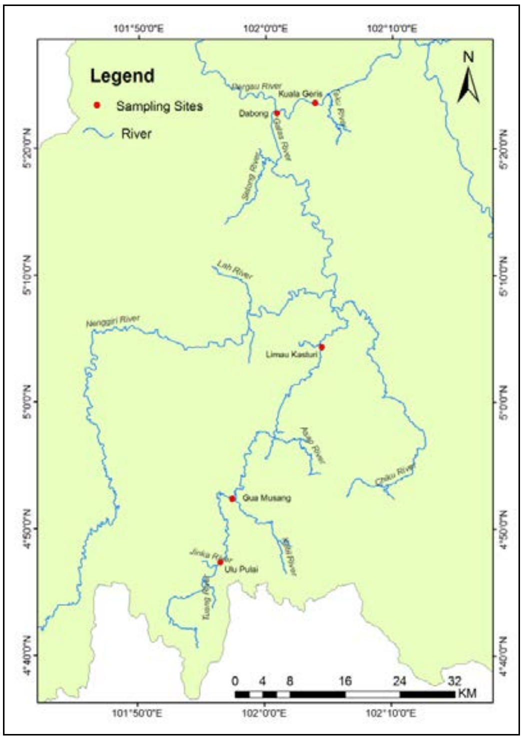
Figure 1: Location of Perak State showing the Perak River, the four dams (along the Perak River) and sampling points (map of Peninsula Malaysia was adapted and modified from http://www.malaysiavacationguide.com/malaysiamap.html) (accessed on January 18th, 2016).

The Kelantan River basin is located in the north eastern part of Peninsular Malaysia; the maximum length and breadth of the catchment are 150 km and 140 km respectively. The Kelantan River is about 248 km long and drains an area of 13,100 km^2, occupying more than 85% of the State of Kelantan. The Kelantan River is a unique river in Southeast Asia since it is the only river known to flow northwards (Fig. 2). The main river from source to river mouth has four tributaries; originating from the Betis River (the first 30 km from the source), then the Nenggiri River, followed by the Galas River, before meeting the Lebir River to form the Kelantan River. The Kelantan River regularly overspills its bank from November to February during the northeast monsoon season. The river flows past four important towns: Kuala Krai, Pasir Mas, Tumpat, and Kota Bharu, the latter being the state capital, which lies near the mouth of the river. Overall, the Kelantan River was classified as a clean river after the Water Quality Index (WQI) for the river was found to be above 80. Over a five-year period, the WQI value for the Kelantan River increased from 84 (year 2005) to 85 (years 2007 to 2009). The WQI value was 82 in both 2011 and 2012 (Department of Environment, 2011, 2012). The Kelantan River has been used heavily by the local people for domestic use, transportation, agriculture, plantation irrigation, small-scale fishing and sand mining (Fig. 1). The Kelantan River's water has been turbid since the early 1990s due to high levels of suspended solids and siltation. This has been caused by logging in the upstream areas (Lojing Highlands) (Department of Environment, 2009; Ambak and Mohd Zaidi, 2010) and sand mining (Tan and Rohasliney, 2013).

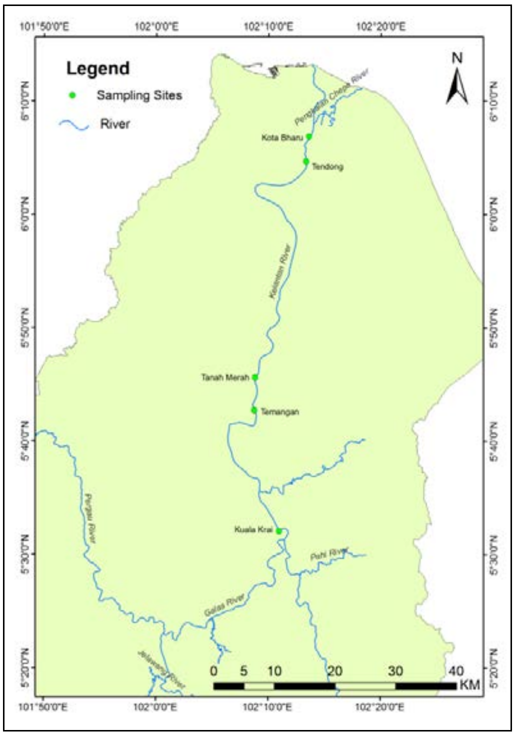
The Galas River is the main tributary of the Kelantan River. The length of the river is 178 km and its catchment area is about 7,770 km^2 with main geological features of shale, mudstone, and limestone (Fig. 2). The Galas River flows northward passing through Ulu Galas Forest Reserve to villages and towns, such as Ulu Pulai, Limau Kasturi, Dabong, Gua Musang and Kuala Krai, before joining other rivers (i.e., the Nenggiri and Pergau rivers) to form the Kelantan River. Land use along the Galas River was observed through groundtruthing. Out of three rivers sampled and monitored, the Galas River tended to reflect the most natural state, especially the upstream part of the river. However, downstream, oil palm and rubber plantations can be found nearby. In addition, the Galas River has been used heavily by local people for domestic purposes, transportation, agriculture, plantation irrigation, and small scale fishing.

Data collection

Fish were collected using experimental gill nets along the Perak River (Bersia to Kenering Reservoir), the Galas River, and the Kelantan River. The gill nets were set at each sampling site for 24 hours. More than 20 sites in the main rivers were sampled. Fish were also caught using gill nets with separate mesh sizes of two, three, and four inches, in order to enhance the fish catch. Each fish net was inspected every day for five days from morning until afternoon. The fish nets (measuring 30 m length, 1.5 m depth) were set up along stretches of river that covered most river pools. In the case of the Perak River, a cast net and a portable backpack electrofishing unit, Model LR-24 (400 V; 60 Hz) (Smith-Root Inc., Washington, USA), were also used, depending on the suitability of sampling locations. All captured fish were labelled accordingly and placed in an ice-chest. Each fish was then measured for its total length (cm) and weight (g), and were carefully preserved in 10% ethanol for further studies. Voucher specimens were retained for laboratory verification. These voucher specimens were preserved, labelled, and stored in the Faculty of Environmental Studies, UPM. Fish data from the Kelantan and Galas rivers were collected from 14 March 2015 to 18 March 2015, whereas fish sampling at the Perak River was conducted from 22 May 2015 to 28 May 2015.



a.



b.

Figure 2: Maps of Kelantan showing two river basins (from south to north: (a) Galas River basin and (b) Kelantan River basin), showing sampling stations along the two rivers.

RESULTS AND DISCUSSION

Overall, 31 fish species representing 14 families (Tab. 1) were caught throughout the study. Two families of fish were found throughout the three systems; the Cyprinidae with 19 species and the Bagridae with three species. Perak River had the highest number of fish caught with 10 families and 22 species (Tab. 1). From 213 individuals, the *Clupeichthys perakensis* made up 27.2% with a total length of 3-4.7 cm. This may have been partly due to the equipment used, namely, a modified scoop net and partly due to behaviour of this species (swimming near the banks of the river). The accumulation of *Clupeichthys perakensis* at the edge of the Perak River made this species an easy fishing target. In contrast with the findings of Zarul (2013), using the same gear (gill nets), three new species were found: *Channa marulioides*, *Leptobarbus hoevenii* and *Osphronemus goramy*. All three species were caught at Air Ganda, a lower stream of Perak River, which has a bigger catchment area compared to that of the Rui River. According to Chew and Zulkafli (2007), *Leptobarbus hoeveni* and the *Osphroremus gouramy* are considered to be rare species that only inhabit undisturbed river conditions.

Different groups of fish were caught between headwater (Rui River) and downstream (Rui and Perak rivers). Zarul (2013) recorded a total of 21 species that were not caught during this study; these species were: *Hemibargus nemurus*, *Hemibargus planiceps*, *Clarias batrachus*, *Cyclocheilichthys armatus*, *Labiobarbus fasciatus*, *Labiobarbus festivus*, *Labiobarbus leptocheilus*, *Labiobarbus lineatus*, *Osteochilus melanopleurus*, *Osteochilus microchepalus*, *Oxygaster anomalura*, *Poropuntius deauratus*, *Rasbora sumatrana*, *Thynnichthys thynnoides*, *Tor* spp., *Oxyeleotris marmorata*, *Mastacembelus erythrotaenia*, *Chitala chitala*, *Chitala lopis*, *Trichogaster trichopterus* and *Pseudolais micronemus*. The difference in the timing of our study and Zarul's study may explain this outcome. Zarul (2013) took samples from 2009 to 2010 (17 months), while our study was conducted only during May 2015 (one month). However, in the Bintang Hijau area, five new species from various families were found upstream of the river, adding up the checklist made by Zarul (2013) which was composed of *Amblyceps foratum*, *Betta pugnax*, *Monopterus javanensis*, *Devario regina*, *Barbodes binotatus* and the *Neolissochilus soroides*. The electro-shocker gear that was used in this study may explain the condition. The pristine condition of the river upstream, as a result of minimal human engagement, has made the river habitable for such sensitive species. One non-native species was also found in the Perak River (i.e. *Oreochromis niloticus*) that could have escaped from a pen culture at the Temenggor Reservoir (upper reservoir) during flooding. Although only a small number of this non-native species was found, if the current situation is not studied and controlled well, predation by these non-native invasive species may be the most likely outcome (Clavero and Hermoso, 2011).

Table 1: Fish species captured from the Perak River (PR), the Galas River (GR), and the Kelantan River (KR) in February, March, and May of 2015 (after major flood occurred in December 2014).

Family	Species	PR	GR	KR
Amblycipitidae	*Amblyceps foratum* (Ng and Kottelat, 2000)	+	–	–
Ariidae	*Arius maculatus* (Thunberg, 1792)	–	–	+
Bagridae	*Hemibagrus capitulum* (Popta, 1960)	–	+	–
	Mystus castaneus (Ng, 2002)	+	–	+
Channidae	*Channa marulioides* (Bleeker, 1851)	+	–	–
	Channa micropeltes (Cuvier, 1831)	+	–	–
Cichlidae	*Oreochromis niloticus* (Linnaeus, 1758)	+	–	–
Clariidae	*Clarias gariepinus* (Burchell, 1822)	–	–	+
Clupeidae	*Clupeichthys perakensis* (Herre, 1936)	+	–	–
Cyprinidae	*Barbonymus gonionotus* (Bleeker, 1849)	+	–	–
	Barbonymus schwanenfeldii (Bleeker, 1854)	+	+	+
	Cyclocheilichthys apogon (Valanciannes, 1842)	+	+	–
	Devario regina (Fowler, 1934)	+	–	–
	Hampala macrolepidota (Kuhl and Van Hasselt, 1823)	+	+	–
	Leptobarbus hoevenii (Bleeker, 1851)	+	–	–
	Mystacoleucus obtusirostris (Valenciennes, 1842)	–	+	–
	Neolissochilus soroides (Duncker, 1904)	+	–	–
	Osteochilus enneaporos (Bleeker, 1852)	–	+	–
	Osteochilus vittatus (Valenciennes, 1842)	+	+	–
	Oxygaster anomalura (Hasselt, 1823)	–	+	–
	Puntioplites bulu (Bleeker, 1851)	+	–	–
	Barbodes binotatus (Valenciennes, 1842)	+	–	–
	Rasbora tornieri (Ahl, 1922)	+	–	–
Mastacembelidae	*Mastacembelus erythrotaenia* (Bleeker, 1850)	–	+	–
Nandidae	*Pristolepis fasciata* (Bleeker, 1851)	+	–	–
Notopteridae	*Chitala lopis* (Bleeker, 1851)	–	–	+
	Notopterus notopterus (Pallas, 1769)	+	–	–
Osphronemidae	*Betta pugnax* (Cantor, 1849)	+	–	–
	Osphronemus goramy (Lacepède, 1801)	+	–	–
Pangasiidae	*Pseudolais micronemus* (Bleeker, 1846)	–	+	–
Synbranchidae	*Monopterus javanensis* (Lacepède, 1800)	+	–	–
	Number of families collected	10	4	5
	Number of species collected	22	10	5

A total of 245 individual fish were collected from the Kelantan River, comprising of five families and five fish species (Tab. 1). *Barbonymus schwanenfeldii* was the most abundant species (42.9% of total catch) and had a total length ranging between 13 and 27.8 cm. Evidently, *Mystus castaneus* was only caught during this sampling period (35.7% of the total average catch with a total length of 16-25 cm) and was not observed between 2010 and 2012 (Rohasliney et al., 2015). *Chitala lopis*, the *Clarias gariepinus*, and the *Arius maculatus* were almost equally abundant throughout the river (7.1%). There was a higher number of catches at the downstream areas (10 individuals) compared to the middle reaches of the river (four individuals). This may have been caused by the change in habitat, as a result of flooding, such as significant bank erosion, enormous bed-load transfer, change in velocity of current and also depth of pool. All of these factors most probably affected the spawning, feeding, and shelter areas of the fishes in that area. Compared to the Galas River and the Perak River, the Kelantan River has undergone enormous human intervention such as sand mining and logging activities, which have caused benthic habitat destruction and water turbidity (Tan and Rohasliney, 2013). The Kelantan River is now under increasing pressure from sand mining activities. There are about 128 sand mining companies operating in the river from Kuala Krai down to Kelantan’s estuary (Ambak and Mohd Zaidi, 2010). Hence, the Kelantan River has started to become very turbid due to a high suspended solids content and siltation. The Kelantan River upstream is threatened by logging activities which correspondingly lead to very turbid water downstream (Tan and Rohasliney, 2013). In some parts of the river, tremendous stress from drought and erosion of the stream bank was observed. Rapid rural development from Tanah Merah to Kota Bharu has led to over-grazing, deforestation and erosion. The conversion of floodplains and riparian zones to agriculture and urban development (i.e., TESCO, a series of waterfront hotels and houses) has had detrimental effects on plants and animals in riverine wetlands. Discontinuity of the floodplain would affect the population of fish adversely. In addition to the general loss of production, changes in the composition of the fish fauna have occurred downstream of the Kelantan River. In some cases, a decline in the swamp-dwelling and herbivorous fishes was noted, and their predators have increased in abundance. Such a disproportionate number of predators cannot persist for any length of time and will eventually act to reduce the abundance of the stock as a whole (Noor Syuhadah and Rohasliney, 2011).

In the Galas River, a total of 48 fish individuals were collected, comprising of four families and 10 fish species. The only species that was high in abundance in the Galas River was the *Cyclocheilicthys apogon* (41.7% of the total average catch with a total length of 15.8-28.1 cm). The high abundance of Temperas in the Galas River may be related to its feeding habit, which is omnivore-detritivore, and the Galas River is known for its rich and varied food sources. Kah-Wai and Ali (2001) found that this species was the second most abundant species in the Chenderoh Reservoir where it made up 22% of the total catch. This species was followed by *Barbonymus schwanenfeldii*, *Hemibagrus capitulum*, and *Mystacoleucus obtusirostris* (14.6%, 12.5%, 8.3% of the total catch with a total length of 16.8-28.1, 22.5-32.6, and 17.2-18.5 cm, respectively). There were six fish species caught in this study that were not observed during sampling between 2008 and 2010: *Hemibagrus capitulum, Mystacoleucus obtusirostris, Osteochilus enneaporus, Osteochilus vittatus, Mastacembelus erythrotaenia* and *Pseudolais micronemus* (Rohasliney et al., 2015).

In contrast to the Kelantan River, the Galas River is located in the Ulu Galas Forest Reserve where the river still has an intact forest and riparian zone so that it remains a good habitat and source of food for most fish species (Rohasliney, 2010). Although there has been a declining trend in the abundance of the *Hampala microlepidota* species (three individuals) (6.25% average catch), Rohasliney et al. (2015) found more than 20 individual fish from 2008 to 2010. The declining trend may be related to destruction of spawning habitat and drifting of fry that are less capable of overcoming strong currents during the flood (Welcomme, 1985b; Reichard and Jurajda, 2004). In contrast, the Galas River downstream is subjected to anthropogenic influences and the fish communities observed are mostly dominated by non-native species. It has been hypothesized that the numerically dominant non-native species are better adapted for altered water quality, habitat, and hydrological condition (Bennet and Moyle, 1996). However, interestingly, species richness is quite high at Dabong's sampling site which is considered to be downstream of the Galas River (Tab. 1) (Mohd Rezza and Rohasliney, 2009). This is partly because Dabong remains a remote area with minimal development. Furthermore, additional fish species from the Pergau River enter the Galas River when the two rivers meet just below the sampling point at Dabong site. The Galas River is categorized as a Class II river using the WQI chemical classification, which was developed by the Department of Environment (2006). Class II can be used for recreation with body contact and may contain sensitive aquatic species. As for domestic water supply, a Class II river may need conventional treatment.

The number of fish species in the Kelantan River was the lowest (Fig. 3a) compared to that found in the Galas and Perak rivers. This is because of a distinctive feature of the potamon area (wide and slow-moving river characteristics) especially at the middle and lower reaches of Kelantan River, characterised by higher in turbidity caused by active sand mining activities along Kelantan River. This action has also changed the natural habitat of the river, resulting in the deepening of the pool, loss of gravel on the riverbed, and the introduction of extensive mud and silt. All of these consequences affect the fish community directly in regard to their spawning, feeding, and protection areas. Species evenness (Fig. 3c) and Simpson's dominance (Fig. 3d) revealed that fish diversity for the Kelantan River sample was moderate at 0.47 and 5.62, respectively. On the other hand, concerning the Galas River, the high number of species shown in figure 3a, along with the unequal distribution of individuals among species, has resulted in lower species diversity, as revealed by the Shannon-Weiner index (2.09) (Fig. 3c) and Simpson's dominance (6.52) (Fig. 3d). The highest number of species out of all the rivers studied was observed in the Perak River (Fig. 3a). This is likely due to the nature of the reservoir river where plenty of allochthonous materials from lower order streams are brought together. The existing vegetation and trees are the source of these autochthonous materials. The Shannon-Weiner index at the Perak River also yielded the highest value of 2.54 (Fig. 3a) compared to Galas River (2.09) and the Kelantan River (1.62). The species diversity for the Perak River was the highest, as indicated by species evenness (0.73) (Fig. 3c) and Simpson's dominance (8.93) (Fig. 3d). This indicates that the species were log distributed normally, which means that certain species are more abundant than others.

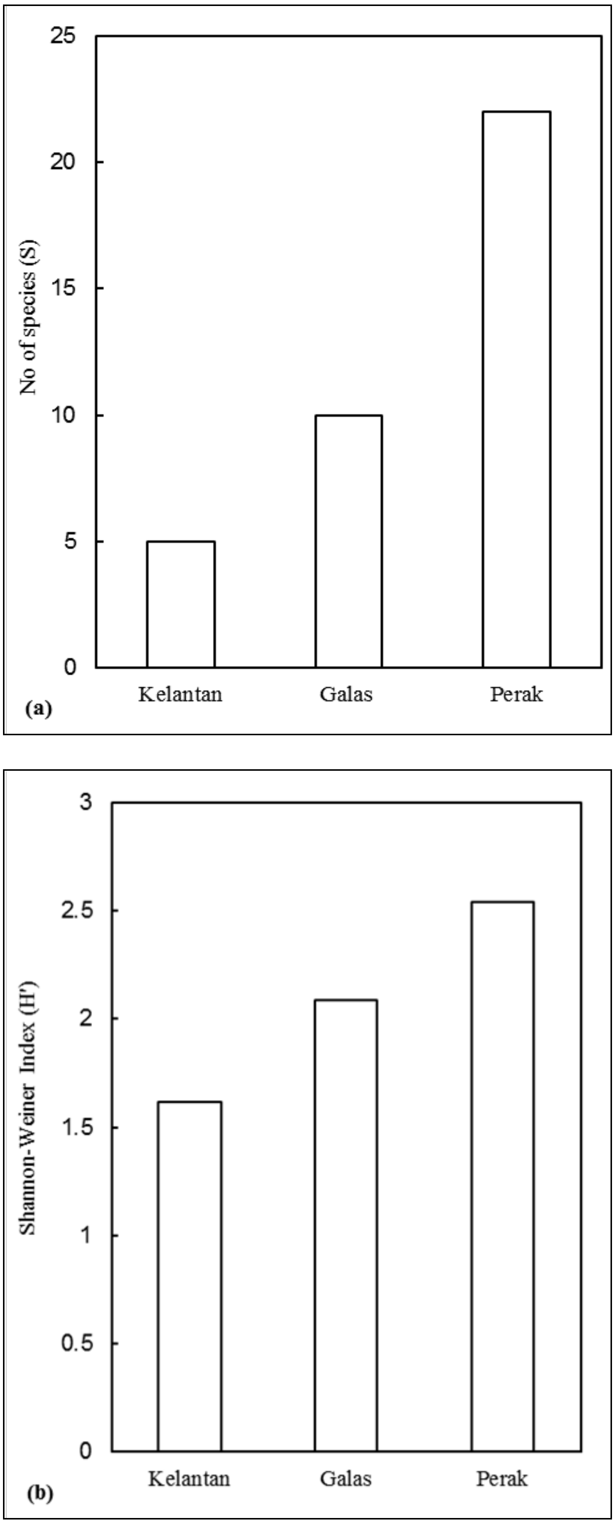


Figure 3a, b: Total number of species (a), Shannon-Weiner index (H’) (b) from samples collected at the KR, the GR, and the PR in February, March, and May of 2015.

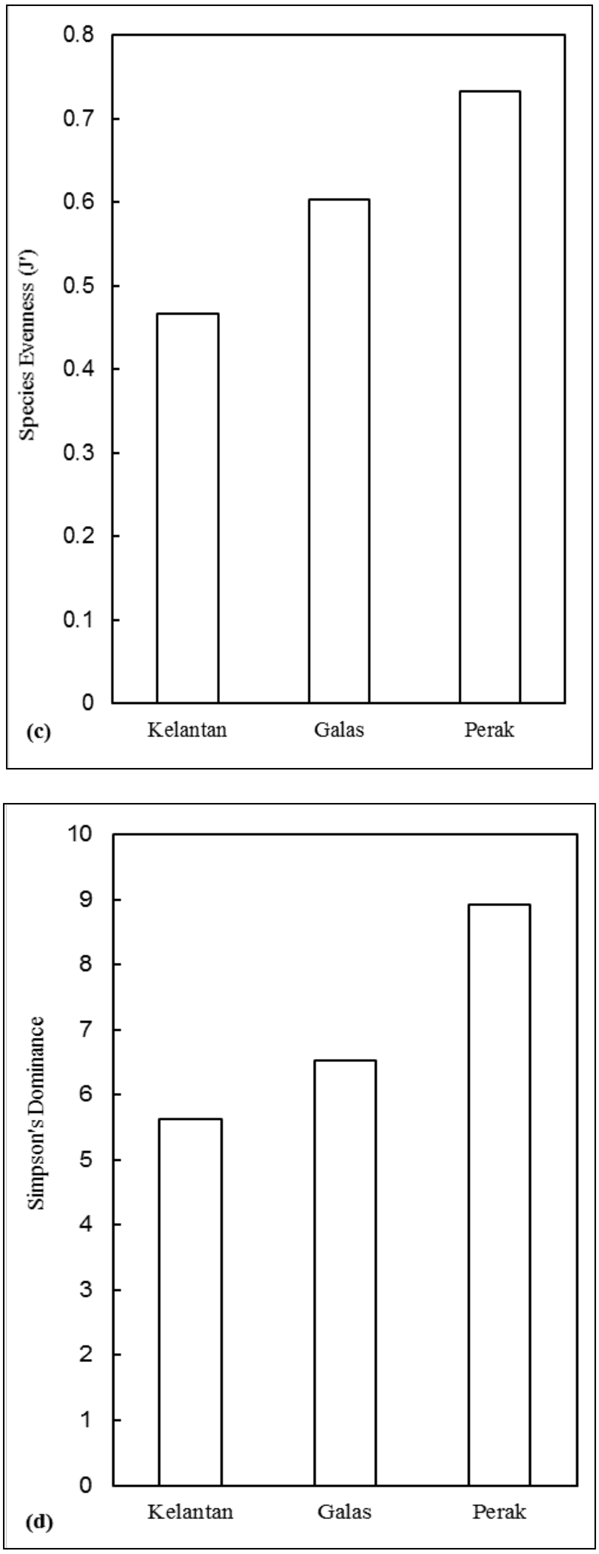


Figure 3c, d: Total Species evenness (J') (c) and Simpson's dominance (d) from samples collected at the KR, the GR, and the PR in February, March, and May of 2015.

CONCLUSIONS

The richness of fish species at the Perak River indicates that the river is in good condition. Meanwhile, the low rate of production from fisheries production along the Kelantan and Galas rivers is at an alarming state. Human activities that cause habitat degradation and water pollution should be managed properly via appropriate action from relevant authorities. Requirements for sustaining biodiversity and fisheries in rivers are integrally linked through a mutual need for improved management of both habitats and their exploitation.

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FISH FAUNA STATUS OF THE NATURA 2000 SITES PROPOSED AS NEW OR FOR EXTENSION IN THE RIVERS SOMEȘ AND MUREȘ (ROMANIA)

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KEYWORDS: fish ecological parameters, fish community interest, anthropogenic impact, Mureș and Someș rivers SCIs.

ABSTRACT

In autumn 2015 the fish fauna of river watersheds from six sites of community interest (SCI) Natura 2000 were investigated as follows: river Mureș (two SCIs) and Someș River in four SCIs from Someșul Mic, Someșul Mare and Someș. The researches were focused on the fish species of community interest (Annex II of Habitats Directive), its position in the ichthyocoenose (fish species richness, abundance and ecological parameters) and anthropogenic pressure. In investigated SCIs rivers researchers found 27 fish species including 11 species of community interest. IBI index shows moderate to good, or excellent levels of the evaluation integrity degree in fish fauna ecosystems.

ZUSAMMENFASSUNG: Zustand der Fischfauna in neu oder zur Erweiterung vorgeschlagenen Natura 2000-Gebieten der Flüsse Someş und Mureş im nördlichen und westlichen Grenzgebiet Rumäniens.

Im Herbst 2015 wurde die Fischfauna von sechs Fluss bezogenen Natura 2000-Gebieten (SCI), zwei am Mureş und vier am Someş (Kleiner Someş, Großer Someş und vereinigter Someş-Fluss) untersucht. Die Forschungen waren ausgerichtet auf die Fischarten von gemeinschaftlichem Interesse (Anhang II der FFH-Richtlinie), ihre Stellung in der Fischzönose (Artenreichtum, Abundanz und ökologische Parameter) sowie den Einfluss menschlichen Druckes auf die Fischfauna. In den untersuchten Gebieten wurden 27 Fischarten festgestellt, darunter 11 Arten von gemeinschaftlichem Interesse. Der IBI-Index zeigt einen mäßigen bis guten, sogar bis hin zu einem exzellenten Integritätsgrad der Fischfauna und ihrer Ökosysteme.

REZUMAT: Starea faunei piscicole din situri Natura 2000 propuse ca noi sau spre extindere în râurile Someş şi Mureş, pe teritoriul României.

În toamna anului 2015 a fost investigată fauna piscicolă din şase SCI-uri după cum urmează: râurile Mureș (două SCI-uri) și Someș (în patru SCI-uri pe Someșul Mic, Someșul Mare și Someșul Unit). Cercetările au fost concentrate asupra speciilor de pești de interes comunitar (Anexa II din Directiva Habitate), poziția lor în ihtiocenoză (bogăția speciilor de pești, abundența speciilor și câtiva parametrii ecologici) totodată și influența presiunii antropice. În SCI-urile investigate au fost găsit 27 specii de pești, inclusiv 11 specii de interes comunitar. Indicatorul IBI arată un nivel moderat până la bun spre excelent privitor la evaluarea gradului de integritate în ecosistemele faunei piscicole.

INTRODUCTION

Before 2015, Romanian Ministry of Environment promoted 273 sites of community interest (SCIs) that took 13.21% from Romanian territory in 2007. After revision in 2011 more sites were enlarged, and new sites were designated thus reaching to 383 SCIs. Therefore, SCI's now occupy 16.76% of Romanian territory (Brânzan, 2013; Oțel and Năstase, 2010; Năstase and Oțel, 2016) according to Romanian Order 2,387/2011 modifying Order 1964/2007. Therefore, at the end of 2015 a total number of 434 SCIs or others ones enlarged were reported to the EU. The total surface of SCIs and SPAs cover about 25% of Romania.

A reference list of fish species of community interest from Romania contains 26 types of fish (Tatole et al., 2009) in accordance with Habitats Directive no. 92/43/1992 and Romanian Law 49/2011 (completing Romanian OUG 57/2007).

Previously, Standard List Form of Natura 2000 in studied SCIs (ROSCI0367, ROSCI0368, ROSCI0394) contained nine species of community interest as follows: *Aspius aspius*, *Barbus meridionalis*, *Cobitis taenia*, *Gobio albipinnatus*, *Gobio kessleri*, *Gobio uranoscopus, Rhodeus sericeus amarus, Sabanejewia aurata, Zingel streber* (in accordance with the nomenclature adopted under the Habitats Directive species lists). We must emphasise that current nomenclature is modified to eight fish species, except *Zingel streber*. European Union requested for *Aspius aspius, Gobio albipinnatus* and *Rhodeus amarus* related with the mammal *Lutra lutra* all considered In Mod (In Moderate) to design new SCIs, or enlarge some pre-existing SCIs on the Mureș and Someș rivers.

The total number of fish species present in the basin of the river Someş is 62 (49 of them native and 13 introduced) (Bănărescu et al., 1999). There is strongly anthropogenic impact mostly located in Someşul Mic and Someş rivers downstream Baia Mare locality. There are also major changes in fish assemblages because of water polluation confirmed in the Hungarian section of Someş River (Antal et al., 2013).

From Mureş River were captured 56 fish species, but the pollution led to negative impacts and led to the loss of some species (Nalbant, 1993, 1994, 1995; Köhler et al., 2005, 2007; Curtean-Bănăduc et al., 2007, 2017; Sandu et al., 2008).

The new proposals for SCI lists were completed for the first time with native fish species in the studied area. Enlarged SCIs added new fish species of community interest.

MATERIAL AND METHODS

Depending on the morphology of water bodies and the flow velocity, we used different gear for fish sampling as follows: an inflatable boat of two-person, Nordic gillnets (with 12 panels 2.5 m each panel, with multiple meshes size six-55 mm), also electric fishing device SAMUS 725MP with accumulator 12V and 5-60 Amps output 600 W. Also angling and data from local fishermen were used. Electric fishing was carried out on day and gillnets fishing on night (12 hours stationary). It was assessed the presence of community interest species (Annex II/Habitats Directive no. 92/43/EC), quantitative structure (numerical abundance, biomass), specimen dimensions, analytical and synthetically ecological indicators, overall status of aquatic habitats in terms of existing anthropogenic pressures.

The camps were installed as close as to the banks of water bodies, about the middle sites. At each site, we performed fishing at least one point (approximately to the middle of SCI sites) or in two points each with a length of approximate 100 m river beds (according to the methodology specified in the Habitats Directive no. 92/43/EC).

The catch was sorted by species (fish identification according to the latest systematic reviews after Bănărescu (1964) with updates after Bănărescu (1994, 2004), Kottelat (1997), Kottelat and Freyhof (2007), Froese and Pauly (2016); weighing and measuring of lengths are performed. The abundances and biomass were determinate to each species and site, in order to find the status of species in the fish community. After measurements, the remaining individuals were released into the river. The human impact was also assessed. Few specimens were collected and preserved in alcohol for species that we had doubts of correct identification.

It was assessed fish fauna community, especially presence of community interest species, quantitative structure (numerical abundance), ecological indices (D = dominance, C = constancy, W = ecological significance, table 1), IBI (biological integrity index in tables 2 and 3).

Table 1: Frequency (C = constancy), dominance (D) and ecological significance (W) classification (Odum, 1975; Schwerdtfeger, 1975; Botnariuc and Vădineanu, 1982; Muhlenberg, 1993; Gomoiu and Skolka, 2001; Șindrilariu et al., 2002; Sârbu and Benedek, 2004).

Dominance (D)		Constancy (C)		Ecological significance (W)	
Class	%	Class	%	Class	%
sporadic D1	< 1	very rare	C1 = 0-10	accidental	W1 < 0.1
subrecedent D2	1 (2^0) – <	rare	C2 = 10.1-25	accessory	W2 = 0.1-1
recedent D3	2 (2^1) – <	widespread	C3 = 25.1-45	associate	W3 = 1-5
subdominant D4	4 (2^2) – <	frequent	C4 = 45.1-70	complementa	W4 = 5-10
dominant D5	8 (2^3) – 16	very frequent	C5=70.1-100	characteristic	W5 = 10-
eudominant D6	> 16 (2^4)			main, leading	W6 > 20

Table 2: Criteria of fish determining IBI (biological integrity index) (Miller, 1985; Karr et al., 1986; Battes, 1991).

Parameters categories	Parameter	Evaluation integrity class		
		5 (abund.)	3 (const.)	1 (rare)
Composition and abundance of species	1. Total number of fish species	> 90%	50-90%	< 50%
	2. Total number of Cyprinidae sp.	> 45%	20-45%	< 20%
	3. Total number of Salmonidae sp.	> 5%	1-5%	< 1%
	4. Others fish sp.	> 20%	5-20%	< 5%
	5. Total number of native fish species	> 68%	35-67%	< 34%
	6. Total number of non-native species	< 1%	1-10%	> 10%
	7. Total no. of disappearing fish species	< 1%	1-10%	> 10%
Composition of the food fish populations	8. Proportion (%) of zoobentophagous	> 45%	20-45%	< 20%
	9. %of carnivore sp.	> 5%	1-5%	< 1%
	10. % of carnivore and planctonophagous	< 20%	20-45%	> 45%
	11. % of herbivorous and detritivores sp.	< 25%	25-50%	> 50%
Stock and general state of fish populations	12. Numerical Stock (ex./100 m²) (ex./100 m linear/collectors)	> 100 ex (> 20 ex)	10-100 (5-20)	< 10 (< 5)
	13. Gravimetrical Stock (g/100 m²) (g/100 m linear/collectors)	> 1000 g (> 5000 g)	100-1000 (500-5000)	< 10 (< 5)
	14. Proportion of hybrid individuals	0%	0-1%	> 1%
	15. Proportion of ill individuals	0%	0-1%	> 1%

Table 3: Framing levels of the evaluation integrity degree in fish ecosystems (Miller, 1985; Karr et al., 1986; Battes, 1991).

No.	Appreciation	Score			Evaluation integrity class
		Small rivers	Medium and big rivers and reservoirs		
		(Miller, 1985)	(Karr et al., 1986)	(Battes, 1991)	
1.	Excellent	37-40	57-60	70-75	I
2.	Excellent-good	34-36	53-56	66-69	II
3.	Good	30-33	48-52	59-65	III
4.	Moderate-good	28-29	45-47	55-58	IV
5.	Moderate	23-27	39-44	47-54	V
6.	Poor-moderate	21-22	36-38	43-46	VI
7.	Poor	16-20	28-35	35-42	VII
8.	Poor-very low	12-15	24-27	20-34	VIII
9.	Very low	< 12	< 23	< 20	IX

The research was conducted during September-October 2015 in six Natura 2000 sites: two SCIs in Mureș River (ROSCI0367 Mureșul between Morești and Ogra and ROSCI0368 Mureș River between Reghin and Deda) and four SCIs in Someș River (Someșul Mic River – ROSCI0394 near Gherla and Miniu Gherlii localities; lower Someș River – ROSCI0435 Ardusat locality to Romania-Hungary border; Someș River between Rona-Țicău ROSCI0436; and Someșul Mare River – ROSCI0437 Someș between Mica and Beclean) (Fig. 1).

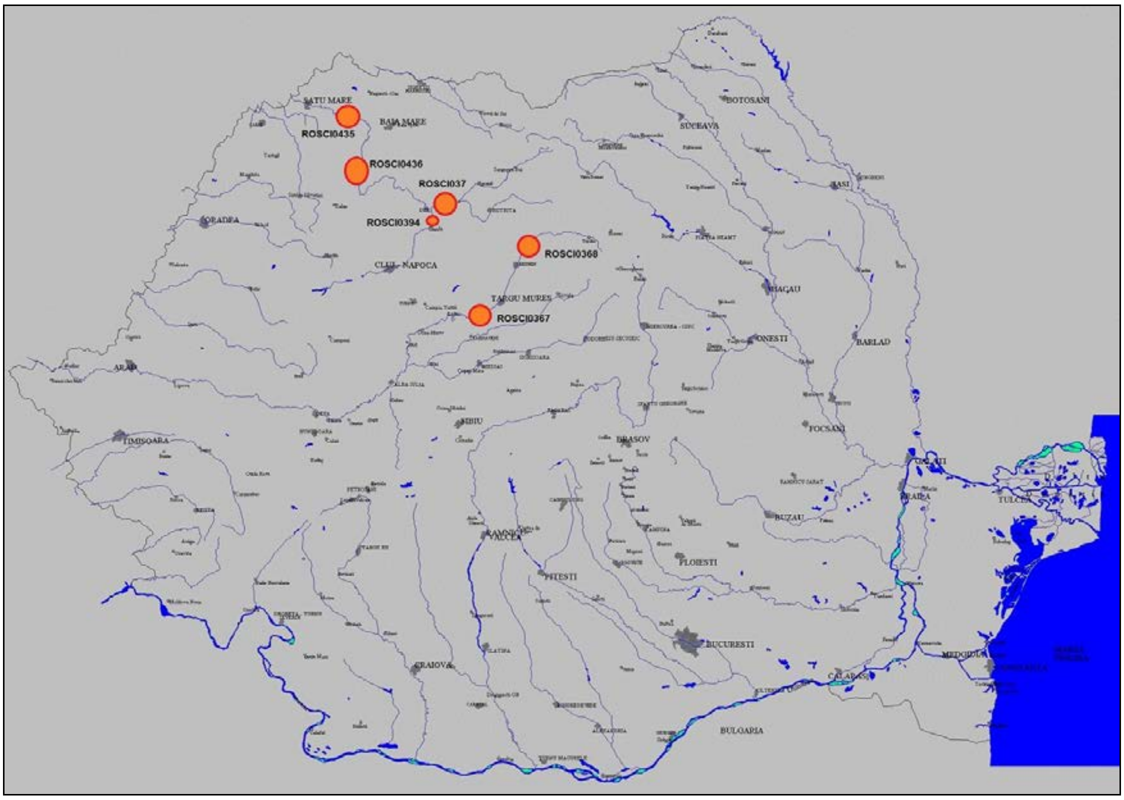

Figure 1: Overview of the project region, localisation of the SCIs points and SCIs codes.

RESULTS AND DISCUSSION

In autumn of 2015 were captured 27 fish species including 11 ones of community interest, from these 11 species three are new added in Standard Form List N2000 of studied SCIs, meaning *Hucho hucho, Cottus gobio* and *Zingel zingel*, but uncaptured was one species in 2015 *Zingel streber* (Tab. 4).

We have to emphasis the first recording of more specimens of *Neogobius fluviatilis* (monkey goby) in Someșul Mare River upstream Dej locality, after first record of species in Someș River in 2014 downstream Dej locality (Cocan et al., 2014), confirming the hypotheses that this species is in a continuous expansion in Danube Basin. We also captured many *N. fluviatilis* individuals in Someș River downstream Dej locality, till to Romania-Hungary border.

Regarding fish abundance percentage in studied SCIs are presented in figures 2-7, with black column for fish species community interest and greyish column for others captured fish species.

Concerning ecological significance (Tabs. 5 and 6) *Alburnus alburnus* is main species in plain area of mostly studied SCIs (except Mureș River Reghin-Deda sector), but in submontain area (in Mureș River in Reghin-Deda sector) *Alburnoides bipunctatus* is main, dominant species. After these species, follows characteristic or complementary species like *Squalius cephalus, Rhodeus amarus, Gobio albipinnatus* and *Barbus meridionalis* with some differences between sites.

Table 4: Fish species richness captured in six Romanian SCIs from Someș and Mureș rivers in 2015 (1 = present species, bolded are fish species community interest).

No.	Species/Sector	Someșul Mic River	Someșul Mare River	Someș River		Mureș River	
		Gherla	Mica-Beclean	Rona- Țicău	Ardusat-border	Reghin-Deda	Morești-Ogra
1.	*Alburnoides bipunctatus*	1	1	1	1	1	1
2.	*Alburnus alburnus*	1	1	1	1	1	1
3.	*Ballerus sapa*				1		
4.	*Barbatula barbatula*					1	
5.	*Barbus barbus*		1	1	1		1
6.	***Barbus meridionalis***	**1**	**1**	**1**		**1**	
7.	*Carassius gibelio*			1			
8.	*Chondrostoma nasus*	1	1	1		1	
9.	***Cobitis taenia***					**1**	**1**
10.	***Cottus gobio***					**1**	
11.	*Gobio gobio*		1			1	1
12.	***Gobio uranoscopus***					**1**	
13.	***Hucho hucho***					**1**	

Table 4 (continued): Fish species richness captured in six Romanian SCIs from Someș and Mureș rivers in 2015 (1 = present species, bolded are fish species community interest).

No.	Species/Sector	Someșul Mic River	Someșul Mare River	Someș River		Mureș River	
		Gherla	Mica-Beclean	Rona-Țicău	Ardusat-border	Reghin-Deda	Moresti-Ogra
14.	*Lepomis gibbosus*	1					
15.	***Aspius aspius***	**1**	**1**	**1**	**1**	**1**	**1**
16.	*Neogobius fluviatilis*		1	1	1		
17.	*Perca fluviatilis*			1			1
18.	*Pseudorasbora parva*	1		1	1		
19.	***Rhodeus amarus***	**1**	**1**	**1**	**1**	**1**	**1**
20.	***Gobio kessleri***		**1**	**1**			
21.	***Gobio albipinnatus***	**1**	**1**	**1**	**1**	**1**	**1**
22.	*Rutilus rutilus*	1		1	1		1
23.	***Sabanejewia aurata***		**1**	**1**		**1**	
24.	*Silurus glanis*				1		
25.	*Squalius cephalus*	1	1	1	1	1	1
26.	*Vimba vimba*		1	1	1		1
27.	***Zingel zingel***				**1**		
	TOTAL	11	14	17	14	15	12
	Fish community interest	**4**	**6**	**6**	**4**	**8**	**4**

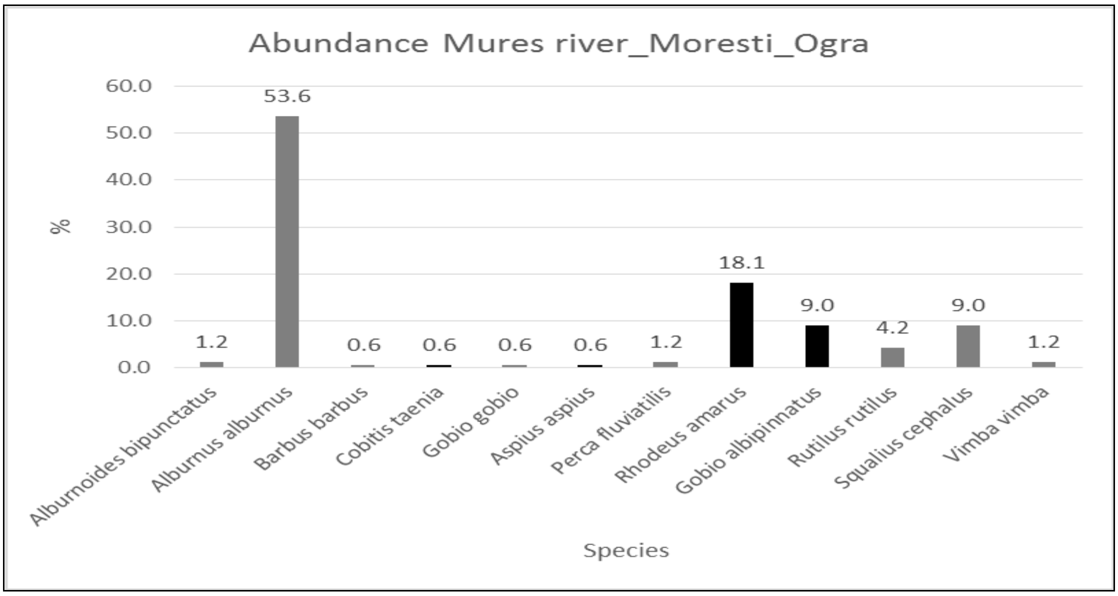

Figure 2: Fish abundance (percent) from Mureș River in ROSCI0367 (Mureș between Morești and Ogra).

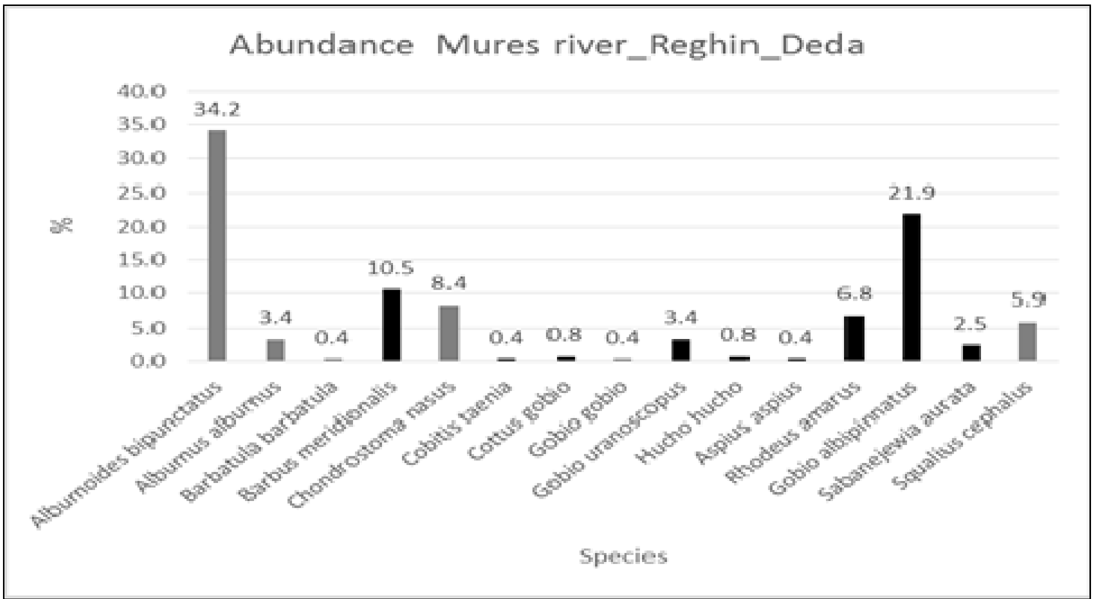

Figure 3: Fish abundance (percent) from Mureș River in ROSCI0368 (Mureș between Reghin-Deda).

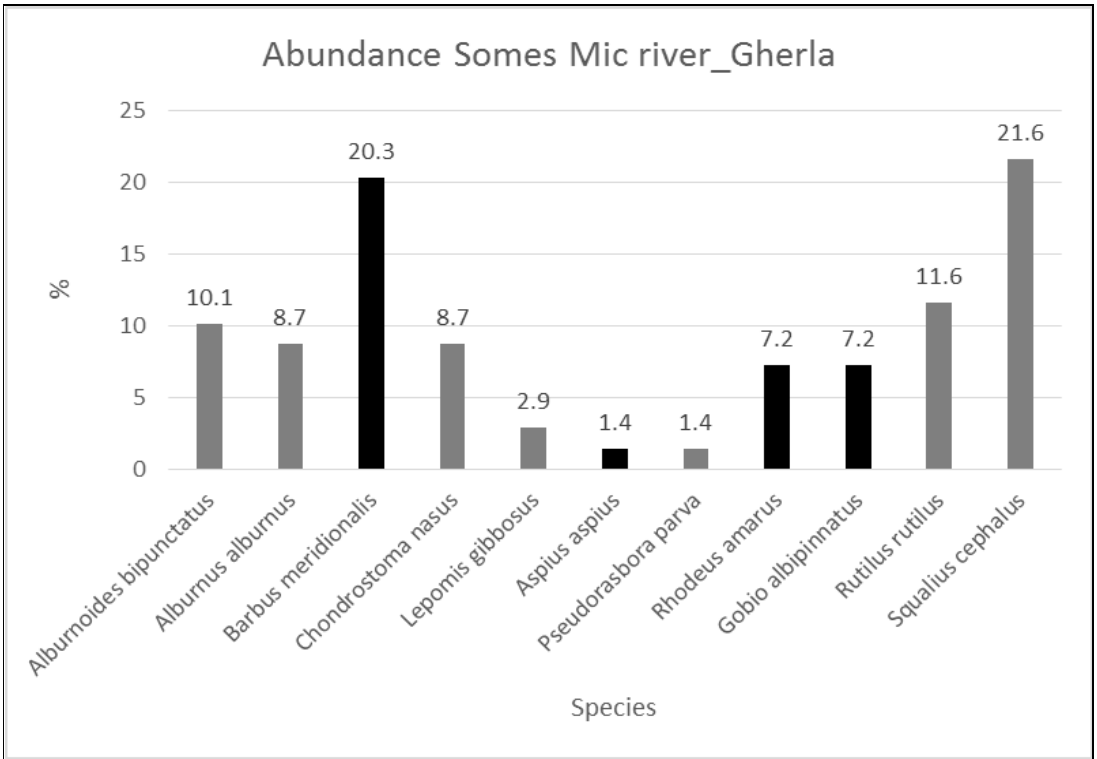

Figure 4: Fish abundance (percent) from Someș Mic River in ROSCI0394 (Someșul Mic).

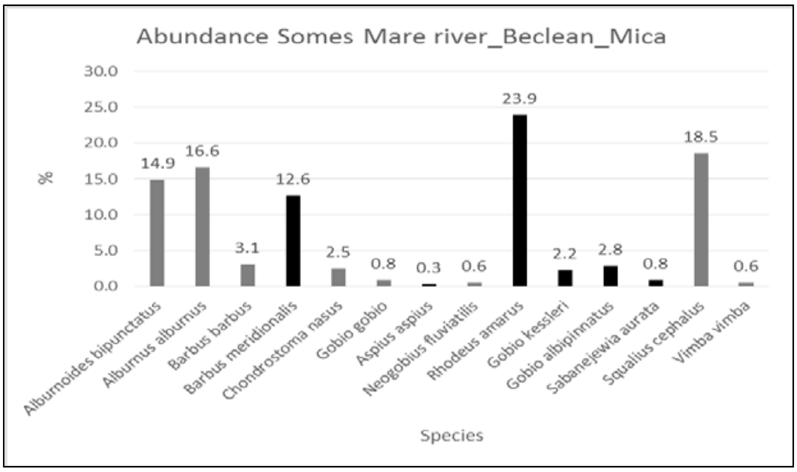

Figure 5: Fish abundance (percent) from Someș Mare River in ROSCI0437 (Someșul Mare between Mica-Beclean).

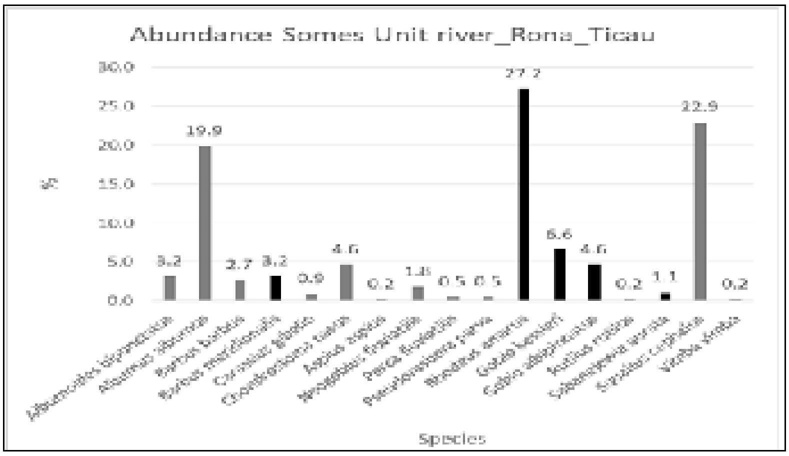

Figure 6: Fish abundance (percent) from United Someș River in ROSCI0436 (Someș between Rona-Țicău).

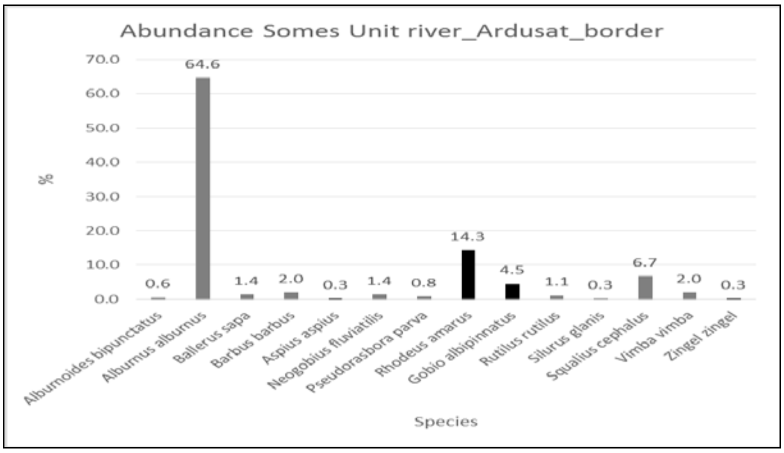

Figure 7: Fish abundance (percent) from Someș River in ROSCI435 (lower Someș River) Ardusat locality to border.

Table 5: Ecological significance from three extended SCIs in 2015.

Species	Someșul Mic River			Mureș River					
				Reghin-Deda			Morești-Ogra		
	D class	C class	W class	D class	C class	W class	D class	C class	W class
Alburnoides bipunctatus	D3	C2	W2	D6	C5	W6	D2	C2	W2
Alburnus alburnus	D3	C3	W3	D2	C2	W2	D6	C5	W6
Ballerus sapa									
Barbatula barbatula				D1	C1	W1			
Barbus barbus							D1	C1	W1
Barbus meridionalis	D6	C4	W5	D6	C4	W5			
Carassius gibelio									
Chondrostoma nasus	D3	C2	W2	D2	C4	W3			
Cobitis taenia				D1	C1	W1	D1	C1	W1
Cottus gobio				D1	C1	W1			
Gobio gobio				D1	C1	W1	D1	C1	W1
Gobio uranoscopus				D2	C3	W2			
Hucho hucho				D1	C1	W1			
Lepomis gibbosus	D1	C1	W1						
Leuciscus aspius				D1	C1	W1	D1	C1	W1
Neogobius fluviatilis									
Perca fluviatilis							D2	C2	W2
Pseudorasbora parva	D1	C1	W1						
Rhodeus amarus	D2	C3	W3	D3	C2	W2	D5	C3	W4
Romanogobio kessleri									
Romanogobio vladykovi	D3	C3	W3	D5	C4	W5	D5	C4	W5
Rutilus rutilus	D4	C3	W4				D2	C4	W3
Sabanejewia a. balcanica									
Silurus glanis									
Squalius cephalus	D6	C5	W6	D4	C4	W4	D4	C3	W4
Vimba vimba							D2	C2	W2
Zingel zingel									

Table 6: Ecological significance from three new designed SCIs in 2015.

Species	Someșul Mare River			Someș River					
				Rona-Țicău			Ardusat-border		
	D class	C class	W class	D class	C class	W class	D class	C class	W class
Alburnoides bipunctatus	D5	C3	W4	D2	C2	W2	D1	C2	W2
Alburnus alburnus	D6	C5	W6	D4	C5	D5	D6	C5	W6
Ballerus sapa							D1	C2	W1
Barbatula barbatula									
Barbus barbus	D2	C4	W3	D3	C4	W3	D2	C3	W3
Barbus meridionalis	D5	C3	W4	D3	C2	W3			
Carassius gibelio				D2	C2	W2			
Chondrostoma nasus	D2	C3	W3	D4	C4	W4			
Cobitis taenia									
Cottus gobio									
Gobio gobio	D2	C2	W2						
Gobio uranoscopus									
Hucho hucho									
Lepomis gibbosus									
Leuciscus aspius	D1	C1	W1	D1	C1	W1	D2	C1	W2
Neogobius fluviatilis	D1	C1	W1	D2	C3	W3	D2	C3	W2
Perca fluviatilis				D2	C2	W2			
Pseudorasbora parva				D2	C2	W2	D2	C1	W1
Rhodeus amarus	D6	C3	W5	D6	C5	W6	D6	C4	W5
Romanogobio kessleri	D2	C4	W2	D3	C2	W3			
Romanogobio vladykovi	D2	C2	W2	D3	C4	W4	D3	C4	W4
Rutilus rutilus				D1	C1	W1	D2	C3	W2
Sabanejewia a. balcanica	D1	C2	W2	D2	C2	W2			
Silurus glanis							D1	C1	W1
Squalius cephalus	D6	C4	W5	D6	C4	W5	D4	C3	W4
Vimba vimba	D1	C2	W2	D1	C1	W1	D3	C3	W3
Zingel zingel							D1	C1	W1

Regarding IBI all six studied SCIs has Scores between 57-67 which are included in II-IV Evaluation integrity class, means moderate-good to excellent-good appreciation in fish ecosystem (Tab. 7).

Table 7: IBI results for fish fauna from six SCIs from Someş and Mureş rivers.

Parameters	Someșul Mic		Someșul Mare	Someșul Unit		Mureș
	Petrest-Gherla	Beclean-Dej	Rona- Țicău	border-Baia Mare	Reghin-Deda	Moreşti-Ogra
1.	5	5	5	5	5	5
2.	5	5	5	5	5	5
3.	1	1	1	1	1	1
4.	3	3	3	3	5	3
5.	5	5	5	5	5	5
6.	1	5	3	3	5	5
7.	3	3	3	1	5	5
8.	5	5	5	5	5	5
9.	5	5	5	5	5	5
10.	3	3	3	3	3	3
11.	5	3	3	5	3	3
12.	3	5	5	5	5	3
13.	3	3	3	5	5	3
14.	5	5	5	5	5	5
15.	5	5	5	5	5	5
Score	57	61	59	61	67	61
Evaluation integrity class	IV	III	IV	III	II	III
Appreciations	Moderate-good	good	Moderate-good	good	Excellent-good	good

Mureș River (ROSCI0367 Mureș between Morești-Ogra) – In this extended SCI (Morești-Ogra) we have identified 12 fish species including four ones community interest species present, none in addition to the Standard Form N2000 List, but three fish species of community interest (*S. aurata, G. kessleri* and *Z. streber)* did not find. Main and characteristic or complementary species, dominated in abundance are *Alburnus alburnus, Rhodeus amarus, G. albipinatus, Squalius cephalus* (Fig. 2; Tab. 5). This sector of Mureș River is affected mostly by industrial and domestic pollution, but in researches time period with low intensity.

Mureș River (ROSCI0368 Mureș between Reghin-Deda) – In this extended SCI we have identified 15 fish species including nine ones community interest species, with new captured species *Hucho hucho* (Fig. 8) and *Cottus gobio* compared with Standard Form N2000 List, but was not found in studied period *Gobio kessleri*. Main and characteristic or complementary species dominant in abundance are *Alburnoides bipunctatus, Gobio albinnatus, Barbus meridionalis* and *Chondrostoma nasus* (Fig. 3; Tab. 5). In this sector of Mureș River low human impact is present: angling, tourists and rocks exploitations.

Someșul Mic River (ROSCI0394 Someșul Mic) (sampling near Gherla and Mintiu Gherlii localities) – It is an extended SCI with 11 captured fish species including four ones community interest, with *G. albipinnatus* and *B. meridionalis* new added in Standard Form N2000 List, but *C. taenia* and *G. kessleri* missing in captures in studied period. Main and characteristic or complementary species dominant in abundance are *B. meridionalis, S. cephalus* and *R. rutilus* (Fig. 4; Tab. 5). Someșul Mic River is affected mostly by industrial, agricultural and domestic pollution, also dams, but actual with medium intensity.

Someș River (ROSCI0435 Someșul Inferior) – New designed SCI on Someș River between Ardusat locality and Romania-Hungary border, designated for 14 fish species including four ones of community interest. Main, characteristic and complementary or complementary species are *Alburnus alburnus, R. amarus, G. albipinatus* and *S. cephalus* (Fig. 7; Tab. 6). Here were found many individuals of *Neogobius fluviatilis* (Fig. 9) new recorded species in Someș River first time recorded in Someș Unit, upstream between Jibou-Dej localities in 2014 (Cocan et al., 2014). Someș River is affected mostly by industrial, agricultural and domestic pollution, also ballast exploitation, but actual with medium intensity.

Someș River (ROSCI0436 Someș between Rona-Țicău) – It is new designed SCI for fish fauna with 17 fish species including six ones community interest, main and characteristic or complementary species dominant in abundance are *R. amarus, S. cephalus* and *A. alburnus* (Fig. 6; Tab. 6). Also in this sector of Someș River was found many individuals of *Neogobius fluviatilis,* new recorded gobies in river (confirming Cocan et al. (2014) records). This part of Someș River is affected mostly by industrial and domestic pollution, also forestry exploitation and angling, but actual with low intensity.

Someșul Mare River (ROSCI0394 Someșul between Mica-Beclean) – Is new SCI designed for fish fauna with 14 fish species including six ones community interest, main and characteristic or complementary species dominant in abundance are *R. amarus, S. cephalus, A. alburnus, A. bipunctatus* and *B. meridionalis* (Fig. 5; Tab. 6). Very important is the presence of some individuals of *N. fluviatilis* in Someșul Mare River between Mica-Beclean, new recorded species in this tributary of Someș River, not present in fish list of Bănărescu et al. (1999). This species booming population in all Danube Basin including Someș tributaries, first record in united Someș Unit (Cocan et al., 2014). Someșul Mare River is affected mostly by ballast exploitation follow by agricultural and domestic pollution, also angling and fish expansion of *N. fluviatilis* competitor to other fish species, but with low intensity.

Figure 8: *Hucho hucho* critical endangered fish species captured in Mureș River, near Brâncovenești locality (two individuals), quickly released in water.

Figure 9: New record of *Neogobius fluviatilis* in Someșul Mare River, upstream Dej locality (10 individuals near Uriu locality), in expansion in all Danube Basin.

CONCLUSIONS

In 2015 were designed three new SCI for fish fauna and other three SCI were extended, all six for solving IN MOD stage of *Aspius aspius, Gobio albipinnatus* and *Rhodeus amarus* related with mammal *Lutra lutra*.

This three IN MOD fish species have stable population with favourable development conditions in all six studied SCIs.

In these six investigated SCIs were captured 27 fish species including 11 ones of community interest with three more species (*Hucho hucho, Cottus gobio* and *Zingel zingel*) that was in Standard Form N2000 List, but *Zingel streber* missed from capture in 2015.

Main and characteristic or complementary fish species dominant in abundance are *Alburnus alburnus* in plain area of studied sectors and *Alburnoides bipunctatus* in submontain area, followed by *Squalius cephalus, Rhodeus amarus, Gobio albipinnatus* and *Barbus meridionalis* with some differences between sites.

The most important fish species captured was two individuals of *Hucho hucho* in Mureș River, near Brâncovenești locality, very rare species critical endangered, its presences is encouraging.

Neogobius fluviatilis (monkey goby) was first recorded by us far upstream in Someșul Mare than its first record in Someș (2014), which indicates its expansion in Someș River system. Its expansion is the same as in all Danube Basin.

Dams, river rocks extraction, ballast and forestry exploitation or agricultural, industrial, touristic, urban pollution and expansion of competitor *Neogobius fluviatilis,* all of these factors have negative impact in Mureș and Someș rivers, disturbing pristine/foremost fish conditions, demaging initial wildlife habitats.

Advanced investigations in time (more seasons) and space (more sampling sites) for SCIs evolution and for finding very rare species are necessary in future.

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THE CAVEFISH *OREONECTES JIARONGENSIS* CAN BE INDUCED TO DIFFERENTIATE AND RECOVER UNDER THE LIGHT CONDITION

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ABSTRACT

This research indicated that one cave fish species of *Oreonectes jiarongensis* can recover the transparent to black under the light condition, this species belongs to the *Oreonectes*, Nemacheilinae, and distributes in Libo County, Guizhou Province, China. The changing process time was 14 days. This is the first time that suggests the cave vertebrates which lived in the dark environment not longer time could change the body color in the light environment, and has a new adaptive strategy for the darkness condition. The result may indicate that this species entrance the underground river not so long time, and the genes not mutation, which control the melanin express, it still has the physiological regulation mechanism under the light condition.

RESUMEN: El pez de las cavernas *Oreonectes jiarongensis* puede ser inducido a la diferenciación y recuperación bajo condiciones de iluminación.

En esta investigación se encuentra que una especie de pez que habita en cavernas, *Oreonectes jiarongensis*, puede alternar su apariencia de transparente a negra, bajo distintas condiciones de iluminación. Esta especie pertenece al género *Oreonectes*, Nemacheilinae, y se distribuye en el condado Lobo, provincia de Guizhou, en China. El proceso de cambio tomó 14 días. Esta es la primera vez que se sugiere que un vertebrado que habita en cavernas, viviendo en un ambiente de oscuridad, puede cambiar de color bajo condiciones de iluminación, lo que puede representar una una nueva estrategia adaptativa. Los resultados pueden indicar que esta especie ingresa no por mucho tiempo al ambiente subterráneo en los rios, de manera que los propios genes, y no una mutación, que controlan la expresión de la melanina, siguen siendo el mecanismo fisiológico de regulación bajo condiciones de iluminación.

REZUMAT: Inducerea diferențierii și a recuperării pigmentării la lumină la peștele cavernicol *Oreonectes jiarongensis*.

Prezentul articol arată că una din speciile cavernicole de pește *Oreonectes jiarongensis* poate să își recupereze la lumină pigmentarea pielii, de la transparent la negru. Această specie aparține genului *Oreonectes*, fam. Nemacheilinae și se întâlnește în ținutul Libo, provincia Guizhou, China. Procesul de refacere a pigmentării a durat 14 zile. Este prima citare care sugerează că vertebratele cavernicole ce trăiesc în întuneric de puțină vreme își pot modifica culoarea corpului în cazul expunerii la lumină și au o strategie adaptativă nouă pentru viața în lipsa luminii. Rezultatul poate indica că această specie a pătruns în râul subteran de relativ puțină vreme, mutația genelor care controlează expresia melaninei neavând timp să se fixeze, mecanismul de reglare fiziologică în condiții fotice fiind încă prezent.

INTRODUCTION

Body colour of teleost fish plays a role in concealing, disguising, alerting, and mating by Salopek and Jimbow (1996). Fish body colour is determined by melanocytes, xanthophore, erythrophores, and iridocytes in the dermis by Feng et al. (2014). Therefore, freshwater teleost fishes present a variety of body colours. Of these, a majority of fishes are black, owing to the presence of abundant, stable melanocytes in their skin that selectively absorb light of specific wavelength and reflect light of other wavelengths by Ye et al. (2003).

In typical cave-dwelling fishes, body colour disappears, and they appear white or translucent, in order to adapt to completely dark environments by Zhao and Zhang (2006). The pathway for production of melanin in freshwater teleost is Phenylalanine → L-tyrosine → 3,4-dihydroxyphenylalanine (dopa) → dopaquinone → melanin by McCauley et al., 2004; Jeffery et al. (2016). Two ligands of MC1R, α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH), bind to MC1R on the melanocyte membrane. This makes the G-protein coupling to the receptor transform from inactive guanosine diphosphate (GDP) to active guanosine triphosphate (GTP), thereby activating the adenylate cyclase system present on the membrane, and transforming adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Further, cAMP activates tyrosine kinase and tyrosinase (Tyr). Tyr catalyzes tyrosine in the melanocytes to produce dopa, and subsequent release of melanin after it accumulates in the melanocytes to a certain extent by Yu et al. (2010). Studies on *Astyanax fasciatus mexicanus* found that it failed to form melanin the mutation of *occuloalbinism 2* (*Oca2*) by McCauley et al. (2004) and *melanocortin 1 receptor* (*Mc1r*) genes by Gross et al. (2009). The main function of *Oca2* gene is to code for tyrosinase related transporter protein by Gross and Wilkens (2013). Meanwhile, *Mc1r* mutation significantly reduces the melanin content and decreases the number of melanocytes by Gross et al. (2009). Tyr is the major rate-limiting enzyme for the conversion of tyrosine to melanin by Jeffery et al. (2016). Too little Tyr will cause tyrosine to transform into cysteamine dopa, thereby hindering the production of melanin.

There also were some studies demonstrated that melanocyte proliferation and melanin secretion were positively correlated with ultraviolet radiation in embryonic *Xenopus laevis* (Yu et al., 1987). Thus, melanin expression in vertebrates might be subject to a combined action of molecular basis and external light environment.

MATERIAL AND METHODS

Experimental species

In 6th-14th, 2016, nine individuals of *Oreonectes jiarongensis*, body length 52-116 mm, were collected from a cave in Shuijingwan Village, Latan group, Jiarong, Libo, Guizhou, China (25°28’12.89” N, 108°06’34.35” E; altitude-634 m) and reared in a dark condition. Lasted for 30 days in the ecology laboratory of the College of Life Science, Guizhou Normal University, From April 15 to May 15, 2016, during which three fish died.

Feeding conditions

Five fish tanks (45 cm × 30 cm × 29 cm), numbered 1, 2, 3, 4, and 5, were used for feeding the *O. jiarongensis*. Another ultra clear glass tank (22 cm × 15 cm × 18 cm) was used for photographing the fish body colour change every day.

The feeding conditions of this cave fish species cited from Richard (2008) mainly, and a little rearrangement based on the origin condition that the fish live in. In order to make the fish were alive. After being collected from the cave environment, the water was applied 24 h with cycle oxygen. During feeding, the water was oxygenated and circulated continuously.

According to Ye et al. (2003), Earthworms, procured from the flower and bird market in Youzha Street, Guiyang, were kept alive before being fed to the fish. During feeding, the earthworms were cut into one-two mm segments. The fish were fed once a day, in the evening, and the unconsumed earthworms were taken out the following evening to maintain water quality.

Experimental methods

Light and other environmental conditions

The fluorescent lamp was used as the experimental light instead of the normal sunlight, and turned on at 08:30 am and turned off at 17:30 pm everyday during the experiment. Meanwhile, the sunrise time was 06:27-06:07 h and the sunset time was 19:17-19:31 h. Illumination was monitored using an illuminometer (VICTOR 1010A, SHENZHEN VICTOR HI-TECH CO., LTD, CHINA), and the illumination interval was 94.0-130.3 lux. The air temperature was 8-28°C and the atmospheric pressure was 1000.9-1014.1 hPa.

Grouping

Nine specimens were divided into two groups by the standard body length of the fish, which body length > 70 mm (range: 73-116 mm) were assigned to group A, and coded number as A1, A2, A3 and A4 (numbered by the decreasing order of body length); if body length < 70 mm (range: 52-63 mm), then belonged to group B, also numbered by the decreasing order of body length as B1, B2, B3, B4, it might reveal that the different body size fished might have the recovery speed on the body colour. In addition, one spared fish with a body length of 67 mm was assigned to B group and denoted as B5 (Fig. 1; Tab. 1).

Nine *O. jiarongensis* were included before and after the experiment. At the start of the experiment, A1 and B1, A2 and B2, A3 and B3 as well as A4 and B4 were fed in tanks 1, 2, 3, and 4 (Tab. 1). Four days later, A2, A3 and B3 died successively. Therefore, B5 that was simultaneously fed under the light environment was considered as the observatory object.

Table 1: Feeding in fish tanks by Body length of fish.

Group	Tank number				
	1	2	3	4	5
A (> 70 mm)	A1 (116 mm)	A2 (95 mm)	A3 (80 mm)	A4 (73 mm)	
B (< 70 mm)	B1 (63 mm)	B2 (61 mm)	B3 (56 mm)	B4 (52 mm)	B5 (67 mm)

Observation and record

An ultra-clear glass aquarium with a scale plate (length 22 cm, least count 0.5 cm) was used for observing and photographing. Each fish was observed once every other day or at noon on two consecutive days in the following order: A1 → B1 → A2 → B2 → A3 → B3 → A4 → B4 → B5. The blackened part of the body was carefully observed. At 20:30-21:00 h on the same day, changes in body colour was photographed and recorded in the same order as that at noon. Each fish was taken out from the tank with a hand-held net, and was placed into the ultra clear glass aquarium. The fish were photographed using a camera (Nikon D810, Nikon Corporation, Japan) when its head turned left, tail turned right and body was parallel to the scale plate.

Determination of degree of blackening

From the photographs of *O. jiarongensis*, taken on the 14th day of light exposure, the degree of blackening at selected points were measured using Adobe Photoshop CC2014 (32bit) operation system based on windows7, use Photoshop CC2014 to measure the extent of blackening with the following settings: graphics mode: CMYK colour, 32 bit; and sample size: average 101*101.

RESULTS AND DISCUSSION

We observed the blackening process in six individuals of *O. jiarongensis*, all of which gradually blackened to resemble surface fish after receiving light exposure for 14 days. In group B (body length < 70 mm), the rate of blackening was generally fast, and the extent of blackening was remarkable. Meanwhile, in group A the rate of blackening was faster than that in group B during the first three days, before becoming slower than that in group B. In addition, the overall extent of blackening was lighter than that in group B (Tabs. 2 and 3). The blackening process progressed in the following order: upper part of the snout → cranium → back → tail → caudal fin → lower part of the snout → dorsal fin → pectoral fin and other appendages.

Table 2: Change in body colour of *O. jiarongensis* with different sizes under light condition.

Time	Group A	Group B
1st day	Translucent	Translucent
5th day	Nostril outside, cranium, upper part of the snout, central part of the head, dorsal fin base, and maxillary barbell base became black; black spots were visible on the back.	Nostril outside, cranium, upper part of the snout, central part of the head, dorsal fin base, most part of maxillary barbell base and caudal-peduncle became black.
8th day	Superior and inferior caudal fins appeared black, whereas the back, cranium, and upper part of the snout became significantly black.	Superior and inferior caudal fins and barbells became black, whereas caudal-peduncle, upper part of the snout, cranium, and back were significantly black.
14th day	Black spots appeared at the lower part of the snout, black spots increased at the base of pectoral rays and caudal fin, cranium, upper part of the snout, the back became black as a whole, the pelvic fin and caudal fin became black.	Blackening became significant as a whole, where dorsal part of the head and the back become completely black, black spots increased in the lower part of snout, the black spots on the caudal fin increased and deepened; the fish looked like a surface fish.

Table 3: Comparison of the extent of blackening in *O. jiarongensis* with different sizes on the 14th day of light exposure.

Part	Group A	Group B
Nostril outside	K = 45%	K = 64%
Cranium	K = 40%	K = 56%
Upper part of snout	K = 44%	K = 63%
Back	K = 36%	K = 53%
Central part of the head	K = 37%	K = 55%
Gill	K = 33%	K = 52%
Middle part of the lateral side	K = 27%	K = 52%
Lower part of the lateral side	K = 18%	K = 50%
Lower part of the snout	K = 40%	K = 61%
Base of dorsal fin	K = 41%	K = 58%
Junction of caudal-peduncle and caudal fin	K = 63%	K = 70%
Caudal-peduncle	K = 62%	K = 68%
Barbell	K = 47%	K = 64%

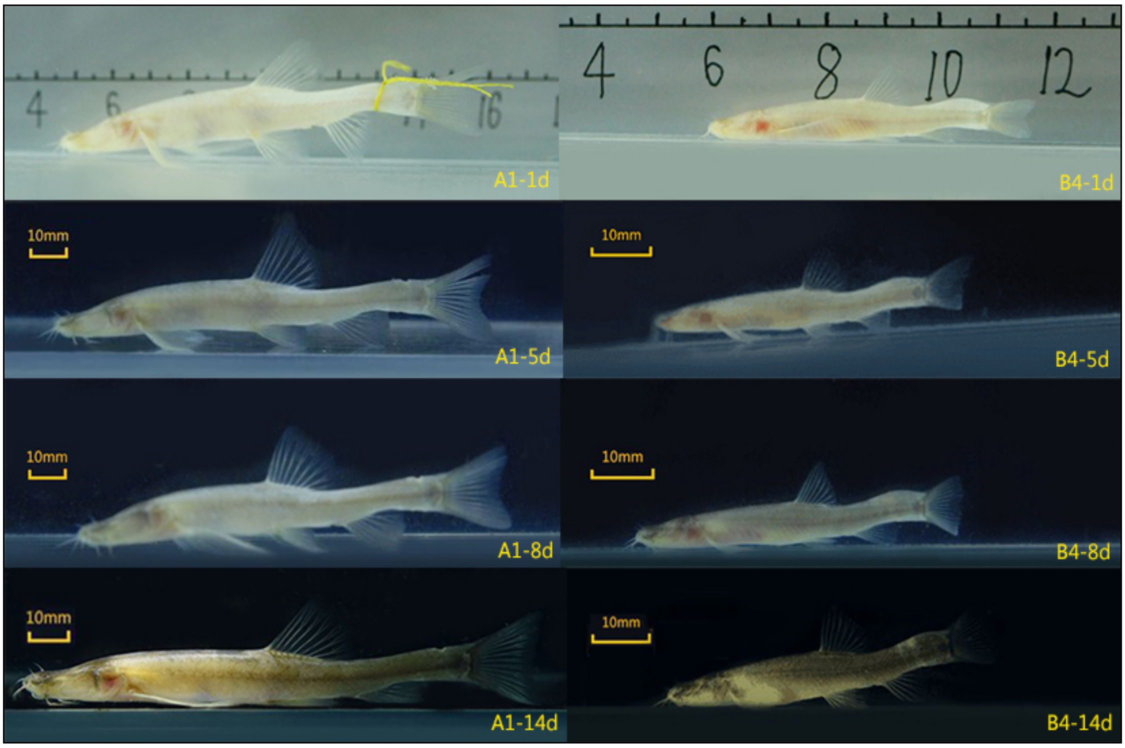

Figure 1: Body colour changes of *O. jiarongensis*
with different sizes before and after light exposure.
A-group A (body length > 70 mm), B-group B (body length < 70 mm), d-days.

There are some studies on the body colour of the vertebrates animals recently, such as: fishes (Maan and Sefc, 2013; Sköld et al., 2013; Nyboer et al., 2014), amphibians (Kindermann and Hero, 2016a, b), Reptiles (Junko and Tsutomu, 2009; Takeo et al., 2009) these researches all indicated that the external body colour changes in amphibians and other colour changing animals are possible due to different distributions of pigment cells (chromatophores) and the movement of pigment within them, meanwhile, also have some genetic foundation. It was suggested that the fishes body colour regulation mechanisms have two kinds, one was the physiological regulation, this mechanism means that the pigment granules could gather and disperse rapidly by the nerve regulating (Leah and Catherine, 1994); another one was the morphological regulation mechanism, that means, the body colour was regulated by the endocrine system (Tripathi et al., 2008). More studies often concern on the river surface or ground vertebrate animals, just a few researches focused on the cave vertebrate animals, for example, the cave fish of *A. fasciatus mexicanus*. As for this fish species which dwelling in the different underground rivers in Mexico, more studies were done on the synthesis and express pathway of the *A. fasciatus mexicanus* based on the biochemistry and genetic, and showed if the main control genes Mc1r and Oca2 had mutated, then this species cannot has the black colour on its' skin (Gross and Wilkens, 2013; Bierbach et al., 2013).

As for the fresh water fish species, the body colour has the very important means on the phenotypic and the fitness in the different subpopulations. In the present study, the body colour of *Oreonectes jiarongensis* was found to transform from translucent to black when exposed to light. It was inferred that the melanin-controlling genes did not undergo mutation. At the same time, the genetic study did not show the genes mutations occurred in the species of *O. jiarongensis* by amplification of *Mc1r*, *Oca2*, and *Tyr* genes, which controlling the synthesis and express of the melanin, meanwhile, it indicated that these three genes could express normally by transcriptome analysis (different study which belongs to the same project, the paper submitted), the results showed the three genes of *O. jiarongensis* had the normal functions. In dark environments that lack ultraviolet radiation, it appears translucent, owing to decreased function of the melanocytes. In contrast, *Mc1r* and *Oca2* genes in *Astyanax fasciatus mexicanus*, incur mutations, thereby reducing the number of melanocytes significantly. In addition, transport of the substrate of melanin, i.e., tyrosine, into the melanocyte nuclei fails, thus preventing melanin synthesis. That means the *Astyanax fasciatus mexicanus* was unable to recover its body colour under light condition, because of the genes mutations.

O. jiarongensis started blackening after being exposed to the selected light regime for three days, and its body colour changed completely after 14 days. The order of blackening was: upper part of the snout → cranium → back → tail → caudal fin → lower part of the snout → dorsal fin → pectoral fin → other appendages. Owing to physiological regulation, this species appeared white when living in the cave but turned black after being exposed to light for a short time. This physiological regulation might reflect imperfect adaptation to the dark cave environment, probably owing to the fact that this species has not been living in the dark cave for a long time. The cave for sampling was formed 91,000-163,300 years ago by Zhang et al. (2000). Meanwhile, *A. fasciatus mexicanus* started to live in caves 2,800,000-6,700,000 years ago by Gross and Wilkens (2013), which implies that the time was sufficiently long for the mutation of their *Oca2* and *Mc1r* genes controlling synthesis of melanin. Thus, the body colour degradation in *A. fasciatus mexicanus* is the morphological regulation mechanism. In summary, two different fish species, *Oreonectes jiarongensis* and *Astyanax fasciatus mexicanus*, present albino phenotypes in a dark environment, but their mechanisms of body colour changing are considerably different.

CONCLUSIONS

In the present study, we investigated the process of body colour change in cave-dwelling fishes under regulated light conditions, which indicates that it takes a long time for the mutation of the functional genes controlling body colour. Regarding the body colour, a significant difference exists in the mechanism of adaptation to the dark cave environment among different fish species.

It is the first time that report and demonstrate the cave fish species can recover the body colour similar to the surface species in short time under the light condition, and suggests that the gene mutation of the body colour expressing of the cave fishes need more longer time, meanwhile, the melanin express of the fish need the environment stimulation factor.

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ACUTE TOXICITY OF BUTACHLOR TO *RUTILUS RUTILUS CASPICUS* AND *SANDER LUCIOPERCA* IN VIVO CONDITION

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ABSTRACT

Herbicides are used to control weeds and are usually targeted to sites that are specific to plants. Butachlor is an herbicide of the acetanilide class, which is widely used in agricultural fields. At the present study, lethal concentration (LC50) of butachlor was calculated for *Rutilus rutilus caspicus* and *Sander lucioperca* with a mean weight of 4 ± 1 g (mean ± SD). Our results indicated that LC50 96 h butachlor for *R. rutilus caspicus* and *S. lucioperca* were 0.342 and 0.760 ppm respectively. These findings suggest that butachlor is moderately toxic and moderately irritating for these two species. Clinical symptoms included irregular protrusion of the eyes and irregular swimming.

RESUMEN: Toxicidad aguda in vivo en la pez (*Rutilus rutilus caspicus*) y la lucioperca (*Sander lucioperca*) al butacloro.

Los herbicidas se utilizan para controlar el crecimiento de hierba mala y suelen ser dirigidos a los procesos y sitios de destino que son específicos de las plantas. El butacloro es un herbicida de la clase acetanilida que se utiliza comúnmente en campos agrícolas. En el presente estudio se calculó la concentración letal (LC50) de butacloro para especímenes de *Rutilus rutilus caspicus* y *Sander lucioperca* cuyo un peso medio fue de 4 ± 1 g (media ± DE). Nuestros resultados fueron que se obtuvo 0.342 y 0.760 ppm de LC50 96 h butacloro en R. rutilus caspicus y S. lucioperca, respectivamente. Estos hallazgos sugieren que el butacloro es moderadamente tóxico y moderadamente irritante para estas dos especies. Se observaron síntomas clínicos incluyendo protrusión irregular de los ojos y natación irregular.

REZUMAT: Toxicitatea acută in vivo a butaclorului la *Rutilus rutilus caspicus* și *Sander lucioperca*.

Erbicidele sunt folosite pentru a controla buruienile și sunt de obicei orientate spre procesele și site-urile țintă specifice plantelor. Butaclor este un erbicid din clasa acetanilida, care este utilizat pe scară largă în terenuri agricole. În prezentul studiu, concentrația letală (CL50) de butaclor a fost calculată pentru *Rutilus rutilus caspicus* și *Sander lucioperca* cu o greutate medie de 4 ± 1 g (valoare medie ± SD). Rezultatele noastre au indicat că LC50 96 h butaclor pentru *Rutilus rutilus caspicus* și *Sander lucioperca* sunt 0,342 și 0,760 ppm. Aceste rezultate sugerează ca butaclor este moderat toxic și moderat iritant pentru aceste două specii. Au fost observate simptome clinice, inclusiv proeminența neregulată a ochilor și înot neregulat.

INTRODUCTION

Aquatic ecosystems are environments rich in biodiversity (Paltenea et al., 2008; Pekarik et al., 2009; Lengyel et al., 2012; Ong'oa et al., 2013; Sanguila et al., 2015; Balasaheb et al., 2017) but constantly faced human activities threats. However, these environments are not the target for herbicide; nevertheless some studies sighted the presence of herbicides and their metabolites in surface water (Mansingh and Wilson, 1995; Tsuda et al., 1996). Herbicides are the most prevalent environmental pollutants worldwide (Khoshnood, 2017). Herbicides are used to control weeds and are targeted to sites specific to plants, exceptions are uncouples of oxidative phosphorylation and herbicides that interfere with cell division (Solomon et al., 2013).

Butachlor is an herbicide of the acetanilide class and is used as a selective pre-emergent herbicide with $C_{17}H_{26}ClNO_2$ molecular formula. Butachlor is one of the most widely used chloro-acetanilide herbicide for the control of annual grasses in rice fields and many broadleaf weeds (Nwani et al., 2013). The herbicide has contaminated river water via the effluents from rice paddy fields. Fish accumulate these chemicals by directly exposure to the chemicals present in water or indirectly through the ecosystem food chain (Ateeq et al., 2002). Butachlor is persistent in agricultural soil and water systems, posing a potential threat to the agro-ecosystem and human health (Debnath et al., 2002; Vajargah et al., 2013).

Rutilus rutilus caspicus (Caspian roach) is one of the most valuable inhabitants of the Caspian Sea, however, the Caspian roach population has recently declined in the Caspian Sea because of overfishing, a degraded habitat, and pollution (Hoseini and Nodeh, 2012). *Sander lucioperca* (Pikeperch) is found in freshwater and brackish water, which is a semi-anadromous, cool-water species in the Caspian Watershed (Ural, Volga, Kura, and Sefid Roud rivers) and in the basins of the Black, Azov, Aral, and Baltic Seas (Abdolmalaki and Psuty, 2007). As a predator and commercially valuable species, pikeperch constitute an important component of the Caspian ichthyofauna, both ecologically and commercially (Abdolmalaki and Psuty, 2007).

The contamination of aquatic ecosystems by butachlor has gained increased attention and several studies have been conducted on acute toxicity and the destructive effect of this herbicide in some fish (Tilak et al., 2007; Geng et al., 2010; Chang et al., 2013; Nwani et al., 2013; Vajargah et al., 2014) but data on acute toxicity of this herbicide on Pikeperch and Caspian roach is scarce. Despite the large use of butachlor in agriculture and the potential ecotoxicological impact, there is a scarcity of data on its effects on many Caspian Basin fish species like Caspian roach or Pikeperch. Sensitivity of various fish species is different on toxic substances, so toxicology tests are needed for different fish. For this purpose, LC50 96 h is required of any ecotoxicology studies.

MATERIAL AND METHODS

231 live specimens of Caspian roach and Pikeperch were obtained from hatchery and ponds in Gorgan Province. Samples weighted 4 ± 1 g acclimatized in 60 × 55 × 30 cm aquarium for 10 days. In order to measure biological capability and determine survival, fish were kept in natural and toxin-free environment to determine natural mortality. Physical and chemical parameters of water are represented in table 1. The fish were fed twice daily with Biomar feed at 2% body weight before the test, but feeding was stopped 24 h prior to and throughout the test. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Commercial butachlor (Machete, EC 60%) was purchased from Exir Keshavarzi Co, Iran. All Experiments were performed with 16-hours of light and eight hours of darkness. Static acute toxicity test was performed following guideline the OECD standard method (OECD, 1989). 10 treated aquariums with concentration ranges 0.11, 0.22, 0.33, 1, 1.66, 2.33, 3.33, 6.66, 13.33, 16.66 and control groups (no toxic concentration) were

performed. Mortality rates were recorded after 24, 48, 72 and 92 hours and dead fish were quickly removed from the aquarium. The nominal concentration of toxin causing mortality (LC1‹LC10 ‹ LC30 ‹LC50 ‹LC70 ‹LC90 and LC99) within 24, 48, 72 and 92 hours for each toxin was calculated separately. LC50 values for 24, 48, 72 and 96 h exposures were computed on the basis of probit analysis version 16/0.

This work was approved by the ethical committee of GAU University. To minimize suffering of the fish, all animals were exposed to clove essence, and a low dose of anesthesia; hypothermia prior to euthanasia and eventually spinal cord dislocation for euthanasia.

Table 1: Physico-chemical properties of the test water.

Physico-chemical indices	24 h	48 h	72 h	96 h
pH	8.22	8.28	8.25	8.30
BOD	69%	68%	67%	68%
Temperature (C°)	17.9	17.8	17.9	17.7
Total hardness ($MgCaCo_3$)	210	210	210	210

RESULTS

No mortality was observed during acclimation (Tabs. 2 and 3). Result showed that within a 96 h test, the LC50 value declined with increasing toxin concentration and duration of exposure. This shows that an LC50 value in the first 24 hours of the experiment was always higher than LC50 96 h. According to the results LC50 96 h Butachlor for *Rutilus rutilus caspicus* and *Sander lucioperca* were obtained 0.342 and 0.760 ppm respectively. Fish had a 100% mortality rate only hour after exposure in 13.33 and 16.66 ppm concentration. The nominal concentration of toxin causing mortality (LC1‹LC10 ‹ LC30 ‹LC50 ‹LC70 ‹LC90 and LC99) within 24, 48, 72 and 92 hours for each toxin was calculated (Tab. 4). It was significantly different between experiment fish, that in all steps, mortality was higher for Caspian roach during exposure to butachlor, Caspian roach is more sensitive in comparison with Pikeperch.

Table 2: Mortality rate in acute toxicity (LC_{50} 96h) rate for Caspian roach (n = 21 each treatment).

Concentration (mg/l)	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.11	0	0	3	5
0.22	0	3	4	7
0.33	2	4	11	17
1	2	3	15	19
1.66	5	9	19	21
2.33	4	9	21	21
3.33	4	21	21	21
6.66	15	21	21	21
13.33	21	21	21	21
16.66	21	21	21	21

Table 3: Mortality rate in acute toxicity (LC_{50} 96 h) rate for Pikeperch (n = 21 each treatment).

Concentration (mg/l)	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.11	0	0	2	4
0.22	0	0	3	7
0.33	1	3	9	13
1	2	4	10	13
1.66	3	6	11	17
2.33	3	11	15	19
3.33	6	15	21	21
6.66	11	19	21	21
13.33	21	21	21	21
16.66	21	21	21	21

Table 4: Lethal concentration of butachlor (mg/l) (95% confidence intervals) depending on exposure time for Caspian roach and Pikeperch.

LC	96 h Exposure time		95% confidence intervals			
			Caspian roach			
	Caspian roach	Pikeperch		Caspian roach	Pikeperch	
LC_{10}	–	–	LC_{10}	–	–	LC_{10}
LC_{20}	0.052	–	LC_{20}	0.052	–	LC_{20}
LC_{30}	0.162	0.247	LC_{30}	0.162	0.247	LC_{30}
LC_{40}	0.255	0.512	LC_{40}	0.255	0.512	LC_{40}
LC_{50}	0.342	0.760	LC_{50}	0.342	0.760	LC_{50}
LC_{60}	0.430	1.008	LC_{60}	0.430	1.008	LC_{60}
LC_{70}	0.523	1.273	LC_{70}	0.523	1.273	LC_{70}
LC_{80}	0.632	1.584	LC_{80}	0.632	1.584	LC_{80}
LC_{90}	0.784	2.015	LC_{90}	0.784	2.015	LC_{90}
LC_{95}	0.909	2.370	LC_{95}	0.909	2.370	LC_{95}

DISCUSSION

Acute and chronic toxicity tests are widely used to evaluate the toxicity of chemicals on non-target organisms (Yalsuyi and Vajargah, 2017). Exposure time is one of the effective factors in toxic ratios (Vajargah and Hedayati, 2014). When fish are exposed to a constant concentration of the toxin, fish tolerance is diminished over time and the toxin has a greater effect (Hedayati et al., 2014). However, when the toxin accumulates in fish tissue it increases the adverse effects on the body and causes a decrease in LC_{50} 96 h. Overall LC_{50} for butachlor in Caspian roach and Pikeperch showed a decreasing trend over 96 hours and in listed physicochemical conditions. Result of LC_{50} 96 h for toxin showed that the rate of LC_{50} decreases with increasing toxin concentration and duration of exposure. The results of the acute toxicity of diazinon and Deltamethrin on *Cyprinus carpio* (common carp) concedes shows decreasing trend in LC_{50} 96 h (Svoboda et al., 2001, 2003).

Herbicides can influence aquatic organisms directly and indirectly. Indirect effects are mediated by herbicide-induced changes in food webs or in the physical environment. Indirect effects can only occur if direct effects occur first and would be mediated by the killing of plants by herbicides (Solomon et al., 2013). Results are limited on acute toxicity of butachlor in fish. The influence of butachlor on fish toxicity was evident. Chang et al. (2013) studied the effects of butachlor on reproduction and hormone levels in adult zebra fish (*Danio rerio*). They declared that butachlor adversely affected the normal reproductive success of zebra fish and disrupted the thyroid and sex steroid endocrine systems. Ateeq et al. (2002, 2006) stated that butachlor causes anisochromasia and anisocytosis of erythrocytes in *Clarias batrachus*. They reported that this herbicide is genotoxic and cytotoxic and is able to induce apoptosis both at molecular as well as cytological level. Lasheidani et al. (2008) reported that butachlor has adverse effect on density and volume of Caspian Kutum *Rutilus frisii kutum*. They stated that the number of abnormal sperm increased by 28.6 ± 1.92% in fish exposed to high butachlor toxicity (75% of its LC_{50} value).

Previous studies demonstrated LC_{50} values of butachlor for *Heteropneustus fossilis* (Ateeq et al., 2006) and *Channa punctatus* (Tilak et al., 2007) 2.34 ppm and 247.46 ppb, respectively. Also Geng et al. (2005) determined the LC_{50} 96 h values of butachlor in *Rana japonica* 1.40 mg/l while Gobic and Gunasekaran (2010) obtained 96 h LC_{50} of 0.515 mg/l for *Eisenia fietida*.

Dissolved oxygen, pH, size and age, type of species, water quality, concentration and formulation of test chemicals are the major factors in affecting toxicity of chemicals in aquatic organisms (Nwani et al., 2010; Vajargah et al., 2013). The safe level obtained for butachlor in the present study varied from 0 to 0.157 mg/l concentration for *Rutilus rutilus caspicus* and *Sander lucioperca* in 68% BOD, pH 8.28, 17 ± 1°C temperature and 210 ppm hardness. However these values are determined in vivo condition.

Herbicides are rapidly absorbed in fish gills which cause respiratory limitations (Masud and Singh, 2013). Fish that were exposed to butachlor had respiratory disorders which quickly opened and closed their gill cover. Fish were anxious, had a harmonic breathing and unusual semi-circular swimming. These findings coincide with studied acute toxicity of butachlor in other fish species, such as *Tilapia zillii* (Nwani et al., 2013) and *Oreochromis niloticus* (Bekeh et al., 2011).

According to the results and Toxicity category rating inhalation LC_{50}, butachlor for *Rutilus rutilus caspicus* and *Sander lucioperca* are lying in toxicity category II: is Moderately toxic and Moderately irritating (Toxicity Category, 2009). Dou to the vicinity of these two species location to farmland and orchards, further studies should be conducted on acceptable level of this herbicide and the usage must be restricted to avoid the severe risk associated with the use of the pesticide.

CONCLUSIONS

Few surveys have focused on the effects of butachlor on mortalities indices of various fish species, the current investigation examined LC_{50} of this herbicide on Caspian roach and Pikeperch. Since the species is edible, infections in turn affect human health. Furthermore, the results show that butachlor is too poisonous for these edible fishes and also Caspian roach is more sensitive than pikeperch.

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ALIEN SPECIES OF EU CONCERN IN ROMANIA

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ABSTRACT

Of the 37 species of the European Union concern eight are already present and two present a future potential risk for Romania. This paper brings updated information regarding these species in Romania. The presence of eight invasive alien species of concern to the European Union have already been recorded in Romania: two plant species *Cabomba caroliniana* and *Heracleum sosnowskyi*, two crustaceans *Orconectes limosus* and *Eriocheir sinensis*, two fish species *Pseudorasbora parva* and *Perccottus glenii*, one reptile *Trachemys scripta* and one mammal *Myocastor coypus*. Other two species of Union concern (*Lithobates catesbeianus* and *Procyon lotor*) may soon become invaders in Romania. We emphasize the urgent need to assess their current distribution and impact or potential to establish and possible impact at national level.

RÉSUMÉ: Espèces exotiques préoccupantes pour l'Union Européenne en Roumanie.

Parmis les 37 espèces préoccupantes pour l'Union Européenne, huit sont déjà présentes et deux autres présentent un futur risque potentiel pour la Roumanie. Cet article apporte des informations régulièrement mise à jour sur ces espèces en Roumanie. La présence de huit espèces exotiques envahissantes préoccupantes pour l'Union a été déjà notée pour la Roumanie: deux espèces végétales *Cabomba caroliniana* et *Heracleum sosnowskyi*, deux crustacés *Orconectes limosus* et *Eriocheir sinensis*, deux espèces de poissons *Pseudorasbora parva* et *Perccottus glenii*, un reptile *Trachemys scripta* et un mammifère *Myocastor coypus*. Deux autres espèces préoccupantes pour l'Union (*Lithobates catesbeianus* et *Procyon lotor*) deviendront bientôt des espèces envahissantes en Roumanie. Nous insistons sur le besoin urgent d'évaluer au niveau national leur distribution et leur impact présent ou leur capacité à s'installer et leur impact possible.

REZUMAT: Specii alogene de interes pentru Uniunea Europeană.

Dintre cele 37 specii care preocupă Uniunea Europeană, opt sunt deja prezente și două prezintă un viitor risc potențial pentru România. Prezentul articol aduce informații actualizate cu privire la aceste specii în România. Prezența a opt specii invazive alogene de interes pentru Uniune a fost deja înregistrată în România: două specii de plante *Cabomba caroliniana* și *Heracleum sosnowskyi*, două specii de crustacee *Orconectes limosus* și *Eriocheir sinensis*, două specii de pești *Pseudorasbora parva* și *Perccottus glenii*, o specie de reptile *Trachemys scripta* și o specie de mamifere *Myocastor coypus*. Două alte specii de interes pentru UE (*Lithobates catesbeianus* și *Procyon lotor*) vor deveni în curând specii invazive în România. Insistăm asupra nevoii urgente de evaluare a distribuției și impactului acestora în prezent sau a potențialului de a se stabili și a posibilului impact la nivel național.

INTRODUCTION

Any regional flora and fauna goes through continuous changes yet these changes are, most of the time, difficult to perceive during a human lifetime. Some natural phenomena such as volcanic eruptions, glaciations, meteorite impacts, etc., are accelerating the flora and fauna turn-over. After man's expansion on Earth, the changes in the structure of the flora and fauna occurred at an increasing pace, locally, regionally, and globally. The profound changes of the last decades under the influence of the industrialization and respectively of globalization include also a larger and increasingly worrying "fluidity" in the reduction, disappearance and introduction of species in equally more extensive biogeographical areas. Alien species and damage or loss of natural habitats are the main factors responsible for the disappearance of some species in past centuries. (Strahm and Rietbergen, 2001) Biodiversity conservation elements should include alien species assessment, monitoring, and management elements (Curtean-Bănăduc, 2006). Aquatic ecosystems, especially those already disturbed by various human activities, appear to be particularly vulnerable to these invasions (Lodge et al., 1998).

In July 2016, the EU adopted a list of 37 invasive alien species that are subject to the restrictions and measures set out in the EU Regulation 1143/2014. The list comprises 23 animals (six species of Crustacea, one insect, two fishes from Actinopterygii class, one amphibian, one reptile, three birds, nine mammals) and 14 plant species (Tab. 1) (European Comission, 2016).

As such, all member states are required to implement cost-effective measures to eradicate these species.

There are several major problems regarding the impact of alien species on biodiversity conservation and management at national level. Thus Romanian legislation on alien species (Ministerial Order 979/2009) does not include lists of alien species and refers to the DAISIE list. Also, the National Biodiversity Strategy and Action Plan 2014-2020 (https://www.cbd.int/doc/world/ro/ro-nbsap-v3-en.pdf) were not ratified by authorities, and the European Biodiversity Strategy 2020 is not implemented at the national level. To comply with the implementation of the EU Regulation 1143/2014, it is necessary to gather information regarding the presence of species of interest, and evaluate their introduction pathways, distribution, and invasive status. Difficulties are encountered in such initiatives: there is limited data available in the scientific literature, inconsistencies and errors in the available databases (e.g., CABI, DAISIE, NOBANIS), and lack of national databases and/or public information regarding alien species. Of the 37 invasive alien species of EU concern, 29 species were not yet reported in Romania, and also their invasion risk was not evaluated.

There is a scarcity of data regarding alien species where apart from the first recording in the country, detailed and updated distribution maps and surveys are missing. The goal of the present paper is to illustrate an update on the distribution and known impact of the alien species of EU concern present in Romania.

Table 1: List of invasive alien species considered of European Union concern according to Commission Implementing Regulation (EU) 2016/1141.

Kingdom	Class	Species
Plantae	Magnoliatae	*Baccharis halimifolia* L.
Plantae	Magnoliatae	*Cabomba caroliniana* Gray
Animalia	Mammalia	*Callosciurus erythraeus* Pallas, 1779
Animalia	Aves	*Corvus splendens* Viellot, 1817
Plantae	Liliatae	*Eichhornia crassipes* (Martius) Solms
Animalia	Crustacea	*Eriocheir sinensis* H. Milne Edwards, 1854
Plantae	Magnoliatae	*Heracleum persicum* Fischer
Plantae	Magnoliatae	*Heracleum sosnowskyi* Mandenova
Animalia	Mammalia	*Herpestes javanicus* É. Geoffroy Saint-Hilaire, 1818
Plantae	Magnoliatae	*Hydrocotyle ranunculoides* L. f.
Plantae	Liliatae	*Lagarosiphon major* (Ridley) Moss
Animalia	Amphibia	*Lithobates* (*Rana*) *catesbeianus* Shaw, 1802
Plantae	Magnoliatae	*Ludwigia grandiflora* (Michx.) Greuter and Burdet
Plantae	Magnoliatae	*Ludwigia peploides* (Kunth) P. H. Raven
Plantae	Liliatae	*Lysichiton americanus* Hultén and St. John
Animalia	Mammalia	*Muntiacus reevesi* Ogilby, 1839
Animalia	Mammalia	*Myocastor coypus* Molina, 1782
Plantae	Magnoliatae	*Myriophyllum aquaticum* (Vell.) Verdc.
Animalia	Mammalia	*Nasua nasua* Linnaeus, 1766
Animalia	Crustacea	*Orconectes limosus* Rafinesque, 1817
Animalia	Crustacea	*Orconectes virilis* Hagen, 1870
Animalia	Aves	*Oxyura jamaicensis* Gmelin, 1789
Animalia	Crustacea	*Pacifastacus leniusculus* Dana, 1852
Plantae	Magnoliatae	*Parthenium hysterophorus* L.
Animalia	Actinopterygii	*Perccottus glenii* Dybowski, 1877
Plantae	Magnoliatae	*Persicaria perfoliata* (L.) H. Gross (*Polygonum perfoliatum* L.)
Animalia	Crustacea	*Procambarus clarkii* Girard, 1852
Animalia	Crustacea	*Procambarus fallax* (Hagen, 1870) f. *virginalis*
Animalia	Mammalia	*Procyon lotor* Linnaeus, 1758
Animalia	Actinopterygii	*Pseudorasbora parva* Temminck and Schlegel, 1846
Plantae	Magnoliatae	*Pueraria montana* (Lour.) Merr. (Willd.) *Pueraria lobata* (Willd.)
Animalia	Mammalia	*Sciurus carolinensis* Gmelin, 1788
Animalia	Mammalia	*Sciurus niger* Linnaeus, 1758
Animalia	Mammalia	*Tamias sibiricus* Laxmann, 1769
Animalia	Aves	*Threskiornis aethiopicus* Latham, 1790
Animalia	Reptilia	*Trachemys scripta* Schoepff, 1792
Animalia	Insecta	*Vespa velutina nigrithorax* de Buysson, 1905

MATERIAL AND METHODS

Information regarding distribution and potential impact of the target species was collected from many sources. The authors added to their personal field data, information based on reliable personal communications, literature and databases (CABI ISC, DAISIE, ESENIAS, GISD), questionnaires for game species managers, surveys of pet-shops and field surveys, etc. The data about the distribution and introduction pathways were collected from many reference data. The invasive status was considered (high, medium, low) based on expert opinion.

RESULTS AND DISCUSSION

The 37 invasive alien plant and animal species of EU concern are distributed among the following higher taxa: three Liliatae, 11 Magnoliatae, six Crustacea, one Insecta, two Actinopterygii, one Amphibia, one Reptilia, three Aves, and nine Mammalia.

Plants

In regards to alien plant species, 14 are included on the list of EU concern, but only two have so far been found in Romania: *Cabomba caroliniana* and *Heracleum sosnowskyi* (Anastasiu and Negrean, 2009; Sîrbu and Oprea, 2011). Both are considered naturalized. One location has been reported for both species, but the data are very old and need to be updated.

Cabomba caroliniana A. Gray (family Cabombaceae) (Carolina water-shield) originates from America. In Romania it was acclimatized in the Ochiul Ţiganilor Wetland and Pârâul Peţea near Băile 1 Mai (Bihor County) in 1950 (Ţopa, 1955). This is the single presence point of this plant known in Romania. Ţopa (1955) specifies that it is a beautiful plant and can be easily reproduced by cuttings, and is recommended for freshwater aquariums. The plant has been recently reported as naturalized in Romania (Lansdown et al., 2016), without any further information being provided about its distribution. Its invasion risk in Romania is medium.

The species is not included in Flora Europaea, even though the data reported from Romania preceded the publication of the two editions of Flora Europaea. Uotila (2009) indicates it as a foreign species only in Great Britain (Sîrbu and Oprea, 2011). According to the file available at www.cabi.org, *Cabomba carolianiana* is present only in the following European states: Belgium, France, Greece, Hungary, the Netherlands, Serbia, United Kingdom (England, Scotland, and Wales).

The second plant species of interest for this paper is Sosnowskyi's hogweed (*Heracleum sosnowskyi* Manden., Apiaceae).

According to the file available at www.cabi.org, *Heracleum sosnowskyi* is present in the following European states: Belarus, Denmark, Estonia, Finland, Germany, Hungary, Iceland, Latvia, Lithuania, Poland, Russia, Ukraine. Romania is not featured on this list, although the plant was reported as naturalized in 2001 (Maruşca and Pop, 2001) and is included on the list of neophytes in Romania (Anastasiu and Negrean, 2009). According to Maruşca and Pop (2001), the species was brought to Romania from Poland in 1975, during a presidential visit, whereby it was presented as high quality fodder. Initially cultivated at Fundulea (Călăraşi County), in 1980 it was transferred to Prejmer (Braşov County). The plant did not survive at Fundulea, but it did survive at Prejmer, although in the first years it was small and it did not show any tendency to expand. Twenty years after its introduction in Braşov County, *Heracleum sosnowskyi* was found approximately 300 meters away from the place where it was first sown, nearby Halta Ilieni, with over 900 individuals on a surface of around 750 m^2 (Maruşca and Pop, 2001). The two authors consider that *Heracleum sosnowskyi* is "an invasive and dominant species" which forms a new vegetal association, *Cirsio (oleracei) – Heraclietum*. According to Maruşca and Pop (2001), "the dominant species – *Heracleum* *sosnowskyi*, eliminated almost all the other species. Considering its continuing expansion, this species could represent a real danger for zonal biodiversity as well as for a large area biodiversity.” Nevertheless, subsequent data regarding the expansion and impact of this species have not been published. The invasion risk of *H. sosnowskzi* in Romania is high.

In regards to *Eichhornia crassipes*, another species of interest for the European Union, the species is not mentioned in older papers, but it is recorded as casual in a more recent paper (Lansdown et al., 2016). It constantly enters the country due to horticulture trade.

Invertebrates

The List of EU concerns contains seven invertebrate species, of which six are aquatic, represented by crustaceans, and one is a terrestrial insect. The presence of two of these invertebrate species, namely *Orconectes limosus* and *Eriocheir sinensis*, have previously been recorded in Romania in natural and semi-natural habitats. However, their status and current distribution need to be updated, and the potential impact of the species should also be assessed.

The spiny-cheek crayfish, *Orconectes limosus*, is native to North America and was intentionally introduced in Europe in the late 19th century possibly to compensate for the decline of the native noble crayfish *Astacus astacus* (L.) populations (Holdich, 2002; Holdich and Black, 2007). The species is currently widespread in Europe, expanding its range naturally as well as through human-mediated dispersion. In Romania, *O. limosus* was first recorded in spring 2008 on the shore of the Danube in an area included in the Iron Gates Natural Park (located in SW Romania) and is quickly spreading downstream at an estimated rate of 13-16 km yr^{-1} (Pârvulescu et al., 2009). *O. limosus* competes with native species for resources and it may have an impact on the structure of the invaded habitats (Gherardi, 2007). But it is also involved in the transmission of the “crayfish plague” to native species, a disease caused by infection with the oomycete *Aphanomyces astaci* that is considered a major contributor to the decline of European freshwater crayfish (Schrimpf et al., 2012). The invasion risk of *O. limosus* was evaluated as high.

Following the accidental introduction in Germany in 1912 from its native range in eastern Asia, the Chinese mitten crab, *Eriocheir sinensis*, has spread throughout Europe (Herborg et al., 2003). The presence of *E. sinensis* was first recorded in Romania in the late 90’s (Gomoiu and Skolka, 1998) and subsequently in the Danube Delta (Oțel, 2004; Micu and Micu, 2006). The species is probably found along the entire lower sector of the Danube, as it has been observed in several locations in Serbia (Paunovic et al., 2004; Škraba et al., 2013) and Bulgaria (Kutsarov and Trichkova, 2016). However, the Chinese mitten crab is a catadromous species. The adults migrate to brackish or salt waters to reproduce while the juvenile crabs invade estuaries during their migration upstream and they can travel great distances (i.e. hundreds of km). According to Herborg et al. (2003), the average total distance of upstream migration reached 562 km/year during the peak period 1928-1939 for Northern Europe and 104 km/year for Southern France (1954-1960). The Chinese mitten crabs are omnivorous and may negatively affect native communities through e.g. competition, predation, and nutrient cycling. Rudnick and Resh (2005) suggest that *E. sinensis* feeding habits could influence shifts in the composition of the invertebrate communities towards deeper sediment-dwelling species and that the crabs have an impact on nutrient dynamics as they export biomass out of the freshwater ecosystems when migrating for reproduction. The Chinese mitten crabs can cause riverbank erosion through their burrowing activities. Recently, the Chinese mitten crabs have been identified as vectors of the crayfish plague pathogen *Aphanomyces astaci* (Schrimpf et al., 2014). The invasion risk of *E. sinensis* is high.

The other five invertebrate species included in the List of Union concern have not been recorded in Romania yet, but their arrival might be just a matter of time. The red swamp crayfish, *Procambarus clarckii*, and the signal crayfish, *Pacifastacus leniusculus*, are known to occur in several European countries while the virile crayfish, *Orconectes virilis*, and the marbled crayfish, *Procambarus fallax*, have a more restricted distribution (Kouba et al., 2014; Loureiro et al., 2015). Nevertheless, *P. leniusculus* has been observed in Hungary since the 2000's (Puky et al., 2005) and further expansion coupled with intentional releases or escapees from the aquarium/aquaculture trade increases the chances that various alien crayfish establish in natural and semi-natural habitats. Their impact on native biota and ecosystems can occur through a wide range of mechanisms, like in the case of the red swamp crayfish (Souty-Grosset et al., 2016).

The Asian yellow-legged hornet, *Vespa velutina*, is the only insect currently on the list. In 2004, the subspecies *nigrithorax* was recorded in south-western France (Haxaire et al., 2006) and subsequently spread to other European countries, including Italy (Bertolino et al., 2016). Recent studies suggest that *V. velutina* could spread over a large part of Europe, and that climate change increases the risk of invasion (Rome et al., 2011; Barbet-Massin et al., 2013). As a predator of other insects, particularly the honey bee, the presence of *V. velutina* in Europe causes concern related to its potential impact on bee colonies and pollination, beekeeping, and human health (de Haro et al., 2010; Monceau et al., 2013; Arca et al., 2014). We consider monitoring actions and awareness campaigns mandatory for the early detection of these and other alien species in order to prevent their spread and impact at lowest costs.

Amphibians and reptiles

The American Bullfrog (*Lithobates catesbeianus*) was introduced in several western European countries (France, Italy, Belgium, United Kingdom, Spain) (Stumpel, 1992), but is still scarcely distributed in Eastern Europe: it was only reported from the island of Krete (Ficetola et al., 2007a) and recently from Slovenia (Kirbiš et al., 2016), although the region overall has a high suitability for the species (Ficetola et al., 2007b). There are no distribution records from Romania or its neighbouring countries, although there is a risk of introduction by farming or the pet trade.

The Common Slider, *Trachemys scripta*, is the most widespread alien species in the region, and reports of successful breeding populations are available from several European countries including: Italy (Crescente et al., 2014), Serbia (Đorđević and Anđelković, 2015), Slovenia (Vamberger et al., 2012), Croatia (Jelić et al., 2016), and southern Turkey (Çiçek and Ayaz, 2015). Juveniles are still traded in pet-shops. The presence of this species in Romania was reported only from ponds and lakes within urban areas or their vicinities. Due to its longevity, it can achieve high population densities (Ficetola et al., 2012). It can compete with the native terrapin (*Emys orbicularis*) for resources, can transmit parasites and pathogens, and is a predator of native freshwater fauna. The invasion risk of *T. scripta* is evaluated as medium.

Fish

Among the vertebrates, the freshwater fish species have the largest share in the accidental and by-purpose introductions of alien species. In most of these cases, a negative direct and indirect impact was registered on the native species. In Europe, there are about 40 introduced fish species, and many more were translocated from some other European countries. In most cases a foreign species of fish will not be limited to the basin of initial entry; most often that species will spread into an increasingly expanded territory (Holčik, 1991).

Among the fish species listed by the EU in this study context, *Pseudorasbora parva* is widespread in Romania in the last decades, and *Perccottus glenii* is in a constant trend of increasing its distribution. The potential impact of both of these species should be assessed.

Pseudorasbora parva Temminck and Schlegel, 1846 (Actinopterygii, Cypriniformes, Cyprinidae, Gobininae) it is a freshwater, benthopelagic, small-sized fish (7-12 cm), most abundantly found in well vegetated small channels and ponds and lakes but also in running water, maximum reported age five years fish species with a relatively large distribution: Amur to Zhujiang drainages in Siberia, Korea and China. Introduced in various areas in Asia and Europe, several countries reported adverse ecological impact after introduction. It feeds on small insects, fish and fish eggs, and usually breeds in habitats with still or very slow-flowing water three-four times in a season. (Bănărescu, 1964; Bănărescu and Nalbant, 1965, 1973; Welcomme, 1988; Bănărescu, 1990; Novikov et al., 2002; Bănăduc and Bănăduc, 2008; Verreycken et al., 2011) The invasion risk of *P. parva* was evaluated as high.

P. parva, was accidentally introduced from Yang-Tze Watershed from China in Romania (Nucet, Dâmboviţa and Cefa, Bihor piscicultural stations for aquaculture) in 1960-1962, together with the Chinese cyprinids with economic value (*Ctenopharyngodon idella* Cuvier and Valenciennes, 1848, *Hypophthalmichthys molitrix* Cuvier and Valenciennes, 1848, *Aristichthys nobilis* (Richardson, 1844), *Mylopharyngodon piceus* (Richardson, 1846), *Parabramis pekinensis* (Basilewsky, 1855), *Megalobrama terminalis* (Richardson, 1846)) (Witkowski, 2009)

P. parva is a species with a high dispersion potential, which succeeded in spreading out in almost all the countries of Europe during the 45 years that passed from its admission into this continent. There were several centers in Europe, out of which the *P. parva* then spread out on almost the entire continent. The two major centers were Romania (from where the species naturally spread out in the whole Danube Basin) and Albania (from where the species spread out in the Balkans, still naturally). In the countries of the former Yougoslavia, the species penetrated from both centers; in Hungary, Slovakia and the Czech Republic, the species penetrated naturally, and from Romania, it penetrated artificially, as it was brought straight from China together with some other species of fish of economic interest. In Poland and Northern Bulgaria, the species was seemingly brought from the Ukraine. The origin of the populations in Italy and France is unknown, but these populations probably come from the Danube Basin. We assume that the species arrived in Denmark from Germany. We do not know how the species got on England’s and Spain’s territory, but it was most likely artificially introduced from a European country. (Gavriloaie, 2007)

In Romania and surrounding countries *P. parva* is living already in the Danube River and all the Romanian hydrographic basins: Tisa, Someş, Crişuri, Mureş, Bega, Timiş, Caraş, Miniş, northern Danube Iron Gorge tributaries, Cerna, Jiu, Olt, Vedea, Argeş, Ialomiţa, Mostiştea, Călmăţui, Siret, Prut, and in some of the near Black Sea Dobrogea region water bodies (Giurcă and Angelescu, 1971; Bănăduc, 1999, 2005, 2013; Schiemer et al., 2004; Battes et al., 2005; Oţel, 2007; Hartel et al., 2007; Costiniuc et al., 2006; Moşu et al., 2006; Năvodaru and Năstase, 2006; Vornicu et al., 2006; Ardelean and Wilhelm, 2007; Telcean and Cupşa, 2009; Goia et al., 2014; Ureche and Ureche, 2015; Bănăduc et al., 2016; Takács et al., 2017).

The appreciable dispersal of *P. parva* on the Romanian hydrographical basins, after its accidental introduction, was due to escapes from piscicultural basins and in their adjacent channels and streams and rivers, and its use as living bait.

It thrives in piscicultural and in the natural areas in some lakes and small hill- and plain- rivers, but is also present in large rivers and even lakes. The polluted areas are included in its normal range of distribution (Gavriloaie and Chiş, 2006).

In the background of the intensive trade of Chinese carp species in 1960s, the invasive characteristics of this accidental introduced fish species with a high plasticity and adaptability to lentic and lotic conditions were proved extensively through natural dispersal in the Romanian hydrographical net. The potential management actions are severely limited to early detection and rapid intervention.

A climatic and human impact associated model combined with an introduction pathways analysis could enable accurate prediction on the risk of spread of this species areal in the higher altitude Romanian water bodies, allowing for robust monitoring and fast intervention management actions.

Perccottus glenii Dybowski, 1877 (Actinopterygii, Perciformes, Odontobutidae) is a freshwater, brackish, demersal, fish species that occurs in lentic waters, lakes, ponds, backwaters and marshes with dense underwater vegetation. It avoids lotic sectors with currents, can tolerate poorly oxygenated water, and is able to survive in dried out or completely frozen water bodies by digging itself into mud where it hibernates. Maximum reported age is seven years for this fish species, and it has a large distribution. The Sea of Okhotsk and Amur southward to Yangtze and Fujian is included in its distribution. It was introduced in Europe, and at least one country reports adverse ecological impact after introduction. It is a voracious predatory fish, feeds on invertebrates, tadpoles and fish, posing a most serious threat to aquatic fauna wherever it occurs, in small water bodies known to extirpate almost all other fish species and amphibian larvae. Reproduction starts for the first time at one-three years, with males guarding the eggs and pelagic larvae (Berg, 1965; Novikov et al., 2002; Koščo et al., 2008; Kati et al., 2015). The invasion risk of *P. parva* was evaluated as moderate to high.

P. glenii was introduced in Russia, near Sankt Petersburg at the beginning of the 20th century, and only later, during the past two decades, it started spreading to the west of Europe. Because of its high resistance to extreme environmental conditions and due to the economic loss caused in the fishing ponds, this species became a real threat for the freshwater ecosystems in Asia and Europe. (Luca and Ghiorghiţă, 2014)

Perccottus glenii appeared recently and spread in different parts of the Romanian and neighboring countries' watersheds like: Danube Delta, Danube River, Mureş River basin, Siret Basin, Suceava Basin, Crişuri rivers basin and Timiş River basin (Nalbant et al., 2004; Jurajda et al., 2006; Simonović et al., 2006; Popa et al., 2006; Moşu, 2007; Năstase, 2007; Copilaş-Ciocianu and Pârvulescu, 2011; Covaciu-Marcov et al., 2011; Kvach, 2012; Luca et al., 2014; Bănăduc et al., 2016; Telcean and Cicort-Lucaciu, 2016; Takács et al., 2017).

Birds

None of the alien bird species of Union concern have been reported in Romania, but their presence should be carefully monitored due to the rapid expansion of their range.

Mammals

Nine mammalian alien species are of Union concern, of which only *Myocastor coypus* is present in Romania. *Procyon lotor* might soon become an invader as it is already present in neighbouring countries.

Myocastor coypus, is a large, robust and heavy rat-like rodent, with an average body weight of five-six kg, with males bigger than females reaching up to 10 kg (Bertolino et al., 2012). Coypus are mostly nocturnal, inhabiting aquatic habitats where they feed on vegetation

(Wood et al., 1992). Originating from southern South America, it was introduced in Europe for the fur trade, starting in the 1880's in France (Carter and Leonard, 2002). It is now established in Europe. It was first reported in Romania in 1959 (Murariu and Chișamera, 2004). It has an impact on bank stability due to its burrows; it can impact aquatic vegetation through overgrazing and can prey on the water birds nests (Woods et al., 1992; Angelici et al., 2012). The *M. coypus* presents a high invasion risk for Romania.

A climatic and human impact associated model combined with an introduction pathways analysis could enable accurate prediction on the risk of more extention of this fish species areal in the unaffected by now of Romanian water bodies, allowing for robust monitoring and fast intervention management actions.

CONCLUSIONS

Are the identified data up to date? Is their updating necessary before setting the management measures for these particular species? What are the entry paths? What is the invasiveness status in our country?

The data available so far are not sufficient for developing an adequate management plan for the control, containment, and eradication of these species. A national program to monitor the distribution and impact of these species is required, together with the development of a rapid response and information network of the countries in the region. The pet and horticultural trades require strict regulations regarding the import of species posing high risk of invasiveness, since the most unregulated activities are the pet trade and the horticulture trade.

The studied alien species of interest for the European Union present in Romania were partially reported, the most of them with medium to high invasion risk, yet their present distribution is not known.

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