## TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

# 22.1

The Wetlands Diversity

**Editors** 

Doru Bănăduc & Angela Curtean-Bănăduc

Sibiu – Romania 2020

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*"Lucian Blaga*" University of Sibiu, Faculty of Sciences, Department of Ecology and Environment Protection

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#### **IN MEMORIAM**

#### *Herbert Spencer* (1820 – 1903)

*Herbert Spencer* was an English biologist, anthropologist, sociologist, philosopher, and distinguished classical liberal political thinker of the Victorian period.

He was born in Derby, England, on the 27<sup>th</sup> of April 1820. Spencer's father introduced him to science while the members of the Derby Philosophical Society introduced him to pre-Darwinian ideas of biological evolution, with emphasis on work done by Erasmus Darwin and Jean-Baptiste Lamarck. One of Spencer's uncles taught him mathematics, physics, and Latin but in general he was an autodidact that collected most of his wisdom from readings and conversations.

*Spencer* found it hard to settle on any scholarly or professional area. He worked as an engineer, as an author, and editor for different journals.

He advanced the exhaustive idea of evolution as the continuous progress of the physical world, biological organisms, the human mind, and culture. As a gifted person, he contributed to a large range of subjects: ethics, religion, anthropology, economics, political theory, philosophy, literature, astronomy, biology, sociology and psychology. Throughout his life Spencer achieved exceptional authority, mainly in the context of the academia.

He was one of the most illustrious European intellectuals in the closing decades of the nineteenth century, but his importance declined after 1900.

He presented his evolutionary viewpoint for the first time in his essay, "Progress: Its Law and Cause" (1857), which formed after the basis of the "First Principles of a New System of Philosophy" (1862). In it he explains the theory of evolution with a generalization of the law of embryological evolution. *Spencer* hypothesized that all structures in the cosmos evolve from an 'uncomplicated, undifferentiated, homogeneity to a complex, differentiated, heterogeneity, while being accompanied by a process of higher integration of the separate elements'. This evolutionary process could be identified at work, *Spencer* concluded, in all of the cosmos. It was a comprehensive law that was relevant to the stars and the galaxies as much as to biological organisms; to human social organisation as much as to the human mind. It contrasts from other scientific theories only by its higher generality.

*Spencer* is especially known for the remark "survival of the fittest", which he created in Principles of Biology (1864), after reading Charles Darwin's *On the Origin of Species*. This phrase firmly advocates for natural selection, and yet as *Spencer* extended the evolution concept into the realms of sociology and ethics, he also used the Lamarckism.

*Spencer*, in his book Principles of Biology (1864), proposed a pangenesis theory that included "physiological units" assumed to be analogous to specific body parts and responsible for the transmission of typical features to descendants. These hypothetical hereditary elements were very much alike to Darwin's gemmules.

Spencer's works were translated into many languages and he was offered honours and awards throughout Europe and North America.

He continued formulating questions all his life, trying to give answers, writing, in later years often by dictation, until he succumbed to poor health at the age of 83.

#### **The Editors**

2020

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#### Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand 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 surviving.

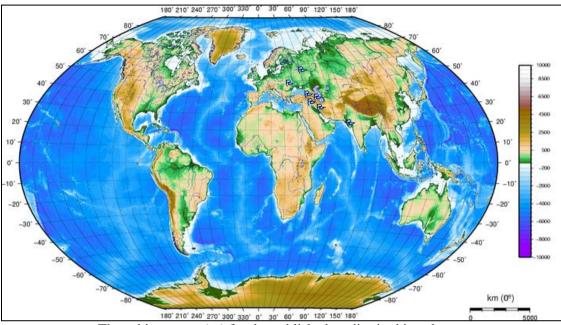
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

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 three annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2017.

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 Seasonal/intermittent saline/brackish/alkaline and Permanent lakes: lakes flats: 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 Excavations; ha); gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

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

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



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

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

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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.

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#### BIOINDICATION OF WATER QUALITY BY DIATOM ALGAE IN HIGH MOUNTAIN LAKES OF THE NATURAL PARK OF ARTABEL LAKES, (GÜMÜŞHANE, TURKEY)

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**KEYWORDS**: diatoms, bioindication, high mountain lakes, Artabel Lakes Nature Park, Turkey.

#### ABSTRACT

The first results of bioindicative analysis of water quality in the high-mountainous lakes of the Artabel Lakes Natural Park are presented using diatom species. A total of 95 diatom taxa collected in August 2013 and 2016 were identified and used as bioindicators for ten environmental variables. Bioindication, statistical methods and comparative floristic results show that the waters in all the lakes studied were fresh, low-saline, with circum-neutral pH and organically uncontaminated. The results of bioindication can be used as etalons for future monitoring of lakes in order to protect species found in the natural park, and can also be included in the national system of water quality standards in Turkey.

**ZUSAMMENFASSUNG**: Bioindikation der Wasserqualität durch Kieselalgen in Hochgebirgsseen des Naturparks Artabel-Seen (Gümüşhane, Türkei).

Die ersten Ergebnisse einer bioindikativen Analyse der Wasserqualität durch Bioindikation in den Hochgebirgsseen des Naturparks Artabel Seen werden anhand von Kieselalgenarten vorgestellt. Insgesamt 95 im August 2013 und 2016 gesammelte Diatomeentaxa wurden identifiziert und als Bioindikatoren für zehn Umweltvariablen verwendet. Bioindikation, statistische Methoden und vergleichende floristische Ergebnisse zeigen, dass es sich bei allen untersuchten Wasserkörpern um Süßwasserseen handelt, die salzarm, mit umlaufneutralem pH-Wert und organisch nicht kontaminiert waren. Die Ergebnisse der Bioindikation können als Grundlage für die künftige Überwachung von Seen verwendet werden, um die im Naturpark vorkommenden Arten zu schützen. Sie können auch in das nationale System der Wasserqualitätsnormen in der Türkei aufgenommen werden.

**REZUMAT**: Bioindicarea calității apei de către alge diatomee în lacurile de mare altitudine ale munților din Parcul Natural Lacurile Artabel (Gümüşhane, Turcia).

Primele rezultate ale analizei bioindicative a calității apei în lacurile de mare altitudine din Parcul Natural Lacurile Artabel sunt prezentate folosind specii de diatomee. Un total de 95 de taxoni de diatomee colectate în august 2013 și 2016 au fost identificate și utilizate ca bioindicatori pentru zece variabile de mediu. Bioindicarea, metodele statistice și rezultatele floristice comparative arată că apele din toate lacurile studiate au fost proaspete, slab saline, cu un pH neutru circumferențial și necontaminate organic. Rezultatele bioindicării pot fi folosite ca etaloane pentru monitorizarea viitoare a lacurilor pentru a proteja speciile găsite în parcul natural și pot fi, de asemenea, incluse în sistemul național de standarde de calitate a apei din Turcia.

#### **INTRODUCTION**

Turkey's high mountain lakes are mostly located in the eastern Black Sea region, and have a high-altitude glacier origin characterizing the land of the region. The algal diversity of high mountain lakes has an important role for the ecological assessment of water quality (Bellinger and Sigee, 2010). These ecosystems have an algal community which have adapted to extreme environmental conditions. Floristic studies in water are very important because the formation of floras are environmental indicators and does infers environmental conditions on the natural, climatic, and economic from the protected areas (Cianfaglione and Di Felice, 2012; Sender and Maślanko, 2017). The study of aquatic algae is of special interest since the formation of their floras occurs under conditions of water flowing from the catchment basin and thus represents an accumulative result of natural and anthropogenic conditions throughout the entire catchment area over many years (Barinova et al., 2006; Necchi, 2016).

One of the components of the aquatic ecosystem used in the ecological evaluation of water quality is algae (Bellinger and Sigee, 2010; Stevenson, 2014). Therefore, many algal species are used as environmental indicators (Paul, 2017). Usually, diatoms represent about one-half of the species richness in the well-studied middle-latitudinal regional algal floras of the Middle East and neighbouring regions (Barinova, 2011).

A bioindication approach has been implemented previously for assessment of lakes and rivers ecosystems in Turkey (Solak et al., 2012; Sivaci et al., 2013; Barinova and Sivaci, 2013; Barinova et al., 2014a, b).

This method yields productive results in the ecological assessment of water quality of other water bodies close to this region, such as those in Israel (Barinova, 2011; Barinova and Krassilov, 2012), Pakistan (Barinova et al., 2013; Barinova et al., 2016a), Kazakhstan (Barinova et al., 2009; Barinova et al., 2011; Jienbekov et al., 2018a, b), China (Barinova et al., 2016b), and India (Barinova et al., 2012). A possibility to develop bioindication methods for alpine lakes ecosystem assessment in a changing environment, like acidification and global warming, can be seen (Tolotti, 2001).

The present work aimed to reveal the diatom species-indicators in the Artabel Lakes Nature Park and to assess the water quality in these lakes with bioindication methods and statistical approach.

### MATERIAL AND METHODS

#### Study area

The Artabel Lakes Nature Park is located between latitude and longitude coordinates 40°21'36" – 40°26'42" North and 39°0'24" – 39°8'23" East. The area of Artabel Lakes Nature Park is 5,859 ha. (Fig. 1). The Artabel Lakes Nature Park has rich biodiversity and endemic species, especially of those included in the International Nature Conservation Union (IUCN) and the Berne Convention Annex I, II and III (Koruma and Müdürlüğü, 2013).

In the Artabel Lakes Nature Park (Fig. 1), there are three stream basins with five different lake sites including 23 lakes, which belong to Artabel Lakes (ARL) (Fig. 2), Acembol Lakes (ACL) (Fig. 3), Beş Lakes (BL), Kara Lakes (KL), and Yıldız Lakes (YL) in an area of approximately 58.2 km<sup>2</sup> (Koruma and Müdürlüğü, 2013). Also studied was a previously unnamed lake (Isimsiz Lake: IL) and a small pond (Yıldız Lakes Pond (YLP)). The Artabel lakes are glacial in origin, covered with ice layer at least eight months of the year and situated in the alpine zone (2,687-3,030 m. a.s.l.). It is not possible to fully (i.e., and seasonally) describe diatom flora because of the difficulties in sampling throughout the year.

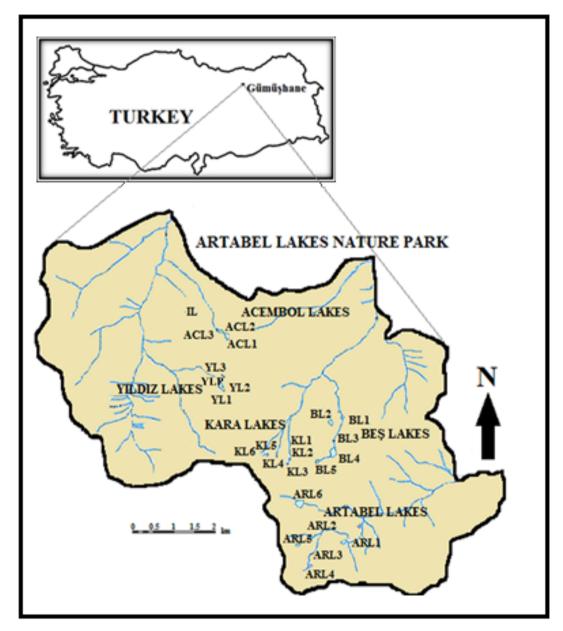


Figure 1: Schematic map of the studied area

(Artabel Lakes, Kara Lakes, Beş Lakes, Yıldız Lakes, Acembol Lakes, İsimsiz Lake and Yıldız Lake Pond) (Koruma and Müdürlüğü, 2013).



Figure 2: Artabel Lakes area characteristic habitat.



Figure 3: Acembol Lakes characteristic habitat.

#### Sampling and identification

The samples of diatoms were taken from 17 lakes and a pond in the Artabel Lakes Nature Park on 15 August 2013 and on 13 August 2016 (Fig. 1). The studied waters were situated between 2,687 and 2,980 m. a.s.l. (Koruma and Müdürlüğü, 2013). The portable devices Orion-4Star and YSI-55 were used to measure dissolved oxygen, water temperature, pH, and conductivity at each lake.

Algal samples were collected from epipelic, epilithic and epiphytic habitats. Samples were taken by glass tube (sediment), scrape (epilithic) and squeezed (epiphytic), placed in 100-ml plastic bottles, and fixed with 4% neutral formaldehyde solution. In the laboratory, thanks to the hydrogen peroxide method (Battarbee, 1986) the shells of the diatoms were cleaned and placed in Naphrax®. Diatoms were examined with the Leica DM 2500 light microscope and photographed with the Leica DFC 290 camera. Identification of the diatom species was made using the relevant handbooks (Hustedt, 1930; Huber-Pestalozzi, 1942; Patrick and Reimer, 1966; Krammer and Lange-Bertalot, 1986, 1988, 1991a, b; Joh, 2010, 2012; Genkal et al., 2008; Buczkó et al., 2013; Jovanovska et al., 2015) and their current scientific names were updated according to algaebase.org (Guiry and Guiry, 2018). The autecological data of the species were taken from our database (Barinova et al., 2006).

The species' frequency was assessed according to the six-score scale (Barinova et al., 2006; Barinova, 2017a): 1 = occasional, 1-5 cells per slide; 2 – rare, 10-15 cells per slide; 3 – common, 25-30 cells per slide; 4 – frequent, one cell over a slide transect; 5 – very frequent, several cells over a slide transect; 6 – abundant, one or more cells in each field of view. The list of the diatom species was combined with an ecological database in the Office Access (Microsoft). Statistica 12.0 program was used to construct the plots of the relationships between biological and environmental variables. The GRAPHS program was used to make diversity indices and comparative floristics (Novakovsky, 2004). The Shannon index (MacArthur, 1960; Rosenberg, 2010) is used in ecology exactly because it takes into account both components of diversity, abundance and equitability value of which are related to indices of saprobity, water quality and aquatic ecosystem state (Protasov et al., 2019). Pearson correlation coefficients were calculated with wessa.net (Wessa, 2018). CANOCO program was used for the calculation of biological and environmental variables relationships (Ter Braak and Šmilauer, 2002). The water quality class is defined according to the EU 5-Classes system based on the species indicators content (Barinova et al., 2006; Barinova, 2017b).

#### **RESULTS AND DISCUSSION** Environmental variables

Water environmental properties in the studied lakes are given in table 1. Lakes altitude varied between 2,668 and 3,015 m. a.s.l., water temperature was low and slightly increased with decreasing of altitude, and water pH was circumneutral and varied in a narrow range (Tab. 1). Water conductivity and total dissolved solids (TDS) are in the narrow range and fluctuated synchronously. Studied lakes water was sufficiently oxygenated, and the oxygen concentration was dependent mostly on the group of lakes rather than to altitude (Tab. 1).

Pearson correlation coefficients for each pair of the lake parameters were calculated, only water temperature had a weak but significant negative correlation with the height of the lake (-0.675, p-value = 0.0001). All other variables did not have a significant correlation.

95 diatom species have been identified from 43 algal samples collected in 17 lakes and one pond of the Artabel Lakes Nature Park. Bioindication properties of all revealed species were found (Tab. 2). Bioindicator species representation was analyzed in each water body.

Natural Park la	Natural Park lakes.									
Lakes	Altitude, m. a.s.l.	Temperature, C	$\mathrm{DO},\ \mathrm{mg}\mathrm{L}^{-1}$	Hq	$TDS, mg L^{-1}$	Conductivity, μSm cm <sup>-1</sup>	No. of species	Abundance, sum of scores	Index Saprobity S	Shannon Index
Artabel Lakes (ARL1)	2,687	17.5	8.71	6.19	16.00	32.6	20	51	0.80	2.93
Artabel Lakes (ARL2)	2,763	16.7	8.42	6.4	22.00	45.5	7	18	0.46	1.87
Artabel Lakes (ARL3)	2,875	16.3	8.76	6.19	24.00	49.9	17	40	0.75	2.74
Artabel Lakes (ARL4)	2,890	15.7	9.45	6.73	10.00	21.5	15	37	0.73	2.64
Artabel Lakes (ARL5)	2,930	12.4	8.95	7.02	13.00	26.2	32	83	1.04	3.40
Artabel Lakes (ARL6)	2,863	15.9	8.97	6.98	10.00	21.3	18	53	1.05	2.87
Beş Lakes (BL1)	2,831	13.9	9.14	7.06	10.00	21.4	21	51	1.01	2.96
Beş Lakes (BL2)	2,863	12.5	9.32	6.75	18.00	36.3	13	42	1.03	2.59
Beş Lakes (BL3)	2,915	10.1	9.20	7.01	16.00	33.1	25	67	1.18	3.18
Beş Lakes (BL4)	2,924	15.5	8.30	7.04	6.00	13.2	22	60	1.09	3.02
Beş Lakes (BL5)	3,015	-	-	-	-	-	1	1	1.30	-
Acembol Lakes (ACL1)	2,713	15.5	3.25	7.04	24.05	30.6	23	52	1.03	3.01
Acembol Lakes (ACL2)	2,712	15.3	2.1	7.09	30.55	38.5	29	74	1.11	3.30
Acembol Lakes (ACL3)	2,711	17.9	2.94	7.52	20.80	27.7	26	71	0.87	3.20
Yıldız Lakes (YL1)	2,980	14.6	2.27	6.85	16.25	19.7	17	51	0.98	2.80
Yıldız Lakes (YL2)	2980	11.5	2.88	6.89	26.66	30.7	28	69	0.86	3.24
Yıldız Lakes (YL3)	2,980	14.1	3.12	7.01	24.05	29.5	26	63	0.98	3.16
İsimsiz Lake (IL)	2,668	19.1	4.25	6.78	9.10	12	37	80	1.07	3.50
Yıldız Lakes Pond (YLP)	2,980	14.5	2.34	7.2	23.4	29.2	21	49	0.91	2.91

Table 1: Mean environmental and biological variables (2013, 2016) in the Artabel Natural Park lakes.

#### **Bioindication properties**

Bioindication properties of the revealed species were summarized for groups of the studied lakes according to altitude (Figs. 4-5).

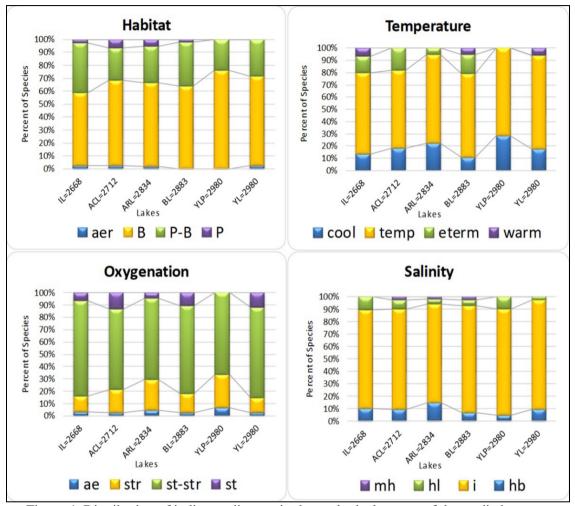


Figure 4: Distribution of indicator diatoms in the ecological groups of the studied waters in the Artabel Lakes Nature Park; abbreviations of ecological groups are as in table 2.

Figure 4 and table 2 show that while benthic species strongly prevailed in all examined lakes, plankto-benthic inhabitants also maintained a presence in all studied waters. Four groups of water temperature indicators were revealed in the studied lakes. Figure 4 shows an increase of cool water inhabitants and a decrease of eurythermic species with altitude. In the studied lakes, bioindication groups were related to standing water. Four groups were found related to oxygenation, water moving, and oxygen saturation. The low-streaming water indicators prevailed in all studied waters (Fig. 4). The results of the pH bioindication show that indifferent species are common in the Artabel Lakes Nature Park, except for Beş Lakes (BL). In Beş Lakes, indicator groups of alkaliphiles and alkalibiontes were dominant (Tab. 2). The acidophiles and alkalibiontes groups were also represented in all studied waters. As a whole,

indicator groups of indifference and alkaliphiles represented about 90% of indicator species in each lake community (Fig. 4). Bioindication of water salinity indicated that the indifferent group was dominant in all waters with decreasing of halophilic and mesohalophilic species with altitude (Fig. 4), which shows more freshwater properties with increasing altitude of the lakes. Salinity is the most important part of the total ion content in the water that affected the algal community. Light and temperature are the basic climatic variables affecting photosynthesis. According to Barinova et al. (2014c) these global climatic factors define life and evolution also.

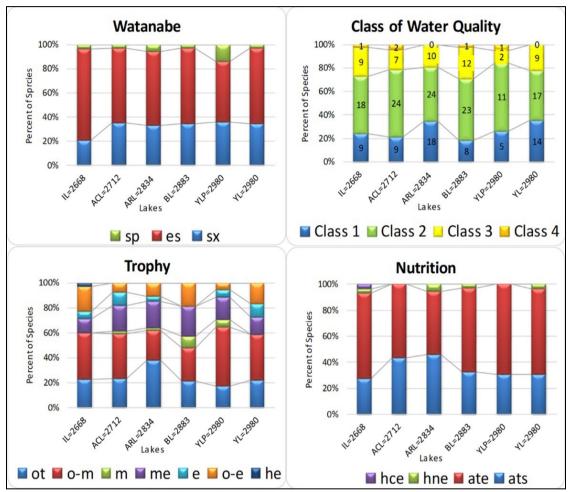


Figure 5: Distribution of indicator diatoms in the ecological groups of the studied waters in the Artabel Lakes Nature Park. The colours in the Class of Water Quality according Sládeček histogram are with number of each Class species-indicators in EU colour code; abbreviations of ecological groups are as in table 2.

Table 2: Diversity of diatom species-indicators in the studied waters in Artabel Lakes Nature Park with autecological characteristics and species-specific indices of saprobity. Substrate preferences (Hab): P - planktonic, P-B - plankto-benthic, B - benthic, aer aerophilic. Temperature preferences (T): temp – temperate temperature, eterm – eurythermic, warm - warm-water, cool - could water inhabitants. Oxygenation and streaming (Oxy): st standing water, str – streaming water, st-str – low streaming water, ae – aerophiles. Acidity degree (pH): alf - alkaliphiles, ind - indifferents; acf - acidophiles, alb - alkalibiontes. Halobity degree (Sal): I – oligohalobes-indifferent, hl – halophiles, hb – halophobes, mh – mesohalobes. Organic pollution indicators according to Watanabe (D): sx - saproxenes, es eurysaprobes, sp – saprophiles. Species-specific index of saprobity S (Sap). Trophic state (Tro): ot - oligotraphentic; o-m - oligo-mesotraphentic; m - mesotraphentic; me - mesoeutraphentic; e – eutraphentic; o-e – oligo-eutraphentic; he – hypereutraphentes. Nitrogen uptake metabolism (Aut-Het): ats – nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate - nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne – facultative nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; hce mixotrophes permanently in need of nitrogen load.

Таха	Hab	Т	Oxy	pН	Sal	D	Sap	S	Tro	Nutr
Achnanthes semiaperta Hustedt	В	_	_	_	_	_	0	1.00	_	_
Achnanthidium minutissimum (Kützing) Czarnecki	P-B	eterm	st-str	ind	i	es	x-b	0.95	о-е	ate
Amphora ovalis (Kützing) Kützing	В	temp	st-str	alf	1	SX	o-b	1.50	me	ate
Amphora pediculus (Kützing) Grunow	В	temp	st	alf	i	es	b-o	1.70	o-m	ate
Aulacoseira lacustris f. tenuior Houk, Klee and Passauer	Р	cool	Ι	acf	hb	Ι	Х-О	0.50	ot	_
Aulacoseira muzzanensis (F. Meister) Krammer	P-B	_	st-str	alf	hb	Ι	b	2.00	me	_
Aulacoseira subarctica (Otto Müller) E. Y. Haworth	Р	_	st-str	ind	i	_	b-o	1.70	0-m	ats

saproony.										
<i>Caloneis silicula</i> (Ehrenberg) Cleve	В	_	st	ind	i	sp	0	1.30	o-m	ats
<i>Chamaepinnularia</i> <i>hassiaca</i> (Krasske) Cantonati and Lange-Bertalot	В	_	str	acf	hb	es	0	1.00	ot	ats
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	P-B	temp	st-str	alf	i	SX	0	1.30	o-m	ate
Craticula hemiptera (Kützing) D. G. Mann	В	temp	st-str	alf	i	es	b-a	2.45	me	_
<i>Craticula</i> <i>halophilioides</i> (Hustedt) Lange-Bertalot	В	_	_	_	hl	es	_	_	_	_
Cyclotella ambigua Grunow	Р	_	-	alf	i	_	0	1.00	_	_
<i>Cymbella affinis</i> Kützing	В	temp	st-str	alf	i	SX	0	1.10	ot	ats
<i>Cymbella aspera</i> (Ehrenberg) Cleve	В	_	st-str	neu	i	es	Х	0.30	o-e	ats
<i>Cymbella cistula</i> (Ehrenberg) O. Kirchner	В	_	st-str	alf	i	SX	0	1.20	e	ats
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer	В	_	st-str	ind	i	es	0	1.20	o-m	ate
<i>Cymbopleura</i> subcuspidata (Krammer) Krammer	P-B	_	str	acf	i	_	0	1.00	ot	ats
<i>Diatoma vulgaris</i> Bory	P-B	_	st-str	ind	i	SX	b	2.20	me	ate
Didymosphenia hemiptera (Lyngbye) Mart. Schmidt	В	_	st-str	ind	i	SX	O-X	0.70	ot	_

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in Artabel Lakes Nature Park with autecological characteristics and species-specific indices of saprobity.

saproony.										
<i>Aulacoseira</i> <i>valida</i> (Grunow) Krammer	P-B	_	_	ind	i	es	0	1.30	o-m	_
<i>Caloneis</i> <i>alpestris</i> (Grunow) Cleve	В	_	str	alf	i	1	X	0.10	ot	ats
Diploneis oblongella (Nägeli ex Kützing) Cleve- Euler	В	_	str	ind	i	SX	x-b	0.90	ot	ats
<i>Diploneis parma</i> Cleve	В	cool	-	alf	i	-	o-b	1.40	ot	_
Diploneis petersenii Hustedt	В	_	str	ind	i	_	O-X	0.70	m	ats
Discostella pseudostelligera (Hustedt) Houk and Klee	Р	_	_	ind	_	_	b	2.30	o-m	_
Discostella stelligera (Cleve and Grunow) Houk and Klee	Р	_	_	ind	i	_	o-b	1.40	o-m	_
<i>Encyonema</i> gracile Rabenhorst	В	_	_	ind	hb	SX	Х	0.30	_	_
<i>Encyonema</i> <i>minutum</i> (Hilse) D. G. Mann	В	_	st-str	ind	1	SX	0	1.20	0-e	ate
<i>Epithemia gibba</i> (Ehrenberg) Kützing	В	temp	Ι	alb	i	es	Х-О	0.40	Ι	-
<i>Eunotia arcus</i> Ehrenberg	В	_	st-str	acf	i	I	Х-О	0.50	ot	ats
<i>Eunotia bilunaris</i> (Ehrenberg) Schaarschmidt	В	temp	st-str	acf	i	es	0	1.00	0-е	ate
Eunotia diodon Ehrenberg	В	cool	st	acf	i	-	Х	0.20	ot	ats

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in Artabel Lakes Nature Park with autecological characteristics and species-specific indices of saprobity.

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in
Artabel Lakes Nature Park with autecological characteristics and species-specific indices of
saprobity.

saproony.										
<i>Eunotia</i> <i>mucophila</i> (Lange-Bertalot, Nörpel-Schempp and Alles) Lange-Bertalot	P-B	_	st-str	acf	hb	_	0	1.00	o-m	ate
<i>Eunotia paludosa</i> Grunow	В	_	str	acf	hb	-	X-0	0.40	ot	ats
Eunotia praerupta Ehrenberg	P-B	cool	st-str	acf	i	SX	х-о	0.40	o-m	ats
Fragilaria crotonensis Kitton	Р	_	st-str	alf	i	es	o-b	1.50	m	ate
<i>Fragilaria tenera</i> var. <i>nanana</i> (Lange-Betalot) Lange-Bertalot and S. Ulrich	P-B	_	str	ind	hb	Ι	0	1.00	me	ats
<i>Frustulia</i> <i>crassinervia</i> (Brébisson ex W. Smith) Lange- Bertalot and Krammer	В	_	str	acf	hb	es	х-о	0.50	ot	ats
<i>Frustulia</i> <i>vulgaris</i> (Thwaites) De Toni	P-B	_	st	alf	i	es	o-a	1.80	me	ate
Gomphonema acuminatum Ehrenberg	В	_	st	ind	i	es	o-b	1.40	o-m	ats
Gomphonema angustatum (Kützing) Rabenhorst	В	-	st-str	ind	i	es	0	1.30	o-m	_
<i>Gomphonema</i> <i>augur</i> Ehrenberg	В	_	str	ind	i	es	o-b	1.50	me	ats
<i>Gomphonema</i> <i>calcareum</i> Cleve	В	_	st-str	alf	i	I	b	2.30	o-m	ate
Gomphonema olivaceum (Hornemann) Brébisson	В	_	st-str	alf	i	es	o-b	1.45	e	ate

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in
Artabel Lakes Nature Park with autecological characteristics and species-specific indices of
saprobity.

saproony.										
Gomphonema parvulum (Kützing) Kützing	В	temp	str	ind	i	es	b	2.35	o-m	hne
Halamphora coffeiformis (C. Agardh) Levkov	В	_	st-str	alf	mh	_	a	3.00	e	ate
Hannaea arcus (Ehrenberg) R. M. Patrick	В	temp	str	alf	i	es	X	0.30	o-m	ats
Hantzschia amphioxys (Ehrenberg) Grunow	В	temp	st-str	ind	i	es	o-a	1.90	0-е	ate
<i>Iconella linearis</i> (W. Smith) Ruck and Nakov	P-B	_	_	ind	i	es	х-о	0.50	o-m	_
Mayamaea atomus (Kützing) Lange-Bertalot	В	_	st-str	alf	i	es	a-o	2.60	he	hce
<i>Melosira varians</i> C. Agardh	P-B	temp	st-str	ind	hl	es	b	2.10	me	hne
Meridion circulare (Greville) Agardh	В	_	str	ind	i	es	0	1.10	o-m	ate
Navicula cryptocephala Kützing	P-B	temp	st-str	ind	i	es	b	2.10	о-е	ate
Navicula radiosa Kützing	В	temp	st-str	ind	i	es	0	1.30	me	ate
Navicula rhynchocephala Kützing	В	_	_	alf	hl	_	o-a	1.95	o-m	ate
<i>Navicula veneta</i> Kützing	В	-	_	alf	hl	es	a-o	2.70	_	-
Neidiomorpha binodis (Ehrenberg) M. Cantonati, Lange-Bertalot and N. Angeli	В	_	str	alf	i	_	0	1.00	me	ate
<i>Neidium affine</i> (Ehrenberg) Pfitzer	В	_	str	ind	i	_	O-X	0.70	ot	ats

saprobity.										
Neidium iridis								0.40		
(Ehrenberg) Cleve	В	-	st-str	ind	hb	es	O-X	0.60	ot	ats
Nitzschia										
perminuta										
(Grunow)	P-B	temp	str	alf	hl	sp	b-o	1.75	o-m	ats
M. Peragallo										
Odontidium										
mesodon	D	,			11			0.40		
(Kützing)	В	cool	st-str	neu	hb	SX	х-о	0.40	ot	ats
Kützing										
Orthoseira										
dendroteres										
(Ehrenberg)	aer	-	_	_	i	_	х-о	0.50	_	-
Genkal and										
Kulikovskiy										
Orthoseira										
roeseana	P-B		_	ind	i	SX	Х-О	0.50	ot	_
(Rabenhorst)	I -D			mu	1	57	х-0	0.50	01	
Pfitzer										
Pinnularia										
abaujensis	В	_	_	acf	_	_	_	_	_	_
(Pantocsek)	D			uer						
R. Ross										
Pinnularia	В	_	_	_	i	_	_	_	_	_
aestuarii Cleve	2				-					
Pinnularia	_									
borealis	В	-	ae	ind	i	es	Х-О	0.40	o-m	ate
Ehrenberg										
Pinnularia	Ð							0.00		
divergens	В	-	st	ind	i	—	x-b	0.90	ot	-
W. Smith										
Pinnularia	В	_		ind	i	_	0	1.00	ot	_
<i>episcopalis</i> Cleve										
Pinnularia										
hemiptera	В	_	str	ind	i	_	0	1.00	ot	_
(Kützing) Rabenhorst										
Pinnularia										
	В			ind	i					
<i>interrupta</i> W. Smith	Б	_		ma	1	_	_	_	_	_
<i>Pinnularia lata</i>										
(Brébisson)	P-B		str	acf	i	_	0	1.00	ot	
W. Smith	1-D	_	su	acı	1	_	0	1.00	01	_
w. Sillul		1								

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in Artabel Lakes Nature Park with autecological characteristics and species-specific indices of saprobity.

saproony.										
Pinnularia major (Kützing) Rabenhorst	В	temp	st-str	ind	i	I	O-X	0.60	me	ate
Pinnularia mesogongyla Ehrenberg	В	-	-	ind	i	_	Х	0.30	ot	_
Pinnularia obscura Krasske	B, aer	_	ae	ind	i	_	0	1.00	ot	ats
Pinnularia rupestris Hantzsch	В	_	str	acf	i	_	X	0.30	ot	_
Pinnularia subcapitata W. Gregory	В	_	st-str	ind	i	sp	O-X	0.60	o-m	ate
<i>Pinnularia</i> <i>viridiformis</i> Krammer	В	_	_	_	_	1	O-X	0.70	_	_
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	P-B	temp	st-str	ind	i	es	X	0.30	о-е	ate
Placoneis hambergii (Hustedt) K. Bruder	В	_	str	acf	_	es	_	_	_	_
Planothidium distinctum (Messikommer) Lange-Bertalot	В	_	_	_	_	_	0	1.00	_	_
Planothidium lanceolatum (Brébisson ex Kützing) Lange- Bertalot	P-B	warm	st-str	alf	i	SX	b-o	1.60	e	ate
Platessa salinarum (Grunow) Lange- Bertalot	P-B	_	st-str	ind	mh	_	b	2.10	me	ate
Psammothidium helveticum (Hustedt) Bukhtiyarova and Round	В	_	st-str	alf	hb	es	X	0.30	m	ate
Sellaphora pupula (Kützing) Mereschkovsky	В	eterm	st	ind	hl	SX	o-a	1.90	me	ate

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in Artabel Lakes Nature Park with autecological characteristics and species-specific indices of saprobity.

suproonty.										
Stauroneis	P			10				1.00		
acuta	В	—	st-str	alf	i	-	0	1.00	o-m	—
W. Smith										
Stauroneis										
anceps	P-B	—	st-str	ind	i	SX	0	1.30	o-m	ate
Ehrenberg										
Staurosira venter										
(Ehrenberg)	P-B	warm	st-str	alf	i	sx	0	1.30	me	ate
Cleve and J. D.	г-D	wanni	51-511	all	1	53	0	1.50	me	ale
Möller										
Staurosirella										
pinnata										
(Ehrenberg) D.	P-B	temp	st-str	alf	hl	es	0	1.20	o-e	ate
M. Williams and		-								
Round										
Surirella angusta	P-B	_	st-str	alf	i	es	b-o	1.70	e	ate
Kützing										
Surirella robusta	P-B		at atm	ind	Ι	2.2		0.50	<u>at</u>	
Ehrenberg	Г-D	_	st-str	ma	1	es	х-о	0.50	ot	-
Surirella										
splendida	חח		at atr	alf	i		0 W	0.70		
(Ehrenberg)	P-B	—	st-str	alf	1	_	O-X	0.70	me	—
Kützing										
Tabellaria										
flocculosa (Roth)	P-B	eterm	st-str	acf	i	es	O-X	0.60	ot	ats
Kützing										

Table 2 (continued): Diversity of diatom species-indicators in the studied waters in Artabel Lakes Nature Park with autecological characteristics and species-specific indices of saprobity.

The bioindication results of organic pollution obtained from the Watanabe and the Sládeček's systems are shown in figure 5. According to these results, it can be stated that all examined lake ecosystems have all indicator groups of Watanab's method. Nevertheless, numbers of species of middle polluted (es) and clear water (sx) indicators are common (Fig. 5). Saproxenic species increase with altitude, and all studied lakes can be assessed as naturally clear with slightly more organic pollution influence to the low-altitude lakes IL group where eurysaprobes increased, and the YLP group was found to have more saprophilic species. While the indicators of Class 2 in the Sládeček system contained a significant portion of the diatom community in all of the studied waters, Class 1 was second. Even the indicators of middle pollution Class 3 were not as high in number as Class 2, except for Beş Lakes (BL). The members of Class 4 were represented by an only one species in Beş Lakes (BL), Acembol Lakes (ACL), İsimsiz Lake (IL), and Yıldız Lakes Pond (YLP), while indicators of polluted water Class 5 have never been determined (Fig. 5).

This means that the studied lakes of the researched area have natural unpolluted and natural undisturbed water ecosystems. Index Saprobity S that has been calculated for each lake community (Tab. 1) refers to water quality Class 1 and 2. Organic pollution of waters is related to the trophic level of its ecosystems. It can be seen in figure 5 that different groups of indicators of trophic level represent the communities of each aquatic ecosystem. It can be seen that the indicators of the oligotrophic and oligo-mesotrophic prevailed in all lake ecosystems.

The eutrophic indicators were represented by a few species in all the studied waters, except in Beş Lakes (BL). So, eutrophic indicators category e, he, and o-e in the Beş Lakes (BL) are in total 12 taxa where in the Yıldız Lakes (YL) are 10 taxa, and only seven high trophy indicators are in the Acembol Lakes (ACL). Therefore, the group of the Acembol Lakes (ACL) looks like most clear. For this reason, we can say that the studied lakes have oligo-mesotrophic waters, which are found in the unpolluted protected area because of the oligo- and oligo-mesotrophic species represented about 60% of the diatom lake communities. The effectiveness of photosynthesis in the examined waters was determined when the nutrition type indicator species prevailed in communities of all investigated lakes (Fig. 5). Distribution of trophic and nutrition indicators did not show correlation with the lakes altitude.

In an overall view of the high mountain lakes of Europe (Tolotti, 2001; Robinson and Kawecka, 2005; Marchetto et al., 2009; Ognjanova-Rumenova et al., 2009; Kryvosheia and Tsarenko, 2018), Asia (Wang et al., 2015), and North America (Saros et al., 2012), it can be seen that altitudes of the studied lakes varied mostly between 1500-2600 m. a.s.l., whereas we started from this level up to 3,015 m. a.s.l. in this research. Few groups of lakes have altitudes above 2,600 m. a.s.l. like in the Alps (Feret et al., 2017), Tropical Andes (Van Colen et al., 2017), North America (Saros et al., 2003), and Pamir (Barinova and Nivatbekov, 2017: Niyatbekov and Barinova, 2018a, b; Blanco et al., 2019). However, oligotrophic state, circumneutral pH, and low-alkaline water are mentioned for alpine lakes using all of these references. This set of environmental properties is characteristic of all of the studied highmountain lakes, both in the boreal (Marchetto et al., 2009; Ognjanova-Rumenova et al., 2009) and subtropical-tropical zones (Wang et al., 2015; Van Colen et al., 2017). Relationships between environmental variables and diatom community productivity estimators, such as Chla, abundance and biomass are studied but bioindication methods were implemented in only a few studies water bodies in the Carpathian Mountains lakes (Kryvosheia and Tsarenko, 2018), and Pamir (Barinova et al., 2015a; Nivatbekov and Barinova, 2018c, d).

#### **Comparative floristics**

A comparative floristic approach was used for the grouping of algal communities with respect to their taxonomic similarity. A similarity tree of floristic composition that was constructed for the Artabel Lakes Nature Park communities showed three species diversity clusters at the similarity level of 50% (Fig. 6). Cluster 1 includes communities of ARL and BL. Cluster 2 comprises ACL and YL, while cluster 3 includes IL and YLP.

According to species distribution and a comparative floristic analysis, five clusters with a similarity level of 50% were determined (Fig. 7). The red cluster contained the ARL1, ARL3, ARL2, BL, and ARL lakes. The navy blue cluster had three lakes, which were ARL5, ARL6, and BL4. The blue cluster included YL2, YL3 and IL lakes, while the pink cluster consisted of BL1, YLP, BL2, YL1, and BL3 lakes. The green cluster comprised of ACL, ACL2, and ACL3 lakes. Therefore, can be seen in figure 7, that algal communities were most similar in each group of lakes and conformed the different clusters. It allows us to conclude that the landscape position of the groups of lakes related to hydrology play the major role in the historical forming process of them algal flora.

Clustering maps provide important contributions to our understanding of the distribution of algal communities. According to the taxonomic overlap, the Yıldız Lakes (YL) represents the floristic core in which other lakes are included, too (Fig. 8).

As a result of floristic comparison of diatom species richness in the benthic habitats of the lakes in the Artabel Lakes Nature Park, benthic communities can be divided into two groups (Fig. 9). The relationships between two floristic groups with cores in ARL5 and ACL3 lakes are about 60%. It can be seen that all studied floras are represented by one closely related cluster in regional diversity and also have some similarities in their evolutionary development.

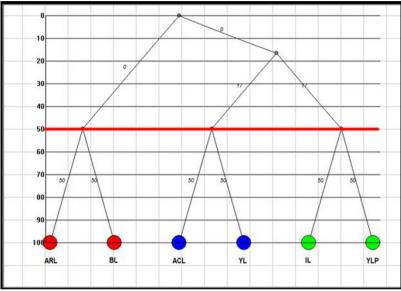


Figure 6: Dendogram of comparative floristic of algal species in the lakes of the Artabel Lakes Nature Park.

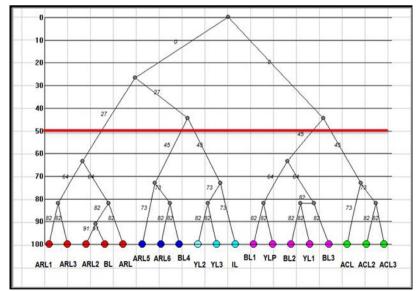


Figure 7: Dendrogram of species similarity in the studied lakes in the Artabel Lakes Nature Park.

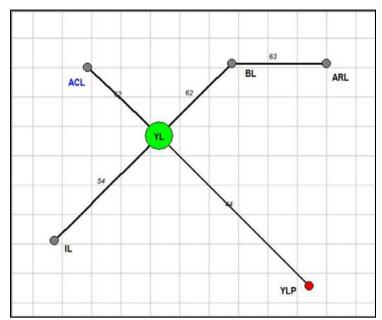


Figure 8: Dendrite of species richness overlapping in studied lakes communities in habitats groups of the Artabel Lakes Nature Park.

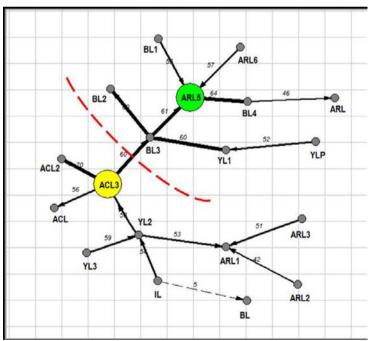


Figure 9: Dendrite of including-crossing of species richness in the algal communities of the studied lakes in the Artabel Lakes Nature Park. The thickness of connected lines is corresponding to the percent of similarity on the lines. The arrows point to the directions of species richness incuding. Large circles are marked by yellow or green color are represent the floristic cores. Red dashed line is the border between communities related to each floristic core.

#### Statistical analysis of species and environmental variables relationships

We used the statistical method of Distance Weighted Least Squares for clarifying relationships of the revealed algal diversity and the environmental variables in the Artabel Lakes Nature Park. Surface plots were constructed according to environmental (altitude, TDS, pH, DO, temperature) and biological (abundance, number of species, Index saprobity S) data represented in table 1. Figure 10a shows that, on the one hand, species richness in revealed diatom communities tend to decrease with altitude when the water temperature is low. On the other hand, species richness changes in waters with high pH and temperature (Fig. 10b). The oxygen saturation decreases with altitude, and we can see two different types of diatom communities: one of them is formed in well-saturated high mountain waters and the second one preferred low-altitude lakes with medium-to-low oxygen content (Fig. 10c). Figure 10d demonstrated two different communities also, but if we compare it with the distribution of species richness in figure 10c, we can conclude that altitude is a regulating factor for species richness but not for water pH and conductivity.

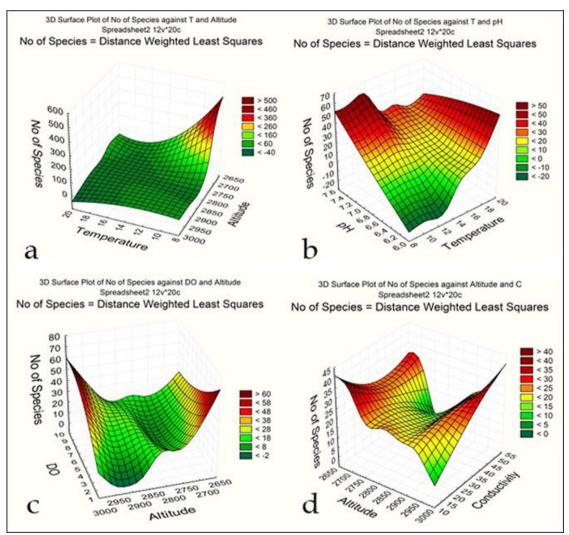


Figure 10: Statistical surface plots of diatom species richness and environmental variables in the studied lakes in Artabel Lakes Nature Park.

Pollution factors (e.g., Index Saprobity S) also divide diatom diversity into two groups with preferences for high mountain low polluted waters and low altitude more organically enriched waters (Fig. 11a). Therefore, this conclusion can be related to a previous one in which the importance of altitude in the algal community forming process is highlighted.

The important variables for diatom communities are the abundance of species and the community structure. Figure 11b shows that abundance and organic pollution are the factors in which community structure was more complex with increasing Shannon index. When we try to reveal the factors in which the algal community in studied lakes flourish, we find the TDS of water and increases of organic pollution are stimulating variables (Fig. 11c). However, abundance shows a similar tendency with species richness in altitude/organic pollution coordinates with forming the same two communities (Fig. 11d).

Often, the ecosystem of the alpine lakes can be impacted by some local factors when the community changes in the same altitude belt (Van Colen et al., 2017), but usually the lake community is sensitive to atmospheric loads of nitrogen or temperature change (Tolotti, 2001). Therefore, water alkalinity, pH, and conductivity are the most important factors determining the taxonomic diversity of the diatom benthic communities in high mountain lakes (Ognjanova-Rumenova et al., 2009).

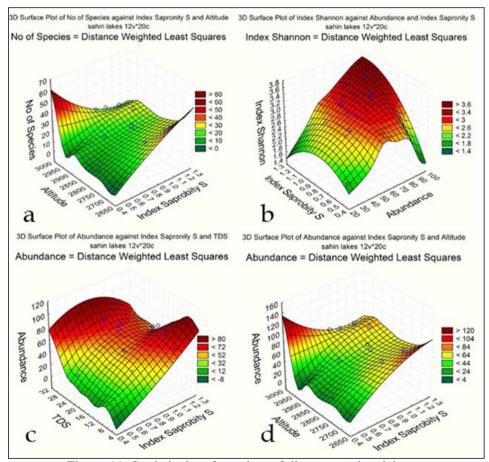


Figure 11: Statistical surface plots of diatom species richness and environmental variables of the studied lakes in Artabel Lakes Nature Park.

#### **Canonical Correspondence Analysis**

To continue the statistical analysis of species and environment relationships, we constructed Canonical Correspondence Analysis (CCA) plots based on environmental and biological properties of the studied lakes water (Tabs. 1 and 2). A triplot of the most abundant species in the diatom communities of the Artabel Lakes Nature Park and environmental variables of each studied lake demonstrated three different clusters of the lakes that correlated with groups of variables (Fig. 12). This can be seen in the red cluster, which combines lakes ARL1, ARL2, and ARL3 in respect to increasing water conductivity and decreasing water pH. The water temperature is indifferent to this cluster of communities. The blue cluster combines lakes YL1, YL2, YL3, and YLP, which indicates increasing altitude with decreasing oxygen saturation. A black cluster included lakes ARL5, IL, BL1, and BL3 communities, which preferred low altitude, well oxygen saturated waters, with high pH.

Dominating species preferences can be seen in figure 13. A CCA plot divided abundant diatom species into three different clusters: A red circle represents two species *Eunotia praerupta* and *Pinnularia borealis*, as indicators of high-mountain, low organically polluted lakes, with low pH and dissolved oxygen. A blue circle cluster included two species, *Encyonema minutum* and *Amphora ovalis*, which preferred waters with high pH, low conductivity, and low temperatures in high altitude habitats with species rich communities with complicated structure. Third cluster species, *Tabellaria flocculosa* and *Stauroneis anceps*, were indicators of well-oxygenated waters and developed in species-rich abundant communities with complex structure. This distribution is similar to diatom species preferences in the high mountain lakes of European Alps and American Yellowstone National Park (Marchetto et al., 2009; Saros, et al., 2012).

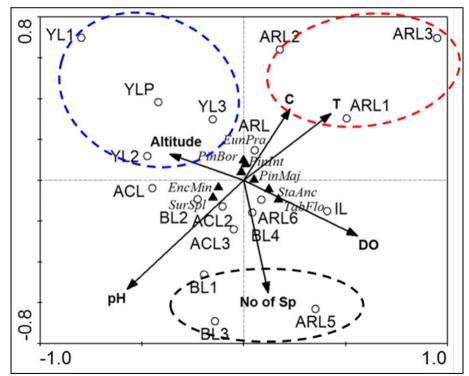


Figure 12: CCA plots of diatom species and environmental variables relationships in the studied lakes in the Artabel Lakes Nature Park.

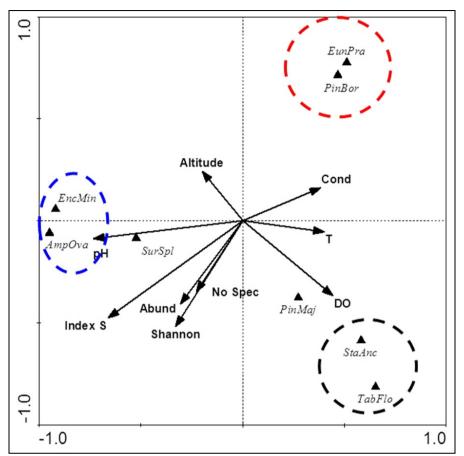


Figure 13: CCA plots of diatom species and environmental variables relationships in the studied lakes in Artabel Lakes Nature Park.

#### CONCLUSIONS

All revealed diatom taxa were used as bioindicators of water quality according to ten environmental features of species autecology. Statistical programs and bioindication that has been done for the first time revealed that in the studied lakes was fresh, circumneutral, and has enough oxygen saturation for the survival of diatom communities with species, with a wide amplitude of richness from 1 in the highest altitude (3,015 m. a.s.l.) in lake Beş 5 (BL5) to 37 in the lowermost altitude (2,668 m. a.s.l.) İsimsiz Lake (IL).

Pearson correlation coefficients demonstrated that only water temperature has a significant negative correlation with the height of the lake. It complied with the bioindication of water properties where more freshwater was found in high- altitude lakes. Because light and temperature are the basic physical variables affecting photosynthesis and is one of the most important factor for diatom algae development (Barinova et al., 2014c), these global climatic factors also define the algal flora forming results in studied high mountain lakes. Therefore, species richness and abundance, and indicators properties of diatoms in the studied lakes of the Artabel Lakes Nature Park demonstrated that studied lakes have natural unpolluted and natural undisturbed water ecosystems.

Detailed bioindication measures also show that the studied lakes have oligomesotrophic waters, which are found in the unpolluted protected area because the oligo- and oligo-mesotrophic species are represented by 60% of the lakes' communities. The autotrophic photosynthetic diatom species have prevailed in the communities of all investigated lakes.

Statistical methods helped us to reveal the relationship between the landscape group of the lakes have its own hydrology and the taxonomic contents of algae.

Comparative floristics analyses showed that all studied floras are represented by one closely related cluster in regional diversity and have some similar evolutionary ways of development when species richness of diatom communities tend to decrease with altitude when the water temperature is low. Therefore, lake altitude can be a regulating factor for species richness but not for other variables, e.g., water pH and conductivity. On the other hand, altitude, as a varying local factor, determines the freezing period and therefore impacts the activity and function in high-altitude lakes (Feret et al., 2017).

In respect to organic pollution, the studied lakes were divided into two groups of highmountain, low polluted waters and low-altitude more organically enriched waters. This conclusion can highlight the importance of altitude in the algal community forming process that is more active in the high-altitude fresh unpolluted waters. In contrary, abundance and organic pollution are factors in which the community species richness and structure were more complex with an increasing Shannon index.

As shown by statistical analysis of total environmental and biological variables relationships, the studied lakes were represented by three different groups: (1) Artabel Lakes where there was increasing water conductivity and decreasing water pH; (2) Yıldız Lakes that indicated a decrease in oxygen saturation with increasing altitude; (3) Beş Lakes and some other, like Artabel Lake 5 and İsimsiz Lake communities, which preferred low-altitude well-oxygen saturated waters with high pH.

Three groups of species were defined as the most abundant indicators for future environmental monitoring: (1) *Eunotia praerupta* and *Pinnularia borealis* as indicators of high-mountain, low organically polluted lakes with low pH and dissolved oxygen; (2) *Encyonema minutum* and *Amphora ovalis*, indicators of high pH, low conductivity, and low temperature in high altitude species rich communities with complicated structure; (3) *Tabellaria flocculosa* and *Stauroneis anceps* as indicators of well-oxygenated waters, which inhabited species rich and abundant communities with complicated structure. This conclusion can be used as a reference for the future monitoring of changing environments and anthropogenic processes on the protected lakes in the Artabel Lakes Nature Park and as an instrument for an algal indication of climatic gradients (Barinova et al., 2015b). Bioindication results can also be included in the development of the National Water Quality Standard system, which is in the development stage now in Turkey.

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# SPECIES AND THALLUS STRUCTURE DIVERSITY OF CHLOROPHYTA IN SHORE PLATFORM OF DWARKA (GUJARAT COAST, INDIA)

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**KEYWORDS**: macroalgae, Chlorophyta, shore platform, species, thallus structure, diversity.

# ABSTRACT

Present study shows the species and thallus structure diversity of Chlorophyta in shore platform of Dwarka, Gujarat Coast. Chlorophyta were surveyed based on systematic random sampling for two years: April 2013 to April 2015. Total 27 species of Chlorophyta were identified through intensive fieldwork survey based on line transect and quadrate based methods. This study identifies four orders, nine families, 13 genera and 27 species among the green macroalgae population inhabiting the rock shore platform of Dwarka. Total 27 species of Chlorophyta have been found with major nine thallus types and 19 sub types of thallus structure. Ramiform and filamentous types of thallus structure are most common in this study.

**ZUSAMMENFASSUNG**: Diversität der Arten und Thallusstruktur bei Grünalgen (Chlorophyta) an der Dwarka Küstenplattform (Gujarat-Küste, Indien).

Vorliegende Studie befasst sich mit der Arten- und Thallustrukturdiversität von Chlorophyten der Küstenplattform von Dwarka im Bereich der Meeresküste Gujarat, Indien. Die Grünalgen wurden mit Hilfe systematischer Zufallsstichproben über einen Zeitraum von zwei Jahren, von April 2013 bis April 2015 untersucht. Insgesamt wurden bei intensiver Feldarbeit durch in situ Methode mit Linientransekten sowie in Quadraten 27 Arten von Grünalgen festgestellt. Diese sind vier Ordnungen, neun Familien und 13 Gattungen von Makroalgenpopulationen der Felsküstenplattform von Dwarka zugeordnet. Unter den insgesamt 27 Arten von Grünalgen wurden bezüglich Thallusstruktur neun Haupt- und neunzehn Subtypen vorgefunden. Dabei wurde deutlich, dass die verzweigten und die fadenförmigen Thallusstruzkturen am häufigten waren.

**REZUMAT**: Diversitatea speciilor și a structurii talului la alge verzi (Chlorophyta) pe platforma marină Dwarka (coasta Gujarat, India).

Prezentul studiu se ocupă de diversitatea speciilor și a structurii talului algelor clorofite de pe platforma marină Dwarka de pe coasta Gujarat, India. Clorofitele au fost investigate luându-se probe prin sondaj pe o perioadă de doi ani și anume din aprilie 2013 până în aprilie 2015. În total au fost găsite, în cadrul activităților intensive de teren, cu ajutorul metodei transectelor lineare și a cvadratelor, 27 de specii de alge clorofite. Ele fac parte din patru ordine, nouă familii 13 genuri de macropopulații ale platformei marine de la Dwarka. Cele 27 de specii de clorifite identificate se prezintă din punct de vedere a structurii talului cu nouă tipuri principale și 19 subtipuri. Cele mai comune structuri de tal înregistrate au fost cele ramificate și filiforme.

# **INTRODUCTION**

Coastal areas all over the world are tremendously important and valuable for human society but they are largely inappropriately managed due to the high impact of human activity in the inner watersheds and marine areas (Novac and Shurova, 2008; Sabai and Sisitka, 2013; Afkhami et al., 2013; Ghasemi, 2014; Sosai, 2015; Dimpal et al., 2015; Bănăduc et al., 2016; Balasaheb et al., 2017).

Algae is one of the largest group of organisms, with significant ecological importance for a high variety of aquatic ecosystems (Round, 1981; Lee, 2008; Barinova, 2018).

Seaweeds or macroalgae comprise a diverse group of lower plants that represent various growth forms in algae (\*). In general, the macroalgae organisms are divided by specialists into three groups based on their colors: green, brown, and red (MacArtain et al., 2007). They appear similar to land plants, however, they lack complex reproductive structure and functional tissues as found in seagrass and land plants (\*\*). They constitute one of the most important living resources of the ocean and are found attached to solid substrates such as rocks, dead corals, pebbles, shells, and plants (Sahayaraj et al., 2014).

As photosynthetic organisms, this group plays a key role in the productivity of seas and oceans and constitutes the basis of these aquatic habitats food chain (Shalaby, 2011). They contribute substantially to the carbon budget of the coastal ecosystems (Dhargalkar et al., 2001). Macroalgae play significant ecological roles in the ecosystem (Satheesh et al., 2017). They provide habitat and nutrients to marine organisms (Dhargalkar et al., 2001). Macroalgae are used as food, fodder, fertilisers, as raw materials in industries, medicines, etc. (Dhargalkar et al., 2001). They are particularly useful organisms for studying diversity patterns and planning the conservation and sustainable use of inshore marine resources and are useful as indicators of climate change (Van der Strate et al., 2002).

Green algae contain chlorophyll a and b and store food as starch in their plastids. The green colour of the Chlorophyta is derived from amounts of chlorophyll a and b in the same proportion as the higher plants, giving them a bright green colour, as well as the accessory pigments  $\beta$ -carotene and xanthophylls (yellowish or brownish pigments). Most of the green algae have firm cell walls composed of cellulose along with other polysaccharides and proteins and they store carbohydrate in the form of starch. Chlorophyta are usually found in the littoral zone where there is strong sunlight. Chlorophyta ranges from the singled-celled to moderately complex structures. They appear in different forms: filamentous, membranous, cylindrical, globular or coenocytic, depending on the way their cell divides. (Guiry, 2000-2019)

The study was carried out with the following principal objectives: to study the species diversity of Chlorophyta in Dwarka, and to study the thallus structure diversity of Chlorophyta on shore platform in Dwarka.

## MATERIAL AND METHODS

#### Study area

Geographical, geological, topographical and physical nature of the shore is important for the macroalgae's growth. Rocky coasts have vertical zonation (Woodward, 2003). Shore platform provides stable coastal environment as compared to soft sediment coast like beaches and spits. This represents an environment where majority of macroalgae species grow with a firm substratum attachment (Sanghvi et al., 2015). The coastline of Gujarat State covers a length of 1,600 km. It represents the north-western most part of peninsular India. This coastline occurs within the geographical limits of 20°00' to 24°45'N and 68°00' to 78°30'E. Gujarat coast extends in the form of four major coastal ecological components from North to South: I) Kori Creek; II) Gulf of Kachchh; III) Saurashtra Coast from Okha to Porbandar, and IV) Gulf of Khambhat (Sanghvi et al., 2015). Gujarat coastline is reportedly rich with diversity of macroalgae (Chakraborty and Bhattacharya, 2012). The substratum of Gujarat Coast is rocky in many components, which provides felicitous environment for macroalgae growth. The Saurashtra Coast, which runs for an approximate length of 985 km, is characterized by rocky, sandy, and muddy intertidal zones, harboring affluent and varied flora and fauna (Gohil and Kundu, 2012).

The present research was carried out completely on the shore platform of Dwarka, located on the Saurashtra Coast, Gujarat, India  $(22^{\circ}14'22'' - 22^{\circ}14'38''N)$  and  $68^{\circ}57'15'' - 68^{\circ}57'25''E)$  (Fig. 1). Total length of the study area is 572.28 m, maximum width of the shore platform sampled is 143.8 m, and it covers a surface area of 82,293.86 m<sup>2</sup> (Sanghvi et al., 2015). In this research area three groups of macroalgae: Chlorophyta, Phaeophyta, and Rhodophyta are found. From these three groups, Chlorophyta was selected for the present study.

# Field data collection

The research area was divided into three sections in North-South directions: I) Northern; II) Central; III) Southern sections for systematic-field sampling. Field sampling of macroalgae was done from April 2013 to April 2015. Field surveys/samplings were performed during the low tide periods. For qualitative and quantitative assessment, GPS (Spheroid and Datum: WGS 84) tagged Line Intercept Transect (LIT) were carried out. The length of the transect lines depended on the tidal exposure of the shore platform during the survey. Maximum depth of the subtidal zone sampled for macroalgae in the present study is 0.5 m. For quantitative assessment of the macroalgae in the given area, line transect was laid perpendicular to the coast in a seaweed direction with the help of a long rope of 50 m (Dhargalkar and Kavlekar, 2004). A sampling point along the rope is marked depending on the gradient and exposure of intertidal and subtidal areas. In Saurashtra Coast, the tidal amplitude is guite high as compared to other parts of the Indian coast and the west coast in particular. Growth of macroalgae in intertidal and shallow subtidal regions can be easily observed in this area as the spring tides expose the intertidal area up to a maximum length of one km (Jha et al., 2009). Each of the three sections on the shore platform was represented by one transect line: thus resulting 33 transect lines wherever the algae growth, density and diversity were high. 258 quadrates were done on the thirty three transect lines. Seaweed species present within the quadrates were sampled.

## Field data analysis

Morphological criteria and reproductive structure were analyzed for taxa identification.

A cladogram was prepared for identifying algae species in order to generate classification statistics of Chlorophyte i.e. number of genera and species pertaining to different classes as shown in figure 2.

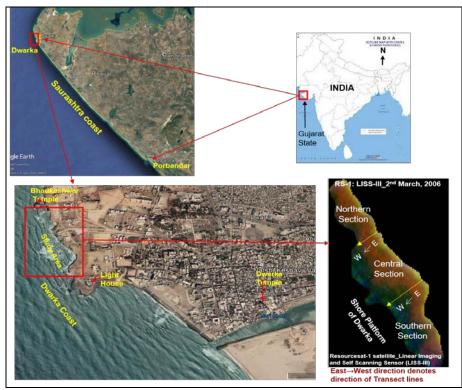


Figure 1: Location map of study area: Shore platform, Dwarka, Gujarat Coast, India; (Source: India outline map- mapsofindia.com and others: google.com/intl/en\_in/earth/).

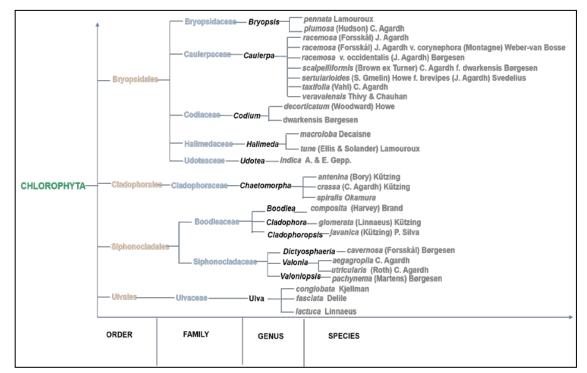


Figure 2: Cladogram of Chlorophyta species found from shore platform, Dwarka.

## **RESULTS AND DISCUSSION**

Macroalgae on the shore platform were surveyed based on systematic random sampling for two years (April 2013 to April 2015). A total of 27 species of Chlorophyta (Tab. 1) were identified from this site through intensive fieldwork based on Line Intercept Transect (LIT) and Quadrate based sampling methods.

From this study, the species and thallus structure diversity of Chlorophyta in shore platform, Dwarka, Gujarat Coast was reported (Tab. 1). Chlorophyta encompasses four orders, nine families, 13 genera and 27 species among the macroalgae population inhabiting the shore platform of Dwarka as shown in the cladogram (Fig. 2). Each species was studied with respect to their occurrence observed through quadrate survey within the study sites. Out of four orders, Bryopsidales order includes five families: Bryopsidaceae, Caulerpaceae, Codiaceae, Halimedaceae, and Udoteaceae. Bryopsidaceae is found to have two species: Bryopsis pennata Lamouroux and Bryopsis plumose (Hudson) C. Agardh having ramiform and feathery thallus (Fig. 3A). Caulerpaceae is found to have seven species which belongs to Caulerpa genus. Out of seven species, six species Caulerpa racemosa (Forsskål) J. Agardh, C. racemosa (Forsskål) J. Agardh v. corynephora (Montagne) Weber-van Bosse, C. racemosa v. occidentalis (J. Agardh) Børgesen, C. sertularioides (S. Gmelin) Howe f. brevipes (J. Agardh) Svedelius, C. taxifolia (Vahl) C. Agardh and C. veravalensis Thivy and Chauhan having ramiform and creeping thallus (Fig. 3B) and one species C. scalpelliformis (Brown ex Turner) C. Agardh f. dwarkensis Børgesen have rhizomatous with flat assimilator thallus (Fig. 3C). Codiaceae is recorded to have two species Codium decorticatum (Woodward) Howe and C. dwarkensis Børgesen have ramiform and spongy thallus (Fig. 3D). Halimedaceae is found with two species Halimeda macroloba Decaisne and H. tuna (Ellis and Solander) Lamouroux have articulated and calcified form thallus (Fig. 3E). Udoteaceae is recorded one species Udotea indica A. and E. Gepp. Has fan like blades thallus (Fig. 3F). Cladophorales order includes one family: Cladophoraceae where three species Chaetomorpha antennina (Bory) Kützing has filamentous and brush-like tufts in flocks thallus structure (Fig. 4A), Chaetomorpha crassa (C. Agardh) Kützing has filamentous and entangled mass thallus structure (Fig. 4B) and Chaetopmpha spiralis Okamura has filamentous and coiled thallus structure. Siphonocladales order includes two families: Boodleaceae and Siphonocladaceae. Booodleaceae have three species, Boodlea composita (Harvey) Brand has spongy, branched cushion (Fig. 4C), Cladophora glomerata (Linnaeus) Kützing has erect or prostrate filaments and sparsely branched (Fig. 4D) and *Cladophoropsis javanica* (Kützing) P. Silva has spongy, filamentous and prostrate thallus structure (Fig. 4E). Siphonocladaceae includes four species Dictyosphaeria cavernosa (Forsskål) Børgesen has Hollow, spherical, irregularly lobed or globose type of thallus (Fig. 4F), Valonia aegagropila C. Agardh has compact vascular mass thallus structure (Fig. 5A), V. utricularis (Roth) C. Agardh has loose vesicle like patches thallus (Fig. 5B) and Valoniopsis pachynema (Martens) Børgesen has cushions or spongy patches type of thallus structure (Fig. 5C). Ulvales order includes one family: Ulvaceae which has three species Ulva conglobata Kjellman has leafy and undivided thallus, U. fasciata Delile has leafy and ribbon-like thallus (Fig. 5D) and U. lactuca Linnaeus has leafy and membranous thallus structure (Fig. 5E).

A total of 27 species of Chlorophyta have been found with nine major thallus types and 19 sub types of thallus structure. Ramiform and filamentous types of thallus structure are most common on shore platform of Dwarka.

Many of the phycologists and researchers have studied on the diversity and distribution (Yedukondala Rao et al., 2005; Palanisamy, 2012; Sahayaraj et al., 2014), economic importance (Dhargalkar, 2014; Yadav et al., 2015; Jayaprakash et al., 2017; Swamalatha, 2018), frequency and biomass (Satheesh and Wesley, 2012), growth cycle (Kotiya et al., 2011), etc., of macroalgae of India but so far no one has studied thallus structure diversity of Chlorophyta in such species level on shore platform from Indian Coast.

Waghmode (2017) has studied diversity and distribution of macroalgae from the west coast of Maharashtra. He has recorded 73 species of macroalgae. Out of 73 species, 21 belongs to Chlorophyta, 17 to Phaeophyta, and 33 to Rhodophyta. He has reported red algae found more as compared to Chlorophyta and Phaeophyta. Pawar (2017) has worked on distribution pattern and species diversity of macroalgae at URAN (Navi Mumbai), West Coast of India. He has found a total of 19 species of macroalgae which is representing 16 genera, 15 families, and 13 orders. Out of these, seven species belongs to Chlorophyte and Rhodophyta each, two each to Phaeophyta and one to Charophyta. At all study sites, maximum species diversity was recorded during pre-monsoon and post-monsoon periods. Macroalgae from Uran Coast are under stress due to this region industrial pollution and port operations. Sahayaraj et al. (2014) have worked on distribution and diversity assessment of the marine macroalgae at four southern districts of Tamil Nadu, India. They have reported 57 taxa belonging to 37 genera representing 18 taxa of Chlorophyta, 14 taxa of Phaeophyta, and 25 taxa of Rhodophyta. Species wise, a higher number of algae have been recorded from Idinthakarai (84.21%). They have recorded a maximum of (59%) species from Idinthakarai and a minimum of (1%) species from Keelvaipaar.

Roy et al. (2015) have worked on diversity and distribution of macroalgae in selected reefs and island of Gulf of Kachchh. They have found 70 species of macroalgae. Out of these, 24 species belongs to Chlorophyta, 15 species of Phaeophyta, and 31 species of Rhodophyta. The numbers of macroalgae found in Chaad, Dedeka-Mundeka, Goose, and Narara were 44, 49, 33, and 31 respectively. Nirmal Kumar et al. (2017) have worked on distribution and biochemical constituents of different macroalgae collected from Okha Coast, Gujarat, India. They have observed that macroalgae were not found continuously during the study period but some species were observed only for short periods whereas other species occurred for two to three months. They have recorded a total of 70 species of macroalgae with the highest number of Rhodophyta (51.42%) species, then Phaeophyta (25.71%) and lastly Chlorophyta (22.85%). From the recorded groups of macroalgae, carbohydrate and chlorophyll contents were higher in Chlorophyta while protein was maximum in Phaeophyta, respectively Rhodophyta. On the other hand, phenol, flavonoids and carotenoids were recorded at higher rates in Phaeophyta. Jha et al. (2009) have studied the diversity and distribution of the macroalgae of Gujarat Coast. They have given the species level characteristic of the Gujarat Coast. They have recorded a total of 198 species belonging to 101 genera of macroalgae. Among these, 24 species are new reported from Gujarat and three from the Indian Coast. The Rhodophyta contained the maximum number of species 109 belonging to a total of 62 genera, followed by the Chlorophyta with 54 species belonging to a total of 16 genera, and Phaeophyta with 35 species belonging to a total of 16 genera. They have also reported thallus form and type, substratum, occurrence season and abundance of 198 species of macroalgae.



Figure 3: A) Ramiform and feathery thallus; B) Ramiform and creeping thallus;C) Rhizomatous with flat assimilator thallus; D) Ramiform and spongy thallus;E) Articulated and calcified form thallus; F) Fan like blades thallus.

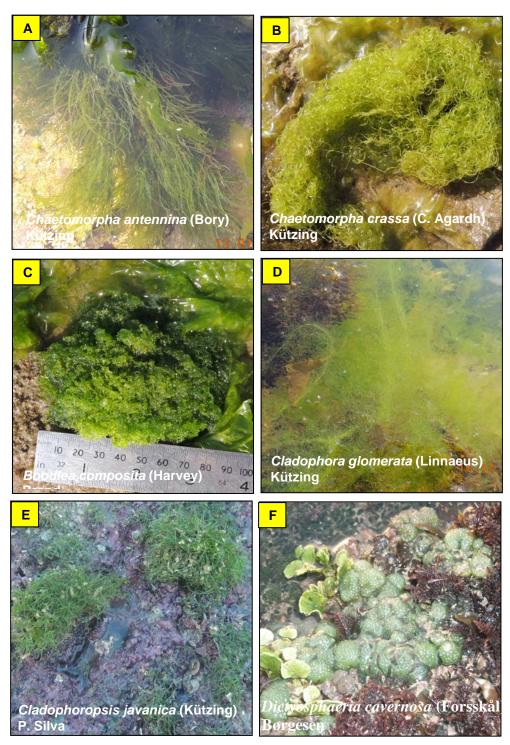


Figure 4: A) filamentous and brush-like tufts in flocks thallus;B) Filamentous and entangled mass thallus; C) Spongy, branched cushion thallus;D) Erect or prostrate filaments and sparsely branched; E) Spongy, filamentous and prostrate thallus; F) Hollow, spherical, irregularly lobed or globose type of thallus.

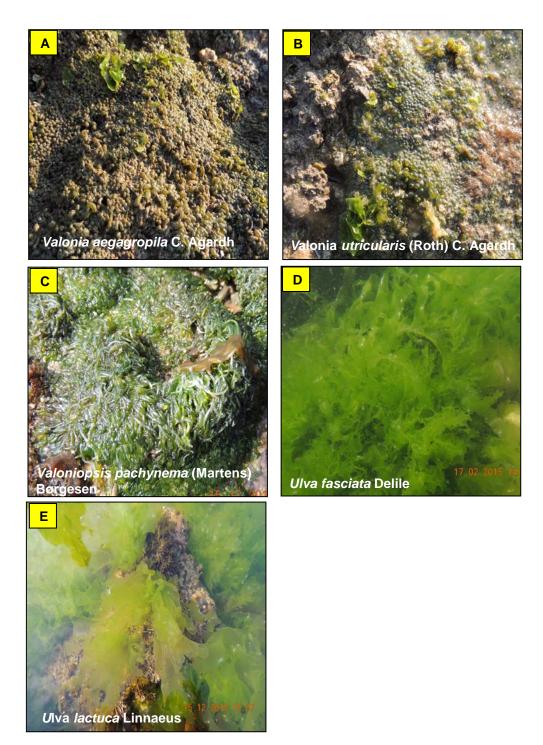


Figure 5: A) compact vascular mass thallus; B) Loose vesicle like patches thallus;C) Cushions or spongy patches type of thallus; D) Leafy and ribbon-like thallus;E) Leafy and membranous thallus structure;F) Hollow, spherical, irregularly lobed or globose type of thallus.

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Group	Order	Family	Genus	Species	Thallus form and type
		Bryo-	D .	pennata Lamouroux	Ramiform and feathery
		psidaceae	Bryopsis	<i>plumosa</i> (Hudson) C. Agardh	Ramiform and feathery
				<i>racemosa</i> (Forsskål) J. Agardh	Ramiform and creeping
				<i>racemosa</i> (Forsskål) J. Agardh v. corynephora (Montagne) Weber-van Bosse	Ramiform and creeping
				racemosa v. occidentalis (J. Agardh) Børgesen	Ramiform and creeping
	Bryo- psidales	Cauler- paceae	Caulerpa	<i>scalpelliformis</i> (Brown ex Turner) C. Agardh f. dwarkensis Børgesen	Rhizomatous with flat assimilator
yta				sertularioides (S. Gmelin) Howe f. brevipes (J. Agardh) Svedelius	Ramiform and creeping
Chlorophyta				<i>taxifolia</i> (Vahl) C. Agardh	Ramiform and creeping
Chlo				veravalensis Thivy and Chauhan	Ramiform and creeping
		Codia- ceae Halime- daceae	Codium	<i>decorticatum</i> (Woodward) Howe	Ramiform and spongy
				dwarkensis Børgesen	Ramiform and spongy
			Halimeda	macroloba Decaisne	Articulated and calcified fronds
				<i>tuna</i> (Ellis and Solander) Lamouroux	Articulated and calcified fronds
		Udote- aceae	Udotea	<i>indica</i> A. and E. Gepp.	Fan like blades
	Clado- phorales			<i>antennina</i> (Bory) Kützing	Filamentous and brush-like tufts in flocks
		Clado- phoraceae	Chaetomorpha	<i>crassa</i> (C. Agardh) Kützing	Filamentous and entangled mass
				spiralis Okamura	Filamentous and coiled

Table 1: Species and thallus diversity of macroalgae in shore platform, Dwarka, Gujarat, India.

Group	Order	Family	Genus	Species	Thallus form and type
	Siphono- cladales		Boodlea	<i>composita</i> (Harvey) Brand	Spongy, branched cushion
		Boodle- aceae Siphonocl adaceae	Cladophora	glomerata (Linnaeus) Kützing	Erect or prostrate filaments and sparsely branched
			Cladophoropsis	<i>javanica</i> (Kützing) P. Silva	Spongy, filamentous and prostrate
Chlorophyta			Dictyosphaeria	<i>cavernosa</i> (Forsskål) Børgesen	Hollow, spherical, irregularly lobed or globose
Chlo			Valonia	aegagropila C. Agardh	Compact vasicular masses
			vaionia	<i>utricularis</i> (Roth) C. Agardh	Loose vesicle like patches
			Valoniopsis	<i>pachynema</i> (Martens) Børgesen	Cushions or spongy patches
				conglobata Kjellman	Leafy and undivided
	Ulvales	Ulvaceae	Ulva	fasciata Delile	Leafy and ribbon- like
				lactuca Linnaeus	Leafy and membranous

T	able 1	(continued):	Species	and	thallus	diversity	of	macroalgae	in	shore	platform	,
Dwarka, O	Gujarat,	, India.	-					_			_	

# CONCLUSIONS

This study reports species and thallus structure of macroalgae sampled in shore platform, Dwarka, India.

This research offer data about which type of macroalgae grow on hard substratum like the studied shore platform.

Nine major types of thallus and 19 sub types of thallus structure are identified. Ramiform and filamentous types of thallus structure are most common on shore platform of Dwarka. Ramiform type of thallus belongs to the Bryopsidales order and filamentous type belongs to the Cladophorales and Siphonocladales orders.

This study is to help in future studies to identify the macroalgae.

Species and thallus diversity of macroalgae can help to identify the substratum, resource inventory and monitoring of macroalgae.

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# INFLUENCE OF ALIEN SPECIES *PISTIA STRATIOTES* L., 1753 ON REPRESENTATIVE SPECIES OF GENUS *SALVINIA* IN UKRAINE

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**KEYWORDS**: *Pistia stratiotes*, aquatic plants, invasive species, pleuston.

#### ABSTRACT

This work purpose was to find the impact of *Pistia stratiotes* L., 1753 - a new species on the territory of Ukraine, on the vitality of other free-floating hydrophytes so-called pleuston - *Salvinia natans* (L.) All., 1785, *S. laevigatum* (Humb. and Bonpl. ex Willd.) Heine, 1968, to determine the degree of stability and competitiveness of these species for resources in natural ecosystems relatively to the undesirable species. To find out the influence of *P. stratiotes* on other free-floating on the water surface plant species which compete for elements of nutrition among each other, we have investigated changes in the content of photosynthetic pigments in *S. laevigatum* and *S. natans*, which were exhibited with *P. stratiotes* for 14 days. Besides changes in water indices, oxygen content, mineralization and pH, were measured.

**ZUSAMMENFASSUNG**: Einfluss der fremdländischen Art *Pistia stratiotes* L. 1753 auf die einheimischen repräsentativen Arten der Gattung *Salvinia* in der Ukraine.

Vorliegende Untersuchungen hatten zum Ziel den Einfluss von *Pistia stratiotes* L., 1753 - eine neu eingebürgerte Art in der Ukraine – auf die Vitalität anderer schwimmender Hydrophyten, dem so genannten Pleuston, wie *Salvinia natans* (L.) All., 1785, *S. laevigatum* (Humb. and Bonpl. ex Willd.) Heine, 1968 zu ermitteln sowie den Stabilitätsgrad und die Konkurrenzkraft dieser Arten als Ressourcen in natürlichen Ökosystemen im Verhältnis zu der neueingebürgerten zu bestimmen. Um den Einfluss von *Pistia stratiotes* L. auf andere Wasserwurzler zu denen sie in einem Konkurrezverhältnis bezüglich der Nährstoffe stehen, herauszufinden, wurden Veränderungen im Gehalt photosynthetischer Pigmente bei *Salvinia laevigatum* und *S. natans*, untersucht und die Arten zusammen mit *P. stratiotes* für 14 Tage in Versuchskonditionen zusammengestellt. Neben Veränderungen in den Wasserzeigerwerten wurden auch Sauerstoffgehalt, Mineralisierung und pH-Werte gemessen.

**REZUMAT**: Influența speciei adventive *Pistia stratiotes* L. 1753 asupra speciilor indigene reprezentative din genul *Salvinia* din Ucraina.

Lucrarea a avut ca obiectiv studierea impactului speciei *Pistia stratiotes* L., 1753 – o specie neofită pe teritoriul Ucrainei – asupra vitalității altor specii plutitoare hidrofite, așa zisul pleuston, cum ar fi *Salvinia natans* (L.) All., 1785 și *S. laevigatum* (Humb. and Bonpl. ex Willd.) Heine, 1968, de asemenea a fost determinat gradul de stabilitate și de competivitate a acestor specii ca resurse în ecosisteme naturale, în comparație cu specia neofită. Pentru a determina influența speciei *Pistia stratiotes* L. asupra altor specii plutitoare pe suprafața apei, cu care se află în relații de concurență în ceea ce privește nutrienții, au fost analizate schimbări în conținutul de pigmenți fotosintetici la speciile *Salvinia laevigatum* și *S. natans*, ele fiind expuse împreună cu *P. stratiotes* timp de 14 zile în condiții de experiment. Pe lângă schimbări în indicatorii apei, a fost măsurat și conținutul în oxigen, mineralizarea și valorile de pH.

# **INTRODUCTION**

Non-aboriginal invasions are one of the greatest environmental problems of the present, which is acute due to the active processes of biotic globalization (\*, 2001; \*\*, 2016; Anastasiu et al., 2017). Problem of extrusion of indigenous species of aquatic plants from the places of their natural existence by invasive plants, which came from artificial growing conditions (technical reservoirs, aquariums) to natural reservoirs, becomes a large scale (May, 2006). Usually, the tropical plants die at low temperatures in winter, but the presence of numerous heat and power plants, industrial enterprises in Ukraine which operate water with a further dumping it in hot water reservoirs, increases the temperature of water in natural reservoirs, creating conditions for hibernation of invasive species (Kazarinova, 2014).

The penetration of the tropical species water lettuce, *Pistia stratiotes*, into the territory of Ukraine and its massive development, which is associated with climate change and rising winter temperatures, is already a real ecological threat to hydrosystems in some regions of Ukraine. Since 1976, this tropical plant began to reclaim water reservoirs in Europe (May, 2006; Sajna et al., 2007; Neuenschwander, 2009; Soloviova, 2009; CABI Invasive Species Compendium online data sheet, 2011), and since 2010 it has been seen in the water bodies of Ukraine, namely in the Kyiv and Kharkiv regions on the rivers Dnipro, Udai, and Siversky Donets (Chorna, 2006; Lushpa, 2009; Mosiakin and Kazarinova, 2014a; Mosiakin and Kazarinova, 2014b). In the summer of 2013, on the river Siversky Donets, a large-scale phytoexpansion of this species was conducted on the aquatic ecosystem, which caused an ecological catastrophe, taking in a short time the channel part and the flood plain basins connected with the main water artery of the region (Vasenko et al., 2013a; Vasenko et al., 2013b). *Pistia stratiotes* are considered malicious weeds (Martins, 2003; Camargo et al., 2003) because in a short period of time, it can completely cover the surface of a small reservoir, dooming it to drain.

*Salvinia laevigatum* does not grow in Ukraine, though it is common in North and South America. The plant has a high invasive potential. Imported from California U.S.A., it has become widespread, displacing local aquatic plants (Haynes, 2000). In Ukraine, this plant is popular among aquarists (Cyrling, 1991), that is why it has a high risk of getting into natural aquatic reservoirs.

In order to preserve the biological diversity of Ukrainian water reservoirs, it is necessary to investigate and control the processes of spreading of aggressive tropical species and their impact on indigenous flora. Therefore, the purpose of the work was to find out the impact of *Pistia stratiotes* (the new species) on the territory of Ukraine, and on the vitality of other free-floating hydrophytes called pleuston (*Salvinia natans* and *Salvinia laevigatum* – the latter also an introduced species) to determine the degree of stability and competitiveness of these species for resources in natural ecosystems relative to the undesirable species.

# MATERIAL AND METHODS

The researched elements were three species of aquatic pleuston plants: invasive species, the effect of which was studied – *Pistia stratiotes* L. (Fig. 1) and two species of the genus *Salvinia* – *Salvinia natans* (L.) All. (Fig. 2) and *Salvinia laevigatum* Humb. And Bonplex Willd. (Fig. 3). *S. natans* is an intrinsic species for Ukraine that occurs in waters and valleys of the Dnipro, Desna, Siversky Donets, Dniester, Danube, Uzh, Latorytsia, and Borzhava rivers, estuaries, artificial reservoirs of Dnipro cascade, forest-steppe and steppe ponds (Dubyna et al., 2003), and belongs to rare species that needs protection (Didukh, 2009).



Figure 1: Pistia stratiotes.



Figure 2: Salvinia laevigatum.



Figure 3: Salvinia natans.

Cultures of aquatic plants were grown in the aquaculture complex of the NSC "Institute of Biology and Medicine" in aquariums of 40-60 liters of settled water at optimum conditions: illumination of 6,000 lux, water temperature 18-25°C, pH 6-7.5.

During the experiment, plants approximately equal in appearance, size and without visible damages were placed in special three liters aquariums filled with settled tap water, and exposed for 14 days under optimal lighting conditions (5,000 lux), water temperature (19-20°C), pH (6-7) and initial mineralization (237 mg/l). According to the experiment design, plants of *P. stratiotes* were also placed into aquariums.

Options for the first experiment:

First option/control – the area of the water surface (S) in the aquarium no *P. stratiotes*; Second option -  $\frac{1}{4}$  part, or 25% S covered by *P. stratiotes*;

Third option -  $\frac{1}{2}$  part or 50% S covered by *P*. stratiotes.

The content of pigments measurement in experimental plants and quantitative indices of oxygen content in water, pH and mineralization were carried out at one, seven, and 14 days.

Determination of the content of pigments was carried out by spectrophotometric method (Havrylenko et al., 1975) on a spectrophotometer Shimadzu UV-1800, for the following wave lengths 440, 644 and 662 nm, and calculated on one g of dry weight (DW).

The oxygen content was measured using a portable oximeter – Hanna, pH – using pH-Meter PH-107, mineralization – using TDS Meter TDS-2.

All biological repetitions and analytical replicates in the experiment were performed at least in triplicate. The data were subjected to statistical analysis using the Microsoft Office Excel software, and were considered significant (according to the Student's t-criterion) at the significance level of  $p \le 0.05$ . Data are expressed as means of replicates  $\pm$  standard deviation.

# **RESULTS AND DISCUSSION**

To find out the influence of *P. stratiotes* on other free-floating water surface plant species which compete for elements of nutrition among each other, we have investigated changes in the content of photosynthetic pigments in *S. laevigatum* and *S. natans*, which were grown together with *P. stratiotes* for 14 days. Besides, we measured changes in water indices, included oxygen content, mineralization and pH.

Since in our previous studies (Olkhovich et al., 2017) the evident changes in the content of photosynthetic pigments by the effects of *P. stratiotes* on submerged water plants were observed only on the 14th day, the evaluation of the content of pigments in pleuston was also carried out on the 14th day.

Yellowing of leaves in the experimental plants (*S. laevigatum* and *P. stratiotes*) and the absence of yellowing and even more intense green coloration of *S. natans* leaves, which were kept together for 14 days, were admitted after visual observations.

According to the results of our studies, the effect of *P. stratiotes* on the content of chlorophyll a and b in *S. laevigatum* on the 14th day of the experiment in both options decreased (from 0.01 mg/g to 0.008 mg/g for 25% S covered by *P. stratiotes*, and from 0.01 mg/g to 0.009 mg/g for 50% S covered by *P. stratiotes*), and content of carotenoids remained unchanged and constant at 0.004 mg/g for 14 days (Fig. 1).

In all variants the chlorophyll a content was predominant, and the ratio of chlorophyll a/ chlorophyll b was doubled, which corresponds to the normal state of the pigment apparatus and the optimal vitality of aqueous plants.

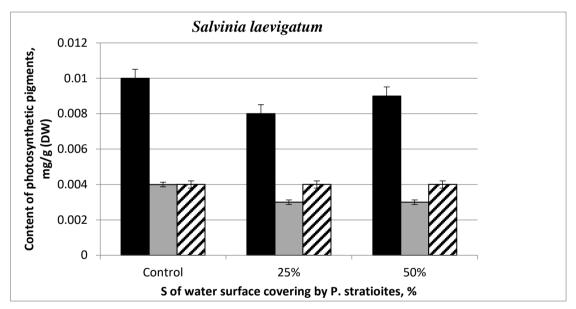


Figure 1: Content of photosynthetic pigments in *S. laevigatum* effected by *P. stratiotes* at 14th day.

The unexpected changes occurred on the 14th day in the content of pigments of *S. natans.* The content of chlorophyll a increased twice in 25% S and 1.5 times in 50% S covered by *P. stratiotes.* The content of chlorophyll b decreased by two times in 25% S, but doubled in 50% S by *P. stratiotes.* The content of carotenoids did not change for option 25% S and increased by 1.5 times in 50% S (Fig. 2). Thus, in the case of wider spreading on the water surface by *P. stratiotes,* an increase of carotenoids content in *S. natans* was observed, and therefore protective mechanisms associated with caroteinoid pool were included. In the case of covering the water surface area by *P. stratiotes* on 25%, protective mechanisms were not used. Probably, this effect was not strong enough for triggering reparatory mechanisms, so it can be considered not threatening for studied species.

Such unexpected increase in the total content of chlorophylls in *S. natans* indicates a positive effect of *P. stratiotes* on this plant. Moreover, it was stronger in the case of fewer plants in the aquarium. But in the case of increasing ratio of biomass per 1 l of water, the positive effect was reduced, as evidenced by the significant rising of the carotenoid content under these researched conditions. It is known that *S. natans* is not a water contentdemanding plant, but more demanding to light, and possibly even a slight decrease in the amount of light that has fallen on the plant due to shading has led to such a rapid increase in the content of chlorophyll a, which is the main photosynthetic pigment. Such an interesting effect may also be caused by changes in water parameters for the long stay of *P. stratiotes*, primarily oxygen content, pH, and mineralization level, which we will discuss in more detail later.

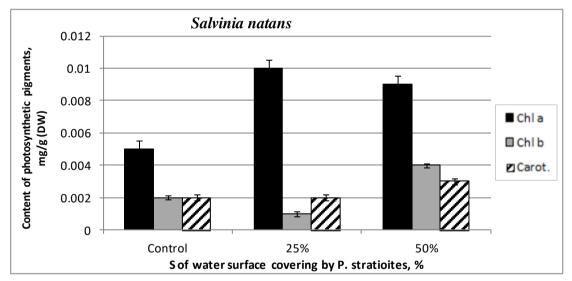
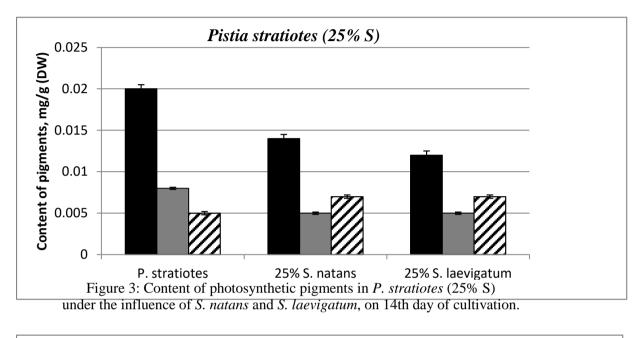
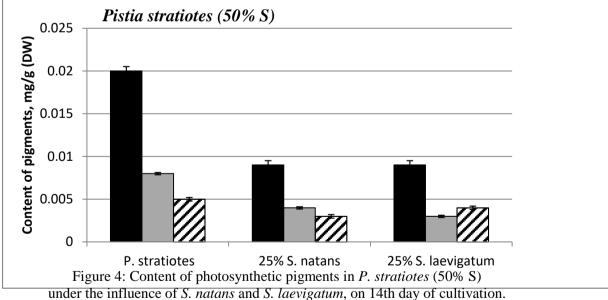


Figure 2: Content of photosynthetic pigments in *S. natans* effected by *P. stratiotes* at 14th day.

Our previous studies showed the negative effects of *Pistia* on the content of pigments and the overall productivity of the submerged water plants – *Vallisneria spiralis* L. and *Ceratophyllum demersum* L. and positive on *Elodea*  $\Box$  *canadensis* Michx. (Olkhovich et al., 2017). By current research, we tried to confirm or deny the negative influence of *P. stratiotes* on pleuston water plants. The obtained results of this influence were not unequivocal.

Since visual observations of morphological changes in the studied free-floating water plants revealed yellowing of leaves not only in *S. laevigatum*, but also in *P. stratiotes*, whose effect on experimental plants was evaluated, we have analyzed the changes in content of photosynthetic pigments in *P. stratiotes* under conditions of co-cultivation of these plants. The results of these studies are presented in figures 3 and 4.





Quantitative indices of the content of pigments convincingly indicate the influence of *S. laevigatum* and *S. natans* on *Pistia* itself. So, the total content of chlorophylls due to contents of chlorophyll a and b were increased, however carotenoid content was reduced. The trend of changes, for both studied species – *S. laevigatum* and *S. natans*, was similar. With increasing density of *P. stratiotes* on the water surface, the impact of other species on it was stronger. Probably, in this case, the competitive impact on the supply of nutrients increases with the increase of *P. stratiotes* biomass per 1 of water, and not in favour of *P. stratiotes*, as evidenced by an increase in the content of pigments in *S. natans*, which was discussed earlier. Since carotenoids expanded the spectrum of photosynthesis, providing absorption of 10 to 20% of the energy of solar quanta, with about 50% of energy absorbed in the short-wave region – the high energy region, these pigments act as auxiliary light absorption by transferring the energy of their electron-excited state to chlorophyll a.

According to the results of our studies, significant changes in the influence of P. stratiotes on the content of the pigments of the studied species in any of the variants were not observed. Even the most significant changes were admitted in the pigment content of P. stratiotes itself, especially in the case of 50% of water surface coverage by P. stratiotes. This testifies to the resistance of the studied pleuston species to P. stratiotes, in contrast to the submerged plant species, primarily due to the absence of direct influence of shading of these species and the decreasing of water temperature under the cover of P. stratiotes.

It is known that in natural water basins of Ukraine, *S. natans* is a rare plant that is even included into the Red Book, its resistance to the influence of *P. stratiotes*, and even the probable increase in productivity in its presence, is impressive. The results of our research indicate that in the case of prolonged competition for energy resources, the possible change in the biodiversity of artificial ecosystems takes place in favour of *S. natans*, which can not be considered as a negative consequence of the after effect of *P. stratiotes*.

In order to assess the influence of *P. stratiotes* on water quality and its suitability for other pleuston species, we have carried out determination of oxygen content, pH of the medium and degree of mineralization of water.

Soluble in water, oxygen is a compulsory component for the existence of most aquatic organisms, including macrophytes. The concentration of oxygen in water influences on the all hydrobionts and the direction of biochemical and hydrobiological processes. When the surface of the water reservoir is covered with plants or a chemical film, and there is no water circulation, then stagnation develops, which leads to the replacement of species of hydrobionts with a narrow ecological amplitude for species with a wide amplitude, which simplifies the species diversity of water basin.

The content of oxygen in water depends on the ratio of two opposite processes: oxygenation and reduction of oxygen in it. Water enrichment with molecular oxygen occurs due to the flow from the atmosphere and its allocation during photosynthesis by water plants (Romanenko, 2001; Bezmaternyh, 2009).

Coastal and aquatic plants, allocating oxygen during photosynthesis, positively affect the oxygen regime of the coastal zone of the water reservoir. Bacteria and algae, which live on the surface of plants, are actively involved in purifying water. In the thickets of coastal and aquatic plants, the phytophilic fauna develops, which also takes part in the self-purification of the water and bottom sediments, with benthic organisms utilizing the organic matter of the silt. The influence of these processes in water increases as well as the content of dissolved oxygen and nutrients increases, its transparency leads to decreasing of the water mineralization and the number of intermediate products of decomposition of organic matter. Photosynthesis in water reservoirs occurs at shallows, mainly in surface layers of water, well-lit and warmed, where most of the aquatic plants are concentrated (Bezmaternyh, 2009).

Free-floating plants on the water surface have advantages over the availability of oxygen in relation to the submerged species, and they are located closest to the light source and have the ability to absorb the largest spectrum of rays of physiologically active radiation. Strongly expanding and intensely covering the water surface, pleuston can block the access of oxygen from the layers of the atmosphere and obscure not only the immersed species that grow under them, but also those growing near these species on the surface, reducing the intensity of photosynthetic processes and the overall productivity of reservoirs. This causes a lack of oxygen in the water basin. At the same time, free-floating species are also actively photosynthetic plants, which probably saturate with oxygen, although stomata, through which oxygen is primarily released, are located on the upper side of the leaf that comes in direct contact with the atmosphere, makes this issue uncertain. In any case, as a positive effect, namely an increase in the level of oxygen, and a negative effect – a decrease in the level of oxygen, will lead to a series of events that will lead to a change in the balance in the reservoir, the consequences of which may be totally unexpected.

We tried to find out what is the oxygen regime in water, where pleuston grow in the presence of a species not typical for Ukrainian reservoirs -P. *stratiotes*. The results of the studies are presented in tables 1 and 2.

Duration of the	Oxygen content, mg/l				
experiment,	Control (pure <i>S. natans</i> )	25%	50%		
day/days		S covered by	S covered by		
uay/uays		P. stratiotes	P. stratiotes		
1	$6.24 \pm 0.01$	$6.24\pm0.01$	$6.24\pm0.02$		
7	$6.29\pm0.04$	$6.82\pm0.01$	$6.7\pm0.05$		
14	$6.85\pm0.03$	$7.73\pm0.01$	$7.52\pm0.04$		

Table 1: Oxygen content in water during cultivation of *S. natans* (25% S) in presence of *P. stratiotes*.

The oxygen content gradually increased from 6.24 mg/l to 6.85 mg/l in the water where only *S. natans* grew (control) for 14 days. The same happened with experimental variants, and the oxygen content in the presence of *P. stratiotes* increased significantly in relation to control. So on the 14th day of the experiment, the oxygen content for 25% coverage of surface area by *P. stratiotes* was 7.73 mg/l, and 50% coverage by *P. stratiotes* was 7.52 mg/l. An increase in the oxygen content in water, which coexisted with *S. natans* and *P. stratiotes*, is well matched with an increase in the content of pigments in *S. natans*. Oxygen enrichment in experimental variants is associated with an increase in the intensity of photosynthesis of *S. natans* influenced by *P. stratiotes*.

Duration of the	Oxygen content, mg/L				
experiment, day/days	Control (pure <i>S. laevigatum</i> )	25% S covered by <i>P. stratiotes</i>	50% S covered by <i>P. stratiotes</i>		
1	$6.24\pm0.01$	$6.24\pm0.01$	$6.24\pm0.02$		
7	$7.93 \pm 0.04$	$7.62\pm0.01$	$7.75\pm0.05$		
14	$7.60\pm0.03$	$7.84\pm0.01$	$7.81\pm0.04$		

Table 2: Oxygen content in water during cultivation of *S. laevigatum* (25% S) in presence of *P. stratiotes*.

At the exposure of pure culture *S. laevigatum* on the 7th day of the experiment the oxygen content increased sharply by 1.69 mg/l, and at the 14th day, it decreased slightly (by 0.33 mg/l). Under the influence of *P. stratiotes*, oxygen content during two weeks also gradually increased from 6.24 mg/l to 7.84 mg/l and from 6. 24 mg/l to 7.81 mg/l for 25% S and 50% S coverage by *P. stratiotes*. Thus, in the presence of *P. stratiotes*, the oxygen content was slightly higher (0.21-0.24 mg/l) than in the control option. Such changes in the content of oxygen in water can not be considered threatening for the existence of other hydrobionts, including the studied pleuston species, because they are normal for natural reservoir fluctuations. It is known that near the thickets of *Potamogeton*, *Vallisneria*, *Elodea* and other immersed plants, the concentration of dissolved oxygen in water on summer days can rise to 10-12 mg/l, and to 6-7 mg/l in non-growth regions (Korotkevich, 1976; Ipatova and Dmitrieva, 2006). Pleuston also emit oxygen from the leaves to the roots and stems, thus influencing the formation of the gas regime of the surface layers of water.

The pH indicator characterizes the active reaction of the medium, on which the development of hydrobionts depends. During intense photosynthesis, the pH can rise to 10 and above, due to almost complete disposal of water from free carbon dioxide by water plants, and increasing the alkalinity of the medium with carbonates (Bezmaternyh, 2009). The pH of water changes throughout the day in natural reservoirs. At night, the water is acidified as a result of separation during the breathing of carbon dioxide. In the afternoon, the alkalinity of water increases due to the consumption of  $CO_2$  by plants (Romanenko, 2001). The pH is reflected in the water-salt metabolism of hydrobionts. The concentration of hydrogen ions not only determines the limits of the distribution of aquatic organisms, but also affects their life.

We tried to find out what is the pH of the water in which the pleuston is exposed in the presence of a new invasive species -P. *stratiotes*. The results of the studies are presented in tables 3 and 4.

Duration of the	Oxygen content, mg/L				
experiment, day/days	Control (pure <i>S. natans</i> )	25% S covered by	50% S covered by		
uay/uays		P. stratiotes	P. stratiotes		
1	$7.7\pm0.04$	$7.7\pm0.5$	$7.7\pm0.02$		
7	$8.6\pm0.01$	$8.4\pm0.03$	$8.6\pm0.01$		
14	$8.7\pm0.01$	$8.4\pm0.02$	$8.3\pm0.02$		

Table 3: pH of the water during cultivation of *S. natans* (25% S) in presence of *P. stratiotes*.

The initial level of pH to the plant exposure was 7.7. During the week it reached the level of 8.3-8.6, namely, the studied species, due to the primarily photosynthesis and respiration, contributed to the sublimation of the environment. There was no significant difference in *Salvinia* exposure separately and compatible with *Pistia*. It can be stated that the effect of *Pistia* on *Salvinia* does not occur due to changes in the pH of the medium.

Table 4: pH of the water during cultivation of *S. laevigatum* (25% S) in presence of *P. stratiotes*.

Duration of the	рН				
experiment, day/days	Control (pure <i>S. laevigatum</i> )	25% S covered by <i>P. stratiotes</i>	50% S covered by <i>P. stratiotes</i>		
1	$7.7\pm0.04$	$7.7 \pm 0.5$	$7.7\pm0.02$		
7	$8.4\pm0.01$	$8.3\pm0.03$	$8.3\pm0.01$		
14	$8.2\pm0.01$	$8.4\pm0.02$	$8.2\pm0.02$		

Similar processes were observed at the exposure of *S. laevigatum*. The pH level of 7.7 (the water on which the plants were originally grown) in seven days changed to 8.2-8.4 and remained stable for the next week. The alkalinization of water in the presence of plants occurs due to the consumption of  $CO_2$  by plants during photosynthetic activity. No significant difference between the experimental variants was observed, and therefore the influence of *P. stratiotes* on the change in pH of the medium did not occur.

An important role in the life of hydrobionts is played by salt dissolved in water. The values for aquatic organisms have the following indicators: ionic composition of water and salinity – the total amount of water dissolved in mineral salts. The bulk of the saline composition of natural water is represented by the ions:  $HCO_3^-$ ,  $CI^-$ ,  $SO_4^{-2-}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ , and  $K^+$ . Fresh and salty waters differ in the ratio of these ions. In fresh waters, hydrocarbons number about 60% of the total amount of salts, and less than 10% are chlorides. The level of mineralization of water is an important indicator of the status of the water reservoir and the possibility of existence in it certain species of aquatic plants. As the mineralization of water increases, its density and viscosity also increase (Bezmaternyh, 2009; Kurilov, 2009).

We tried to find which changes of the level of mineralization would occur in water for the compatibility of the studied pleustons. The results of the studies are presented in tables 5 and 6.

Duration of the	Water mineralization, mg/l				
Duration of the experiment, day/days	Control (pure <i>S. laevigatum</i> )	25% S covered by <i>P. stratiotes</i>	50% S covered by <i>P. stratiotes</i>		
1	$240 \pm 2.0$	$240 \pm 4.2$	$240 \pm 2.7$		
7	271 ± 6.1	$254\pm7.8$	$260 \pm 5.3$		
14	$313 \pm 7.5$	$232 \pm 3.1$	$227 \pm 4.1$		

Table 5: Water mineralization at exposure of S. natans (25% S) in the presence of P. stratiotes.

The mineralization of water in aquariums in which the *S. natans* were exhibited, has changed in the control variant from one day to 14 days from 240 mg/l to 313 mg/l, and under the influence of *P. stratiotes:* from 240 mg/l to 232 mg/l, and from 227 mg/l to 276 mg/l) at 25% S and 50% S respectively. So, on the 14th day a significant decrease in mineralization in experimental variants relative to the control was marked, which may be the evidence of intense absorption of mineral elements by invasive species that's characterized by high growth potential. In the control variant (pure *S. natans*), during two weeks a gradual increase in mineralization (240 mg/l – one1 day, 270 mg/l – seven days, 313 mg/l – 14 days) was observed, and in the experimental group an increase was admitted at the 7th day (14 mg/l at 25% S and 20 mg/l at 50% S), and then, on the 14th day of exposure a sharp decline in mineralization (on seven mg/l at 25% S and 13 mg/l at 50% S) in relation to the starting value (240 mg/l).

Table 6: Water mineralization at exposure of *S. laevigatum* (25% S) in the presence of *P. stratiotes*.

Duration of the	Water mineralization, mg/l				
experiment, day/days	Control (pure <i>S. laevigatum</i> )	25% S covered by <i>P. stratiotes</i>	50% S covered by <i>P. stratiotes</i>		
1	$240 \pm 2.0$	$239\pm4.2$	$240 \pm 2.7$		
7	$268\pm6.1$	$234\pm7.8$	$236\pm5.3$		
14	$239 \pm 7.5$	$233 \pm 3.1$	$221 \pm 4.1$		

In studies with *S. laevigatum* the level of mineralization increased by 18 mg/l on the 7th day, but on the 14th day returned to the original value (239 mg/l). The influence of *P. stratiotes* was manifested in reduction of the mineralization level. Besides, in the case of an increase of the number of *P. stratiotes* this effect was stronger, thus, at 14th days at 25% S it decreased by six mg/l, and at 50% S – by 19 mg/l. In the control variant, the level of mineralization on the 14th day was the highest (239 mg/l), and at 50% S by *P. stratiotes* – the lowest (221 mg/l).

# CONCLUSIONS

Significant negative changes due to the influence of *P. stratiotes* on the content of pigments of the studied species (*S. natans*, and *S. laevigatum*) did not occur, while there was a decrease in the content of pigments in *P. stratiotes* itself in the presence of *S. natans*.

In *S. natans* plants the content of chlorophyll a increased 1.5-2 times, and the content of chlorophyll b increased twice in 50% S by *P. stratiotes*. The content of carotenoids did not change and increased 1.5 times at 25% S and 50% S by *P. stratiotes* respectively.

In the presence of *P. stratiotes*, the content of chlorophyll in *S. laevigatum* during the studied period did not significantly decrease (from 0.01 mg/g to 0.008 mg/g), and carotenoids did not change at all. In all variants the chlorophyll a content was predominant, and the ratio of chlorophyll a/chlorophyll b was greater than two, which corresponds to the normal state of the photosynthetic pigment apparatus and the optimal vitality of aqueous plants.

The oxygen content in water on which only *S. natans* grew had gradually increased from 6.24 mg/l to 6.85 mg/l for 14 days, and in the presence of *P. stratiotes* at 25% S of coverage to 7.73 mg/l, and at 50% S – up to 7.52 mg/l. In the presence of *P. stratiotes* in variants with *S. laevigatum*, the oxygen content was also higher (by 0.21 and 0.24 mg/l, respectively) than in the control version.

It has been established that in the presence of *S. natans* and *S. laevigatum*, the level of mineralization of water has been increased, and on the contrary in the presence of *P. stratiotes* decreased. The pH of water remained unchanged in the presence of *P. stratiotes* with the species studied.

In the case of prolonged presence (during 14 days) during competition for energy resources, alteration of the species composition of artificial ecosystem in favour of *S. natans* is possible, which can be considered as a positive consequence of the after effect of *P. stratiotes*. Since *S. natans* is a rare plant in the natural waters of Ukraine, which is even included into the Red Book, its resistance to the influence of *P. stratiotes* and even the probable increase in productivity under its presence is pleasantly impressive.

Extension of the range of distribution and acceleration of the course of invasive *P. stratiotes* in the territory of Ukraine, which have been observed in recent years, will unequivocally cause changes in groups of pleuston species. This species, in cases of rapid spreading in Ukraine's water basins, can lead to environmental problems associated with the alterations in the composition of aquatic ecosystems, as well as the simplification of their biodiversity.

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# RANGE MAP AND DISTRIBUTION OF *LUCIOBARBUS BARBULUS* HECKEL 1847 IN THE TIGRIS AND EUPHRATES RIVER BASINS

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**KEYWORDS**: *Luciobarbus barbulus*, type locality, geographical distribution, status, Iran.

### ABSTRACT

*Luciobarbus barbulus* Heckel, 1847 was described from water bodies around Shiraz and the Qara Aqaj River, one of the main rivers in Shiraz City, Iran. In the present study localities, a geographical range map of *Luciobarbus barbulus* and new data on the present status of this species are presented. In our latest sampling we assessed for the first time the presence of this species in the Lesser Zab River where no specimen had been collected in the past on this part of the river. The detailed distribution of *Luciobarbus barbulus*, based on the captured specimens, and literature records of this species are mentioned.

**ZUSAMMENFASSUNG**: Verbreitungskarte und Verteilung von *Luciobarbus barbulus* Heckel, 1847 in den Einzugsgebieten der Flüsse Tigris und Euphrat.

Die Art *Luciobarbus barbulus* Heckel, 1847 wurde aus dem benachbarten Gewässersystem Shiraz, im Qara Aqaj-Fluss, einem der Hauptflüsse der Stadt Shiraz im Iran festgestellt. Vorliegende Arbeit umfasst die Untersuchungen zu den Fundorten, die Verbreitungskarte von *Luciobarbus barbulus* sowie neue Angaben zum gegenwärtigen Zustand der Populationen der Art. In unserer letzten Bestandaufnahme wurde ein neues Vorkommen der Art im Leaser Zab Fluss festgestellt und zwar in einem Abschnittp, in dem sie bisher nicht vorgefunden wurde. Außerdem sind Einzelheiten zur Verbreitung von *Luciobarbus barbulus* angegeben, die auf den Sammelbelegen und auf Fachliteraturangaben beruhen.

**REZUMAT**: Harta de răspândire și distribuția speciei *Luciobarbus barbulus* Heckel, 1847 în bazinele hidrografice ale râurilor Tigris și Euphrat.

Luciobarbus barbulus Heckel, 1847 a fost descris din corpuri de apă din jurul Shirazului și din râul Qara Aqaj, unul dintre râurile principale din orașul Shiraz, Iran. Pentru localitățile luate în considerare, au fost prezentate o hartă a distribuției geografice pentru Luciobarbus barbulus și noi date asupra statutului prezent al acestei specii. În ultima noastră campanie de prelevare am evaluat pentru prima dată prezența acestei specii în râul Lesser Zab, în care nici un specimen de acest fel nu a mai fost colectat în trecut în această parte a râului. A fost menționată distribuția detaliată a speciei Luciobarbus barbulus, bazată pe indivizii capturați și semnalările din literatură.

# **INTRODUCTION**

Fish, a limited resource, are not only a source of food for human consumption but also have many additional benefits, such as ornamental, hygienic, and industrial uses (Coul, 2002, Bănăduc et al., 2016; Del Monte-Luna et al., 2016). Furthermore, they are an important resource for the drug industry (Moyle, 1988). Despite their value, freshwater fish are probably the most threatened of all aquatic vertebrates (Moyle, 1988). The threat against them is likely to worsen as the demand for food and conflicts over their use increase, a consequence of climate change and drought (Valiallahi, 2015). Moyle and Leidy (1992) estimated that 20% of the world's freshwater fish fauna is extinct or in danger of becoming extinct in the near future.

The study of the ichthyofauna of the big rivers of the world is a principal concern among researchers in aquatic biology and ecology (Greenwood, 1976; Rainboth, 1996; Rinne et al., 2005; Tweddle, 2010). The barbel species of Western Iran are environmentally and economically important. Some of them are the largest lotic freshwater species, which will grow in length and weight to 170 cm and 120 kg, respectively. There is little information on the taxonomy, biology, geographical distribution or environmental status of this species. *Luciobarbus barbulus* Heckel, 1847 is one of the important large lotic freshwater fish species in the study area. One of the main spawning habitats and nursery grounds of the genus *Barbus sensu lato* is the Tigris-Euphrates River basin in Iran, Iraq, Turkey, and Syria, from where there are relatively few reports on the fish fauna. (Valiallahi and Shakiba, 2015)

The genus *Barbus sensu lato* Cuvier and Cloquet, 1816 (Cuvier and Valencienes, 1828-1849) has been split into a number of genera which are now finding general acceptance (Coad, 2019). Some of these genera or subgenera are: *Barbus, Luciobarbus, Carasobarbus,* and *Arabibarbus.* The aim of this paper is to outline the geographical range map of *Luciobarbus barbulus.* Like many other Cyprinids, the *Barbus* genus was long included in *Barbus.* It appears to be a very close relative of the typical barbells – which include that genus type species *Barbus barbus,* and may well warrant inclusion in *Barbus.* Many modern authors prefer to consider it a subgenus instead. It is, moreover, not entirely clear what species to place in *Luciobarbus* if it is deemed valid. The IUCN argues for a rather inclusive circumscription. Notwithstanding the taxonomy and systematics of this ill-defined assemblage, their closest living relative is probably *Aulopyge huegelii* (Casal-Lopez et al., 2015).

There are relatively few papers or new information available about *Luciobarbus* barbulus or other related species, except some checklists in the databases of the California University Catalog of Fishes (\*, 2018). These papers have been focused on the distribution of these taxa in Tigriss and Euphrates basin in Iran, Iraq, Syria, and Turkey.

Despite the economic and conservation importance of this fish species there are some contradictions in the geographical distribution and differentiation of this species and other similar species. During recent decades *Luciobarbus barbulus* has been reported and described several times or has been placed into synonymy with other related species. Also, there have been conflicting views on the morphological character of this species. (Valiallahi, 2000a, b)

The possible syntype of *Luciobarbus barbulus* (NMW 53957) was examined by Almaça (1986). He states that a probable syntype of *Luciobarbus barbulus* (from Kara Agatsch, Iran) was found, but it is in such bad condition that nothing can be recognised by its examination; curiously, all the specimens which could be identified with the original description of *Luciobarbus barbulus* proceed from the Mediterranean coast of Western Asia: Lake Tiberias (Israel), Orontes River and Lake Homs (Syria), and Lake Antakya (Turkey). As *Luciobarbus barbulus* was described from Iran there is here an intriguing geographical

problem which cannot be solved without more information (Almaça, 1983). Regarding similarity and overall morphology, it was realised that there is a close affinity between *Luciobarbus barbulus* and the *bocagei* group (Almaça, 1984b, 1991). Later Almaça (1984a, 1984b, 1986, 1991) retained *barbulus* as a full species known only from the Levant, despite Heckel's record from the Qarah Aqaj (Mand) of Fars, Iran (Coad, 2014). The aim of the current article is to give some real records of catchment for these important taxa.

In 2000 the *Barbus* species of Iran were revised. The revision was based on nearly all *Barbus* specimens that were held at the Canadian Museum of Nature (CMN) and sampled fishes that were caught in water bodies of western Iran from 1996 to 2000. The same fishing programme was repeated from 2013 to 2016 in order to find new records on the present status of *Barbus* species (Valiallah, 2015).

The details of distribution and historical biogeography of *Luciobarbus barbulus* were plotted on maps based on the captured specimens, and available data from literature records.

### MATERIAL AND METHODS

Between the years 1995-2000, samples were collected in water bodies of western Iran. The same fishing programme was repeated from 2013 to 2016 in order to find new records on the present status of *Barbus* species. The sites where *Luciobarbus barbulus* was found and the sites from literature review have been recorded together in tables 1 and 2.

The specimens were preserved in 10% formalin for 24 hours then transferred to 70% alcohol, labeled and deposited in the Collection of Environmental Science Laboratory in Sahid Rjaee Teacher Training University (SRTTU) Lavizan, Tehran, Iran.

After identifying the specimens, the longitude and latitude of the sampling stations were transferred on to maps and the longitude and latitude coordinates of fishing stations were plotted.

The distribution of this species is based on the localities of newly caught specimens as well as available data on:

– samples from western Iran, collected by Valiallahi et al. since 1985 (J. V. C. – Jalal Valiallahi Collection).

- specimens in the Canadian Museum of Nature collections (C. M. N.)

The plotting of *Luciobarbus barbulus* distribution has been done based on all specimens that have been caught in the Tigris and Euphrates basins or reported from literature by different authors, and 34 specimens preserved in C. M. N. (the details on catalogue number can be available on request).

## **RESULTS AND DISCUSSION**

The original describer of *Luciobarbus barbulus* Heckel, 1847 (Heckel, 1847) stated that the water systems around Shiraz yielded 15 fish species. *Luciobarbus barbulus* was one of 15 species among specimens collected by Theodor Kotschy (1841-1842), in the water system around Shiraz and was sent to Heckel in the Vienna Museum (N. M. W.). Heckel described these 15 fish species before as barbels of Syria. These nominal species were: *L. mystaceus* Heckel, 1843, *L. scheich* Heckel, 1843, *L. kersin* Heckel, 1843, *L. pectoralis* Heckel, 1843, *L. rajanorum* Heckel, 1843, *L. esocinus* Heckel, 1843, *L. xanthopterucs* Heckel, 1843, *Barbus grypus* Heckel, 1843, *Labeobarbus kotschyi* Heckel, 1843, *Barbus lacerta* Heckel, 1843, *Barbus scincus* Heckel, 1843, *Barbus luteus* Heckel, 1843, *Barbus plebejus* Bonaparte, 1832, *Barbus sharpeyi* Günther, 1868, and *Barbus subquincunciatus* Günther, 1868.

# **Description of the type locality**

In the literature for *Luciobarbus barbulus* localities, occurred errors during transcriptions and translations, for example Geré is incorrect because around Shiraz there is the name of Gereh that is a mountain with lat./long.  $31^{\circ}52'$  N –  $50^{\circ}21'$  E that actually is not the type locality. There is a population in the centre and a branch of a river with the name of Jereh ( $29^{\circ}15'$  N –  $51^{\circ}58'$  E) that most presumably is the correct name. Pronunciation of Kara-Agatsch in English may be Gharah Aghaj = Qarah Aqaj (means black wood or black tree in Turkish). The type locality of *Luciobarbus barbulus* is near the village of Jereh, the Qarah-Agaj = or Mond River, Fars. Heckle, for the Syrian specimens' locality, refers to the Quwayq, Naher = Strem near Aleppo  $35^{\circ}59'$  N –  $37^{\circ}02'$  E. (Heckel, 1846-1849)

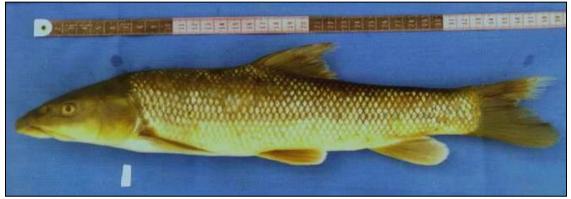


Figure 1: L. barbulus TL 402 mm, Gamasiab River; photo Mohammad Ghazi – 1996.

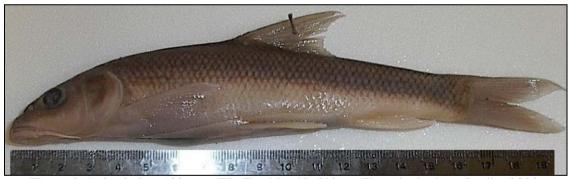


Figure 2: L. barbulus, 182 mm, TL, Tag No. 40, Shiraz, Firozabad; photo Izadi – 2000.

## Shiraz water bodies

About the local waters in the type locality, Heckel refers to a short introductory note by Kotschy. Heckel states that Shiraz ( $29^{\circ}36 \text{ N} - 52^{\circ}32 \text{ E}$ ) is located at an altitude of 1,219.2 m behind two mountain ranges that run parallel to the Persian Gulf; in its vicinity there are three different river systems: Kor, Fahliyan, and Aghaj.

The smallest includes in its area the town of Shiraz (29°45 N – 53°00 E) itself. It is formed by the Kokn Abad Creek and most of its waters flow into the many irrigation canals of the plain, and therefore its actual riverbed dries up completely during the summer. Downstream of the town, this creek combines with the Sadi (29°37 N – 52°35 E), which arises from Cliffside springs, and together they drain into the southward salt lake Namak Daria

(Tabs. 1 and 2). East of Shiraz, the Band-E – Amir, (known as Araxes) flows with its small tributaries through the higher Persepolis Plain. Their waters flow into another salt lake, which according to some information, is supposed to have a subterranean connection with the previously mentioned neighbouring lake. In the high mountains of Kuh-Noor, Northwest of Shiraz, arises the Qareh Agaj River; it flows in a southward arc into the Persian Sea. On the chord of this arc there is a high alpine lake, Dariya-e Kaserun or the lake of Kaserun, and a second lake named Parishan. The waters of both lakes flow into the Qhara-Aqaj. Parishan and Dariya-e Kaserun or Lake Kazerun are both alternative names for Lake Famur (Heckel 1846-1849).

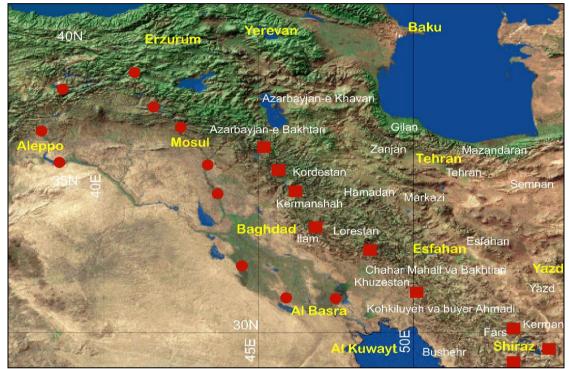


Figure 3: Type locality, and geographical range distribution of *Luciobarbus barbulus*, reported in literature •, the localities where *Luciobarbus barbulus* has been caught by Valiallahi et al.<sup>■</sup>; this map has been prepared by: ArcGIS<sup>®</sup> 10.3, 2018.

Also, Izadi (2000) and Moradi Esmaeil (2013) reported this species in Shiraz, and Shkiba et al. (2015) reported it in Laser Zab, sampling this species from Zab River. This sampling indicated the wide distribution of this species from the north-west to south-west of Iran (Tab. 1, Figs. 1 and 2). Izadi (2000), carried out a fishery programme to identify the status of the freshwater fishes of Shiraz (Fars Province) during 1998-2000. He reported *Luciobarbus barbulus* at Noor Abad (Pol E, Totestan, Garab Village), Sepedan (Shesh per River), Firoz Abad (Ghaleh Doghtar), Ney Riz (Pahnaveh), and Darab (Cheshmeh Golabi). Noor Abad and Sepedan are in the Zohreh River Basin and Firoz Abad is located in the Mond Basin, and Ney Riz and Darab are located in the Kol Basin (Izadi, 2000). Moradi Esmaei (2013), who carried out another programme of catching fish species at Salman Dam basin and Qara Aqaj River territory and he reported *Barbus* species. The Qarah Aqaj River is one of the most important rivers in Shiraz, its water used for drinking and agricultural purposes. Constrictions of the Salman Farsi Dam in Gher and Karzeun districts and plans for construction of the Kovar Dam on this river show the importance of this river in Fars Province (Valiallahi and Moradi Kochi, 2013, 2015, unpublished data).

The Zagros Mountain river system in western Iran at some places holds the upper branches of Tigris River and finally joins and flows as the Shatt Al-Arab to the Persian Gulf. The area surrounding the Shatt Al-Arab consists largely of alluvial lands and swamps in the south of Iran (Fig. 3), and is one of the main habitats, spawning, and nursery grounds of *Barbus* species including *Luciobarbus barbulus* and other related species.

Table 1: type locality and geographical range distribution of *Luciobarbus barbulus*, reported in Iran in literature, latitude and longitude are in decimal degrees (DD).

	Province and	Sample locations	Collector		
	river	with GPS coordinates	and date		
	Fars,	Kokn Abad Creek, Sadi Spring, East of Shiraz, known	Heckel,		
	Qarah Aqaj	as Araxes, Band-e-Amir, lat.: 29.777532, long.:	1846-		
		52.851913, Qarah Aqaj, Fars, Siakh Darengan Rd,	1849;		
		Iran, lat.: 29.475921, long.: 52.346403, Kazerun, or	Theodor		
Iran		lake Famur. Fars, Famour-Parishan, Iran lat.: 29.521189	Kotschy		
		Long.: 51.813498 Dariya-e Kaserun or Lake	1841-1842		
	Fars,	Noor Abad, (Pol E, Totestan, Garab Village), Sepenan			
	Zohreh Basin	(Shesh per River), Fars, Yasuj-Shiraz Rd, Iran lat.:	Izadi, 2000		
		30.173625, long.: 52.043288			
	Fars,	Firoz Abad, (Ghaleh Doghtar) Fars, Kavar-Firuzabad	In di 2000		
	Mond Basin	Rd, Iran, lat.: 28.92026, long.: 52.528762	Izadi, 2000		
	Fars, Kol	Ney Riz, (Pahnaveh); Darab (cheshmeh Golabi).	Ind: 2000		
	Basin		Izadi, 2000		

#### Zoogeography and distribution

*Luciobarbus barbulus* was reported by different authors, who have tried to describe it. Some examples are: Almaça (1991), Günther (1874), based on Heckel's original description (Heckel, 1846), Sauvage (1884), and Pellegrin (1923), based on specimens from the Orontes (Lake Homs, Hammah, and Antakya), and Khalaf (1961) on specimens from Iraq, all present descriptions of *Luciobarbus barbulus*. Berg (1949a, b), based on Heckel's original description, reported four specimens from Karun Basin in Khuzistan, Iran, that were apparently gathered by Zarudnyi in his travels throughout Iran in 1896, 1900-1901 and 1903-1904. Khalaf (1961) presents *Luciobarbus barbulus* on specimens from Iraq. The Iraqi fish fauna including *Luciobarbus barbulus*, were described by Khalaf (1961) who recorded 45 species, while Mahdi (1962) described the same number of species (Al-Hamed, 1966; Mahdi and Georg, 1969; Al-Nasiri and Shamsul-Hoda, 1976; Al-Daham, 1977; Faddagh et al., 2012).

The Iraqi fish fauna was recently reviewed by Coad (1991, 2009). Faddagh et al. (2012) for their DNA analysis via RAPDs have collected 1,200 specimens of cyprinids, including *Luciobarbus barbulus*, and related species from three separate sites in the Shatt Al-Arab River in the governorate of Basrah contain Al Qurnah 31°017.928 N – 47°26 31.242 E, Garmat Ali and Abul-Khaseeb 30°2652.029 N – 28°455.848 E (Faddagh et al., 2012). Mahdi (1962) from Iraq reported *Luciobarbus barbulus* in Qezilja River (Leaser Zab). Saadati (1977) reported eight specimens from the tributaries of the Saymareh and Tigris rivers.

Jawad (2012a, b), and some other Iraqi researchers has reported the presence of this species in Iraq. Erdoğan et al. (2015), and some other Turkey's researchers report *Luciobarbus barbulus* in Keban,  $38^{\circ}4815.479$  N –  $38^{\circ}4538.367$  E. Kralkizi Dam Lake,  $38^{\circ}2124.243$  N –  $40^{\circ}14.338$  E, Hazar Lake, and the Euphrates and Tigris basins in Turkey (Fig. 3; Tabs. 1 and 2). Kefah Naser and Fatima (2017) have caught and examined *Luciobarbus barbulus* along the Tigris River near Al-Graiat region in Baghdad Province during July 2015 – March 2016 (Kefah Naser and Fatima, 2017). Also Laith et al. (2016) have found *Luciobarbus barbulus* in Himreen Lake in summer of 2014 to spring of 2015 (Laith et al., 2016).

Table 2: Localities where *Luciobarbus barbulus* individuals were caught by Valiallahi J. et al.

J. et a	al.		
	Province and River	Stations	Collector and date
	Kermanshah	Mir Azizi, AH2, Iran, Gharban, Cham Heydar, Gorgiwand,	Valiallahi
	Ghamasiab	Samanghan, Hashem Abad Sofla, Ghzarud, Polcheher,	1995-1997
		Qozevand, Farash, Biston, Amalh, Ali Abad, Nazeliyan, Ghaross	
	Harsin	Polcheher, lat. 34.335685, long. 47.425455 Sorkhehdeh,	Valiallahi
	Ghamasiab	Shahmaliky, Azez Abad, Zangichga, Shan Abad	2013-2015
	Kermanshah	Khezer Zendeh, Kolocheh, lat. 34.700625, long. 46.890614	Valiallahi
	Razavar	Kholocheh Dam lat. 34.69378, long. 46.889756	1995-1997
	River		
	Kermanshah	Ravansar, Rika, Sarab Nelofar, Galanjeh, Karam Ali Bag,	Valiallahi
	Gharahso	Brimavand, Jafar Abad, Gandomiyan, Sarab Jaberi, Garab	1995-1997
	or	Sofla, Jan Jani, Pol-e-Naderi, Shorbelagh, Doro Faraman,	
_	Quraso River	Dinavar, Boz Abad, Ghozivand	
Iran	Kermanshah	Kashabah, Jafar Abad, Shakabayan, Poshtareza Bala,	Valiallahi
Ι	Mereg River	Rashbarg, Chahar Zabar Bala	1995-1997
	Kermanshah	Ghasershirin, lat. 34.46, lon. 45.5925	Valiallahi
	Alvand River		1995-1997
	Lorestan,	Darb Gonbad, lat. 33.706491, long. 47.152376	Valiallahi
	Saymareh		1995-2018
	River		
	Lorestan,	Roberoy Gombad Saymareh dam	Valiallahi
	Saymareh		1995-2018
	Pyranshaher	West Azerbaijan, Mirabad – Piranshahr Rd, Iran lat. 36.555706, long., 45.249081	Valiallahi
	Leaser Zab	Sakiba,	
			2014
	Khuzestan,	Karkheh	Valiallahi
	Karun River		1995-2018
	Dez	Dezful, Shohada Blvd., Iran, lat. 32.411922, long.	Valiallahi
		48.432938	1995-2018

Table 2 (continued): Localities	where	Luciobarbus	barbulus	individuals	were	caught
by Valiallahi J. et al.						

	Quwayq, Naher	Quwayq, Naher = Strem near Aleppo	Heckel, 1846-1849
Syria	Orontes, Lake Homs	Orontes (Lake Homs, Hammah, and Antakya Qattinah, Syria lat. 34.644507, long. 36.588593	Sauvage, 1884 and Pellegrin 1923
Iran	Pyranshaher Leaser Zab	Qezilja River West Azerbaijan, Iran Kani eshkot, lat. 36.575353, long. 45.240197	Mahdi Nuri 1962
Iraq	Basrah, Basrah	Qurnah Iraq, lat. 31.00498, long. 47.442012 meters Garmat ali, Abul-Khaseeb Basrah, Iraq, lat. 30.458736, long. 48.033428	Faddagh, et al. 2012
Turkey	Upper Tigris	Keban, Değirmenbaşı Keban/Elâzığ, lat. 38.8043, long. 38.760656, Kralkizi Dam Lake, Hazar Lake D885, 23900 Sivrice/Elâzığ, lat. 38.476707, long. 39.321785 and Euphrates and Tigris river basins part of Turkey	Çiçek et al 2015, Cüneyt Kaya1 2016, Ünlü and Balcı 1993a

## CONCLUSIONS

Luciobarbus barbulus was one of the 15 species among the specimens collected by Theodor Kotschy 1841-1842, in the water system around Shiraz and was sent to Heckel in the Vienna Museum (NMW). Heckel 1848 named Luciobarbus barbulus when he encountered the samples from Shiraz although he had seen the samples from Kueik or Quiek near Halab or Aleppo in Syria. So for the first time he described the new species (Luciobarbus barbulus) from Shiraz, which was similar to the previously described species Luciobarbus mystacus. In conclusion Luciobarbus barbulus is a valid species and the type locality of this species is Qarah Aqaj River, Shiraz, Iran.

This species has been identified and verified before; in this paper we have reported the recently collected specimens from new localities. The recent record of *Luciobarbus barbulus* shows that this species has a wide distribution range in western Iran, also according to the literature reviewed, from west and north-west Turkey, Iraq and Syria.

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# THE FISH FAUNA OF THE ZARINEH RIVER (URMIA LAKE BASIN) DOWNSTREAM SECTOR – CONSERVATION AND MANAGEMENT

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**KEYWORDS**: ichthyofauna, exotic species, threats, anthropogenic activities, Iran. **ABSTRACT** 

This study aimed to investigate the fish fauna in the downstream sector of the Zarineh River, Urmia Lake basin. Seven stations were sampled along the Zarineh River before and after Shahin-Dezh City to help gain a better understanding of human activities on its fish fauna. The results revealed 11 fish species, which belong to three families, including Cyprinidae, Nemacheilidae and Gobiidae. Nine of them were Cyprinidae. Out of 11 species, five species, *Carassius gibelio, Rhodeus amarus, Pseudorasbora parva, Hemiculter leucisculus* and *Rhinogobius lindbergii* were exotic that were unintentionally introduced to the Zarineh River along with Asian carps. The threats to fish fauna are categorized into two categories: natural and anthropogenic. According to the observations, anthropogenic activities such as overfishing, pollutions, sand and gravel extraction, dam construction and introduction of exotic fish species were among the main threats for the ichthyofauna of the Zarineh River.

**RÉSUMÉ**: Etude de la faune piscicole en aval de la rivière Zarineh (Bassin du lac Urmia) – conservation et gestion.

Le but de cette étude était d'examiner la faune piscicole en aval de la rivière Zarineh, dans le bassin du lac Urmia. Dans l'objectif de cette étude, sept stations étaient situées tout au long de la rivière Zarineh (Shahin-Dezh) pour réaliser des échantillons. Le résultat montre qu'il y a 11 espèces de poissons y compris Cyprinidae, Nemachelidae et Gobiidae. D'après les résultats la famille Cyprinidae était la plus diverse en aval de la rivière Zarineh. Les menaces concernant la faune piscicole sont classées en deux catégories: naturel et anthropique. Parmis ces menaces, les innodations, les maladies, les plantes aquatiques, la sécheresse et les maladies naturelles ont été mentionné. Aussi, les menaces anthropiques sont: la surpêche, les différents types de pollution, l'extraction de sable et de gravier, la construction de barrage et l'introduction de poissons exotiques.

**REZUMAT**: Studiul faunei de pești din partea de aval a râului Zarineh (bazinul lacului Urmia) – conservare și management.

Scopul studiului a fost investigarea ihtiofaunei din partea de aval a râului Zarineh, din bazinul lacului Urmia. Pentru aceasta, șapte stații pentru prelevare de probe au fost amplasate de-a lungul râului Zarineh (Shahin-Dezh). Rezultatele arată că sunt 11 specii de pești, aparținând la trei familii: Cyprinidae, Nemachelidae și Gobiidae. Pe baza rezultatelor, familia Cyprinidae cu nouă specii, a fost cea mai diversă familie. Amenințările asupra faunei de pești sunt împărțite în două categorii: naturale și antropice. Dintre amenințările antropice au fost menționate cele mai abundente, și anume: supraexploatarea peștilor, poluarea, extragerea de nisip și pietriș, construirea de baraje și introducerea de pești exotici.

### **INTRODUCTION**

Rivers are one of the most important sources of fresh water in the world (Radkhah et al., 2018a). These ecosystems have been ecologically degraded due to anthropological activities (Niazi, 2004). They are the best water resources for human use, but nonetheless, they are used as a place for the disposal of human wastes (Nyairo et al., 2015; Traoré et al., 2016; CLC, 2019). The excessive expansion of agriculture, urbanization, industrialization and construction of dams are considered as main human activities that disturb the ecological status and integrity of river systems (Schmutz and Sendzimir, 2018).

Many aquatic organisms of rivers, suffer from human interventions (Angeler et al., 2014). The bio-communities of the rivers such as macrobenthos and fish are considered as biological indicators of quality and change in aquatic systems (Oberdorff et al., 2002). These organisms respond to physico-chemical disturbances in aquatic ecosystems, i.e. the ecosystem's flexibility seems to depend on the diversity of its inhabitants (Muhammad et al., 2017).

Endemic fish are an important part of a country's natural heritage. Hence, it is essential to protect them (Çiçek et al., 2018). Iran has a high diversity of freshwater fish (Radkhah et al., 2018b). Most of the freshwater fish of Iran have a Palearctic origin, some are elements from the Ethiopian and Oriental regions. Therefore, there is a wide variety of fish species in Iranian inland waters (Ghasemi and Ramin, 2012).

According to available references (Saadati, 1977), the studies on inland water fish of Iran were started about 166 years ago by various ichthyologists such as Hackel, Kessler, Nikolsky, and Berg (Taghavi-Nia and Velayatzadeh, 2015). Subsequently, many studies have carried out to identify and investigate the fish fauna in inland freshwaters of Iran (Esmaeili et al., 2018), but, it is still necessary to assess and monitor many aquatic ecosystems in terms of the distribution of endemic, native and exotic species, since many new species or records are described or reported every year.

According to Esmaeili et al. (2018), 297 fish species have been recorded in 109 genera and 30 families from different basins of Iran. Of these species, 95 species (32%) are endemic and 29 species (9.76%) are exotic. In addition, Cypriniformes (59.3%), Gobiiformes (14.1%), Cyprinodontiformes (6.4%), and Clupeiformes (3.7%) are the most rich orders (Esmaeili et al., 2018).

Zarineh River, with 302 km length, originates from the Chehel-Cheshmeh Mountains in Saqqez (Kurdistan Province, northwest of Iran). It flows into the Urmia Lake after passing through the cities of Bukan, Shahin-Dezh and Miandoab (Fallah, 2009).

The shrinking of lake Urmia is one of the worst ecological disasters of recent decades and Zarineh River is the main feeding source of this lake that is affected by various human activities, hence understanding its fish fauna can provide useful information about its ecological state along it toward downstream. Therefore, the present research was conducted and implamented to investigate the fish fauna in the downstream of the Zarineh River in the vicinity of Shahin-Dezh City. The findings of this study can provide valuable information for environmental experts and policymakers to better understanding of human influence in order to apply conservation and management strategies.

### MATERIAL AND METHODS

Seven stations were sampled along the Zarineh River, Urmia Lake basin, in September 2018 (Tab. 1; Fig. 1), including four stations before the Shahin-Dezh (stations 1-4), one station in the vicinity of this city (station 5) and two others after the city (stations 6 and 7). Field observations were carried out on the river's margin, which was influenced by various human activities such as agricultural activities, urban, and industrial development, and so on (Fig. 2).

Fish sampling was carried out using an electrofishing device (Samus Mp750). The level of fishing effort was similar for all stations. After sampling, fish species (native and exotic) were identified using identification keys such as Coad (2019) and Esmaeili et al. (2018). Then, the samples were fixed into 10% formalin solution and transferred to the laboratory for further studies.

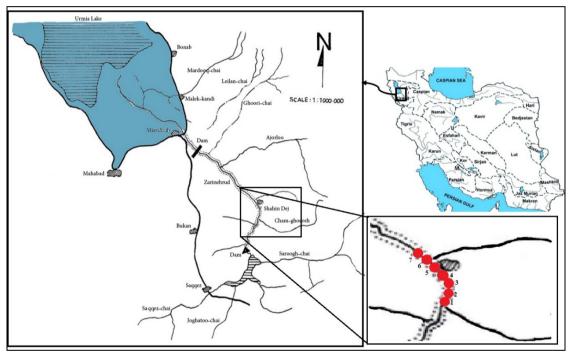


Figure 1: Map of the study area in the Urmia Lake basin; sampling stations 1-7.

Table 1: The geographical location of sampling sites in the Zarinen River.							
Sampling site	Site code	Latitude and longitude					
Station 1	St-1	36°39'57.58''N, 46°32'45.88''E					
Station 2	St-2	36°39'32.93''N, 46°33'5.88''E					
Station 3	St-3	36°38'13.36''N, 46°33'20.90''E					
Station 4	St-4	36°35'33''N, 46°33'4.59''E					
Station 5	St-5	36°35'28.46''N, 46°33'22''E					
Station 6	St-6	36°42'46.7"N, 46°9'48.68"E					
Station 7	St-7	36°40'42.3"N, 46°32'29.3"E					



Figure 2a: Zarineh River – sampling station 1.



Figure 2b: Zarineh River – sampling station 2.



Figure 2c: Zarineh River – sampling station 3.



Figure 2d: Zarineh River – sampling station 4.



Figure 2e: Zarineh River – sampling station 5.



Figure 2f: Zarineh River – sampling station 6.



Figure 2g: Zarineh River sampling station 7.

# **RESULTS AND DISCUSSION**

A total of 404 specimens belonging to 11 fish species were collected during sampling (Tab. 2, Fig. 3). Nine species belong to the Cyprinidae family. Two species, including *Oxynoemacheilus elsae* and *Rhinogobius lindbergii* were members of the families Nemacheilidae and Gobiidae, respectively. Out of 11 species, five species, *Carassius gibelio*, *Rhodeus amarus*, *Pseudorasbora parva*, *Hemiculter leucisculus* and *R. lindbergii* were exotic i.e introduced to the Zarineh River (Eagderi and Moradi, 2017).



Figure 3a: Rhodeus amarus.



Figure 3b: *Pseudorasbora parva*.



Figure 3c: Hemiculter leucisculus.



Figure 3d: Gobio perseus.



Figure 3e: Capoeta capoeta.



Figure 3f: Barbus lacerta.



Figure 3g: Squalius turcicus.



Figure 3h: Oxynoemacheilus elsae.



Figure 3i: Alburnus atropatenae.



Figure 3j: Carassius gibelio.



Figure 3k: Rhinogobius lindbergii.

At St-1, the most numerous species was *H. leucisculus*, at St-2 *A. atropatenae*, at St-3 *B. lacerta*, at St-4 *A. atropatenae*, at St-5 *A. atropatenae*, at St-6 *C. gibelio* and at St-7 *A. atropatenae*.

The results showed that A. atropatenae as most frequent species found in all the sampling stations.

No.	Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7
1.	Carassius gibelio		_	5	2	4	6	1
2.	Squalius turcicus	_	_	_	3	2	_	_
3.	capoeta capoeta	—	—	25	—	—	4	
4.	Gobio perseus	—	5	14	1	2	—	
5.	Albrunus atropatenae	2	24	15	40	23	3	24
6.	Rhodeus amarus	_	_		1	9	3	3
7.	Barbus lacerta	—	4	39	24	15	3	16
8.	Oxynoemacheilus elsae	—	—	34	1	—	4	21
9.	Pseudorasbora parva	—	—	4	—	—	—	
10.	Hemiculter leucisculus	16	_	_	_	_	_	_
11.	Rhinogobius lindbergii	_	_	_	_	1	1	_
	Total		33	136	72	56	24	65

Table 2: List and number of fish species collected from the downstream of the Zarineh River, Urmia Lake basin.

Ghasemi and Ramin (2012) investigated the fish diversity and species richness in the eastern rivers of Urmia Lake basin, including Mardough-Chai, Aji-Chai, Sufi-Chai and Qhale-Chai. They reported same native and endemic species along with two exotics *Pseudorasbora parva* and *Carassius gibelio*, as we reported in the Zarineh River. In addition, *A. atropatenae*, an endemic species of this basin, was the most frequent one as well. Most fish elements of this basin are similar to that of Caspian Sea basin (Esmaeili et al., 2018). Jalili et al. (2017) stated that the presence of *O. bergianus* in the Caspian Sea basin (Aras River drainage) and lake Urmia basin indicates the connection between these two basins in the past that have been separated from each other due to geological events.

The fish populations of Zarineh River have declined dramatically due to anthropogenic activities such as overfishing, pollution (industrial, agricultural, urban, etc.) (Ahmadi, 1998, Fallah, 2009), sand and gravel extraction, dam construction, and introduction of exotic fishes during last decade (Ahmadi, 1998; Fallah, 2009; Radkhah et al., 2016). Among the human factors, the effect of overfishing, especially unauthorized fishing is a major threat. Field observations of the present study revealed that sewages of Saqqez, Miandoab, and Shahin-Dezh cities are the most important urban pollutants sources of this river. The industrial and agricultural pollutants, including pesticides and agricultural fertilizers are transferred to the river due to rainfall (Ahmadi, 1998; Radkhah et al., 2017). Since there is a huge amount of farmland alongside the river, the entrance of agricultural pollutants could be one of the main concerns of this river as reported killing of many fishes (Ahmadi, 1998). In addition, Fallah (2009) reported that sand extraction, river bed destruction and dam construction are also effective in changing river topography and destruction of fish habitats. Therefore, these anthropogenic activities are serious threats to fish populations of the Zarineh River.

Based on the results, the most accentuated changes in the river landscape were observed at sampling station five somewhat after entrance of the wastewater of Shahin-Dezh City. In this sampling station, the high volume of wastewater along with sedimentation of the suspended materials have created many spots due to enriching water and eventually led to the growth of many aquatic plants in the river' banks. Despite the high volume of wastewater release, presence of aquatic plants along the river up to station six shows a proper self-purification process. The results also showed that the fish composition community changes in stations before the city about 10 km after the city i.e. station seven. But at a distance about 1.5 km from the city (station 6), fish composition is disturbed and those exotic species like *C. gibelio* and *R. amarus* were dominant, because a high rate of aquatic plants has provided a proper habitat for these species. Furthermore, at station seven, conditions almost return to normal state and river landscape, and composition and richness of fish were similar to those upstream the city.

The present study showed that nearly half of the collected fish were exotic, hence, it is necessary to pay special attention to control and management of these exotic species. The introduction of exotics into aquatic ecosystems is one of the main threats to native fish diversity in Iran (Esmaeili et al., 2014b; Radkhah et al., 2015; Radkhah et al., 2016; Radkhah et al., 2017). According to previous reports, the presence of these species will have negative impacts on the endemic species such as *A. atropatenae* and *O. elsae* (Radkhah and Eagderi, 2015; Radkhah et al., 2017). Exotic species have different mechanisms after introducing into aquatic ecosystems. Some of them cannot expand and even fail to create sustainable populations in the environment (Radkhah et al., 2018a) whereas, invasive species e.g. *P. parva*, spread quickly and occupy different parts of the aquatic habitats (Esmaeili et al., 2014a, b). In fact, a wide range of feeding, short generation time, early reproduction, high spawning, and high reproductive effort have led an increase in the density and colonization of these exotic fishes in many aquatic ecosystems (Simon et al., 2011; Radkhah et al., 2018b).

#### CONCLUSIONS

The present study showed that the present fish fauna of the Zarineh River is strongly affected by various anthropogenic activities. Therefore, it is imperative that management measures are taken to protect its fish diversity. In addition, the native fish of this river are influenced by exotic species making it necessary to control their populations. The present study suggests that special monitoring studies should be carried out on the negative impacts of exotic fish species on the Zarineh River.

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# INNOVATIVE ON-SITE ADAPTED SYSTEM FOR FISH MIGRATION WITH FLOW DIVIDER AND GLASS COLLECTOR BASIN

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# **KEYWORDS**: fish migration, weir, passage, Bistrița River, Transylvania, Romania. **ABSTRACT**

The authors designed a new site-adapted fish passage system for upstream and downstream migration of small and large fish on the urban sector of the Bistrita River. The longitudinal connectivity of this lotic system is interrupted by numerous transversal hydrotechnical works (weirs). This proposed system was designed to facilitate fish migration, promote fish recolonization of upstream and downstream habitats, allow the expansion of fish range, and increase spawning potential. All components of the proposed system are attached to a concrete girder located to the right stream bank, except a glass basin that is submerged in the riverbed. The positioning of the system in this girder ensures its resistance to high water events, while offering safe passage for fish in both directions.

**RÉSUMÉ**: Système innovant adapté au site à la migration des poissons, avec diviseur de débit et bassin collecteur en verre.

Les auteurs ont conçu un nouveau système de passage des poissons adapté au site (pour la migration des petits et grands poissons en aval et en amont) sur un secteur urbain de la rivière Bistrița. La connectivité longitudinale de ce système lotique est interrompue par de nombreux ouvrages hydrotechniques transversaux (barrages). Le système proposé a été conçu pour : faciliter la migration des poissons, favoriser la recolonisation des habitats en amont et en aval, permettre l'expansion de l'aire de répartition des poissons et augmenter le potentiel de ponte. Tous les éléments du système proposé sont construits sur une poutre en béton située sur la rive droite du cours d'eau, à l'exception d'un bassin en verre qui se trouve dans le lit du fleuve (submergé). Le positionnement du système dans cette poutre assure sa résistance aux crues, et la conception permet le passage des poissons dans les deux sens.

**REZUMAT**: Sistem inovator de migrare a peştilor cu separator de debit şi bazin colector de sticlă.

Autorii au conceput un nou sistem de migrare a peștilor adaptat la sit (pentru migrarea în amonte și în aval a peștilor mici și mari) pe un sector urban al râului Bistrița. Conectivitatea longitudinală a acestui sistem lotic este întreruptă de numeroase lucrări transversale (praguri). Sistemul propus a fost conceput pentru: facilitarea migrării peștilor, suportul repopulării cu pești a habitatelor din amonte și aval de prag, permite extinderea distribuției peștilor și creșterea potențialului de reproducere. Toate componentele sistemului sunt construite într-o grindă de beton existentă pe malul drept al râului, cu excepția unui bazin de sticlă, care se află în albia râului (submers). Poziționarea sistemului în această grindă, îi asigură rezistență la inundații, iar designul oferă trecerea în siguranță a peștilor, în ambele direcții.

### **INTRODUCTION**

The security first principle has shown many times really insufficient interest for the environmental elements (Mehlhom, 2019; Jägerskog et al., 2014; Myers, 1986; Brown, 1977). Surface waters are given priority especially in contacts where there are limited water resources to meet the needs of humans and other living beings (Kilic and Yucel, 2008).

Human-dominated management of streams and rivers in the Anthropocene is not impossible if the management approach is integrated and on-site adapted (Bănăduc et al., 2020, 2011; Adom et al., 2019; Curtean-Bănăduc et al., 2019, 2018; Marić et al., 2019; Deng et al., 2014; Wetzel 2001). Traditional river management is purpose-oriented to achieve water security, economic benefits, or habitat modification and focuses only on relatively short periods of time (Wang et al., 2015; Cianfaglione, 2009). There are numerous reasons as to why streams and rivers are modified and/or even destroyed. Some modifications for example are to protect human settlements from floods, to offer water supply for industry, agriculture and human settlements, or to evacuate use or waste water, etc.; but many of these activities negatively affect the connectivity and quality of watercourses (Enachi et al., 2019; Popa et al., 2019, 2017; Piria et al., 2018; Marić et al., 2017; Curtean-Bănăduc et al., 2007; Dynesius and Nilsson, 1994). Aquatic systems connectivity is a fundamental element of the biophysical structure of natural capital, which plays a key role in providing a wide range of natural resources and services (Badura et al., 2018; Sender et al., 2017: Vădineanu and Preda, 2008). EU countries report significant proportions of water quality degradation, with as much as 50-90% of water bodies being adversely affected (Castro et al., 2002).

Due to the fact that the lotic systems represent both permanent habitats and migration corridors, they are mainly characterized by their water-mediated complex connectivity (Pringle, 2003; Wiens, 2002). Four principal elements are important to the ecological functioning of lotic ecosystems; longitudinal connectivity, lateral connectivity, vertical connectivity, and temporal connectivity (Jungwirth et al., 2003; Ward, 1989).

River and stream biotopes and biodiversity are in general highly threatened by habitat fragmentation (Popa et al., 2016; Trichkova et al., 2009; Nilsson et al., 2005; Zwick, 1992), i.e. in Europe only 28% of large rivers remain free flowing (WWF, 2006), the situation of medium and small rivers in this respect being more or less unknown.

Rehabilitation of longitudinal connectivity of lotic systems is improving around the globe, and ecological studies put a lot of effort into conservation technical solutions to assure connectivity (O'Hanley, 2011; Mesa and Magie, 2009).

The evolution of aquatic organisms occurred in relationship to habitat variability, accessibility, and distribution. They adapted their life history characteristics in reaction to their space and time connectivity (Schmutz and Mielach, 2013). Therefore, fish achieve habitat shifts to use different habitats (Mader et al., 1998). Human activities induced environmental changes which modify the structure and diversity of the aquatic communities including fish (Bănăduc et al., 2020; Ćaleta et al., 2019; Joy et al., 2019; Tutman et al., 2019; Curtean-Bănăduc et al., 2016; Curtean-Bănăduc 2015; Lenhardt et al., 2009). When habitat fragmentation appears, fish populations decline and can vanish (King et al., 2017; Aarts et al., 2003), but also other organisms can react similarly (Bănăduc et al., 2020; Curtean-Bănăduc et al., 2016; Branco et al., 2014; Simian et al., 2009, 2008).

For all this reasons, this paper goal is related with a significant issue, and is of high priority including in European Union regulations, specifically those that deal with river management under the Water Framework Directive (Voicu et al., 2018; Voicu and Merten, 2014; Kay and Voicu, 2013).

It is clear that the connectivity of streams and rivers can be affected by the construction of hydrotechnical works (Gevorgyan et al., 2017; Bănăduc et al., 2016; Lenhardt et al., 2016, Voicu et al., 2015). Furthermore, it is also clear that new, specific on-site adapted methods must be developed to protect connectivity, which implicitly extends to lotic ecosystems (McKay et al., 2016; Kemp and O'Hanley, 2010).

Rehabilitation of watercourse connectivity can be accomplished by equipping hydrotechnical constructions with specific new on-site adapted migration systems for fish, which can be used for their continuous upstream and downstream access. Many old systems need to be updated altogether, and researchers in this field must develop new ones that can be implemented in Europe and around the world (Voicu and Baki, 2017).

From the technical point of view it is possible to use some solutions to help fish passage along already engineered river reaches. Such solutions are for example boulder block ramps when considering them as fish migration systems with increased roughness (Radecki-Pawlik et al. 2018; Voicu et al., 2018; Rowinski and Radecki-Pawlik, 2015; Teppel and Tymiński, 2013; Oertel, 2013; Pagliara and Palermo 2012, 2013; Radecki-Pawlik, 2013b; Radecki-Pawlik and Ślizowski, 1998) or fish passes (Tymiński and Kałuża 2013; Hassinger 2009). The most common designs for technical structures of this kind are as follows: conventional pool passes, slot pool passes, Denil (baffle) fish passes and modular meander-type fish passes (Mokwa and Tyminski, 2018). However, there is still a need for innovative on-site adapted designs and the construction of structures for fish migration in lotic sectors with specific natural and anthropogenic restrictive conditions.

The research objective of this paper is to design a functional on-site adapted fish passage system to restore the possibility of migratory fish to move upstream and downstream. The construction of this system will lead to the restoration of the biological footprint that has largely disappeared due to the construction of this first spill threshold (as well as the other spill thresholds) on the Bistrita River in the city of Bistrita (Romanian Carpathian Basin).

The new proposed technical system was designed to face natural, sometimes daily, fast and repeated floods; the classic technical systems usually clogged in such situations.

Lack of sufficient space for the river owing to nearby anthropogenic structures, make impossible the proposal of a classic by-pass. This was replaced by an innovative glass basin which can solves the fish migration problem induced by the difference of water level between the upstream and downstream heads of the technical construction.

# MATERIAL AND METHODS

### Study area

The River Bistriţa originates in Romania's Călimani Mountains at an altitude of about 1,870 m. The confluence of the Bistriţa River with Şieu River takes place in the town Sărăţel, at an altitude of 312 m. According to the Atlas of Water Cadastre in Romania (1992), the stream covers a total of 67 km, the total catchment area is 650 km<sup>2</sup>, and its average altitude is 815 m. River regulation began at the beginning of the 20th Century, along with the development of local socio-economic systems.

The river sector on which the water spillway/weir is located passes through the city of Bistrița, Bistrița-Năsăud County. This weir is downstream at about 40 m at Budacu Bridge (Figs. 1a, b, c). The riverbed is principally composed of a mixture of boulders, sand and gravel.



Figure 1a: weir image from the left river bank.

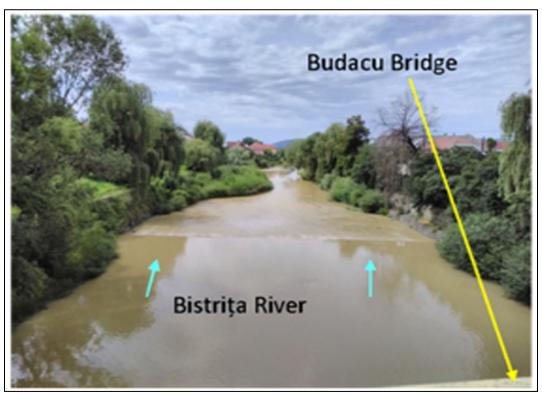


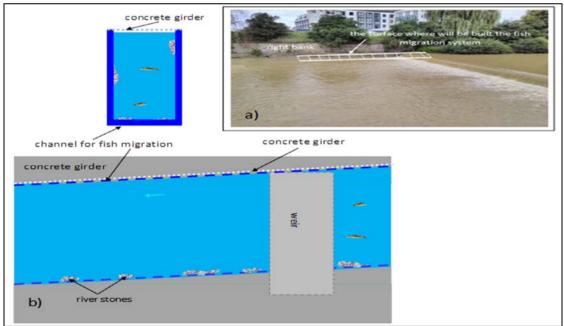
Figure 1b: weir image from the Budacu Bridge.



Figure 1c: location of "weir one" (source: www.googleearth.com).

#### Location of the proposed migration system

Before describing the proposed migration system, it is important to explain why the river right bank has been selected as the migration system location. At the base of the right bank there is a concrete girder/beam built over the overflow weir and fixed to the stony right stream bank (Figs. 2a, b), which has the following dimensions: approximately 1.5 m wide and 2.5 m high extending below the Budac Bridge. The height of the "weir one" is 1.10 m and the length is about 35 m (across the width of the riverbed).



Figures 2a, b: the proposed migration system; a - 10cation of the system; b - channel slope for fish migration - cross section.

#### Local fish community

The local fish species, including four of particular conservation interest, which will benefit from this new on-site adapted technical proposal that will diminish the local lotic sector fragmentation, are: the Chub – *Squalius cephalus* (Linnaeus, 1758), the Eurasian Minnow – *Phoxinus phoxinus* (Linnaeus, 1758), the Common Bleak – *Alburnus alburnus* (Linnaeus, 1758), the Schneider – *Alburnoides bipunctatus* (Bloch, 1782), the Common Nase – *Chondrostoma nasus* (Linnaeus, 1758), the Gudgeon – *Gobio gobio* (Linnaeus, 1758), the Danubian longbarbel gudgeon – *Romanogobio uranoscopus* (Agassiz, 1828), the Common Barbel – *Barbus barbus* (Linnaeus, 1758), the Mediterranean barbell – *Barbus meridionalis* Risso, 1827, the Stone loach – *Barbatula barbatula* (Linnaeus, 1758), the Balcan spined loach – *Sabanejewia balcanica* (Karaman, 1922), and the European Bullhead – *Cottus gobio* Linnaeus, 1758.

The fish species of the Grayling and Mediterranean barbell, and Nase ichthyological zones of the Romanian Carpathian basin (Bănărescu, 1964) are under constant pressure and threats of human impact in the Romanian Carpathians Basin (Bănăduc, 2012) and in our study area, and are in urgent need of specific technical solutions for on-site adapted conservation management for river fragmentation mitigation.

# **PROPOSED METHODOLOGY**

At present, there are many technical solutions (for example, fish ladder, Denil fish pass, fish ramps) and non-technical or nature-like fish ways (i.e. rock-ramps, classical bypass channels) for fish migration and movements. They can be used according to the particularities of the analyzed area (i.e. type/size/volume of river, hydrological conditions) and the target fish species (i.e. for large species, smaller fish, potamodromous or catadromous species, period of migration) (Baki et al., 2017; Katopodis and Williams, 2012).

Some of the existing fish ladders are relatively selective, being suitable for individuals of certain sizes, i.e. Denil pass for individuals > 30 cm of salmon, sea-run trout, marine lamprey, and barbell. This type of pass has the disadvantage that there are no resting zones for fish. (Larinier and Marmulla, 2004).

The design of nature-like fish ways (NLFs) are site-specific and are based on the principle of simulating the flow of a natural river channel using natural materials (i.e. tree logs, rocks and boulders, etc.) (Baki et al., 2017). In the specific literature they are also called near-nature or close to nature (FAO, 2002). The classifications of NLFs are based on the configuration of structures, such as the arrangement of boulders.

For example in New Jersey, North America, (Musconetcong River) one of these nontechnical solutions was used, which consisted of manipulating the channel bed to create a naturalized riffle, at a distance to two to five or more stream widths (depending on the size of the river) downstream of weir. It used numerous, very large, five to eight foot boulders, interspersed with smaller boulders and cobbles to seal it, excavated into the channel bottom. Depending on the slope of the channel, this technique can raise the water surface elevation upstream anywhere from six inches to four feet. In most cases, this will provide fish passage by increasing water surface elevation and reducing the height of the obstacle. In case of need to get the fish over a higher obstruction, a series of two, three, or four of these riffles are built, spaced apart, to raise the water surface elevation in steps, permanently.

In some cases, both technical and non-technical solutions may be used, depending on the particularities of the analyzed river sector. For example, in some rivers that already have a fish ladder, but have experienced low flows, such solutions can raise the water surface elevation at low-water to a point where fish can access the existing fish ladder. In other cases, when the thresholds have lost their functionality, it is recommended to remove them (Travade and Larinier, 2002).

In our study, taking into account that the currently analyzed threshold "weir one" is functional (for slope breaking and prevents deepening of the talveg), we have excluded the possibility of its removal. Also, the weir under analysis is in an urban area, so there is no space for creating a classic by-pass, because this regularized river sector crosses the city of Bistrita.

This weir is the first upstream one of the three thresholds located at 200 m from each other and is often subject to high flow due to water discharges from the dam accumulation (which supplies water to the city of Bistrița) located upstream. Thus, frequent fluctuations of water level would damage any frontal system of fish migration/vertical fish slots.

Consequently, conventional solutions (by-pass, vertical fishway, removal weir) could not be applied in the analyzed area. In actual particularities of the study area, we have chosen a specific technical solution, more precisely, we proposed the new system of fish migration with flow divider and unbreakable glass collector basin (FMSDGCB). This system was designed as a linear channel made in the concrete beam on the right bank of the Bistrița River.

At 1.5 m upstream of this weir, in the concrete girder/beam described above, it is proposed to drill a breach (crenel) about 42 cm wide. This crenel (breach) will resemble the upstream end of a fish migration channel that will be drilled into the concrete girder.

## The components and the principle of the FMSDGCB

In order to achieve the needed fish migration system, the following components are proposed: i) a fish migration channel made of concrete; (ii) an unbreakable glass separator; (iii) two channels built of metal sheet piles to take over the flows formed by the glass separator; (iv) a water supply pipe for the fish migration channel; (v) an unbreakable clear glass parallel piped basin that communicates directly with the channel positioned under the glass separator; (v) a vertical gate operated by a manual reducer.

The bypass entrance is an opening of 60 cm wide/one m deep. The main component is the fish migration channel made of concrete, which will have a slope the same as the slope of the river in the area, namely 0.4%, the length of about 5.2 m and 2.30 m depth (Fig. 3).

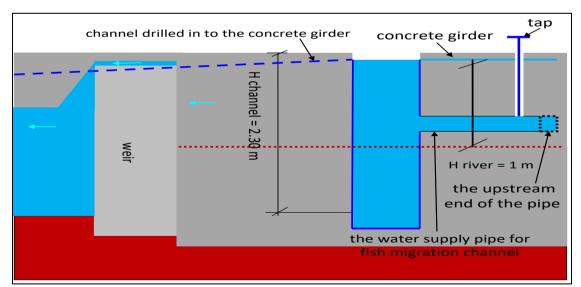
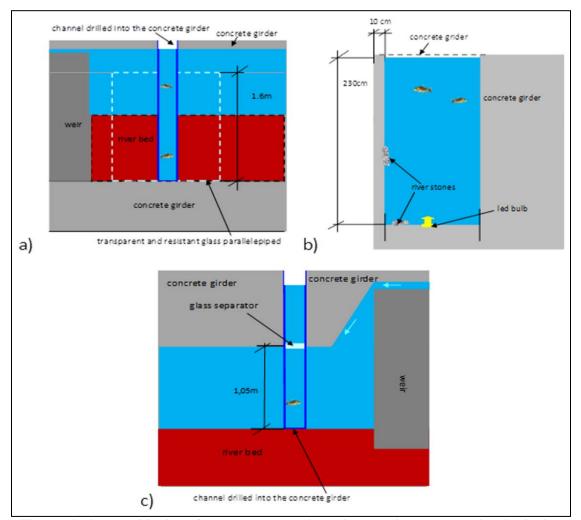


Figure 3: positioning of the channel into the concrete beam/girder – indicative scheme.

The velocity of water in this zone is v = 0.15 m/s. The vertical surface (which is in direct contact with the Bistrita River) of the channel will have a thickness of approximately 14 cm. The channel must be facing up-river (one m in the area), and the water column in the channel must be 2.3 m. Considering that the water level in the channel (about 2.3 m) cannot be assured by direct feed from the river alone, through the breach (crenel), it is proposed to supply the water from upstream through a pressure pipe, also located inside the concrete girder, but with the upstream end outside the concrete girder. At the upstream end of this pipeline (when the pipe becomes parallel to the stony stream bank) there will be a tap (Fig. 3). There can be two pipes if the flow of the first pipe cannot ensure that the water fills the channel.

The fish migration channel will be extended at the upstream end by a transparent and resistant glass parallel piped basin 1.6 m high (Figs. 4a, b, c). The fish can thus swim up on the upstream channel and cross "weir one" through the glass basin whose upper part will be at about half the river level. In the lower part of the channel (the base of the channel) the turbulences are small, which allows the fish to arrive at the glass basin (upstream of "weir one"), and then continue up the Bistrita River.



Figures 4a, b, c: positioning of components: a – the resistant and transparent glass basin; b – concrete girder and river stones – cross-section; c – the glass separator – indicative scheme.

The fish migration channel will be provided with river stones, and some LED light bulbs will be attached to the entire horizontal surface (the base of the channel), which can be powered by either solar panels, generators, or the local power grid. A concrete grid will be mounted above the channel (Fig. 4b).

Approximately three m inside the fish migration channel a glass separator is attached horizontally at a height of approximately 1.05 m from the base of the channel (Fig. 4c). Thus, the fish migration channel will have two compartments. The water from the lower channel will be discharged into a channel of metal sheet piles of about 0.4 m long, and then directly into the Bistrița River. This channel built of metal sheet piles of approximately 0.4 m in length has the approximate height of the river in this area (one m). The upper channel made by the glass separator will open the water in a channel also from metal pallets. In this way the fish can travel through the channel under the glass separator because it has the downstream end directly into the Bistrița River, and after the separator has finished, the fish will migrate to the bottom of the channel because there are smaller turbulences. Thus the fish will reach the glass basin and later in the Bistrița River. For improvements to the fish migration system at the upstream end of the system, a metal frame is fastened, and inside this metal frame is fastened a metal weir which is moved by a reducer. The movement of the metal weir will also be controlled by a metal threaded bar (Fig. 5).

At the upstream end of the fish migration channel, a metal bar fixed to the bearings. On this metal bar there are fixed plastic floats with a diameter of 30 cm. At the downstream end, the water supply pipe has a metallic grille that has two metal hinges that can be folded by an electric winch (with a similar feed to the fish migration channel light bulbs) when the inside of the migration system requires maintenance work (Fig. 5).

Most fish passes are not operational because the fish cannot find the entrance of the fish pass, cannot follow the flow or because the flows exceed swimming capacity (Bowman and Rowe, 2002). The effectiveness of a fish pass is closely linked to the water velocities and flow patterns and must be compatible with fish swimming capacity and behaviour (Larinier and Marmoula, 2004).

The design of our system takes into account the swimming ability and behaviour of the local target fish species and the physical and hydraulic conditions at the water intake. In our study we considered the target species, all species of fish identified in this section. Also, this system can be used for both small and large sized fish species and individuals. The proposed migration system does not present slots or other components that could lead to clogging. The river bed stones that will form the substrate of the channel have the role of dissipaters.

Compared to other types of migration systems (i.e. fish ladder, by-pass channel), our system, with the help of the glass basin placed below the water level (submerged), solves this difference in level, or slope, between the entrance and the exit system. This is necessary because there is not enough space to make a long channel. The bypass channel cannot be adapted to significant variation in upstream level without special devices (gates, sluices) but these control devices may cause hydraulic conditions that make fish passage difficult (Larinier and Marmulla, 2004). The FMSDGCB offers a constant flow rate during flow fluctuations in the Bistrita River.

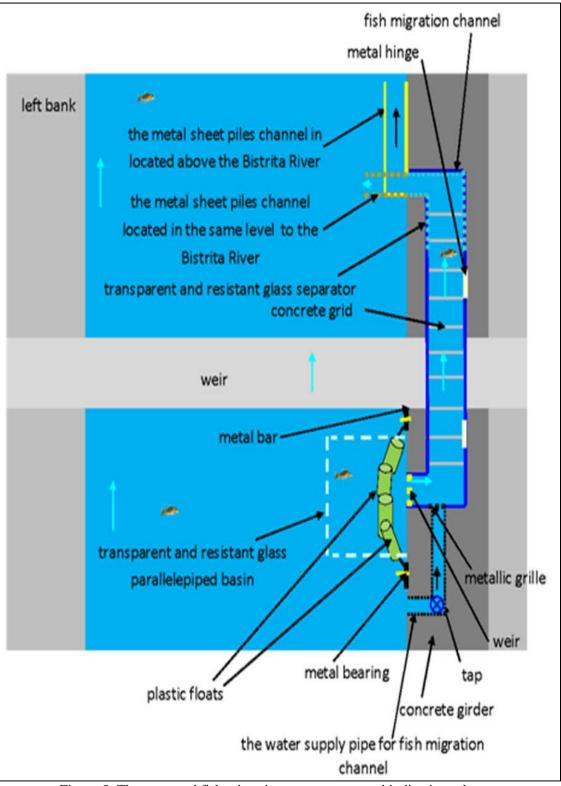


Figure 5: The proposed fish migration system – general indicative scheme.

Regarding the operating period, this system may be functional all year round except in winter. Closing it (in winter or in repairs) will be done with a vertical gate by a manual reducer. At the same time, feeding it with water using a pipe equipped with a tap allows it to be closed in the situations described above. With regard to its maintenance, it is recommended that it is cleaned inside every few months or after the flood.

The FMSDGCB is also expected to survive future flood events because, with the exception of the submerged basin fixed in the river bed, all the components are entirely in the concrete beam situated on the right bank of the river Bistrița. We consider that our technical solution provides a new design which can be implemented, especially for thresholds/weirs in urban rivers, where space is limited.

By implementing our new design (FMSDGCB), we expect that local lotic sector fragmentation will diminish, and that the following native fish species of conservation interest (Habitats Directive, Annex 2) will benefit: Danubian longbarbel gudgeon (*Romanogobio uranoscopus*), Mediterranean barbell (*Barbus meridionalis*), Balcan spined loach (*Sabanejewia balcanica*), and European bullhead (*Cottus gobio*). This will avoid geographic isolation of the fish populations (Schick and Lindley, 2007) as a result of the presence/construction of many thresholds/weirs in rivers.

Considering that the efficiency of a migration system is proved by monitoring the species of fish migrating upstream (telemetry) (Larinier, 2002), we recommend that after this technical construction realisation, it should be implement a monitoring activity.

Regarding the use of this system in other cases, the main disadvantage is that it needs considerable space in the vicinity of the obstacle and cannot be adapted to significant variation in upstream level without special devices (gates, sluices). These control devices may cause hydraulic conditions that make fish passage relatively difficult.

The designed system induces low water velocities, which will be important at the entrance and exit points of fish passes. In the new fish pass, the half-round obstructions in the outer channels were positioned so that they would slow down water velocities to less than about 0.3 m/s (Bowman and Rowe, 2002).

As with any other fishway, it is recommended that the entrance to the bypass channel (i.e. the downstream entrance for the fish) should be located as far upstream as possible and very close to the obstruction. Because of the low slope required in this type of fishway, it is sometimes difficult to position the entrance immediately below the obstruction, and it has to be located well downstream of the fish latter. This will restrict their efficiency, and consequently makes them of limited use on large rivers. On the other hand, this disadvantage can be overcome on smaller waterways by passing a large proportion of the total flow through the facility. Velocities, drops and turbulence in the fish passage facility can be adapted to the swimming capacity and behaviour of the particular species found in the river. (Larinier, 2002b)

#### DISCUSSION

Positioning the fish migration system in the concrete strip (concrete beam) gives it increased resistance to extreme flows. The system can be closed in winter with the aid of the movable shutter, thus protected against potential damage caused by the freeze-thaw phenomenon. The corrosion resistance of the components, but also their quality, gives the system long service life. The slope of the system, the basin and the resistant glass separator ensures the necessary conditions for the fish to ascend upstream of "weir one". This solution was designed taking into account the conditions of the Bistriţa River in the Bistriţa area. On a one km stretch there are six obstacles over one meter tall which are built to dissipate the discharge from the upstream dam at the entrance to the city of Bistriţa. This dam has two buildings and discharges flood waters, ensuring serious damage would not occur to the city of Bistriţa in the case of a flood. Given that flow differences are frequent in this area and that the river carries various floats that can damage any fish migration system positioned frontally on the spill thresholds, we have devised this engineering solution with the downstream end below the water level and with the rest in the right bank, specifically in the concrete beam that stabilizes the cobbled bank. The small difference in level between the upstream end and the downstream end of the horizontal surface of the channel underneath the glass separator facilitates the uptake of fish in the resistant and transparent glass basin located in the water course but below the water level.

This area is important to many migratory fish species, and so river managers (National Administration "Romanian Waters"), in accordance with the European Union Water Framework Directive, want to restore the longitudinal connectivity of the Bistrița River, which is strongly anthropogenically affected.

It is appreciated that the proposed technical solution does not affect the structure and functions of the spill threshold. The fact that the system is built in a durable structure (concrete beam), that it has a closing system for periods of repair and winter, and that it is not positioned on the river front, ensures that this structure will be long-lived and require little maintenance.

When compared with the costs of classic fish migration systems, maintenance costs are minimal, which is an important advantage in supporting the construction of this solution in the study area.

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