TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

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The Wetlands Diversity

Editors

Doru Bănăduc, Teodora Trichkova & Angela Curtean-Bănăduc

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Applied Ecology Research Center,
"*Lucian Blaga*" University of Sibiu

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IN MEMORIAM
Oleksiy DZYZIUK
(1975 – 2022)

Mr. **Oleksiy Dzyziuk** was a Ukrainian zoologist, nature conservationist, and researcher of the vertebrate fauna. He was born on October 13, 1975, into a large family in the nice Cossack village of Novoprokopivka, Tokmatsky district, Zaporizhia region of Ukraine. As a kid, he was excited by nature and interested in his country nature. This determined his life. While studying at school, he was the winner of many competitions in natural sciences.

In the autumn of 1992, *Oleksiy Dzyziuk* studied in the Faculty of Biology at Zaporizhia University. During this period, he conducted active public and environmental work and led a group of young ornithologists for the education of schoolchildren. Oleksiy was able to gather like-minded people around him and was a man of principle and a patriot of his country. In the first year, the University officials wanted to expel him from the university for refusing to take one of the exams in Russian, insisting on his right to Ukrainian. Then his entire course in support also refused to take this subject in Russian. Together with like-minded people, he took an active part in many nature protection and anti-poaching raids. In 1996, Oleksiy was able to turn this activity into an official one, and with his active participation, the Nature Protection Group was founded at Zaporizhzhya State University, which he headed. In addition to community service, he also worked as a biology teacher at school, and published nature articles in a student newspaper. He was married and had two daughters.

After graduating from the university in 1997, *Oleksiy Dzyziuk* began working in the Lviv region, in the Society of Military Hunters and Fishermen of the Western Region of Ukraine. In 2000 he became the director of the Maidan Hunting and Fishing Farm (Vereshchytsia village, Yavoriv district, Lviv region) located within the International Biosphere Reserve "Roztochchya". With his arrival at this job, the nature-based economy began to grow rapidly. For this and other zoological and environmental projects, he successfully involved teachers and young environmentalists and ecologists from the Lviv City Children's Ecological and Naturalistic Center for several years. During this time, he also conducted active scientific work and wrote more than 15 scientific publications on the vertebrate fauna of the International Biosphere Reserve "Roztochchya", in particular in co-authorship with the famous Ukrainian ornithologist Dr. *Ihor HORBAN*. He also actively collaborated with scientists from the Yavorivskyi National Nature Park, the Roztocze Nature Reserve, and the Western Ukrainian Ornithological Society. In 2004 he was elected a deputy of the local self-government body. He held this public office service for three terms.

Oleksiy Dzyziuk was a hard-working, versatile, and erudite scientist, who actively studied and protected the nature of the International Biosphere Reserve "Roztochchya", and successfully helped to educate young zoologists and ecologists, who sought to be useful to the local community. He was a true patriot of Ukraine.

In 2014 he was an active participant in the Russian influenced terrorist events on the Maidan in Kyiv and the Revolution of Dignity in Ukraine. After the armed invasion and annexation of Crimea by the army of Russia's President Putin in the east of Ukraine, Oleksiy volunteered to join the Armed Forces of Ukraine. He served during several rotations in the zone of operations of the Joint Forces of Ukraine in the Donetsk and Luhansk regions, in the newly-started Russian-Ukrainian armed confrontation on Ukrainian territory.

On March 22, 2022, **Oleksiy Dzyziuk** died as a hero, defending Ukrainean civilian lives, his country's freedom, and the corner-stone beliefs of democracy and liberty of the free world. He was killed by Russians near the town of Popasna in the Donetsk region of Ukraine. He is buried in the cemetery of the village of Vereshchytsia in the Lviv region.

Bohdan PROTS and *Andriy KYIKO*

Transylvanian Review of Systematical and Ecological Research

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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 surviwing.

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

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch 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 lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peatswamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland. **Human-made wetlands** – Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue 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.
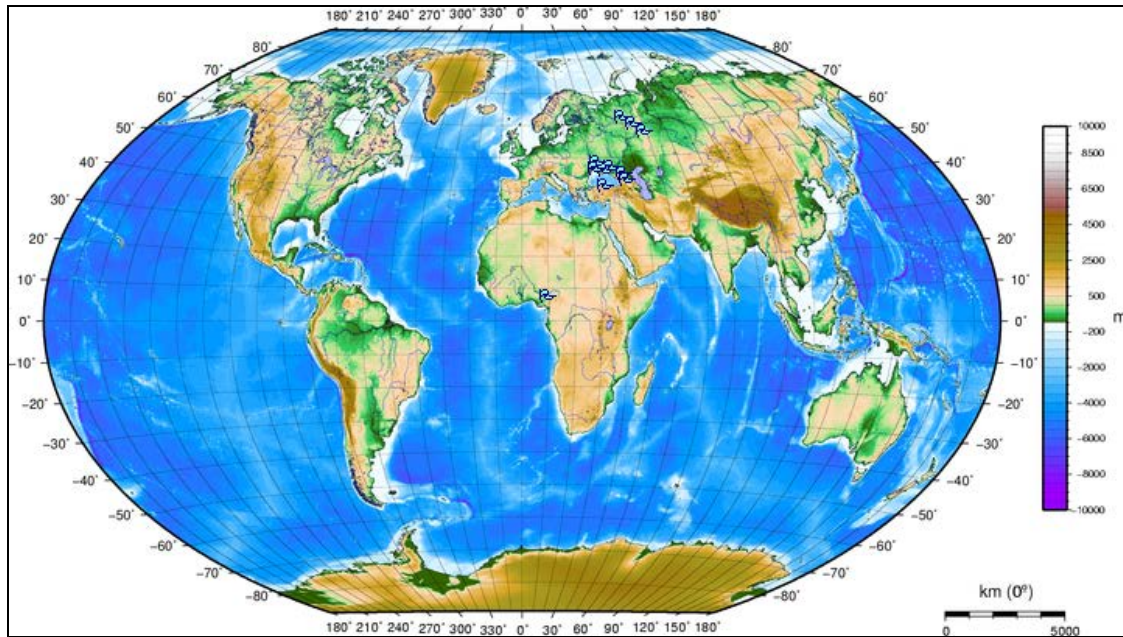
The subject areas (⧉) for the published studies in this volume.

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

HYDRO-DYNAMIC AND GEOCHEMICAL ASSESSMENT OF THREE SUCCESSIVE CUTOFF MEANDERS OF THE DANUBE DELTA

Laura DUŢU *, Dan SECRIERU *,
Florin DUŢU *, Irina CATIANIS * and *Dan VASILIU **

* National Institute for Research and Development on Marine Geology and Geo-Ecology – GEOECOMAR, Dimitrie Onciul Street 23-25, Romania, laura.dutu@geoecomar.ro, ORCID: 0000-0003-3482-6938, dsecrieru@yahoo.com, ORCID:0000-0002-0931-8440, florin.dutu@geoecomar.ro, ORCID: 0000-0002-5393-3125, irina.catianis@geoecomar.ro, ORCID: 0000-0001-8120-736, dan.vasiliu@geoecomar.ro, ORCID: 0000-0002-5899-949X.

KEYWORDS: Danube Delta, cutoff meander, sediment pollutants, sediment quality.

ABSTRACT

Hydrological and sedimentological data were collected on several cross-sections of both natural and artificial canals of three cutoff meanders (Mahmudia, Dunavăţul de Sus, and Dunavăţul de Jos meanders) of the Sfântu Gheorghe branch at the beginning of June 2017. The hydrological data shows a heterogeneous distribution of water discharges: on the meanders of Mahmudia and Dunavăţul de Jos, most of the liquid flow passes through artificial channels. In the case of the Dunavăţul de Sus meander, the cut meander is the main transport channel for the liquid flow. The statistical analysis of chemical data evidenced an excellent quality of the bottom sediments, with few parameters exceeding the limits (for Ni, Cu, and Cr). These results correlated with the hydrological measurements made on each investigated cross-section.

RÉSUMÉ: Evaluation hydrodynamique et géochimique de trois méandres recoupés du Delta du Danube.

Des données hydrologiques et sédimentologiques ont été collectées sur plusieurs sections transversales de chaneaux naturels et artificiels de trois méandres recoupées (méandres de Mahmudia, Dunavăţul de Sus et Dunavăţul de Jos) du bras de Sfântu Gheorghe au début du mois de Juin 2017. Les données hydrologiques montrent une distribution hétérogène des débits d'eau: sur les méandres de Mahmudia et Dunavăţul de Jos, la majeure partie du débit liquide passe par des canaux artificiels. Dans le cas du méandre de Dunavăţul de Sus, le méandre recoupé est le principal canal de transport du flux liquide. L'analyse statistique des données chimiques a mis en évidence une bonne qualité des sédiments de fond, avec peu de paramètres dépassant les limites (pour Ni, Cu et Cr).

REZUMAT: Evaluarea hidrodinamică şi geochimică a trei meandre succesive din delta Dunării.

Date hidrologice şi sedimentologice au fost colectate pe secţiuni transversale situate atât pe canalele naturale cât şi pe cele artificiale ale celor trei meandre ale braţului Sfântu Gheorghe la începutul lui iunie 2017 (meandrele Mahmudia, Dunavăţul de Sus şi Dunavăţul de jos). Datele hidrologice arată o distribuţie heterogenă a debitului de apă: pe meandrele Mahmudia şi Dunavăţul de Jos, cea mai mare parte a debitului trece prin canale artificiale. În cazul meandrului Dunavăţul de Sus, meandrul rectificat este principalul canal de transport pentru debitul lichid. Analiza statistică a datelor geochimice a evidenţiat o bună calitate a sedimentelor de fund, cu câţiva parametri care depăşesc limitele admise (pentru Ni, Cu şi Cr). Rezultatele au fost corelate cu măsurătorile hidrologice de pe fiecare secţiune transversală.

INTRODUCTION

The geochemistry of the bottom sediments is often affected by the chemical composition of their source rocks (Amorosi and Sammartino, 2007), by the anthropogenic influences, such as industrial cities, the presence of reservoirs (Sedláček et al., 2013; Bábek et al., 2015), hydro-technical works on the channel planform (e.g., groins, embankments, jetties, meander cutoff) (Vasiliu et al., 2021) or by the interaction between tectonics and climate (Singh et al., 2016). These pressures produce the segmentation or even interruption of the sedimentary flow downstream (Tiron Duţu et al., 2019).

Extensive characterization of bed sediments along the whole course of the Danube River was carried out in 2001 and 2007 by the International Commission for the Protection of the Danube River (ICPDR) (Woitke et al., 2003; ICPDR, 2008). Other studies with a more detailed resolution and examining pollution by trace elements in the Danube Delta were performed by Secrieru and Secrieru (1996), Vignati et al. (2003), Oaie et al. (2005, 2015), Catianis et al. (2018), Tiron Duţu et al. (2019), and Vasiliu et al. (2021).

The Danube Delta begins at Ceatal Izmail (bifurcation), at Mile 44 from the river mouth, where the Danube divides into two branches: Chilia (going to the North) and Tulcea (going to the South). At Mile 34, the Tulcea Branch is further divided into the Sulina Branch to the North and Sfântu Gheorghe Branch to the South (Fig. 1) (Panin, 2003).

The Sfântu Gheorghe distributary starts at the hydrographic knot at Sfântu Gheorghe Ceatal (km 108.8 from the Black Sea). A major fault system (called the Sfântu Gheorghe fracture zone) at the north border of the North Dobrogean orogenic unit controls the general orientation of the distributary. The North Dobrogean unit represents a hard to erode "wall" that influences the river physiography. It results in the course of the Sfântu Gheorghe Branch subdividing into three sections (Panin, 1976): the Dobrogean section of limited meandering (between km 104 and km 90), the free, meandering segment (between km 90 and km 22) with a succession of six meander loops, and the downstream section of limited meandering (between km 22 and km 0). The Sfântu Gheorghe meander loops were rectified between 1984-1988, leading to a shortening of the distributary by about 31 km. As a result, the Sfântu Gheorghe distributary water and sediment discharges have slowly increased, reaching 26.5% of the water flow and suspended sediment discharge of the Danube River (Panin, 2003; Oaie et al., 2015; Tiron Duţu et al., 2019).

This paper further analyses previously published datasets, by the authors (Duţu et al., 2021), in relationship with the hydrological context of the study area.

MATERIAL AND METHODS
Study area

The survey was undertaken in June 2017 on the Sfântu Gheorghe distributary, the southern branch of the Danube within its Delta (Fig. 1). Three cutoff meanders represent the study area: Mahmudia, Dunavăţul de Sus, and Dunavăţul de Jos named here as M1, M2, and M3, respectively.

The period during which the measurements were made (1-2 June 2017) corresponds to average to high waters. The flow rate entering the study area (on profile A1) was 2,170 m^3/s^{-1} (Fig. 1). During the two days of measurements, the water flow discharge remained constant.

Hydrodynamic data were measured using a powered boat-mounted acoustic Doppler current profiler (Acoustic Doppler Current Profiler – ADCP, RiverRay 600 kHz, from Teledyne). 14 ADCP profiles were measured (Fig. 1).
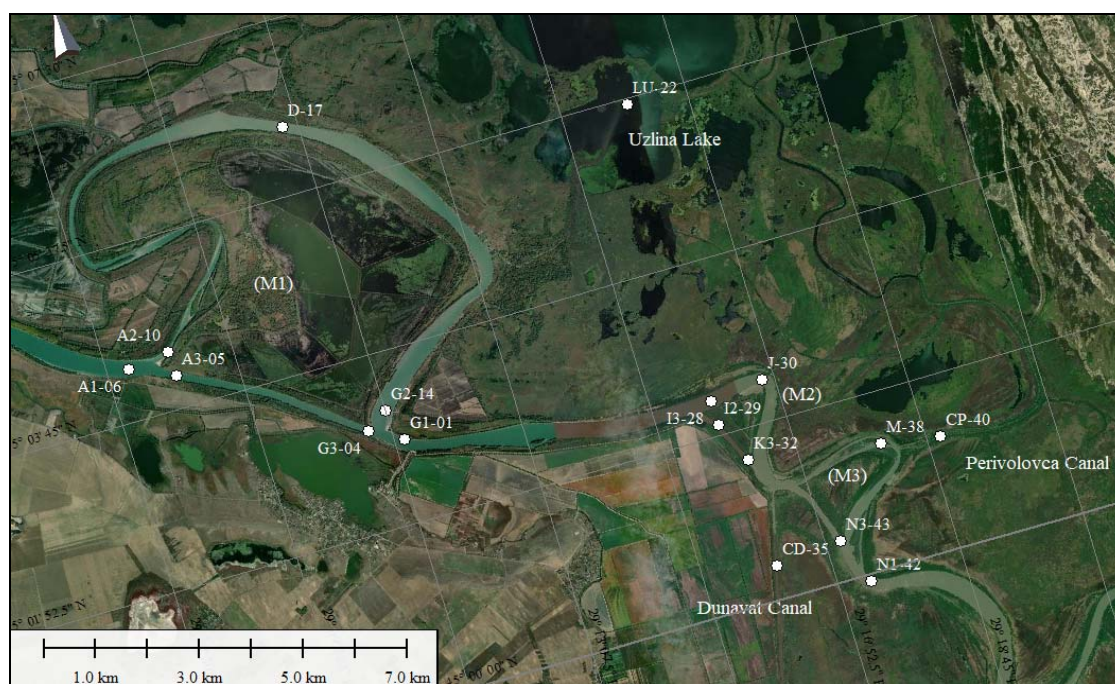
Figure 1: Map of the investigated area with the investigated cross-sections.

Based on the measured parameters such as maximum water depth, water velocity, and discharge, other specific hydrodynamical parameters were calculated, such as specific stream power (ω), mean bed shear stress (τ_0), water slope energy (S), for an overall estimation of water dynamic (Bravard and Petit, 1997; Duțu et al., 2018).

Bed sediment samples (17 samples) were collected from the main channel of the Sfântu Gheorghe Branch (14 samples collected from the center of the channel), from two lateral canals, Perivolovca Canal (one sample) and Dunavăț Canal (one sample), and the Uzlina Lake (one sample) (Fig. 1).

Geochemical analyses used the analytical procedures Secrieru and Secrieru (2002) described in detail. The analyses determined the concentrations of some major components – $CaCO_3$, TOC, Fe_2O_3, and minor ones – TiO_2 and MnO, which exercise significant control over the concentrations of trace elements. Some trace elements with genetic significance – Rb, Sr, Zr or toxic and potentially affected by anthropic influences – Cu, Pb, Zn, Cd, Cr, V, Ni, Co. Volumetric methods were used for analyzing $CaCO_3$ (Black, 1965) and TOC (total organic carbon), (Gaudette et al., 1974). Fe_2O_3 (total), TiO_2, Rb, Sr, Zr, and V have been analyzed by X-ray fluorescence spectroscopy using a VRA-30 sequential spectrometer on compacted powders; Mn, Co, Ni, Cu, Zn, Cr, and Pb have been determined by flame AAS and Cd – by graphite furnace AAS on an ATI UNICAM 939E AA spectrometer, after digestion of the samples with boiling HNO_3.

The accuracy and precision of AAS and XRF analyses were checked with several SRMs from US Geological Survey, NIST, and IAEA. Recovery for AAS varied from 93.2% (Co) to 99.4% (Pb), while for XRF the recovery range was from 90.3% (Zr) to 104.4% (Sr). Precision, expressed as the coefficient of variation for six replicate determinations varied for FAAS between 0.8% (Zn) and 4.5% (Mn); for XRF from 0.2% (Fe_2O_3) to 8.5% (V), the highest variability has been recorded for Cd – 12.5%, GFAAS determination.

RESULTS AND DISCUSSION

The water flux distribution between the natural course of meanders and cutoff canals varies from one sector to another, depending on several factors such as the ratio between the former and the new canal length, the diversion angle, and the bed level difference between the natural channel and the cutoff canal (Tiron Duţu et al., 2014).

The cutoff channel of M1 receives 3.8% of the upstream flow (Fig. 2; Tab. 1). The water discharge decreases progressively along the natural course of the meander, as well as the flow velocities (from 0.44 to 0.05 m/s^{-1}) (Fig. 3; Tab. 1). On the M2, the upstream discharge captured by the natural meander course was over 77.7% (Fig. 2).

The water flow acceleration in the natural channel shows the presence of incision processes at the bifurcation sector (I1-I2) with high-velocity values located in the right bank of the I2 profile (Fig. 3). The water fluxes at the bifurcation are distributed unequally between the natural course of the M3 meander (78 m^3/s^{-1}) and the cutoff canal (2003 m^3/s^{-1}), with a very high flux in the cutoff canal (≈96-97% of total) (Fig. 2). Local morphodynamical processes of the 2006 flood were analyzed through two geomorphological parameters: the specific stream power, indicating the stream's ability to adjust its channel morphology (Biedenharn et al., 2000); and the boundary shear stress indicating flow capacity to mobilize sediment from the bed as suspended-load or bedload.
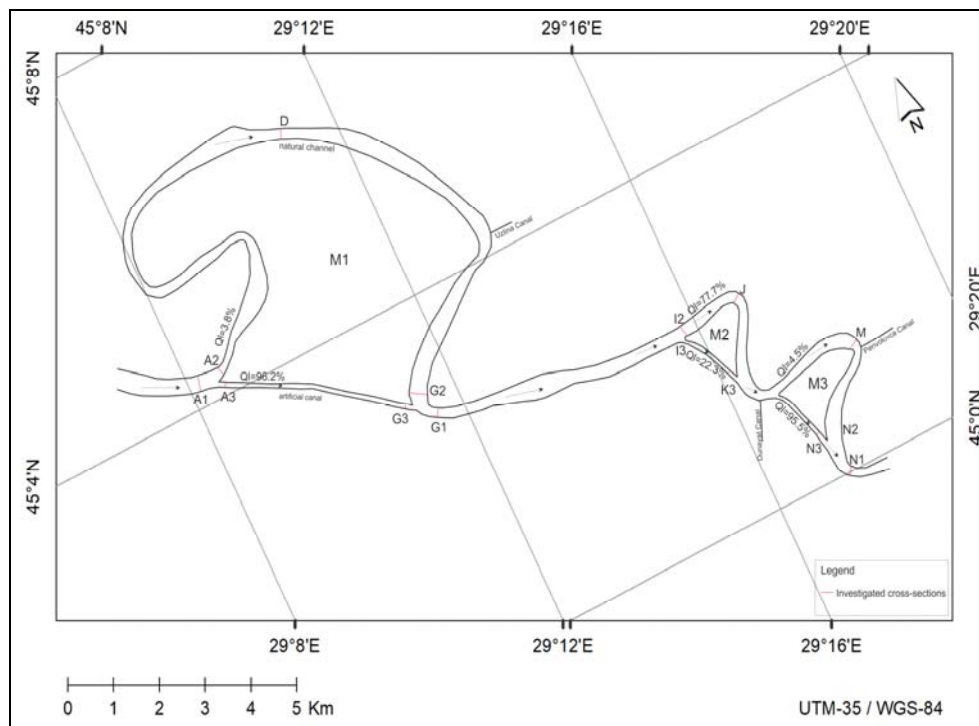
Figure 2: Water discharge repartition between the natural channel and artificial canals along the three investigated cutoff meanders.
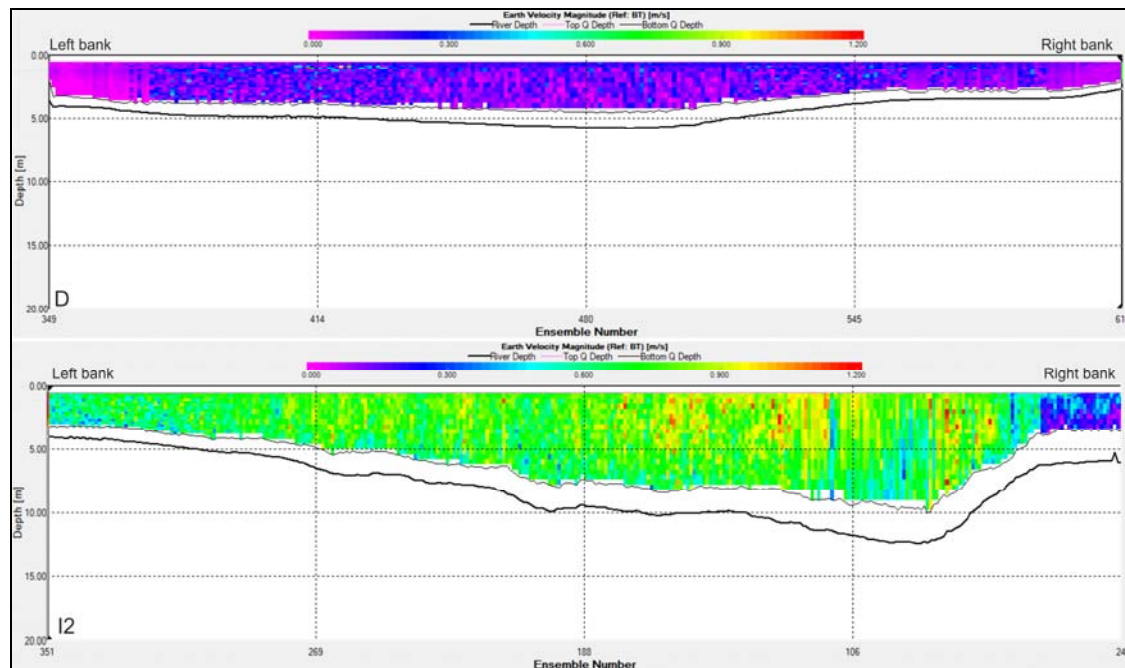
Figure 3: Velocities' magnitude distribution on the former meanders M1 and M2.

Morphodynamical activity is generally assessed by the specific stream power (ω) as an indicator of the river transport capacity (Bagnold, 1966):

$$\omega = \Omega/B \qquad [W.m^{-2}]$$

where B is the bankful channel width and Ω is the stream power that writes as:

$$\Omega = \rho\, g\, Q\, S \qquad [W.m^{-1}]$$

where the representative discharge Q [m³.s⁻¹] is usually taken as the bankful discharge Q_{bf}.

Sectors with high specific stream power (above 4.00 W.m⁻²) are found on the artificial canals (A3, I3, K3, and N3) and the main channel (A1 and G1). Along the former meanders, stream power values are much lower on M1 and M3 because of the lower slope and longer length (between 0.06 and 0.92 W.m⁻²) and higher on M2 (1.2 W.m⁻²) (Tab. 1).

The mean bed shear stress (τ_0) corresponds to the unit tractive force exerted on the bed:

$$\tau_0 = \rho\, g\, R\, S \qquad [N.m^{-2}]$$

Where ρ is the fluid density (1000 kg.m⁻³ for sediment-free water), g is the gravitational acceleration (9.81 m.s⁻²), R is the hydraulic radius [m], and S is the water-energy slope [m.m⁻¹].

The mean bed shear stress (τ_0) generally agrees with the stream power values. The shear stress is significantly higher along the main channel (A1, G1, and N1) (Tab. 1). In the former meander, shear stress values are dramatically lower (between 0.02 and 0.67 N.m⁻² on the profiles A2, G2, and M). The results offer a more continuous view of the complex variability of the hydrodynamic processes along the study reaches.

Chemical characteristics of the sediments

From a chemical point of view, the analyzed sediments can be classified based on their concentration of calcium carbonate as follows: terrigenous non-carbonated sediments – CaCO₃ ≤ 10%, weakly calcareous – 10% < CaCO₃ ≤ 30%, and calcareous sediments – 30% < CaCO₃ ≤ 50%.

Table 1: Location of the cross-sections and sediment samples, site description, and measured and calculated hydraulic parameters.

Profile/ sample	Site description	Depth (m)	Q water ($m^3.s^{-1}$)	Velocity ($m.s^{-1}$)	τ_0 ($N.m^{-2}$)	Ω ($W.m^{-2}$)
A1-06	Upstream bifurcation of M1	10.1	2170	0.85	5.25	4.95
A2-10	Downstream bifurcation of M1	1.5	85.1	0.1	0.14	0.13
A3-05	Artificial canal at the M1 bifurcation	17.4	2063	0.96	5.58	5.96
D-17	M1 natural course	4.5	82.4	0.1	0.10	0.06
G1-01	Downstream M1 confluence	8.8	2130.8	0.78	4.63	6.15
G2-14	Upstream M1 confluence	2.3	70.93	0.05	0.03	0.02
G3-04	Artificial canal at the M1 confluence	20.4	2068.3	0.78	3.49	2.93
I2-29	Downstream bifurcation on M2	9	1643.2	0.7	3.70	2.91
I3-28	Downstream bifurcation on M2 canal	4.4	465.2	0.68	4.43	9.72
J-30	Apex of M2	15.8	1705.1	0.55	1.89	1.20
K3-32	Upstream confluence on M2 canal	7.8	484.5	0.92	6.70	10.19
M-38	M2 natural course	3.1	83.4	0.25	0.67	0.92
N1-42	Downstream confluence of M3	12.5	2052.3	0.79	4.22	4.40
N3-43	Upstream confluence of M3 canal	21.8	1983.9	1.13	7.17	6.87
LU-22	Uzlina Lake	1.0	–	–	–	–
CD-35	Dunavăț Canal	5.6	–	–	–	–
CP-40	Perivolovca Canal	3.0	–	–	–	–

The geochemical characteristics of the sediments

The results of the geochemical analyses are given in tables 2a and 2b. The statistical analysis of the data (Tab. 3) evidenced a high compositional variability, with concentration variation coefficients ranging between 12.9% – Sr and 138.3% – TOC (Total Organic Carbon). From this point of view, the chemically analyzed compounds can be divided into two major groups: group I includes compounds with variability ranging from small to moderately high ($15\% < c_v < 50\%$), and group II, which includes compounds with high variability ($c_v > 50\%$).

Group I includes major compounds ($CaCO_3$, Fe_2O_3), minor compounds (TiO_2), and trace elements (Sr, Rb, V, and Co), with the variation coefficients ranging between 12.9% – Sr and 48.7% – Zr.

It is essential to underline the extremely low variability of the Sr, probably due to the presence of calcium carbonate in the form of calcite, a crystalline form in which Sr cannot replace calcium. Similarly, the variability of the $CaCO_3$ ($c_v = 30.6\%$) concentrations are determined by the exceptional concentration ($CaCO_3 \approx 21.2\%$) in sample LU-22, characterized by the presence of shell fragments and whole shells of *Anodonta*. The elimination of value causes a decrease in the coefficient of variation to 18.6% and a slight reduction of the mean and median concentrations (Tab. 3a). For Zr, the variability of concentrations is determined by its exclusive presence in the form of zircon, whose sedimentation depends on the hydrodynamic environmental factors, which determine the concentration of the mineral in accumulations of heavy minerals, characteristic of coarse sediments.

The II-nd group includes compounds whose variation coefficients range between 50.6% – Co and 138.3% – TOC. Aside from the two components, the group consists of MnO (minor component) and the other determining trace elements. These high variabilities can have different causes. The highest variability recorded by TOC is determined by the biogenic origin of the TOC (especially in the primary productivity), combined with its chemical instability and the dependence of its concentration on its conservation in sediments. TOC preservation depends on sediment granulometry (fine particles much better preserve organic matter) and local physicochemical conditions (dissolved oxygen concentration). High concentrations of total organic carbon (TOC > 0.5%) characterize samples from fine silty sediments with low permeability. Exceptional concentrations (TOC > 1%), found in samples D-17 – 1.15% and especially LU2-22 – 2.6% TOC, are associated with the presence in sediments of easily oxidizable vegetal residues.

Mn is an element with extremely high redox sensitivity. The local physicochemical conditions can determine post-depositional processes that significantly affect the MnO concentrations in the sediments, leading to the increase of its variability.

In the case of heavy metals with high technophilic indices – Cu, Pb, and Cd, it is assumed that the anthropic contributions play a significant part in determining the variability of their concentrations.

As for the frequencies of the appearance of the concentrations of the analyzed compounds, their distribution curves show right-side asymmetries (Sk = 0.460-3.474). $CaCO_3$, TOC, and Sr have strongly leptokurtic distribution curves (K > 5), indicating the concentration values in a very narrow range. Cr, Pb, Cu, and Cd also have leptokurtic distribution curves, but kurtosis values range from 0.312 (Cu) to 1,345 (Cr). The curves are slightly platykurtic for all other components, with kurtosis values between –1,426 (TiO_2) and –0.052 (V).

Based on the values of these two parameters, the distribution curves of Fe_2O_3, TiO_2, MnO, Zr, Rb, Ni, V, and Co are considered normal, symmetrical, and mesokurtik. Important asymmetries (1,500 < K > 1,000) recorded for Zn, Cr, Pb, Cu, and Cd, do not allow the acceptance of the normality of their concentration distribution curves. The Shapiro-Wilk test also confirmed the normality of the TiO_2, V, and Co distributions for a probability p > 0.05. The same test confirms the normality of the distributions at a probability of 0.01 < p < 0.05 for Fe_2O_3, Zr, Rb, and Ni, rejects the normality of the MnO distribution (p = 0.0085) and, instead, allows the acceptance of the normality of the Cr distribution (p = 0.034).

Generally, the chemically analyzed compounds can be divided into two major categories: biogenic compounds – $CaCO_3$, Sr, and TOC, and terrigenous compounds – the rest of the compounds. $CaCO_3$, a generally inferior compound in trace elements, Sr excepted, usually acts as a diluter for the terrigenous compounds. Given the relatively small variability in calcium carbonate concentrations, its role as a diluent in determining the variability in the concentrations of terrigenous compounds is minor. Under these circumstances, the determining role played by the hydrodynamic conditions that determine the gravitational particle differentiation of the sedimentary material.

Table 2a: Geochemical analyses on bed sediments of the three studied meanders during the field campaign from June 2017 to estimate the geochemical properties of bottom sediments and characterize the variation of major and trace elements.

Sample	CaCO₃, %	TOC, %	Fe₂O₃, %	TIO₂, %	Zr, µG/G	Sr, µG/G	RB, µG/G	Zn, µG/G
G1-01	7.22	0.07	2.30	0.48	98	185	55	24.54
G3-04	8.23	0.04	2.80	0.59	112	186	53	20.83
A3-05	11.41	0.02	2.60	0.42	92	199	48	26.07
A1-06	7.66	0.05	2.44	0.51	94	189	51	18.81
A2-10	10.64	0.07	2.73	1.13	192	190	47	19.22
G2-14	10.16	0.95	6.09	0.98	272	196	109	103.40
D-17	9.67	1.15	7.54	1.01	187	185	134	151.10
LU-22	21.18	2.60	5.18	0.45	103	290	91	119.10
I3-28	9.14	0.01	3.17	0.93	117	176	37	16.12
I2-29	8.96	0.06	2.83	0.63	99	187	49	21.88
J-30	12.13	0.24	4.11	0.80	292	194	75	37.78
K3-32	13.20	0.72	6.01	1.02	285	183	104	73.82
CD2-35	12.13	0.53	5.34	0.93	347	210	106	74.98
M-38	10.20	0.06	2.65	0.71	143	203	54	23.47
CP2-40	10.52	0.86	5.61	0.95	316	190	103	96.26
N1-42	6.95	0.06	2.34	0.62	137	191	54	19.90
N3-43	11.38	0.72	5.84	0.83	244	189	105	68.63

Table 2b: Results of geochemical analyses on bed sediments of the three studied meanders during the field campaign from June 2017 to estimate the geochemical properties of bottom sediments and characterize the variation of major and trace elements.

Sample	Ni, µg/g	MnO, %	Cr, µg/g corr	V, µg/g	Co, µg/g	Pb, µg/g	Cu, µg/g	MnO, %	Cd, µg/g
G1-01	23.9	0.024	31.5	46	6.83	9.717	4.367	0.024	0.07
G3-04	18.1	0.021	30.4	44	4.64	8.762	3.86	0.021	0.071
A3-05	23.0	0.033	31.7	66	7.38	10.81	4.211	0.033	0.073
A1-06	17.7	0.019	28.6	43	3.88	9.019	2.934	0.019	0.056
A2-10	14.9	0.021	14.0	77	3.25	8.62	4.064	0.021	0.064
G2-14	50.7	0.107	89.7	114	12.60	24.2	44.84	0.107	0.296
D-17	73.9	0.111	127.0	144	16.28	38.18	69.80	0.111	0.427
LU-22	51.4	0.133	55.6	64	11.81	38.06	69.62	0.133	0.571
I3-28	14.0	0.019	19.2	89	3.31	7.529	3.00	0.019	0.048
I2-29	19.5	0.020	18.0	43	5.46	7.968	3.02	0.020	0.079
J-30	28.2	0.050	48.4	64	6.44	11.8	11.01	0.050	0.125
K3-32	51.8	0.090	75.2	88	12.37	22.52	26.37	0.090	0.182
CD2-35	42.9	0.069	57.7	110	11.24	16.68	28.75	0.069	0.281
M-38	18.8	0.015	19.4	69	4.68	8.368	4.21	0.015	0.079
CP2-40	46.2	0.085	70.7	99	10.87	20.44	41.81	0.085	0.325
N1-42	17.8	0.020	22.1	76	4.09	8.585	3.64	0.020	0.058
N3-43	51.5	0.075	59.9	104	11.59	20.35	27.37	0.075	0.177

Table 3a: Descriptive statistics for the concentrations of the studied variables of the collected sediment samples.

Sample	CaCO$_3$, %	TOC, %	Fe$_2$O$_3$, %	TiO$_2$, %	Zr, µg/g	Sr, µg/g	Rb, µg/g	Zn, µg/g
Mean	10.63	0.48	4.09	0.76	184.11	196.64	75	53.87
Median	10.2	0.07	3.17	0.8	143	190	55	26.07
Standard Deviation	3.25	0.66	1.71	0.22	89.64	25.34	29.95	42.54
Minimum	6.95	0.01	2.3	0.42	92	176	37	16.12
Maximum	21.18	2.60	7.54	1.13	347	290	134	151.1
Cv, %	30.62	138.28	41.85	29.91	48.69	12.89	39.94	78.97
Count	17	17	17	17	17	17	17	17

Table 3b: Descriptive statistics for the concentrations of the studied variables of the collected sediment samples.

Sample	Ni, µg/g	MnO, %	Cr, µg/g corr	V, µg/g	Co, µg/g	Pb, µg/g	Cu, µg/g	Cd, µg/g
Mean	33.19	0.05	47.00	78.82	8.042	15.97	20.75	0.17
Median	23.86	0.032	31.73	76	6.82	10.81	4.36	0.07
Standard Deviation	18.13	0.03	30.72	28.78	4.06	10.05	23.27	0.15
Minimum	13.98	0.01	13.95	43	3.248	7.52	2.93	0.04
Maximum	73.92	0.13	126.95	144	16.28	38.18	69.8	0.57
Cv, %	54.63	73.71	65.37	36.51	50.58	62.91	112.15	87.65
Count	17	17	17	17	17	17	17	17

Geochemical relations between components

Sediments result from mixing between the terrigenous material, biogenic calcium carbonate (generally low in heavy metals), and a low quantity of organic matter (Dutu et al., 2019). Due to this mixing process, a number of relationships are established between the chemical compounds, which are frequently linear and are identified by calculating the linear correlation coefficients (Tab. 4).

The analysis of the matrix of the linear correlation coefficients (Tab. 4) clearly emphasizes the complete lack of calcium carbonate dilution effect for these analyzed samples. Moreover, CaCO$_3$ correlates positively with the vast majority of terrigenous compounds (except TiO$_2$), in some cases significant at the critical statistical confidence level $\alpha = 0.05$ – Zn, Pb, Cu, and Cd. The significant association of calcium carbonate with these heavy metals can only be explained by the abiogenic origin of a considerable amount of calcium carbonate, most likely resulting from the surface alteration of complex sulfide mineralizations, in which calcium carbonate, generally present as calcite, appears as a secondary mineral.

A fascinating aspect revealed by the matrix of linear correlation coefficients (Tab. 4) is the close association of TOC with the vast majority of terrigenous compounds, especially with Zn, Pb, Cu, and especially Cd. The linear correlation coefficients of TOC with these metals have a statistical significance higher than those of Fe$_2$O$_3$ or Rb, with which metals are usually associated.

Table 4a: Correlation matrix (Pearson) for the major constituents, minor constituents and heavy metals ($r_{17;\,0.05;\,95} = 0.482$).

Compound	CaCO$_3$ %	TOC %	Fe$_2$O$_3$ %	TiO$_2$ %	Zr µg/g	Sr µg/g	Rb µg/g	Zn µg/g
CaCO$_3$, %	1							
TOC, %	**0.817**	1						
Fe$_2$O$_3$, %	0.434	**0.689**	1					
TiO$_2$, %	− 0.021	0.051	**0.567**	1				
Zr, µg/g	0.191	0.200	**0.666**	**0.714**	1			
Sr, µg/g	**0.854**	**0.779**	0.161	− 0.359	− 0.112	1		
Rb, µg/g	0.381	**0.668**	**0.969**	0.480	**0.691**	0.180	1	
Zn, µg/g	**0.517**	**0.841**	**0.926**	0.356	0.459	0.394	**0.923**	1
Ni, µg/g	0.457	**0.753**	**0.965**	0.385	**0.529**	0.257	**0.970**	**0.960**
MnO, %	**0.675**	**0.906**	**0.907**	0.303	**0.489**	**0.506**	**0.889**	**0.956**
Cr, µg/g	0.262	**0.610**	**0.938**	0.445	**0.542**	0.068	**0.942**	**0.922**
V, µg/g	0.120	0.378	**0.823**	**0.725**	**0.622**	− 0.091	**0.786**	**0.733**
Co, µg/g	0.458	**0.725**	**0.939**	0.333	**0.531**	0.261	**0.956**	**0.939**
Pb, µg/g	**0.637**	**0.917**	**0.858**	0.220	0.290	**0.528**	0.841	**0.964**
Cu, µg/g	**0.605**	**0.914**	**0.870**	0.272	0.362	**0.524**	**0.858**	**0.984**
Cd, µg/g	**0.707**	**0.950**	**0.788**	0.170	0.334	**0.669**	**0.787**	**0.937**

Table 4b: Correlation matrix (Pearson) for the major constituents, minor constituents and heavy metals ($r_{17;\,0.05;\,95} = 0.482$).

Compound	Ni µg/g	MnO %	Cr µg/g corr	V µg/g	Co µg/g	Pb µg/g	Cu µg/g	Cd µg/g
CaCO$_3$, %								
TOC, %								
Fe$_2$O$_3$, %								
TiO$_2$, %								
Zr, µg/g								
Sr, µg/g								
Rb, µg/g								
Zn, µg/g								
Ni, µg/g	1							
MnO, %	**0.931**	1						
Cr, µg/g	**0.951**	**0.857**	1					
V, µg/g	**0.755**	**0.632**	**0.782**	1				
Co, µg/g	**0.985**	**0.920**	**0.935**	**0.728**	1			
Pb, µg/g	**0.924**	**0.955**	**0.849**	**0.613**	**0.893**	1		
Cu, µg/g	**0.915**	**0.958**	**0.851**	**0.656**	**0.886**	**0.981**	1	
Cd, µg/g	**0.838**	**0.932**	**0.744**	**0.540**	**0.821**	**0.945**	**0.975**	1

The close association is partly explained by the preferential concentration of organic matter in the fine sediments, which allow its preservation and in which the terrigenous compounds are also concentrated. However, this cannot fully explain the extremely high statistical significance of TOC-metal relationships, which suggests that at least some of the metals contained in sediments come from bioavailable forms and reach sediments through organic matter in the form of organo-metallic compounds.

The same metals have the highest values of linear correlation coefficients with MnO, a probable consequence of both their association in the fine material and the adsorption capacities of hydrated manganese oxides, which concentrate metals in soluble forms.

Co, Ni, Cr, Cu, Pb, Zn, and Cd tightly correlate among each other and the Fe_2O_3 and Rb concentrations, indicating their association in the terrigenous material. The linear correlation coefficients of V with Fe_2O_3 and Rb have lower values ($r_{Fe-V} = 0.823$ and $r_{Rb-V} = 0.786$) due to the concentration of some V minerals in the accumulations deposits of heavy metals. This process leads to the partial alteration of its relations with the other two components. Due to their concentration in fine silicate fraction, the linear correlation coefficients between metals and Fe_2O_3 are relatively close to those with Rb.

Sediments quality

Order 161/2006 of the Romanian Ministry of Environment and Water Management sets quality criteria for many chemical compounds, organic and inorganic, in sediments; these include many heavy metals (Tab. 5). Comparing the mean concentrations of the primary metals (Tab. 4) with high toxicity to the criteria in effect in Romania (Tab. 5) proves that overall, at the level of the investigated area, these fit the criteria, indicating a good quality of the sediments.

However, the comparison between the criteria and the maximum values of the concentrations of the investigated metals show exceeding concentrations/ratios for Ni, Cr, and Cu. In the case of Ni and Cu, the exceeding concentrations/ratios are frequent: Ni – 41.2% and Cu – 23.5% of the analyzed samples. Cr and Zn exceed the quality criterion in a single sample (D-17).

Table 5: Quality criteria for several chemical compounds, organic and inorganic, in sediments (Order 161/2006).

Metal	UM	Ord. 161/2006	Mean concentration	Concentrations exceeding Ord. 161 criteria
Cadmium (Cd^{2+})	µg/g	0.8	0.175	0
Total chromium ($Cr^{3+} + Cr^{6+}$)	µg/g	100	47	1
Copper (Cu^{2+})	µg/g	40	20.8	4
Lead (Pb^{2+})	µg/g	85	16	0
Zinc (Zn^{2+})	µg/g	150	53.9	1
Nickel (Ni^{2+})	µg/g	35	33.2	7

Still, the systematic exceeding of the quality criteria does not necessarily mean an intense pollution process, just as compliance with the quality criteria does not necessarily imply the absence of pollution. It needs to be taken into account that these criteria are general, set exclusively based on biological criteria, without considering the regional and local geochemical background.

The natural abundance of nickel in the Earth's crust, which through alteration generates sedimentary nickel, is 84 µg/g, more than twice the median concentration. The same applies to Cr, which has an abundance of 140 µg/g – almost three times more than the average concentration of Cr in the analyzed samples and Cu – an abundance of 55-60 µg/g, mean concentration of 21.8 µg/g.

One more argument favoring the normality of the concentrations of these metals is represented by the very close relationships between the concentrations of the incriminated metals and the concentrations of the non-technophilic chemical compounds Fe_2O_3 and Rb, relations with a very high degree of statistical significance. The linear regression analysis and the calculation of the prediction interval for the concentration of the heavy metal (the dependent variable) subject to the concentration of the independent variable (non-technophile compound) highlights, for instance, the case of the relationship Fe_2O_3 – Ni (Fig. 4), the fitting of the Ni concentrations within the prediction interval of the relationship:
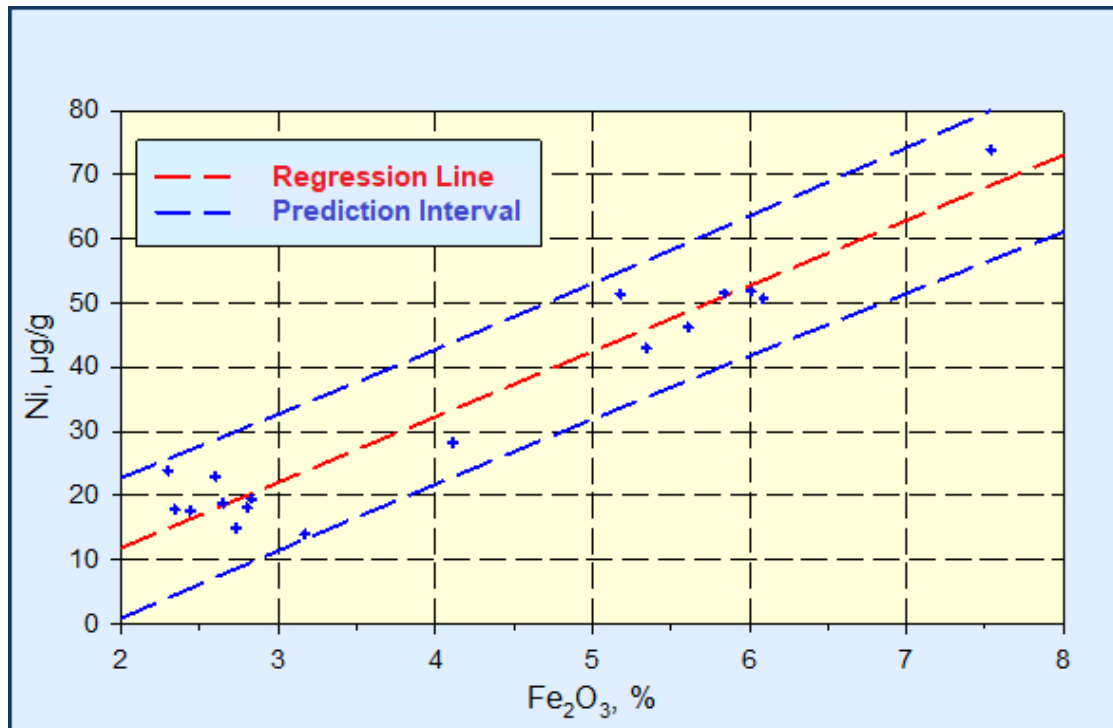
$$C_{Ni} = 10.210 * C_{Fe2O3} - 8.597, R^2 = 0.930$$

Figure 4: Linear regression diagram Fe_2O_3 – Ni,
with the prediction interval
($C_{Ni} = 10.210 * C_{Fe2O3} - 8.597, R^2 = 0.930$)

They can be considered normal; their variation is determined by natural processes leading to either the dilution or the concentration of the nickel-bearing sedimentary material. A similar result was obtained for Cr and shows that only one sample exceeded the quality criterion (127 µg/g Cr, on D-17) but fits in the prediction interval of the relationship (Fig. 5):

$$C_{Cr} = 16.818 \cdot Fe_2O_3 - 21.831, \quad R^2 = 0.879$$
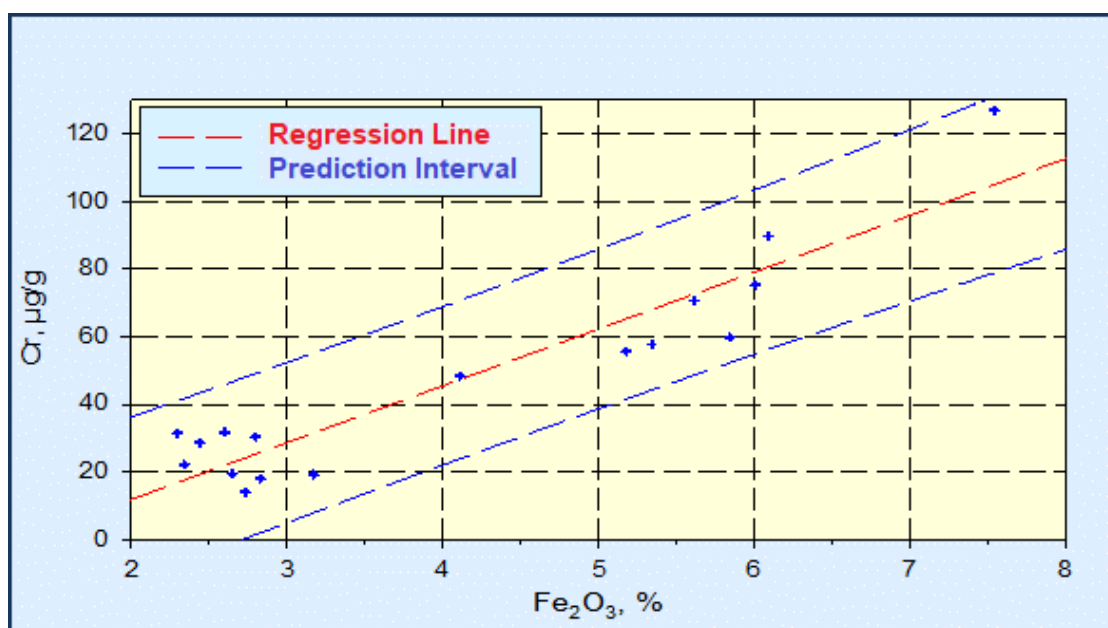
Figure 5: Linear regression diagram Fe_2O_3 – Cr,
with the prediction interval
($C_{Cr} = 16.818 \cdot Fe_2O_3 - 21.831$, $R^2 = 0.879$)

Also, for Zn, the concentration of 151.1 µg/g Zn in sample D-017 can be considered normal (Fig. 6). However, in this case, the analysis reveals that in the sample LU2-22, the Zn concentration, although lower than the quality criterion (C_{Zn} = 119.1 µg/g), is more than that predictable of the Fe_2O_3 – Zn relationship (Fig. 6). It indicates low Zn contributions of possible anthropic origin.
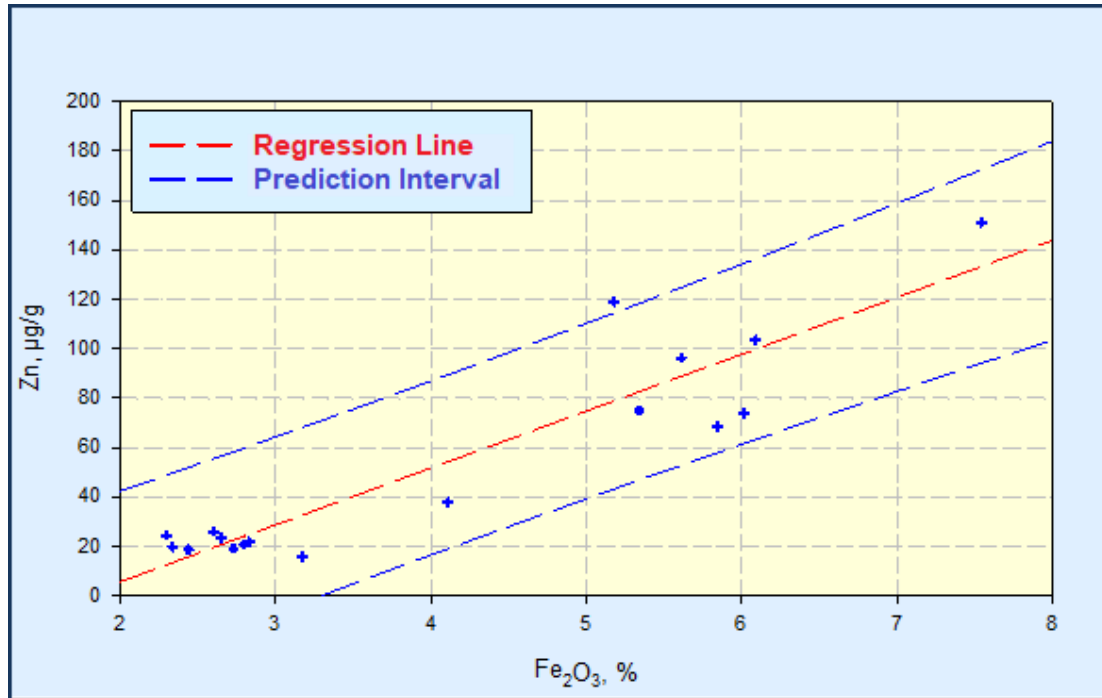
Figure 6: Linear regression diagram Fe_2O_3 – Zn,
with the prediction interval
(C_{Zn} = 23.007*C_{Fe2O3} –0.289, R^2 = 0.858).

In the case of Cu, the analysis reduced the number of abnormal concentrations from four to one, whose concentration exceeds the prediction interval of the relationship Fe_2O_3 – Cu – sample LU-22, Lake Uzlina – 69.6 µg/g Cu (Fig. 7). The excessive concentration of Cu in this sample is also accompanied by concentrations of Pb and Cd that exceed the limits of the prediction range, even if they are lower than the quality criteria, indicating possible influences of mining activities.

In previous considerations, it is concluded that the sediments in the investigated area comply qualitatively. The elevated concentrations of the heavy metals are, for the most part, the result of the sedimentary material originating from the Dobrudjan green schists characterized by high natural concentrations of Ni, Cu, and Cr. At the same time, the analyzes clearly show that compliance with the quality criteria does not exclude anthropogenic or natural contributions independent of the sedimentary material.
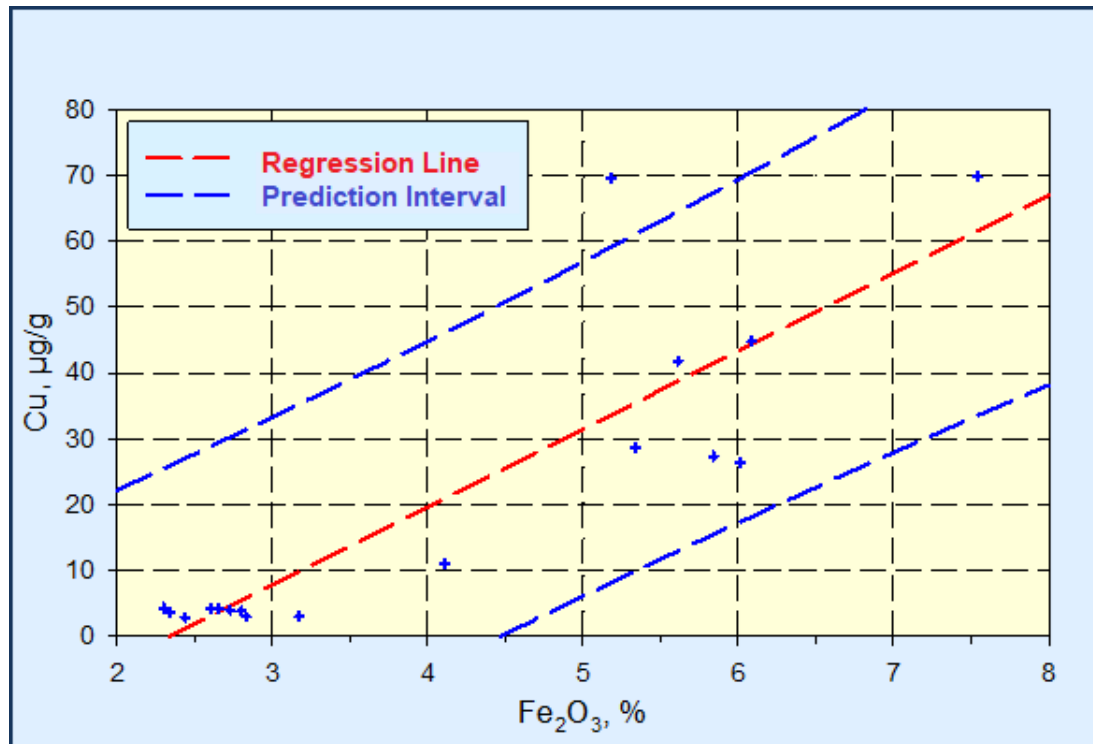
Figure 7: Linear regression diagram $Fe_2O_3 - Cu$, with the prediction interval
($C_{Cu} = 11.826 * C_{Fe2O3} - 27.644$, $R^2 = 0.757$)

CONCLUSIONS

Generally, the highest concentrations of terrigenous compounds appear in the samples collected from the natural canals. Occasionally, high concentrations of these compounds are also encountered at the exits from the rectification canals on the Sfântu Gheorghe Branch (samples K3-32 and N3-43). It is expected that the hydrodynamic conditions, among which the speed of the current is probably the determining factor, will facilitate the accumulation of fine material (silt and clay). The terrigenous compounds are concentrated and favor the conservation of organic matter. In the other sectors, a more active hydrodynamic regime favors the deposition of coarse material (coarse silt and mainly sand), generally quartzitic, poor in terrigenous compounds and organic matter. The coarse sediments are often characterized by enrichment in heavy minerals, clearly evidenced by high concentrations of TiO_2, Zr, and sometimes V – especially the A1-08, A2-11, G1-01, I2-29, and CD-35 samples.

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THE PENNATE BENTHIC DIATOMS
OF THE SAND BEACH OF KRUGLAYA BAY (THE BLACK SEA)

Larisa RYABUSHKO *, *Andrey BEGUN* **, *Sophia BARINOVA* *** and *Denis LISHAEV* *

* Moscow Representative Office of A. O. Kovalevsky Institute of Biology of the Southern Seas of Russian Academy of Sciences, 38/3 Leninskiy Avenue, RU-119991, larisa.ryabushko@yandex.ru, ORCID: 0000-0003-0443-9929, chandler37@yandex.ua, ORCID: 0000-0002-9737-3246.
** A. V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevsky Street, Vladivostok, Russia, RU-690041, andrejbegun@yandex.ru, ORCID: 0000-0002-8383-796X.
*** Institute of Evolution, University of Haifa, Mount Carmel, 199 Abba Khoushi Avenue, Haifa, Israel, IL-3498838, sophia@evo.haifa.ac.il, ORCID: 0000-0001-9915-2503.

ABSTRACT

For the first time in the Kruglaya Bay of the Black Sea, 25 taxa of Bacillariophyta from 17 genera were found, three of them were new for the Crimea and the Black Sea *Halamphora tenerrima*, *Amphora tenuissima*, and *Navicula antonii*. Marine (40%), coastal-brackish (32%) and boreal-tropical (32%), arctic-boreal-tropical (24%), boreal and cosmopolitan (20% and 16%, respectively) prevailed. The morphological characteristics of the species in the SEM (scanning electronic microscope) and LM (light microscope), as well as the occurrence, ecology, phytogeography, and general distribution in various regions of the World Ocean are given.

ZUSAMMENFASSUNG: Die pennaten benthischen Diatomeen des Sandstrandes der Kruglaya-Bucht (Schwarzes Meer).

Erstmals wurden in der Kruglaya-Bucht des Schwarzen Meeres 25 Bacillariophyta-Taxa aus 17 Gattungen gefunden, drei davon neu für die Krim und das Schwarze Meer: *Halamphora tenerrima*, *Amphora tenuissima* und *Navicula antonii*. Marine (40%), küstenbrackig (32%) und boreal-tropisch (32%), arktisch-boreal-tropisch (24%), boreal und kosmopolitisch (20% bzw. 16%) überwogen. Die morphologischen Merkmale der Arten im SEM (Elektronisches Scan Mikroskop) und LM (Licht-Mikroskop) sowie das Vorkommen, die Ökologie, die Phytogeographie und die allgemeine Verbreitung in verschiedenen Regionen des Weltozeans werden angegeben.

REZUMAT: Diatomeele bentonice penate ale plajei de nisip din Golful Kruglaya (Marea Neagră).

Pentru prima dată în Golful Kruglaya al Mării Negre au fost găsiți 25 de taxoni de Bacillariophyta din 17 genuri, trei dintre aceștia fiind noi pentru Crimeea și Marea Neagră *Halamphora tenerrima*, *Amphora tenuissima* și *Navicula antonii*. Au predominat taxoni marini (40%), litoral-salmaștri (32%) și boreal-tropicali (32%), arctic-boreal-tropicali (24%), boreal și cosmopoliți (20% și respectiv 16%). Sunt prezentate caracteristicile morfologice ale speciilor în SEM (Microscop electronic cu baleiaj) și LM (Microscop optic), precum și apariția, ecologia, fitogeografia și distribuția generală în diferite regiuni ale Oceanului Mondial.

INTRODUCTION

The diatoms episamon in shallow coastal waters of Kruglaya Bay are still poorly studied, including in SEM. However, the data from the study of diatoms in electronic scanning microscope (SEM) are important not only for species identification in general, but especially for cryptic ones, i.e. morphologically similar species. In addition to studying the morphological elements of diatoms, data on ecology and phytogeography are needed too.

The aim of this work is to study the species belonging of the epipsammon biraphid benthic diatoms of the Kruglaya Bay of the Black Sea, their morphology in SEM, size, structure, ecology, and phytogeography.

MATERIAL AND METHODS

Description of the study site

Kruglaya Bay (Omega) of the Black Sea is located five km southwest of the entrance to the Sevastopol (Fig. 1). Sampling from the sand beaches was carried out on October 30, 2016 in Kruglaya Bay from a depth of 0.3 m. At the time, water temperature was 13°C and water salinity 17.89‰.

Figure 1: Map of the sampling sites in Kruglaya Bay, Crimea, Black Sea.

Sampling and laboratory study

Sample processing and preparation for analysis by scanning electron microscopy ("Sigma 300 VP" type, Great Britain) was carried out at the A. V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences.

We used the classification of benthic diatom taxa based on Round et al. (1990) with some later additions (Cox, 1988; Levkov, 2009). The morphometric (dimensions in SEM and LM), ecological (the relation of species to water salinity) and phytogeographical characteristics of diatoms were used from the literary sources. The SEM and LM examination of material from Kruglaya Bay reveals that diatom cell sizes are in accordance to our previous data and to the literature (Korotkevich, 1960; Proshkina-Lavrenko, 1963; Karayeva, 1972; Guslyakov et al., 1992; Ryabushko and Begun, 2016).

RESULTS

There are 25 species of Bacillariophyta representing to 17 genera and three species of *Halamphora tenerrima*, *Amphora tenuissima*, and *Navicula antonii* first indicated in the Black Sea (Tab. 1). From all species of marine (40%), marine-brackish (32%), boreal-tropical (32%), arctic-boreal-tropical (24%), boreal and cosmopolites (20% and 16% respectively) were dominated. But brackish, freshwater, indifferents and arctic-boreal marked with fewer (8% each), including notal species (12%), found in the southern hemisphere (Tab. 1).

Table 1: The occurrence of diatoms, their ecological (RS) and phytogeographycal (PhG) characteristics of epipsammon on Kruglaya Bay of the Black Sea; species are listed in alphabetical order; * – A new species for the Crimean coastal waters and the Black Sea; RS – the relation of species to the water salinity: M – marine species, FW – freshwater, MB – marine-brackish, B – brackish, ind – indifferents; PhG – phytogeographic elements: B – boreal species, AB – arctic-boreal, BT – boreal-tropical, ABT – arctic-boreal-tropical, C – cosmopolite, not – notal species, found in the southern hemisphere.

Taxa	RS	PhG	Figs.
Adlafia besarensis (Giffen 1980) L. I. Ryabushko comb. nov.	M	BT	7, a-c
Amphora crassa W. Gregory 1857	M	ABT	8, f-h
Amphora tenuissima Hustedt 1955 *	M	BT	8, k-m
Cylindrotheca closterium (Ehrenberg) Reimer et Lewin 1964	MB	C	9, a-b
Cymbella Odesana Guslaykov 1992	B	B	7, d-g
Diploneis smithii (Brèbesson) P. T. Cleve 1894	MB	C	2, a-h
Entomoneis paludosa duplex (Donkin) Czarnecki et Reinke 1982	B	B	12, d-e
Gyrosigma attenatum (Kützing) Rabenhorst 1853	FW	ABT	6, a-c, e
Halamphora coffeiformis (C. A. Agardh) Z. Levkov 2009	MB	C	8, a-b
Halamphora tenerrima (Aleem et Hustedt) Z. Levkov 2009 *	M	BT	8, c-e
Hantzschia spectabilis (Ehrenberg) Hustedt 1959	MB	B, not	11, a-m
Lyrella abrupta (W. Gregory) D. G. Mann 1990	M	BT	4, b-d
Lyrella atlantica (W. Gregory) D. G. Mann 1990	M	BT	4 a
Lyrella rudiformis (Hustedt) Guslyakov et Karayeva, 1992	M	B	4, e-g
Navicula antonii Lange-Bertalot 2000 *	FW	BT, not	3, f-k
Navicula distans (W. Smith) Ralfs 1861	MB	ABT	4, h-n
Navicula palpebralis Brébisson ex W. Smith 1853	M	ABT, not	3, a-e
Nitzschia cf. *angustata* (W. Smith) Grunow	ind	B	9 f
Nitzschia ovalis Arnott 1880	MB	AB	9 c-e
Plagiotropis lepidoptera (W. Gregory) Kuntze 1898	M	ABT	6, d, f-k
Pleurosigma cuspidatum (P. T. Cleve) Peragallo 1891	M	BT	5 k-o
Pleurosigma elongatum W. Smith 1852	MB	C	5, a-h
Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle 1993	M	ABT	10, a-g
Surirella ovalis Brébisson 1838	ind	AB	12, a-c
Tryblionella coartata (Grunow) D. G. Mann 1990	MB	BT	9, g-m

Information on the taxonomic characteristics of diatoms identified in Kruglaya Bay, the Black Sea, their morphology and morphometric, ecology and phytogeography is presented below.

Biraphid diatoms, Class Bacillariophyceae Haeckel 1878
Order Naviculales Bessey 1907

Family Diploneidaceae Mann 1990
Genus *Diploneis* (Ehrenberg) P. T. Cleve 1894

***Diploneis smithii* (Brébisson) P. T. Cleve 1894. Fig. 2, a-h**
(Basionym: *Navicula smithii* Brébisson 1856; Synonyms: *Navicula elliptica* W. Smith 1853; *Pinnularia scutellum* O'Meara 1875; *Navicula scutelum* O'Meara 1875; *Navicula smithii* var. *laevis* Juhlin-Dannfelt 1882; *Navicula smithii* var. *borealis* Grunow 1884; *Navicula fusca* var. *permagna* Pantosek 1889; *Diploneis major* Cleve 1894; *Navicula smithii* var. *scutellum* Van Heurck 1896; *Navicula smithii* var. *permagna* Peragallo 1897; *Navicula gyrinida* A. Mann 1907; *Navicula smithii* var. *minor* West 1912; *Diploneis smithii* var. *permagna* Cleve 1915; *Diploneis gyrinida* Mills 1934).

Valve elliptical; central nodule not broad; furrows evenly curved on the outer edge, crossed by costae and double oblique rows of alveoli. Variable in size and in the curvature of the furrows. The size of the species varies depending on the geography of its distribution. Dimensions in SEM images: valve 19.4 μm length, 9.8 μm width, 14 ribs in 10 μm. Dimensions in LM images: valve 23-110 μm length, 13-40 μm width; frustule 35.3-50.4 μm width, 6-11 ribs in 10 μm with a double row of areoles reaching the longitudinal canals (Ryabushko and Begun, 2016); 48-60 μm length, 15-20 μm width, 10-11 ribs in 10 μm (Korotkevich, 1960; Karayeva, 1972); 46-54 μm length, 16-18 μm width, 9-10 striae in 10 μm (Al-Yamani and Saburova, 2011).

Ecology, distribution, and phytogeography: marine and brackish waters, benthic, euryhaline and eurythermal, littoral and sublittoral species, cosmopolite and ubiquiste. The species is indicated in the plankton of Amur Estuary, Arctic Ocean, Pechora, Laptev, Kara, Baltic, Barents, North, Norwegian, White, Caribbean, Mediterranean, Black, Azov, Caspian, Japan, East China seas and off the coast of Mexico, Greenland, Iceland, Spitsbergen, Finnmarken, Turkey, Kuwait, China, India, Japan, Northern America, Australia and New Zealand, the Arctic and Antarctic, the Islands of Sri Lanka (Ceylon), Madagascar, Java, Sumatra and Bahamas (Ryabushko and Begun, 2016).
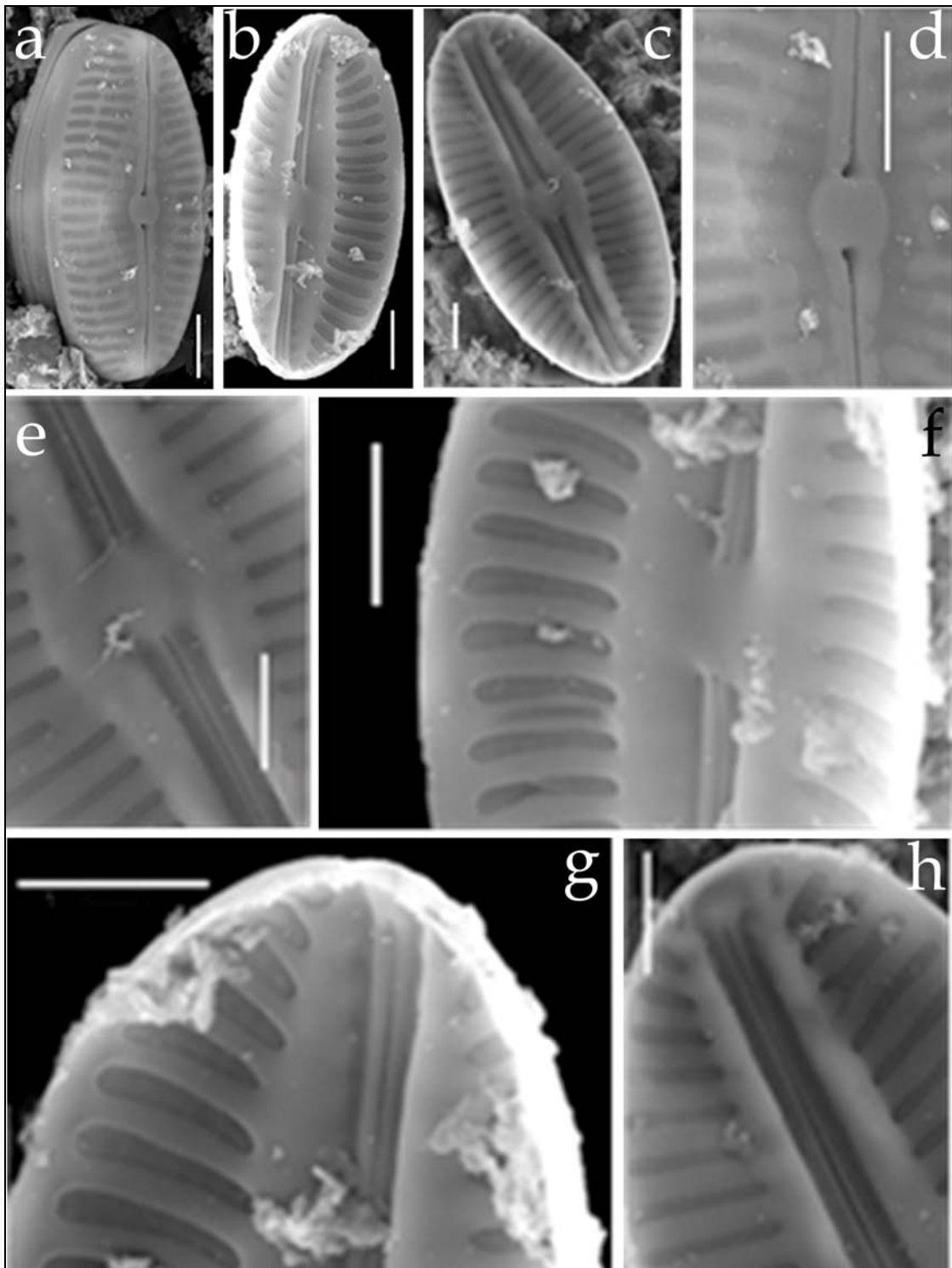
Figure 2: The valves of *Diploneis smithii* view (a-h), their fragments and structure (d-h); scale bar: 2 µm.

Family Naviculaceae Kützing 1844
Genus *Navicula* J. B. M. Bory de Saint-Vincent 1822 emend. E. J. Cox 1988

***Navicula antonii* H. Lange-Bertalot 2000. Fig. 3, f-k**

(Synonym: *Navicula menisculus* var. *grunowii* Lange-Bertalot 1993)

Dimensions in SEM images: valves 22 μm length, eight μm width, 20 punctae of areolae in 10 μm. Dimensions in LM images: valves 10-23.8 μm length, 4.2-7.3 μm width, 16-22 striae of areolae in 10 μm (Rumrich et al., 2000).

Ecology, distribution and phytogeography: freshwater, benthic, boreal-tropic and notal species. Occurrence in the Andes of Venezuela to Patagonia/Tierra del Fuego (Rumrich et al., 2000). This species was first discovered on the sand beach of Kruglaya Bay for the Crimean coastal waters of the Black Sea.

***Navicula distans* (W. Smith) Ralfs 1861. Fig. 4, h-n**

(Basionym: *Pinnularia distans* W. Smith, 1853; Synonym: *Navicula distans* (W. Smith) A. Schmidt 1874 nom. illeg., *N. distans* W. Smith, 1853)

Dimensions in SEM images: raphe valve 33-34.3 μm length, 7.5 μm wide, 13 radial striae in 10 μm. Dimensions in LM images: valves 27.5 μm length, 13-18 μm width, 6-7 striae and 20-21 areolaea in 10 μm (Proshkina-Lavrenko, 1963). 33.6-56 μm length, 14.4-29.7 μm width, 7-8 radial striae in 10 μm (Ryabushko, 1986).

Ecology, distribution, and phytogeography: benthic, sublittoral, marine and brackish water, arcto-boreal-tropical species. Occurrence in the ice of Franz Josef Land, the Laptev Sea, in the Chukchi, Bering, Norwegian, Barents, White, North, Black, East China and Caribbean seas, Arctic Ocean, off the coast of the North America, Greenland, Iceland, Svalbard, Finnmarken, Sweden, China, Canary, and Philippine Islands. In the Sea of Japan in Vostok Bay in the summer on rocky and sand beach at a depth of five m (Ryabushko 1986, 2014), in the periphyton of experimental plates made of plexiglass, asbestocement, high-alloy steel in Amur Bay, Uglovoe, and Golden Horn Bay in the epiphyton of the green alga *Cladophora stimpsonii* and sea flax of *Phyllospadix iwatensis*, the epizoon of the mussel *Mytilus trossulus*, and the barnacle of *Amphibalanus improvisus* (Begun, 2012; Ryabushko and Begun, 2016), as well as this species was found in Kraternaya Bight (Kuril Islands) (Ryabushko, 2020).

***Navicula palpebralis* Brébisson ex W. Smith 1853. Fig. 3, a-e**

(Basinonym: *Schizonema palpebrale* (Brébisson ex W. Smith) Kuntze 1898; Synonym: *Navicula palpebralis* var. *genuina* A. Cleve-Euler, 1953).

Cells biraphid, solitary, free living and motile. Dimensions in SEM images: valve 41.1 μm length, 12.9 μm width, 11 striae in 10 μm. Dimensios in LM images: valves 38-80 μm length, 13-16 μm width, 10 striae in 10 μm (Proschkina-Lavrenko, 1950; Ryabushko and Begun, 2016); 33-64 μm length; 10-26 μm width, 9-11 striae in 10 μm (Proshkina-Lavrenko, 1963); 44-51 μm length, 14-15 μm width, transapical striae radiate 11 in 10 μm (Al-Yamani and Saburova, 2011.

Ecology, distribution, and phytogeography: benthic, marine, arctic-boreal-tropical and notal species. Known in the North, Norwegian, Kara, Barents, Baltic, Mediterranean, Adriatic, Black, and Azov seas; in basins of Europe, Asia, America, the Arctic, Iceland, Norway, Sweden, England, Germany, Romania, Spain, France, New Zealand, the Canary and Galopagos Islands (Ryabushko and Begun, 2016), as well as off the coast of Kuwait (Al-Yamani and Saburova, 2011). In Kazachya Bay of the Black Sea was found on the red alga of *Gracilaria verrucosa* at a depth of 1-3 m at 5.7°C (Ryabushko, 1991) and on the epilithon of stony substrates of Vostok Bay of the Sea of Japan at a depth of 0.5 m (Ryabushko, 2014).
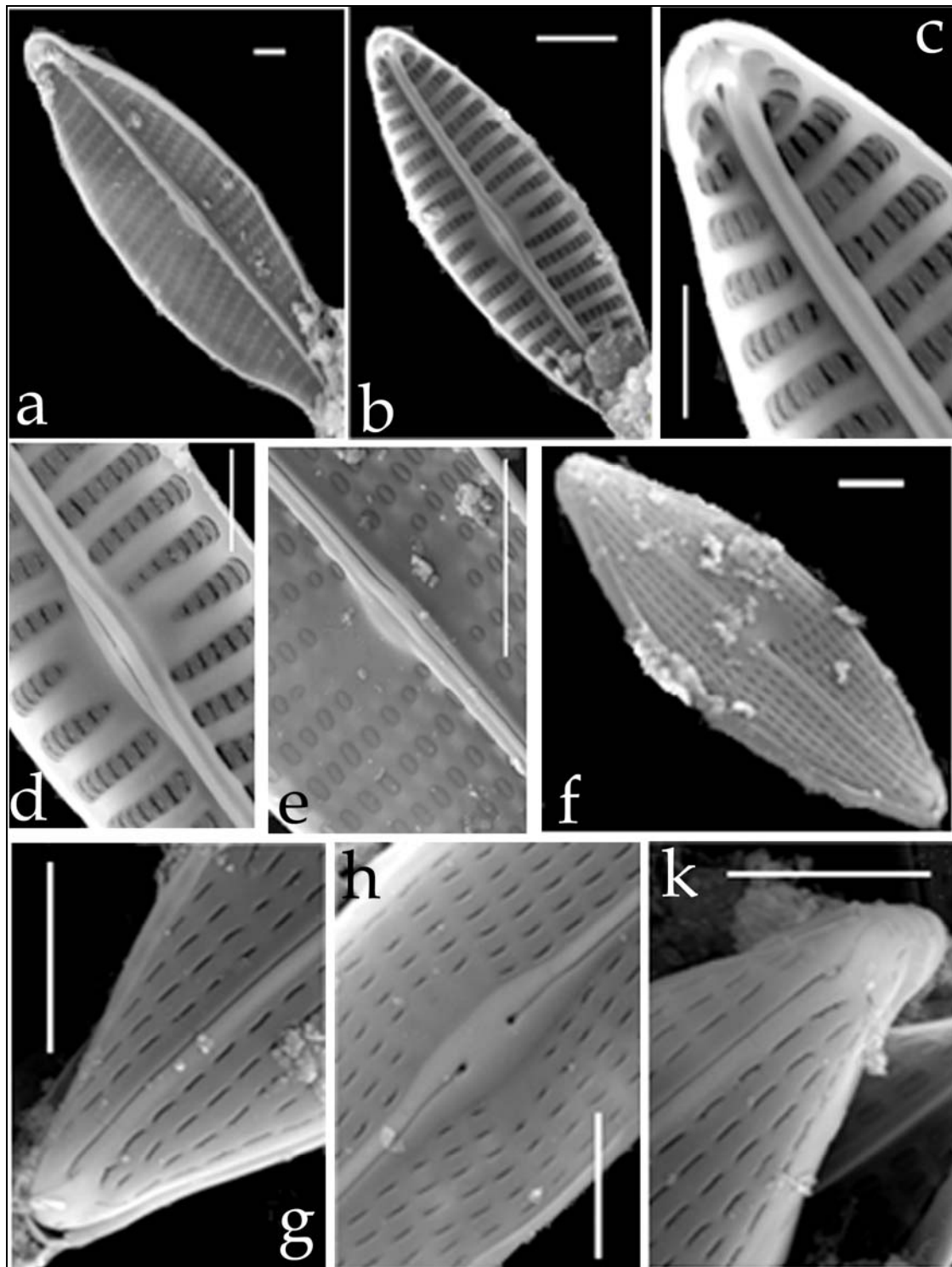
Figure 3: The valves of *Navicula palpebralis* (a-e) and *Navicula antonii* (f-k) view in different angles, with their fragments and structures; scale bar: 1 μm (a-e) and 2 μm (f-k).

Order Lyrellales D. G. Mann, 1990
Family Lyrellaceae D. G. Mann, 1990
Genus *Lyrella* N. I. Karayeva 1978

***Lyrella abrupta* (W. Gregory) D. G. Mann 1990. fig. 4, b-d**
(Basionym: *Navicula abrupta* (W. Gregory) Donkin 1870; Synonyms: *Navicula lyra* var. *abrupta* W. Gregory 1857)
Dimensions in SEM images: valve 27.7 μm length, 18.9 μm width, 10 striae and 25 puncta in 10 μm. Dimensions in LM images: valves 32-85 μm length, 19-35 μm width, 9-10 striae and 22-24 puncta in 10 μm (Proschkina-Lavrenko, 1950; Proshkina-Lavrenko, 1963); 29-46 μm length, 14-21 μm width, 12-14 striae in 10 μm (Al-Yamani and Saburova, 2011).
Ecology, distribution, and phytogeography: marine, eurythermal, littoral and sublittoral, boreal-tropical species. Found off the coast of Mexico, Turkey, Puerto Rico and Kuwait's marine sediments, in the Caribbean, Aegean, Adriatic, Black, Azov and Caspian seas; Canary and Philippine Islands, the Sea of Japan it is indicated in the summer in the sand beach of Vostok Bay at the depth of five m, in October at 10 m by 5.2ºC (Ryabushko, 1986, 2014).

***Lyrella atlantica* (A. W. F. Schmidt) D. G. Mann 1990. fig. 4a**
(Basionym: *Navicula atlantica* A. W. F. Schmidt 1874; Synonyms: *Navicula lyra* var. *atlantica* (Schmidt) Cleve 1895; *Lyrella lyra* var. *atlantica* (Schmidt) Karayeva 1988; *Lyrella lyra* var. *atlantica* (A. Sm.) Gusliakov et Karayeva 1992).
Dimensions in SEM images: valve 27.7 μm length, 18.9 μm width, 10 striae and 25 puncta in 10 μm. Dimensions in LM images: valves 32-85 μm length, 19-35 μm width, 9-10 striae and 22-24 puncta in 10 μm (Proschkina-Lavrenko, 1950; Proshkina-Lavrenko, 1963); 29-46 μm length, 14-21 μm width, 12-14 striae in 10 μm (Al-Yamani and Saburova, 2011).
Ecology, distribution, and phytogeography: marine, eurythermal, littoral and sublittoral, boreal-tropical species. Found off the coast of Mexico, Turkey, Puerto Rico and Kuwait's marine sediments, in the Caribbean, Aegean, Adriatic, Black, Azov and Caspian seas; Canary and Philippine Islands, the Sea of Japan it is indicated in the summer in the sand beach of Vostok Bay at the depth of five m, in October at 10 m by 5.2ºC (Ryabushko, 1986, 2014).

***Lyrella rudiformis* (Hustedt) Guslyakov et Karayeva, 1992. Fig. 4 e-g**
(Basionym: *Navicula rudiformis* Hustedt 1964)
Dimensions in SEM images: valve 30.4 μm length, 17.6 μm width, 10 striae in 10 μm. Dimensions in LM images: valve 28–36 μm length, 22–26 μm width, 10–11 striae in 10 μm (Guslyakov et al., 1992, page 38, table XLVII, figures 7, 8).
Ecology, distribution, and phytogeography: marine, littoral and sublittoral, boreal species. Indicated in fouling of macrophytic algae, as well as on silty-sandy ground in Tendrovsky and Dzharylgachsky gulfs (Guslyakov et al., 1992), as well as on the sand beach of Kruglaya Bay for the Crimea of the Black Sea.
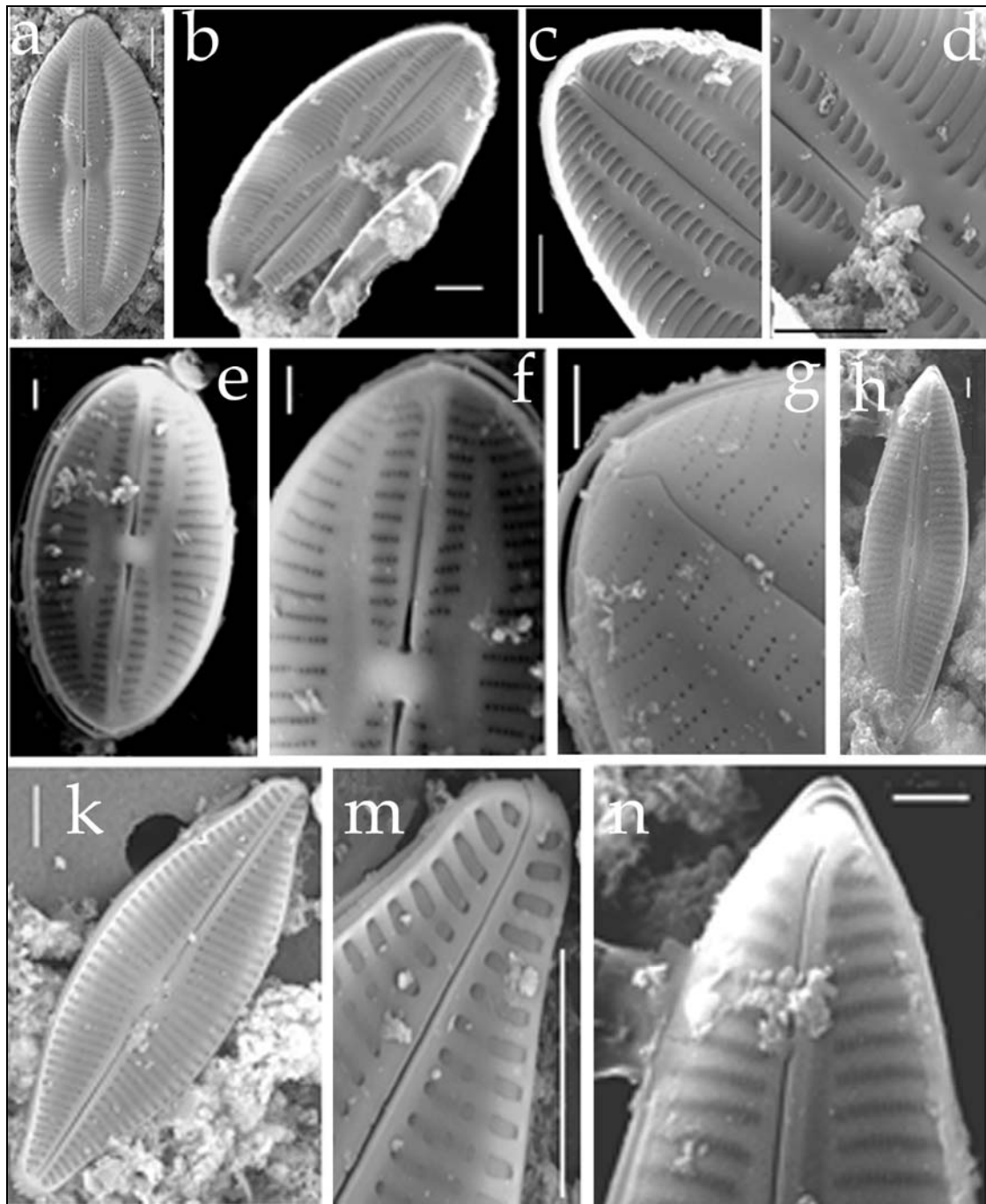
Figure 4: SEM images of the valves of *Lyrella atlantica* (a), *L. abrupta* (b-d),
L. rudiformis (e-g) and *Navicula distans* (h-n) view,
its fragments and structure. Scale bar: 5 µm (a-d) and 2 µm (e-n).

Family Pleurosigmataceae Mereschkowsky 1903
Genus *Pleurosigma* Smith 1852

***Pleurosigma cuspidatum* (Cleve) Peragallo 1891. Fig. 5, k-o**
(Synonym: *Pleurosigma lanceolatum* var. *cuspidatum* Cleve 1881)
Dimensions in SEM images: valves 76.3-86 μm length, 25.3-26.3 μm width, 25 transapical and 26 oblique striae in 10 μm. Dimensions in LM images: valves 85-148 μm length, 21-32 μm width, 19-21 transapical and 18-22 oblique striae in 10 μm (Al-Yamani, Saburova, 2011).
Ecology, distribution, and phytogeography: marine, littoral, sublittoral, boreal-tropical species, indicated in the Black Sea coast of Russia, Romania, also England, France, Sweden and Kuwait in siltflates, sandflates and epiphyton of macrophytes (Merezhkowsky, 1903; Hendey, 1964; Bodeanu, 1987-1988; Al-Kandari et al., 2009; Al-Yamani and Saburova, 2011).

***Pleurosigma elongatum* W. Smith 1852. Fig. 5, a-h**
(Synonym: *Pleurosigma maeoticum* Pantocsek 1902)
Dimensions in SEM images: valve 142 μm length, 20.5 μm width, 18 transapical and 19 oblique striae in 10 μm. Dimensions in LM images: valves 101-386 μm length, 17-65 μm width, 18-20 transapical and 17-19 oblique striae in 10 μm (Proshkina-Lavrenko, 1963).
Ecology, distribution and phytogeography: marine and brackish waters, littoral and sublittoral, eurythermal, *β*-mesosaprobes, cosmopolite, widespread in seas and continental ponds. First discovered in June 1849 and October 1850 off the coast of Britain (Smith, 1853). Occurrs in the ice of the Laptev Sea, in the White, Kara, Barents, Black, Azov, Caspian, East China Seas, off the coast of Romania, Bulgaria, Spain, England, Argentina, Brazil, China, Australia and New Zealand; in the Canary, Balearic and Hawaiian Islands, in ponds of Turkey (Aysel, 2005). The first discovered in the Sea of Japan in July 1921 and November 1923 (Skvortzow, 1932), on near Shikkoku Inland (Oshite, 1955). In the north-western part of the Sea of Japan, this species was found in the different bays and substrates and all seasons of the year, as well as in fouling of macrophytes (Ryabushko and Begun, 2016).

Genus *Gyrosigma* A.H. Hassall 1845
***Gyrosigma attenatum* (Kützing) Rabenhorst 1853. Fig. 6, a-c, e**
(Basionym: *Frustulia attenuata* Kützing 1834; Synonyms: *Sigmatella attenuata* (Kützing) Brébisson and Godey 1835; *Navicula attenuata* (Kützing) Kützing 1844; *Pleurosigma attenuatum* W. Smith 1852; *Scalptrum attenuatum* (Kützing) Kuntze 1891).
Dimensions in SEM images: valves 76.3-86 μm length, 25.3-26.3 μm width, 25 transapical and 26 oblique striae in 10 μm. Dimensions in LM images: valves 85-148 μm length, 21-32 μm width, 19-21 transapical and 18-22 oblique striae in 10 μm (Al-Yamani, Saburova, 2011).
Ecology, distribution, and phytogeography: marine and freshwater, arctic-boreal-tropic species. Occurs in Iberian Peninsula, Balearic Islands, Canary Islands, in the wetlands of Sweden, Iraq, Slovakia, Netherlands, Poland, Romania, Britain, France, Taiwan, Canada, Brazil, India, Pakistan, Tajikistan, Korea, Mongolia, Ireland, Portugal, Alaska, Laurentian Great Lakes, U.S.A., Mexico, Iceland, Albania, Italy, Russia, Ukraine, Spain, Ghana, Egypt, Taiwan, Iran, Turkey, Australia, New Zealand, and Baltic, Adriatic and Black seas (Guiry and Guiry, 2021). This species was first discovered on the sand beach of Kruglaya Bay and for the Crimean coastal waters of the Black Sea.
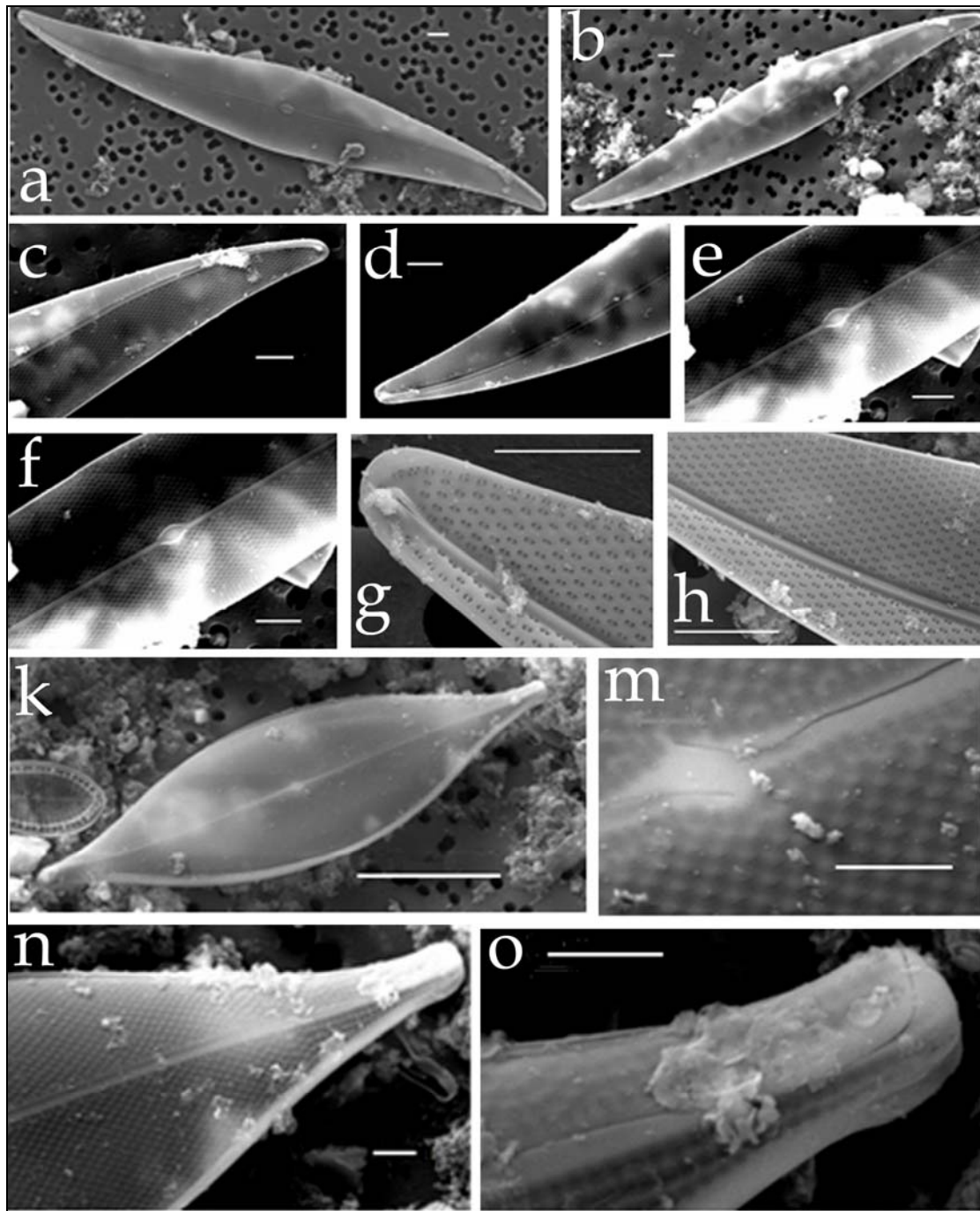
Figure 5: SEM images showing fragments and structures of thevalves
of *Pleurosigma elongatum* (a-h), *Pleurosigma cuspidatum* (k-o) view,
its fragments and structure; scale bar: 5 μm (a-d) and 2 μm (e-n).

Family Plagiotropidaceae D. G. Mann 1990
Genus *Plagiotropis* E. Pfitzer 1871
***Plagiotropis lepidoptera* (Gregory) Kuntze 1898. Fig. 6, d, f-k**

(Basionym: *Amphiprora lepidoptera* Gregory 1857; Synonyms: *Tropidoneis lepidoptera* (Gregory) Cleve 1894; *Orthotropis lepidoptera* (Gregory) Van Heurck 1896; *Plagiotropis lepidoptera* (Gregory) Poulin et Cardinal 1983).

Dimensions in SEM images: frustule 40 µm length, 10.4 µm width, 39 rows and 55 puncta in 10 µm. Dimensions in LM images: valves 120-200 µm length, 18-22 µm width, 20-21 striae in 10 µm (Proschkina-Lavrenko, 1950); valves 66-113 µm length, 14-18 µm width, 20-22 striae in 10 µm (Korotkevich, 1960; Proshkina-Lavrenko, 1963); valves 57-92 µm length, 9-18 µm wide and 23.8-27 µm width frustules, 20-21 rows of areolae in 10 µm, found in the summer in the sandflats and intestines of the Far Eastern sea cucumber in Vostok Bay, the Sea of Japan, at a depth of five m (Ryabushko, 1986, 2014).

Ecology, distribution, and phytogeography: marine, benthic, littoral and sublittoral, *β*-mesosaprobes species, arctic-boreal-tropic species. It is known in the Caribbean, Baltic, Barents, White, Black, Milk Estuary, Azov, Caspian, East China seas, off the coast of Europe, U.S.A., China, Kuwait, India, Bahamas and Canary Islands (Ryabushko and Begun, 2016).
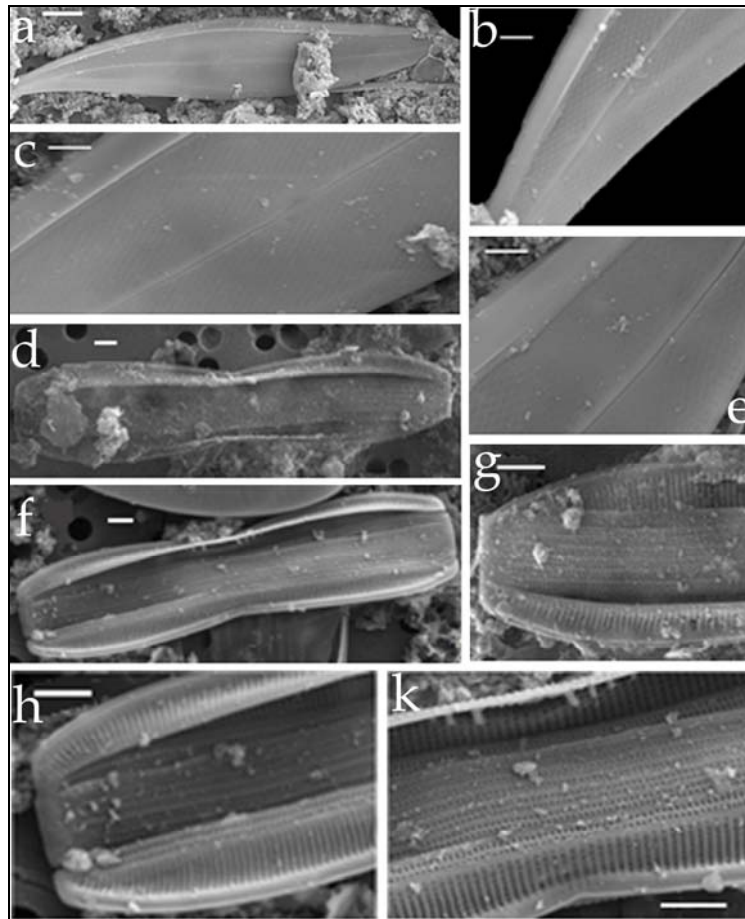
Figure 6: SEM images showing fragments and structures of the frustules
of *Gyrosigma attenatum* (a-c, e) and *Plagiotropis lepidoptera* (d, f-k) view,
its fragments and structure; scale bar: 5 µm (a-c, e) and 2 µm (d, f-k).

Order Cymbella Les D. G. Mann 1990
Family Anomoeoneidaceae D. G. Mann 1990
Genus *Adlafia* G. Moser, H. Lange-Bertalot et D. Metzeltin 1998
***Adlafia besarensis* (Giffen 1980) L.I. Ryabushko comb. nov**. **Fig. 7, a-c** (Synonym: *Navicula besarensis* Giffen 1980**)**

Dimensions in SEM images: lower raphe valve 35 μm length, 15 μm wide, 12 radial striae and 12 puncta in 10 μm. Dimensions in LM images: valve 34-35 μm length, 16-17 μm width, 12-14 radial striae and 12-15 puncta in 10 μm (Al-Yamani, Saburova, 2011). Valve 35 μm length, 15 μm width, 12 radial striae and 12 puncta in 10 μm (Witkowski et al., 2000).

Ecology, distribution, and phytogeography: marine, benthic, a rare and boreal-tropical species. Occurs also in the littoral of the Seychelles (Giffen, 1980), in the Indo-Western Pacific Ocean, Oman on the Arabian Sea, Poland and was noted in the sandflates of Kuwait (Al-Yamani and Saburova, 2011). Indicated for the first time off the coast of Karadag Reserve of the Crimean coastal waters (Nevrova, 2015) and now, for the first time, on the sand beach of Kruglaya Bay of the Black Sea.

Family Cymbellaceae Kützing 1844
Genus *Cymbella* C. Agardh 1830
***Cymbella Odesana* Guslaykov 1992. Fig. 7, d-g**

Dimensions in SEM images: valves 13.4 μm length, 6.7 μm width, 20 rows areolae in 10 μm. Dimensions in LM images: valves 10-15 μm length, 2-4 μm width, 55 lionel and 20-24 rows areolae in 10 μm (Guslyakov et al., 1992).

Ecology, distribution, and phytogeography: brackish waters, benthic, littoral and sublittoral, boreal species. Occurred in the Ukraine in Odessky Gulf of the Black Sea on the rock and macrophytes in May at a depth of 0.5-4.0 m at a water temperature of 15°C with a salinity of 14 ‰ (Guslyakov et al., 1992). This is the first time when this species was found on the sand beach of Kruglaya Bay and for the Crimean coastal waters of the Black Sea.

Figure 7: SEM images of fragments and structures of the valves of *Adlafia besarensis* (a-c) and *Cymbella Odesana* (d-g); scale bar: 2 μm.

Order Thalassiophysales D. G. Mann 1990
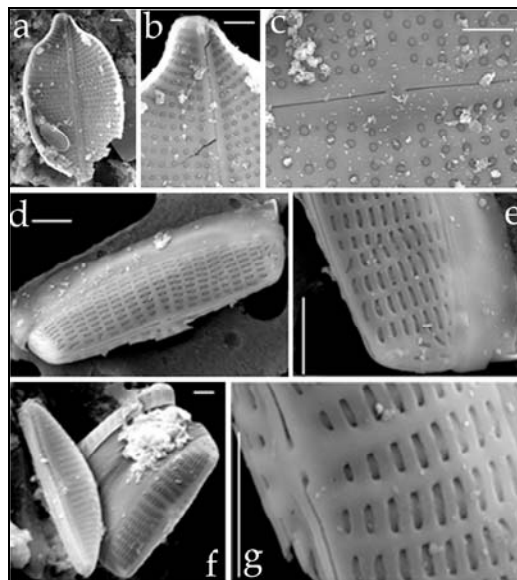Family Catenulaceae Mereschkowsky 1902
Genus *Halamphora* (Cleve) Mereschkowsky 1903

Halamphora coffeiformis (C. A. Agardh) Z. Levkov 2009. Fig. 8, a-b (Bisyonym: *Frustulia coffeiformis* C. Agardh, 1827; Synonym: *Amphora coffeiformis* (C. A. Agardh) Kützing, 1844)

Dimensions in SEM images: frustule 35 μm length, 14.3 μm width, 12 band rims and 22 striae in 10 μm. Dimensions in LM images: frustules 18-42 μm long, 10-15 μm width, 12-14 band rims with 20-22 striae in 10 μm and 24 striae in 10 μm at the ends of the frustule (Proshkina-Lavrenko, 1963); valves 14-35 μm length, 4-6 μm width, 16-24 rows of areolae in 10 μm (Guslyakov et al., 1992); valves 16-30.8 μm length, 16.8 μm width, 12 striae in 10 μm (Ryabushko and Begun, 2016).

Ecology, distribution, and phytogeography: marine and brackish waters, littoral and sublittoral, euryhaline and eurythermal, cosmopolite. Occurrence in the Pechora, North, Kara, Barents, Baltic, Bering, Japan, Tyrrhenian, Caribbean, Mediterranean, Adriatic, Black, Azov, Caspian, Aral, East China and Dead seas, in Siavash, in the reservoirs of the Arctic, Canada, U.S.A., Iceland, Saxony, Poland, Austria, Greece, West India, South Africa, China, Syria, Iraq, Kuwait, Mongolia, Australia, off the Atlantic coast of the North America, in the Sandwich and Bahamas Islands. The species in summer on rocky and sand beach in Vostok Bay (Ryabushko, 1986), in the epiphyton of the red alga *Ahnfeltia tobuchiensis* (Kanne et Matsubara) Makijenko in Stark Strait, Bay of Peter the Great in August 2008 and 2009, in the periphyton of experimental plates of asbestos cement and the epiphyton of green macrophyte in Golden Horn Bay at 23°C and 24‰ were found (Ryabushko and Begun, 2016).

Halamphora tenerrima (Aleem et Hustedt) Z. Levkov 2009. Fig. 8, c-e (Basionym: *Amphora tenerrima* Aleem et Hustedt; Synonym: *Amphora tenerrima* Aleem et Hustedt 1951)

Dimensions in SEM images: frustule 20 μm length, five μm width, 25 striae and 45 areolae in 10 μm. Dimensions in LM images: frustules 7-24 μm length, 3.5-8 μm width, 22-29 striae and 24-40 areolae in 10 μm (Clavero et al., 2000); valve 13.11-13.64 μm length and 3.02-3.11 μm width, 26 striae in 10 μm (Álvarez-Blanco and Blanco, 2014; Kaleli et al., 2017).

Ecology, distribution, and phytogeography: marine, benthic species, boreal-tropical. Occurrence in the European Inland Waters and Comparable Habitats (Levkov, 2009), Baltic Sea (Snoeijs and Balashova, 1998), South Florida, U.S.A. (Wachnicka and Gaiser, 2007), British Islands (Hendey, 1964), Spain (Álvarez-Blanco and Blanco, 2014), England (Simonsen, 1987), and Turkey (Kaleli et al., 2017). Fist time to be observed in Kruglaya Bay and Crimean coastal waters of the Black Sea.

Genus *Amphora* Ehrenberg ex Kützing, 1844

Amphora crassa Gregory 1857. Fig. 8, f-h (Synonym: *Amphora crassa punctata* A. Schmidt)

Dimensions in SEM images: frustule 40 μm length, 30 μm width, eight striae and 15 areolae in 10 μm. Dimensions in LM images: frustules 50-100 μm length, 20-30 μm width, 5-8 striae in 10 μm (Proschkina-Lavrenko, 1950); 45-136 μm length, 18-30 μm width; valves 8-17 μm width, five striae in 10 μm (Proshkina-Lavrenko, 1963); frustules 46-86 μm length, 20-30 μm width, 11-12 areolae in 10 μm (Ryabushko and Begun, 2016).

Ecology, distribution, and phytogeography: marine, benthic, arctic-boreal-tropical species. Occurs in Caribbean, North, Barents, White, Mediterranean, Adriatic, Black, Japan, East China seas, off the coast of Greenland, Spitsbergen, U.S.A., Finnmarken, England, Sweden, Finland, Kuwait, China, Sumatra, Philippines. It was found in the silflates of the Amur Bay, in the epilithon of stony and sandflats in winter and summer at a depth of 5-10 m at 18-19.6°C, in Posyet and Vostok bays of the Sea of Japan (Ryabushko and Begun, 2016).

Amphora tenuissima **Hustedt 1955. Fig. 8, k-m**

Dimensions in SEM images: frustules 11.3-18.3 µm length, 3.5 µm width, 35 striae in 10 µm. Dimensions in LM images: frustules: 9.4-10.7 µm length, 2.2-3.1 µm width, 31-40 striae in 10 µm (Clavero et al., 2000); valves 8-10 µm length, 2-3 µm width, 35-45 striae in 10 µm. Recorded on siltflats and sandflats in the North-West of part the Black Sea (Guslayakov et al., 1992).

Ecology, distribution, and phytogeography: benthic, marine, boreal-tropic species. Observed for the first time in Kruglaya Bay of the Crimean coastal waters of the Black Sea.
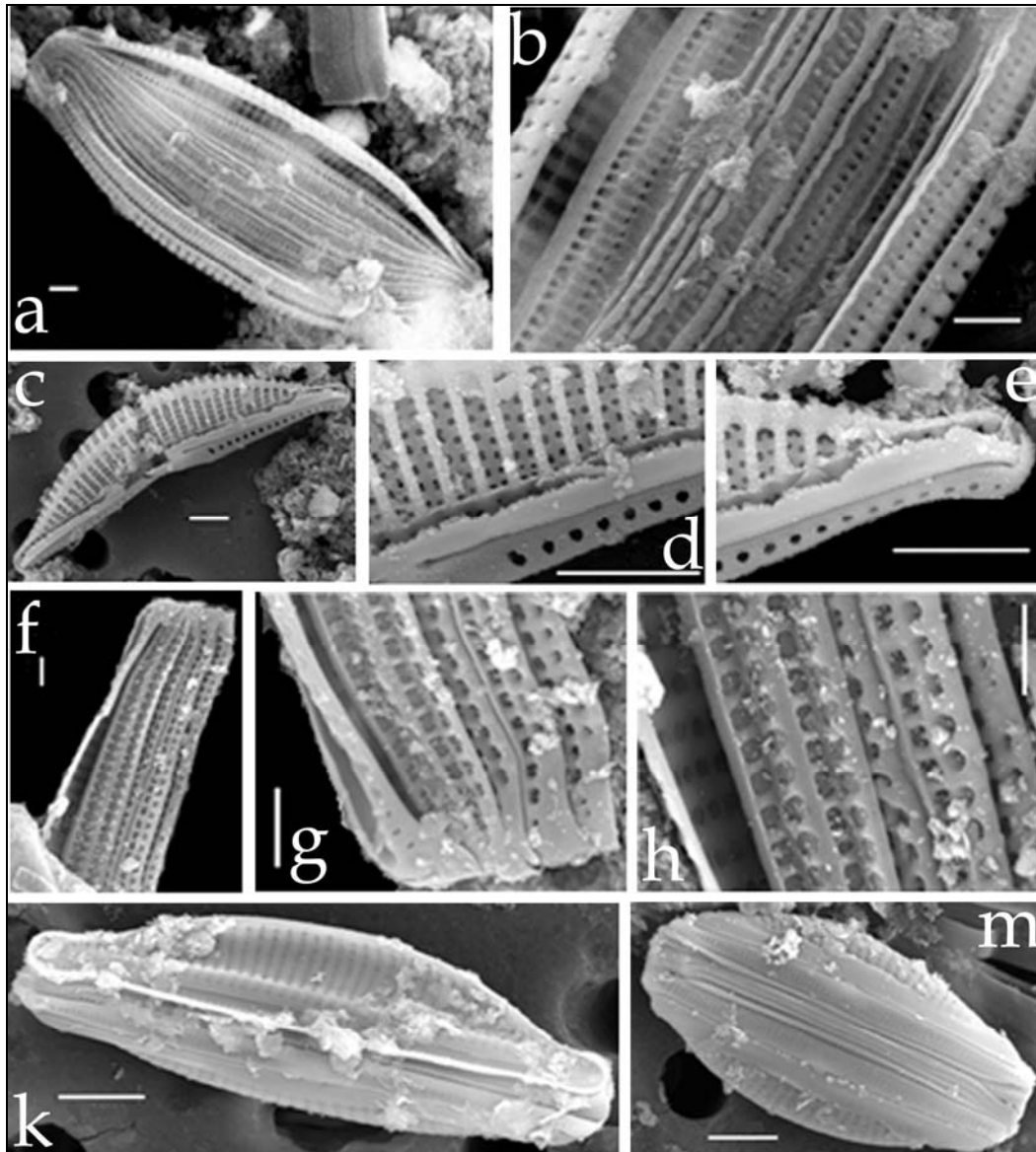

Figure 8: SEM images of fragments and structures of the valves and frustules
of *Halamphora coffeiformis* (a-b), *H. tenerrima* (c-e), *Amphora crassa* (f-h),
and *A. tenuissima* (k-m) view, fragments and structure; scale bar: 2 µm.

Order Bacillariales Hendey 1937
Family Bacillariaceae Ehrenberg 1831
Genus *Cylindrotheca* Rabenhorst 1859 emend. Reimann et Lewin 1964
Cylindrotheca closterium (Ehrenberg) Reimann et Lewin 1964. Fig. 9, a-b
(Basionym: *Ceratoneis closterium* Ehrenberg 1839. Synonyms: *Nitzschia closterium* (Ehrenberg) W. Smith 1853; *N. reversa* W. Smith 1853; *N. closterium* var. *reversa* (W. Smith) Hauck; *Nitzschiella closterium* Rabenhorst 1864; *Nitzschia rostratum* Grunow 1880; *N. longissima* var. *closterium* (Ehrenberg) Van Heurck 1885; *N. curvirostris* var. *closterium* (Ehrenberg) De Toni 1892; *Nitzschiella longissima* var. *closterium* (Ehrenberg) Peragallo et Peragallo 1897; *Homoeocladia closterium* (Ehrenberg) Kuntze 1898; *Nitzschiella tenuirostris* Mereschk. 1901; *Nitzschia longissima* Gran 1930; *N. closterium* var. *recta* Gran 1931).

The species has a broad range of sizes. Dimensions in SEM images: valves 32.2-78.3 μm length, 2.6-5.7 μm width, 25 striae in 10 μm; valve 73.6 μm, 4.8 μm width. Dimensions in LM images: valves 32.5-260 μm length, 2-6 μm wide, striae are gentle, 12-16 keel points in 10 μm (Proschkina-Lavrenko, 1950); valves 42.5-217 μm length, 2.5-5 μm width, 12-16 keel points in 10 μm (Proshkina-Lavrenko, 1963); valves (SEM) 25-180 μm length, 1.5-8 μm width, 12-25 10 μm fibulae (Reimann and Lewin, 1964).

Ecology, distribution, and phytogeography: marine and brackish waters, euryhaline, littoral and sublittoral, benthos plankton species, cosmopolite, found in all geographic zones of the World Ocean. It was first discovered by W. Smith in May 1851 off the coast of England (Smith, 1853), in waters of Turkey (Aysel, 2005). Indicated in the ice of the Laptev Sea, North, Norwegian, Kara, Barents, White, Bering, Caribbean, Mediterranean, Adriatic, Aral, Caspian, Norwegian, Chukotka, Baltic, Black, Azov, East China seas and in the Amur Estuary, off the coast of Greenland, California, Spitsbergen, Brazil, Mexico, Finnmarken and Sweden, Spain, Croatia, Romania, Germany, Denmark, Kuwait, West India, Japan, Australia, New Zealand, Singapore, Sakhalin and Primorye, on the Hawaiian and Canary Islands (Ryabushko and Begun, 2016), as well as in the microphytobenthos of the Antarctic (Ryabushko, 2016).

Found in the Sea of Japan: in the Amur Gulf, Patrokl and Vityaz bays, Posyet Gulf, among fouling stones in Vostok Bay regularly on different substrates and in different seasons at depths of 0.5-10 m, in the stomachs of invertebrates and in the sandflate, fouling of brown alga *Saccharina japonica* in the Kit Bay, on the anthropogenic substrates, of different invertebrates and the epiphyton of macrophytes in the Amur Bay, Uglovoy, Ussuriysky, Rynda, Nakhodka, Slavyanka Gulfs, Tavrichansky Estuary, in Golden Horn and Severnaya bays, an artificial lagoon of Vladivostok (Ryabushko, Begun, 2016; Ryabushko et al., 2019).

Genus *Nitzschia* Hassall 1845
Nitzschia cf. angustata (W. Smith) Grunow 1880. Fig. 9f
Dimensions in SEM images: valves 25.4 μm length, 2.8 μm width, in LM images: valves 32-55 μm length, 5-8 μm width, 13-16 rows of areolae in 10 μm (Guslayakov et al., 1992).

Ecology, distribution, and phytogeography: indifferent, *α*-mesosaprobe, boreal species. Occurs very rare in epiphyton macrophytes and muddy bottom in the estuaries of the Dniestersky and Khadzhibeysky, Barents, Caspian, Aral, White, Red seas and reservoirs of Estonia, Belarus, Russia, Ukraine, France, Canada, U.S.A. and others (Guslayakov et al., 1992). The first listed for Kruglaya Bay of the Black Sea.

Nitzschia ovalis Arnott 1880. Fig. 9 c-e

Dimensions in SEM images: valves 10.3 µm length, three µm width, 30 row in 10 µm; Dimensions in LM images: valves 10-14 µm length, 2.5-3 µm width, 13-14 keel points in 10 µm (Proshkina-Lavrenko, 1963; Guslyakov et al., 1992).

Ecology, distribution and phytogeography: benthic, indicated in macrophytes fouling, stones and silts, marine and brackish waters, arctic-boreal species. Occurrence in bays and estuaries of the northwestern part of the Black Sea and near the Karadag, as well as in the Sivash area (Guslyakov et al., 1992), in the salt water bodies of Germany, indicated in the Kara Sea (Cleve-Euler, 1953). The first listed for Kruglaya Bay of the Black Sea.

Genus *Tryblionella* W. Smith 1853
Tryblionella coartata (Grunow) D. G. Mann 1990. Fig. 9, g-m

(Basionym: *Nitzschia coarctata* Grunow 1880; Synonym: *Nitzschia punctata* var. *coarctata* (Grunow) Hustedt 1921).

Dimensions in SEM images: valve 26.9 µm length, six µm width, in LM: valves 19-34 µm length, 7-13 µm width, 15 striae and 12-15 punctae in 10 µm (Proshkina-Lavrenko, 1963); valves 28-47.6 µm length, 10.2 µm wide, in the wide part 13.6-18 µm width, 8-10 keel punctae in 10 µm, striae in the middle of the valve not joined (Ryabushko, 1986).

Ecology, distribution, and phytogeography: marine and brackish waters, eurythermal, sublittoral, boreal-tropical species, inhabits mainly in the seas of temperate latitudes. Occurs in the Caribbean, Baltic, White, Black, Azov, Aral, Mediterranean, Aegean, Sea of Japan, off the coast of the Atlantic and Pacific oceans, Brazil, Sweden, Romania, Turkey, Kuwait, Korea, Japan (Ryabushko and Begun, 2016). In the Sea of Japan is indicated in the summer in the sandflats and epiphyton the macrophytes of Vostok Bay at a depth of 0.5-5.0 m (Ryabushko, 1986). The first listed for the Kruglaya Bay of the Black Sea.
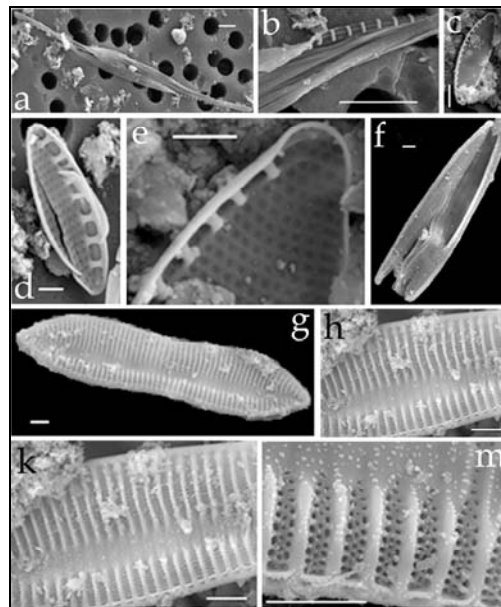
Figure 9: SEM images of fragments and structures of the valves and frustules of *Cylindrotheca closterium* (a-b), *Nitzschia* cf. *angustata* (f), *N. ovalis* (c-e) and *Tryblionella coartata* (g-m) view, its fragments and structure; scale bar: 2 µm, 1 µm (c-e).

Genus *Pseudo-nitzschia* H. Peragallo ex M. Peragallo 1900

***Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle 1993. Fig. 10, a-g**

(Basionym: *Nitzschia pseudodelicatissima* Hasle 1976; Synonyms: *Nitzschia delicatula* Hasle 1965, *Nitzschia haslea* Schoeman 1982).

Dimensions in LM images: valves 59-140 µm length, 1.5-2.5 µm width, more 30 striae and 16-26 fibules in 10 µm (Hasle and Syvertsen, 1997).

Ecology, distribution, and phytogeography: marine, planktonic, arctic-boreal-tropic species. Widespread in the coastal waters of Denmark, Norway, Spain, England, off the coast of eastern Canada, South Brazil, Argentina, Chile, Australia, New Zealand, China, Mexico and Saint-Laurent Gulfs, French, Portugal and Greek coastal waters, Japan, Adriatic seas (Hasle, 2002) and also in the Baltic Sea (Snoeijs and Balashova, 1998), in the ice of the Laptev Sea (Usachev, 1946), phytoplankton of the Black Sea and estuaries Sea of Azov (Ryabushko, 2003). This species causes the water blooms leading to production of domoic acid, which in turn causes amnesic shellfish poisoning (ASP) in humans (Martin et al., 1990; Lundholm et al., 2003; Ryabushko, 2003). The first listed for Kruglaya Bay of the Black Sea.

Figure 10: SEM images of fragments and structures of the valves and frustules of
Pseudo-nitzschia pseudodelicatissima (a-g) view,
its fragments and structure;
scale bar: 2 µm.

Genus *Hantzschia* Grunow 1877

***Hantzschia spectabilis* (Ehrenberg) Hustedt 1959. Fig. 11, a-m**

(Basionym: *Synedra spectabilis* Ehrenberg 1841)

Dimensions in SEM images: valves 58.2-87.0 µm length, 5.4-7.8 µm width, 10 striae in 10 µm. Dimensions in LM images: frustules 67.9-103 µm length, 13,8 µm width, valve six µm width, nine striae in 10 µm (Ryabushko and Begun, 2016).

Ecology, distribution, phytogeography: benthoplanktonic, marine and brackish water, boreal and notal species. Known in the water bodies of the U.S.A., Germany, Britain, Spain, the Far East, and New Zealand (Guiry and Guiry, 2021). Occurs in the Baltic Sea (Snoeijs and Balashova, 1998). In the Japan Sea was first found in March 2014 on the red alga *Mastocarpus stellatus* in Troitsa Bay of Posyet Gulf at depth of 3-6 m at a water temperature of about –1.5°C (Ryabushko and Begun, 2016). The first listed for the Black Sea Kruglaya Bay.
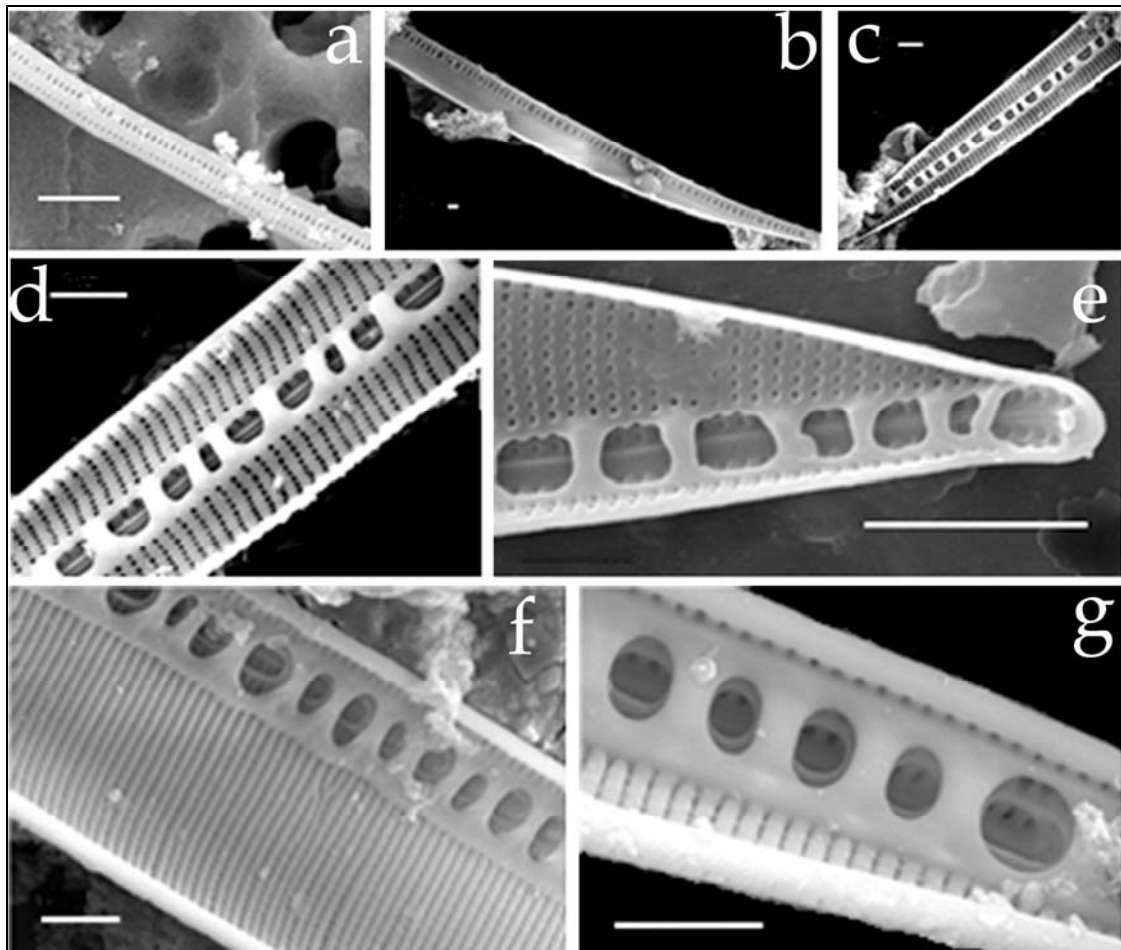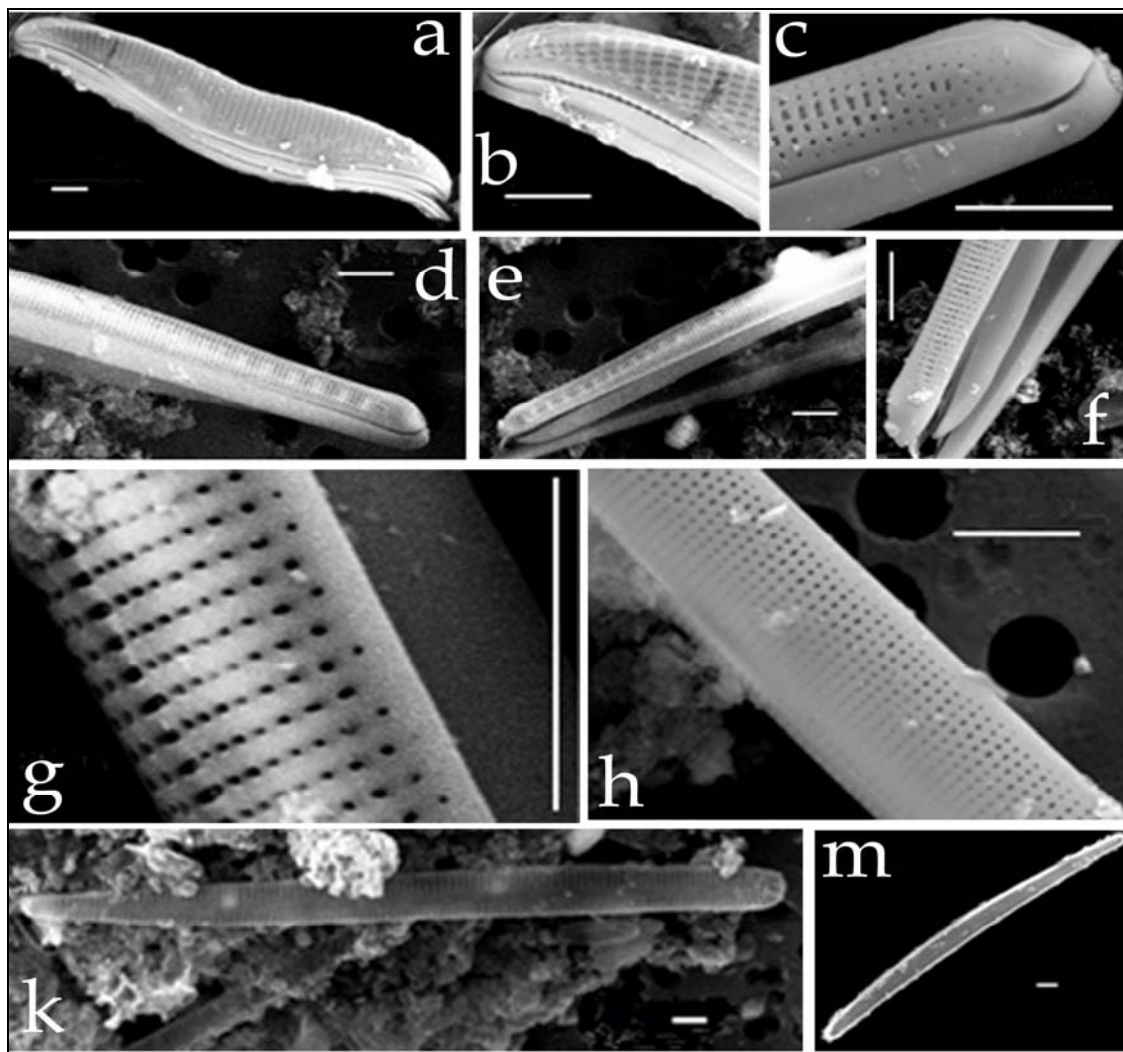

Figure 11: SEM images of fragments and structures of the valves and frustules of *Hantzschia spectabilis* (a-m) view, its fragments and structure; scale bar: 3 µm.

Order Surirellales D. G. Mann 1990
Family Surirellaceae Kützing 1844

Genus *Surirella* Turpin 1828
***Surirella ovalis* Brébisson 1838. Fig. 12, a-c**
(Basinonym: *Surirella ovata* var. *ovalis* (Brébisson) Kirchner 1878; Synonyms: *Surirella ovalis* (Brébisson) Pfitzer 1871; *Surirella ovalis* var. *ovata* (Kützing) Van Heurck 1885, *Surirella ovalis* f. *typica* A. Mayer, *Surirella lanceolata* H. P. Gandhi 1955)

Dimensions in SEM images: valve 26.3 µm length, 15 µm width, 15 rows areolarea in 10 µm. Dimensions in LM images: valves 30-37 µm length, 13-17 µm width, 15-17 rows areolarea in 10 µm (Guslyakov et al., 1992).

Ecology, distribution, and phytogeography: indifferent, benthic, *β*-mesosaprobes, arctic-boreal species. Recorded in epiphyton of macrophytes, epilithon of stones, on siltflates, sandflates and on the surface of mollusk shells, artificial substrates off the coast of the Crimea in Karadag Reverse (Roshchin, 1989) and in the north-western part of the Black, Azov, Baltic, Northern, Barents, White, Kara, Laptev, Japan, Mediterranean, Tyrrhenian, Caspian, Aral seas; Amur Estuary, in the waters of Russia, Ukraine, the Caucasus, Central Asia, as well as off the coast of Greenland, England (Hendey, 1964), Norway, Sweden, Finland, Germany, Belgium, Poland, France, Greece, Turkey, Iraq, China, South Africa, Canada, U.S.A., Colombia (Guslyakov et al., 1992), near of Iberian Peninsula, in Balearic and Canary Islands (Guiry and Guiry, 2021).

Family Entomoneidaceae Reimer 1975
Genus *Entomoneis* (Ehrenberg) C. G. Ehrenberg 1845
***Entomoneis paludosa* var. *duplex* (Donkin) Czarnecki et Reinke 1982. Fig. 12, d-e**
(Basionym: *Amphiprora paludosa* var. *duplex* Donkin 1880; Synonyms: *Amphiprora duplex* Donkin 1858; *Entomoneis paludosa* (W. Smith) Reimer 1975.

Dimensions in SEM images: frustule 30 µm length. Dimensions in LM images: valves 16-24 µm length, 7-13 µm width in the middle (Proshkina-Lavrenko, 1963).

Ecology, distribution, and phycology: brackish waters, littoral, sublittoral, rare boreal species. Occurs in the Black Sea in the northwestern part and in the Crimean coast near Karadag Reserve, as well as in the North, in the Bothnia Gulf of the Baltic Sea (Proshkina-Lavrenko, 1963; Guslyakov et al., 1992; Roshchin et al., 1992). This species was first noted in the Black Sea Kruglaya Bay.
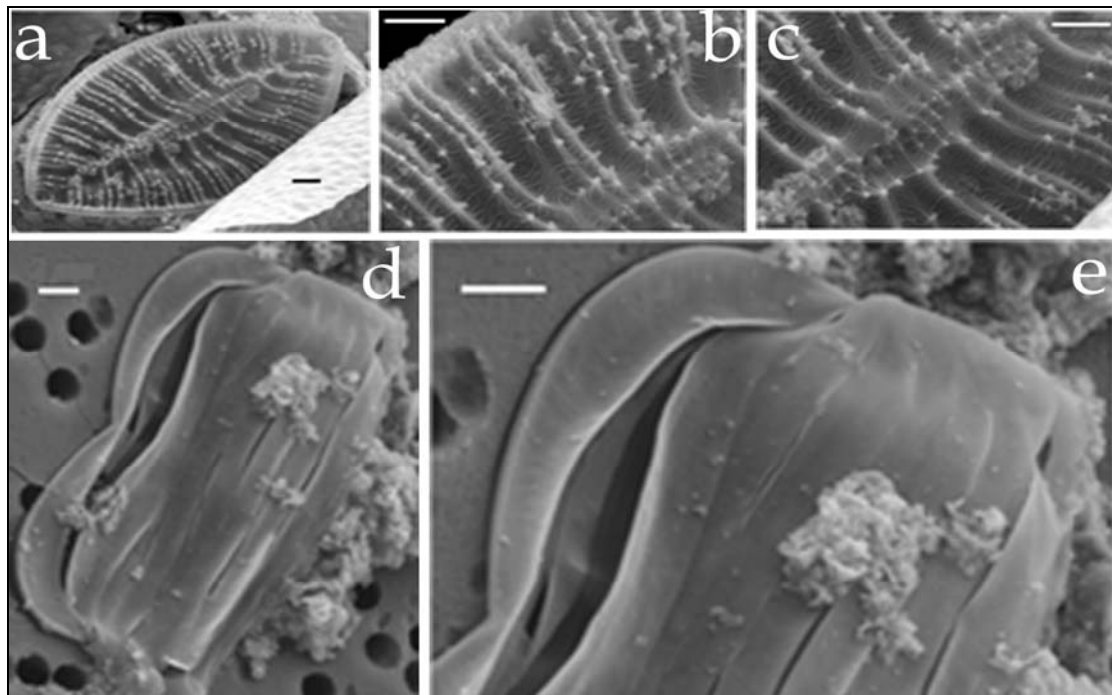
Figure 12: SEM images of fragments and structures of the valve and frustules
of *Surirella ovalis* (a-c) and *Entomoneis paludosa* var. *duplex* (d-e) view;
scale bar: 2 µm.

DISCUSSION

     This is a study of the taxonomic composition of the diatoms of Kruglaya Bay, characterized by the use of SEM. The results show that there is a high biodiversity with a prevalence of the biraphid species. This result is typical for the microphytobenthos of the coastal waters of the Black, Azov, and Japan seas (Ryabushko, 2013; Balycheva, 2014; Ryabushko and Begun, 2015, 2016; Bondarenko, 2017; Barinova et al., 2019). The species composition of diatoms of sand has a rich flora and in some species similar to other ecotopes of the sea. The temperature, light and hydrochemical regimes at the shallow waters of the Black Sea are very favorable for microalgae vegetation. Many of the observed species form different communities on both natural and anthropogenic substrates (Ryabushko, 2009, 2013). We observed some typical species of epipsammon, such as representatives of the genera *Amphora, Halamphora, Lyrella, Diploneis, Hantzschia*, etc. Among them there were three species first recorded in the Crimean coastal waters and the Black Sea. The current study investigated the species of diatoms found on 16 species of brown, red, and green macroalgae epiphyton at a depth of 0-20 m. This study was carried out in May and August 1990 near Cape Omega of Kruglaya Bay (Ryabushko, 1996). In May on 10 species of macrophytes was found 36 species of diatoms, in August on 6 species of macrophytes – 31, from them 15 were biraphid algae. In addition, in February-March 1989, in the closed part of Kruglaya Bay, 100 m from the sand beach, 10 species diatoms were noted in the periphyton of synthetic materials (Ryabushko, 2013). In general, pennate algae predominated, which differed in species composition depending on the ecotope of habitat and on the sandy substrates of the Kruglaya Bay.

CONCLUSIONS

This is the first study conducted on diatoms from the sand beach of Kruglaya Bay of the Black Sea, accomplished in just one day, 30th of September 2016 and analyzed by scanning electron microscopy (SEM). We found 25 taxa to biraphid of Bacillariophyta representing 17 genera. Three new species: *Halamphora tenerrima*, *Amphora tenuissima*, and *Navicula antonii*, were reported for the first time in the Crimea and the Black Sea. The most dominant species were: marine (40%), marine-brackish (32%) and of boreal-tropical (32%), arctic-boreal-tropical (24%), boreal and cosmopolites (20% and 16% respectively) while brackish, freshwater, indifferent, and arctic-boreal were less well represented (8% each), including notal species (12%), found in the southern hemisphere. The morphological characteristics of species in the SEM and LM are given based on original and published data on the variability of the valve and frustule sizes, occurrence, ecology, phytogeography, and the general distribution in different areas of the World Ocean.

ACKNOWLEDGEMENTS

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POPULATION GENETIC INDICES OF VEINED RAPA WHELK GROUPS OF THE BLACK SEA REMOTE REGIONS

Valentin TOPTIKOV *, Tetyana ALIEKSIEIEVA * and Oleg KOVTUN **

* Odesa I. I. Mechnikov National University, Biological Faculty, Department of Genetics and Molecular Biology, Dvoryanska Street 2, Odesa, Ukraine, UA-65082, v.a.toptikov@gmail.com, ORCID: 0000-0002-7881-6726, t.alieksieieva@onu.edu.ua, ORCID: 0000-0002-1308-7673.
** Odesa I. I. Mechnikov National University, Hydrobiological Station, Dvoryanska Street 2, Odesa, Ukraine, UA-65082, hydrobiostation@gmail.com, ORCID: 0000-0001-8820-5606.

ABSTRACT

Genetic structure and genetic distance of *Rapana venosa* populations from remote regions of the Black Sea (Odesa Bay and eastern Crimea, Ukraine) were defined. Despite the significant difference in morphometric parameters, the investigated samples of mollusks are genetically very similar, and the distance between them is at the level of local populations. Common features of observed groups of *Rapana* in the northern part of the Black Sea are the unequal occurrence of some alleles and genotypes, a high level of heterozygosity and nonequilibrium in the Hardy-Weinberg equation. The gene flow is the most significant of the evolutionary factors that form the genetic structure of *Rapana venosa* populations.

ZUSAMMENFASSUNG: Populationsgenetische Indikatoren von geäderten Rapa-Wellhornschnecken Gruppen der abgelegenen Regionen des Schwarzen Meeres.

Genetische Struktur und genetische Distanz von *Rapana venosa* Populationen aus abgelegenen Regionen des Schwarzen Meeres (Bucht von Odesa und Ostküste der Krim, Ukraine) definiert wurden. Trotz des signifikanten Unterschieds in den morphometrischen Parametern sind die untersuchten Exemplare von Schalentieren genetisch sehr ähnlich, und der Abstand zwischen ihnen liegt auf der Ebene lokaler Populationen. Gemeinsame Merkmale der beobachteten Gruppen von *Rapana* des nördlichen Teils des Schwarzen Meeres sind das ungleiche Vorkommen einiger Allele und Genotypen, ein hohes Maß an Heterozygosität und Nichtgleichgewicht in der Hardy-Weinberg- Gleichgewicht. Der Genfluss ist der bedeutendste unter den Evolutionsfaktoren, die genetische Struktur von *Rapana venosa* Populationen bilden.

REZUMAT: Indicii genetici ai populației grupurilor de rapană din regiunile îndepărtate ale Mării Negre.

Au fost definite structura genetică și distanța genetică a populațiilor de *Rapana venosa* din regiunile îndepărtate ale Mării Negre (Golful Odesei și estul Crimeei, Ucraina). În ciuda diferenței semnificative a parametrilor morfometrici, probele investigate sunt foarte asemănătoare din punct de vedere genetic, iar distanța dintre ele este la nivelul populațiilor locale. Trăsăturile comune ale grupurilor de *Rapana* observate în partea de nord a Mării Negre sunt apariția inegală a unor alele și genotipuri, un nivel ridicat de heterozigozitate și dezechilibru în ecuația Hardy-Weinberg. Fluxul de gene este cel mai semnificativ dintre factorii evolutivi care formează structura genetică a populațiilor de *Rapana venosa*.

INTRODUCTION

The veined rapa whelk, the gastropod mollusk (*Rapana venosa*, Valenciennes, 1846) was first discovered in Novorossiysky Bay in 1947. However, it is believed that this species appeared in the Pontic basin earlier, in the last 30-40 years of the last century. In less than two decades, the coastal zone around the entire Black Sea was colonized by this mollusk (Eberzin, 1951; Drapkin, 1953; Stark, 1957; Chukchin, 1961; Băcescu, 1963; Gomoiu, 2005). Currently, *Rapana* is a very dangerous invasive species that attracts the attention of researchers not only in the countries of the Black Sea basin. There are data about these mollusk populations in many areas of the World Ocean: Italy, Great Britain, Netherlands, USA, South America, New Zealand (Mann et al., 2004). All this testifies to the great adaptive capacity of rapa whelk. It should be noted that all *Rapana* populations in the secondary ranges are derived from the Black Sea *Rapana* population, and not from the native range (Chandler et al., 2008).

Various aspects of *Rapana*'s adaptative properties as a species are well studied (Chukchin, 1970; Chukchin, 1984; Zolotarev, 1996; Mann and Harding, 2003; Gaevskaya, 2006; Savini and Occhipinti-Ambrogi, 2006; Shadrin and Afanasova, 2009; Zaika et al., 2010). However, the basics of the considerable adaptability of *Rapana*, in particular the genetic ones are not well-studied.

The elucidation of mechanisms of formation of *Rapana* groups' genetic structure in the Black Sea is necessary to predict the spread of rapa whelk. Such data can elucidate the impact of *Rapana* on existing biocoenosis and justify the nature of protection measures, as well as estimate the opportunities for fishing of rapa whelk and other species.

The priority is given to methods based on DNA analysis using PCR in genetic and population studies. All potential genetic determinants (both active and inactive) are identified by this approach.The results of the "Human Genome" program showed a certain paucity in understanding the genetic information implementation only on the basis of nucleotide sequence determination (Venter et al, 2001; Venter, 2003). The international program "Human Proteome" launched in 2010 testifies the importance of proteomic and transcriptomic research. In this regard, iso-allozyme analysis (as an element of proteome research), which includes only active genetic determinants, seems relevant for studying the structure of populations not only in the genetic but also in the ecological aspect.

In our opinion, the high adaptive abilities of rapa whelk from Black Sea may be due to its significant genetic polymorphism. Mechanisms that ensure a high level of internal genetic diversity of *Rapana venosa* are not well understood. Earlier, we made the assumption that significant genetic heterogeneity and disequilibrium (according to the Hardy-Weinberg equation) of rapa whelk groups in the northwestern part of the Black Sea is associated with a high intensity of migration processes. In our studies it was shown that the different groups of *Rapana* in the northwestern part of the Black Sea from the western coast of the Crimea (Cape Tarhankut) to the island of Zmiinyi can be considered as one population (Toptikov et al., 2017). According to our data, the genetic distance by Nei between the investigated groups of rapa whelks is on average 0.024 ± 0.003, which corresponds to the difference between local populations (Ayala and Kiger, 1984). The role of significant migration activity was also shown in the study of different age rapa whelk groups living in the same biotope (Toptikov et al., 2019). The great importance of migration in the forming of the flexible genetic structure of Black Sea *Rapana* groups can be related to two main causes: the presence of special distinct currents in the Black Sea (Ivanov and Belokopytov, 2011; Belevich et al., 2013) and long pelagic larval stage of the mollusk (Chukchin, 1970). This, combined with the great fertility of the *Rapana*, ensures the motion of a significant number of such organisms into the open sea, their transfer to long distances and crossings of incomers with local individuals.

It should be noted that in different areas of the northwestern part of the Black Sea, hydrological and hydrochemical conditions are quite similar and differ significantly from other regions of the Black sea (Tamaychuk, 2009; Polonsky et al., 2013; Gazyetov et al., 2015; Artamonov et al., 2017). In this regard, the purpose of this study was to determine the genetic indices and the genetic distance of various groups of *Rapana venosa* from remote regions of the Black Sea, which differ in their hydrological and hydrochemical properties.

MATERIAL AND METHODS

The *Rapana venosa* samples were collected manually, by a non-selective method, by divers in July-August 2012 in two remote water areas of the Black Sea from solid substrates at a depth of 5-15 m (Fig. 1). The sampling sites were located in Odesa Gulf (Odesa, the north-western sea area, 46°43'8"N, 30°77'2"E) (23 individuals) and in the Karadagsky Reserve area (the biostation, near Kurortne settlement, northeast aquatorium, 44°90'6"N, 35 19'4"E) (25 individuals). Samples of mollusks were immediately frozen and stored at –28°C until analysis.

Morphometric and age parameters were estimated by Kovtun et al. (2014). For the possibility of mathematical calculation of the mean values of age, the age categories 3+, 4+, etc., adopted in hydrobiology were designated as 3.5, 4.5, etc. The enzymes in the gels were detected according to the current recommendations (Manchenko, 2003). Nephridium and Leiblein gland were used as a source of enzymes. Esterases of EC 3.1.1.-(EST) and DT-diaphorase EC 1.6.99.2 (DT) were determined in the nephridium extract. Acid and alkaline phosphatases of EC 3.1.3.-(ACP i ALP, respectively), NADH oxidases of EC 1.6.99.3 (NOX), alcohol dehydrogenases of EC 1.1.1.1 (ADG), α-amylases of EC 3.2.1.1 (AMI), ATP-ases EC 3.6.1.-(ATPase) and glutathione peroxidases EC 1.11.1.9 (GPX) were determined in the Leiblein gland.
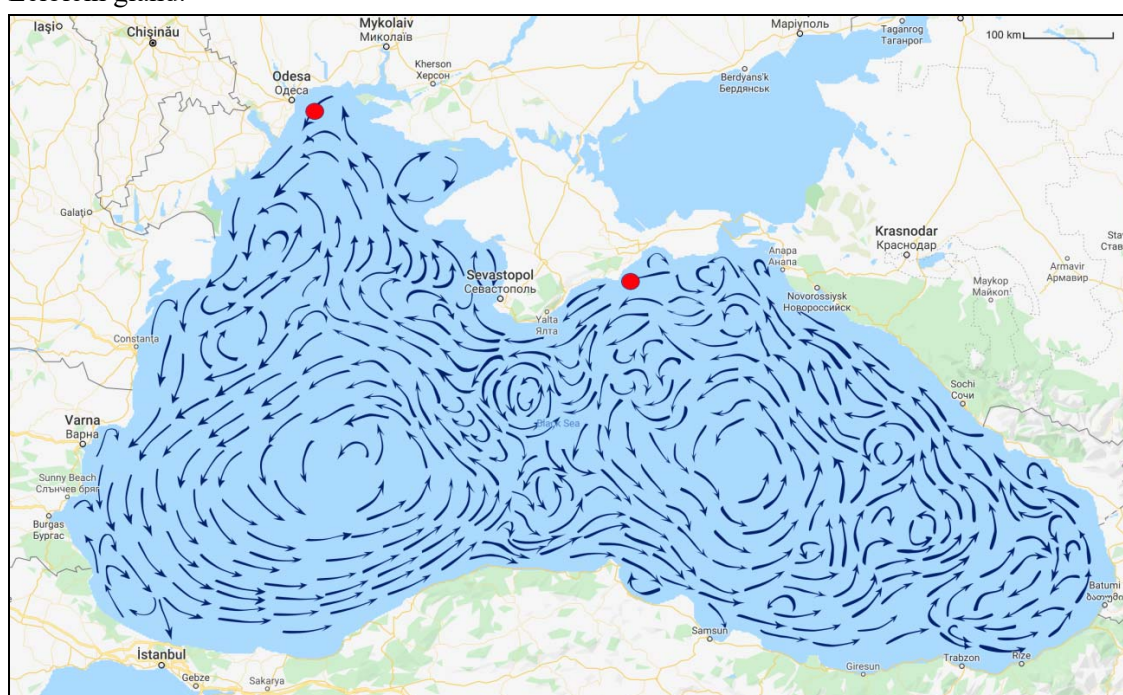
Figure 1: Sampling places (red dots) for collecting stocks of mollusks
and patterns of currents in the Black Sea
(http://blacksea-map.ru/map_black_sea_current_576665.html).

Analysis of the genetic structure of *Rapana venosa* groups was carried out according to generally accepted approaches and formulas (Wright, 1951, 1978; Nei, 1972; Li, 1976; Ayala, 1982; Ayala and Kiger, 1984; Slatkin, 1985; Altukhov, 2003; Kornienko et al., 2005; Kartavtsev, 2008). The analysis was carried out only on polymorphic loci. This approach was used because it makes possible to clearly see the difference in the level of gene diversity in different populations Pudovkin (1998). The investigated groups of *Rapana* were analyzed as described in a previous work (Toptikov et al., 2019).

RESULTS AND DISCUSSION

Morphometric indicators of the investigated mollusk groups are presented in table 1. Minor differences in the sexual structure for the studied *Rapana venosa* samples have been established. Taking into account the insignificant (within the limits of statistical inaccuracy) number of females present in the Odesa sample group, it can be assumed that this difference will not affect the general conclusions. Mollusk samples from the two study sites did not differ significantly in age. The average age of rapa whelks from the water area near Karadag was 3.6 ± 0.1, for mollusks from Odesa Bay it was 3.9 ± 0.2. Thus, we can assume that the differences found between these groups have no fundamental connection with gender and age.

Table 1: The mean values of the morphometric indicators of *Rapana venosa* from the investigated Black Sea water areas.

Indices	Morphometric indicators								
	Shell height, H, mm	Shell width, W, mm	Total weight, Σ, g	Shell weight, M_s, g	Mass of soft body, M_b, g	W/H	M_b/Σ	Solidity of the shell, M_s/H	Coefficient of fatness %
Karadag (n = 25) (♂ = 25)									
Average ± *SE*	**67.11±** *1.18*	**56.55±** *1.01*	**49.57±** *2.76*	**34.45±** *1.65*	**15.12±** *1.37*	**0.84±** *0.01*	**0.30±** *0.01*	**0.51±** *0.02*	**21.99±** *1.49*
Coefficient of variation, %	**8.78**	**8.91**	**27.88**	**23,98**	**45.19**	**4.86**	**21.15**	**17.19**	**33.78**
Odesa (n = 23) (♂ = 21, ♀ = 2)									
Average ± *SE*	**75.67±** *1.58*	**63.23±** *1.41*	**71.34±** *4.69*	**42.31±** *2.94*	**29.03±** *1.95*	**0.84±** *0,01*	**0.41±** *0,01*	**0.55±** *0,03*	**38.00±** *1.97*
Coefficient of variation, %	**8.08**	**8.64**	**25.45**	**26.89**	**25.96**	**2.46**	**8.58**	**19.29**	**20.11**
Reliability of differrences between groups by indicators, *p*	<0.001	<0.001	<0.001	0.03	<0.001	not reliably	<0.001	not reliably	<0.001

As can be seen from table 1, between the two groups of mollusks there is a difference in many morphometric parameters of the shell and body and also in degree of variation of these parameters. But, the question arises whether these differences are a consequence of hereditary determination or they are a result of phenotypic plasticity. To clarify the degree of genetic heterogeneity of these two studied groups, an allozime analysis of their representatives was carried out.

Rapana enzyme systems with the most unequivocal interpretation of their electrophoretic distribution by genetic positions were chosen here. In connection with the purpose of this work to elucidate intraspecific (interpopulation) heterogeneity, only polymorphic loci were considered. As a result 19 loci of nine enzymes were selected for testing genotypes (Tab. 2). As can be seen from the data of table 2, the occurrence of alleles in both groups of rapa whelks is almost similar. Each of the groups has insignificant features of the allele's distribution. Thus, in the sample of *Rapana* individuals from the sample of Karadag, the frequency of the alleles "*a*" of both alkaline phosphatases was almost twice as low compared to the same frequency in Odesa sample. And contrariwise, in case of allele "*a*" of ATP-ase locus its occurrence was twice as high as in the group of mollusks from the Odesa Bay. From the general features of the occurrence of alleles, we can determine the following: the frequency of alleles "*A*" of the loci *ALP-1*, *ALP-2*, *ACP-1*, *ACP-2*, *NOX-2*, and *GPX* is approximately twice as high as the alternative alleles of the corresponding loci. In the distribution of different alleles of the ATPase locus, both alleles "*b*" and "*c*" predominate in both of the investigated *Rapana* groups.

There were no fundamental differences in the occurrence of different genotypes in the studied groups of rapa whelk (Tab. 2). Thus, the number of homozygotes "*AA*" by locus *EST-2* and homozygotes by allele "*b*" of the *ATPase* locus in the group of individuals in the water area near Karadag is lower in comparison with the group of mollusks in the Odesa Bay. A different ratio of homo- and heterozygotes of the "*A*" allele at loci of alkaline and acid phosphatases and α-amylases (*AMI-1*) was also observed. In most other cases, the distribution of genotypes was similar in both *Rapana* populations. Certain features inherent in both rapa whelk investigated groups were identified. No homozygous "*AA*" were found for loci *AMI-3* and *ADG*. The same result – absence of homozygotes "*aa*" was typical for *GPX* locus. Homozygous genotypes of "*AA*" loci *EST-6* and *AMI-2* and also genotypes "*aa*" of loci *AMI-1* and *AMI-2* were found with a low/very low frequency. A very low proportion of the genotypes "*aa*" and "*ac*" at the locus *ATPase* was observed.

As can be seen from the obtained data on the gene pool of studied groups of the Black Sea *Rapana*, not only the prevalence of certain alleles and genotypes is characteristic (Tab. 2, where in bold type are reliable deviations from the Hardy-Weinberg equation). A common feature of the genetic structure of rapa whelk is the significant genetic disequilibrium of its groups, as evidenced by the large number of discrepancies in the alleles distribution to the Hardy-Weinberg equation (Tab. 3) On average, a deviation from the Hardy-Weinberg rule was observed for half the loci. Also, deviations in most loci were common for both groups studied.

In addition, the investigated gene pool of rapa whelks had an increased level of available heterozygosity (H_O, the H_E is expected heterozygosity) and, accordingly, some excess of heterozygotes (D) (Tab. 3). For most loci (82%), this excess was found, more than half of the loci (63%) were common to both groups. The average excess of heterozygous genotypes was within 15-17%. A high level of heterozygosity is consistent with a very low probability of inbreeding (negative values of the corresponding coefficients F', F_{IS}) and a small discrepancy between the effective and predicted number of alleles (Tabs. 3 and 4).

Table 2: The frequency of alleles and distribution of genotypes in the investigated groups of *Rapana venosa*.

Loci	Karadag Allele A	Actual frequencies of genotypes			Odesa Allele A	Actual frequencies of genotypes			χ^2 Karadag	Odesa
		AA	Aa	aa		AA	Aa	aa		
EST-2	0.480	0.04	0.88	0.28	0.500	0.17	0.66	0.17	**15.42**	2.54
EST-3	0.440	0.16	0. 56	0.28	0.457	0.22	0.48	0.30	0.71	0.10
EST-4	0.500	0.24	0.52	0.24	0.457	0.17	0.57	0.26	0.18	0.70
EST-5	0.540	0.24	0.60	0.16	0.543	0.09	0.91	0.00	1.39	**17.21**
EST-6	0.440	0.08	0.72	0.20	0.413	0.09	0.65	0.26	**5.91**	3.24
ALP-1	0.760	0.64	0.24	0.12	0.522	0.26	0.52	0.22	2.30	0.20
ALP-2	0.800	0.60	0.40	0.00	0.609	0.26	0.70	0.04	2.67	**5.55**
ACP-1	0.660	0.52	0.28	0.20	0.587	0.30	0.57	0.13	3.12	0.94
ACP-2	0.640	0.44	0.40	0.16	0.652	0.35	0.61	0.04	0.38	3.31
AMI-1	0.500	0.08	0.84	0.08	0.630	0.35	0.57	0.08	**12.34**	1.45
AMI-2	0.500	0.04	0.92	0.04	0.478	0.00	0.96	0.04	**18.58**	**20.36**
AMI-3	0.440	0.00	0.88	0.12	0.413	0.00	0.83	0.17	**16.37**	**12.30**
NOX-1	0.380	0.12	0.52	0.36	0.457	0.13	0.65	0.22	0.51	2.71
NOX-2	0.660	0.32	0.68	0.00	0.674	0.35	0.65	0.00	**7.52**	**6.29**
ADG	0.440	0.00	0.88	0.12	0.435	0.00	0.87	0.13	**16.37**	**14.55**
DT-1	0.580	0.36	0.44	0.20	0.522	0.26	0.52	0.22	0.24	0.23
DT-2	0.460	0.08	0.76	0.16	0.522	0.17	0.70	0.13	**7.66**	**4.08**
GPX	0.620	0.24	0.76	0.00	0.696	0.39	0.61	0.00	**10.27**	**5.32**

Loci	Alleles	aa	bb	cc	ab	ac	bc	Alleles	aa	bb	cc	ab	ac	bc	Karadag	Odesa
ATPase	a-0.220 b-0.420 c-0.360	0.08	0.08	0.12	0.24	0.04	0.44	a-0.152 b-0.544 c-0.304	0.04	0.26	0.13	0.22	0.00	0.35	6.49	4.03

Table 3: Heterozygosity at the studied loci of rapa whelk groups from different ranges.

Loci	Areas					
	Karadag			Odesa		
	H_O	H_E	D	H_O	H_E	D
EST-2	0.88	0.50	− 0.38	0.65	0.50	− 0.15
EST-3	0.56	0.49	− 0.07	0.48	0.50	0.02
EST-4	0.52	0.50	− 0.02	0.57	0.50	− 0.07
EST-5	0.60	0.50	− 0.10	0.91	0.50	− 0.41
EST-6	0.72	0.49	− 0.23	0.65	0.48	− 0.17
ALP-1	0.24	0.37	0.13	0.52	0.50	− 0.02
ALP-2	0.40	0.32	− 0.08	0.70	0.48	− 0.22
ACP-1	0.28	0.45	0.17	0.57	0.49	− 0.08
ACP-2	0.40	0.46	0.06	0.61	0.45	− 0.16
AMI-1	0.84	0.50	− 0.34	0.57	0.47	− 0.10
AMI-2	0.92	0.50	− 0.42	0.96	0.50	− 0.46
AMI-3	0.88	0.49	− 0.39	0.83	0.49	− 0.34
NOX-1	0.52	0.47	− 0.05	0.65	0.50	− 0.15
NOX-2	0.68	0.45	− 0.23	0.65	0.49	− 0.16
ADG	0.88	0.49	− 0.39	0.87	0.49	− 0.38
DT-1	0.44	0.49	0.05	0.52	0.50	− 0.02
DT-2	0.76	0.50	− 0.26	0.70	0.50	0.20
GPX	0.76	0.47	− 0.29	0.61	0.42	− 0.19
ATPase	0.72	0.65	− 0.07	0.52	0.59	0.07
	0.63 ± 0.05	**0.45** ± 0.01	**− 0.15**	**0.66** ± 0.03	**0.46** ± 0.02	**− 0.17**
F	**0.037**					

Table 4 shows the values of F' (the coefficient of inbreeding of a specific sample), F_{IS} (the measure of the deviation of the actual and expected genotypes frequencies within individual samples), F_{IT} (the measure of the deviation of the actual and expected frequencies of genotypes of a specific sample as compared to a generalized set of samples) as well as F_{ST} (the measure of gene differentiation of the investigated samples) and G_{ST} (relative gene differentiation between the samples studied). The sign * is mean, that data were calculated for a single sample relative to a generalized set of samples.

As can be seen from the presented data (Tab. 4), the F_{IS} and F_{IT} values are significantly larger than the F_{ST} level. This indicates the predominance of individual and intra-group variability over the general genetic heterogeneity of rapa whelks of the northern part of the Black Sea.

Table 4: The indicators of genetic variability of *Rapana venosa* groups investigated

Investigated groups	Indicators of genetic diversity and the degree of inbreeding				
	F'	F_{IS}	F_{IT}	F_{ST}	G_{ST}
Karadag	− 0.295	− 0.324*	− 0.451*	− 0.096*	
Odesa	− 0.321	− 0.294*	− 0.451*	− 0.121*	0.020
Entire set of samples	− 0.450	− 0.292	− 0.283	0.009	

The question of the affinity of rapa whelk groups in various areas of the Black Sea area is of very high practical importance, it is necessary mainly for scientifically based exploitation of marine resources. The use of various indicators of genetic similarity gave corresponding results (Tab. 5). For comparison, data from other sources are given in table 5.

The Nei' genetic distance between the investigated rapa whelk groups from different areas of the Black Sea was not significant (0.017). This level of genetic distance indicator is characteristic for local populations (Ayala, 1982). The genetic similarity index of Jeffries-Matusita also testifies to the lack of a reliable disagreement between rapa whelk groups from Karadag and Odesa (the threshold of reliability of differences is < 0.92). The indicator of genetic distance DH_{XY} in the calculation of heterozygosity shows the same composition of genotypes in the compared samples, because the obtained value of the indicator is very close to zero. Negative value of the indicator is associated with significant deviations from the Hardy-Weinberg equilibrium (Wolf et al., 2001).

What causes the difference in the frequency and spread of alternative alleles and genotypes is difficult to guess. This may be a consequence of the bottleneck effect due to the migration to the Black Sea of a part of *Rapana* individuals from the original range. We cannot exclude the adaptive value of certain allozymes and the preservation of the corresponding alleles as a result of natural selection as well. The elucidation of this question requires additional specific research.

As is known, significant genetic variability is observed under changing environmental conditions and potentially provides a high level of genetic adaptability (Ayala et al., 1973; Johnson, 1974; Leclair and Phelps, 1994). The northern part of the Black Sea, especially the northwestern part, is characterized by significant seasonal fluctuations in temperature and salinity. Under such conditions, it is possible that the high heterogeneity of Black Sea bream populations will lead to significant adaptation and ensure that this particular type of gastropod will be able to spread in the World Ocean. (Chandler et al., 2008) According to Kholina (2005), one of the reasons for the high level of heterozygosity can be a relatively significant life expectancy, which leads to the presence of overlapping generations and increases the effective genetic population number. This mechanism of heterozygous growth can also work for the type of *Rapana* in the northern Black Sea. Here we want to draw attention once again to the fact that the analysis was performed only on polymorphic loci.

Table 5: The genetic relationship of *Rapana venosa* various groups.

Habitats of the investigated rapa whelk groups	Indicators							Source
	Genetic similarity		Genetic differentiation					
	J	GSI	F_{ST}	D	DH_{XY}	Φ_{ST}	Θ	
Odesa and Karadag, Ukraine	0.983	0.961	0.009	0.017	−0.093	−	−	Present work
Odesa and island Zmeinyi, Ukraine	0.988	−		0.012	−	−	−	Toptikov et al., 2017
Island Zmeinyi and Tarkhankut, Ukraine	0.992	−		0.008	−	−	−	
The Yellow Sea: Qingdao, China Inch'on, S. Korea	−	−		−	−	0.007	−	Chandler et al., 2008
Qingdao, China Mikawa Bay (east Japan, the Pacific coast)	−	−		−	−	0.111	−	
South and Central of the Bo Hai Sea	−	−		−	−	−	0.000	Yang et al., 2008
The southern part of the Bo Hai Sea and the northern coast of the Yellow Sea	−	−		−	−	−	0.067	

It should be noted that the low genetic distance between the *Rapana* groups occurs against the background of a significant difference in morphometric parameters. This combination of relative genetic conservatism with significant phenotypic disagreements is characteristic for species that are able to adapt well to various environmental conditions and rapidly spread in their area. In particular, it is known for example about Eastern Asian species of mice (Mezhzherin, 2001). We suppose that *Rapana venosa* can also be attributed to this kind of species.

Returning to the question posed at the beginning of the paper on the nature of the differences in the morphometric parameters between rapa whelk groups from the different parts of the Black Sea, we can assume the following. Taking into account the insignificant genetic difference of the studied *Rapana* groups, it can be assumed that their morphological features belong to the category of modification variability. So the species *Rapana venosa* is characterized by a broad norm of reaction to environmental conditions. It is known that the rapa whelks linear-mass dimensions strongly depend on ecological, primarily trophic conditions (Bondarev, 2010, 2016; Kovtun et al., 2014). It is the insufficient food base in the Karadag area that determines the lower values of the morphometric parameters of individuals of this region.

A very high level of genetic polymorphism has been found in many other animal species, in particular in marine mollusks (Fujio et al., 1983; Yang et al., 2008; An et al., 2013).

The combination of disequilibrium with an excess of heterozygotes is indeed an infrequent phenomenon. However, it takes place in nature. In the work of some Chinese researchers on the development of microsatellite DNA markers, it was shown that at 10 loci out of 23, there was a deviation from equilibrium, and in three cases an excess of heterozygotes was observed (An et al., 2013). In a different study by the same researchers, there was disequilibrium in 24 out of 57 loci with an excess of heterozygotes in 13 loci (Sun et al., 2014). In addition, disequilibrium with an excess of heterozygotes was shown for the European catfish (*Silurus glanis*) population (Kuciñski et al., 2015) and the green lizard (*Lacerta viridis viridis*) (Böhme et al., 2007) and even for the purebred dogs (Shinkarenko et al., 2010).

Significant deviations of the genetic equilibrium at many loci can indicate the presence of pressure of evolutionarily significant factors on the genetic structure of the studied *Rapana* groups. In this connection, the question becomes which of the evolutionary factors determine the features of the gene pool formation of the Black Sea rapa whelk? We are well aware of the complexity of the question posed. On one hand, answering this question is limited by the insufficient base of existing field observations. On the other hand, the use of the known equations of population genetics has its limitations and requires certain conditions to be met. Nevertheless, we hope that our study will allow us to preliminarily assess the role of the environmental factors in the development of the genetic structure of rapa whelk population of northern Black Sea.

Table 6 shows the values of the following indicators: N_e/N – ratio of effective strength of the group to the total, SE – standard error, δ, $V_{\delta q}$ – gene drift indicators, f'_o – f'_e – the difference between the actual (available) and the expected variances. It should be noticed, that the value of the N_e/N indicator can be significantly greater than one in the case of intercrossing generations, which indicates * sign.

As can be seen in table 6, the studied groups of rapa whelk have a high effective N_e value, which is probably due to the overlap in generations in the selected samples. The large value of N_e and the low level of genetic differentiation of the studied mollusk populations indirectly indicate a low level of genetic drift. This conclusion is supported by calculations of the drift indicators.

Table 6: The value of some evolutionarily significant indicators.

Samples	Indicators				
	N_e/N	N_m	$\Delta \pm SE$	$V_{\delta q} \pm SE$	$f'_o - f'_e$
Karadag	0.772	$-2.858\circ$	0.109 ± 0.002	0.003 ± 0.000	0.00001
Odesa	0.757	$-2.317\circ$	0.116 ± 0.001	0.003 ± 0.000	0.00004
Totality	0.765	29.006	0.080 ± 0.001	0.002 ± 0.000	0.00007
The maximum possible value of the indicator	$> 1*$	$\to \infty$	0.353	0.125	$\to 1$

Evaluation of the natural selection role is the most difficult. The genetic heterogeneity of the various groups of rapa whelk taken into study may reflect the existence of selection. As a preliminary solution to this problem, we used the approach proposed in the works of Altukhov (2003). Comparison of the actual and expected variances of groups (Tab. 6) indicates the weak selective environmental pressure on the gene pool as a whole in the observed rapa whelk groups. If $f'_o < f'_e$, then there will be an effect of stabilizing selection, with $f'_o > f'_e$ – a disruptive selection (Altukhov, 2003).

Based on the study of four groups of rapans of the northern part of the Black Sea (this work; Toptikov et al., 2017, 2019) we assume that the main factor determining the characteristics of the *Rapana* groups' genetic structure is the gene flow. The values of the coefficient N_m (Tab. 6) are much larger than 0.5, which according to Wright (1931) indicates a significant advantage of the gene flow over the drift. According to calculations by Kimura and Maruyama (1971), if the migration rate exceeds four, then the population exists as the singular panmictic structure. However, the migration processes between rapa whelk groups in Karadag and Odesa are not as intense as between these mollusks' groups in the northwestern part of the Black Sea. Thus, according to our data, the value of N_m for individual groups of the northwestern water area of the Black Sea averaged 17.827 (Toptikov et al., 2017). This discrepancy between the intensity of gene flow in different sea regions is understandable given the distance between these sea regions and the presence of the Black Sea main stream branch from the Crimean Peninsula from north to south (Fig. 1). This water flow can cut off part of rapa whelk larvae from the transference in a western direction.

CONCLUSIONS

Thus, on the basis of the data obtained, it is possible to establish a definite genetic and significant phenotypic (by morphometric features) heterogeneity of different rapa whelk groups in the northern part of the Black Sea. Despite this, they can be considered local parts of one population. Panmixia is mediated through the migration of *Rapana* larvae in other areas of water. The high intensity of migration processes causes genetic heterogeneity and disequilibrium of the rapa whelk groups in the northern part of the Black Sea. The gene flow has a significant advantage over genetic drift, which does not have a significant effect on the genetic structure formation of rapa whelk groups from the investigated Black Sea water areas. Additional research is needed for further clarification of these problems.

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HABITAT PREFERENCES OF SMALL FISH SPECIES
IN RIVERS AT THE EASTERN EDGE OF EUROPE

*Arthur ASKEYEV **(c.a.)**, Oleg ASKEYEV *, Igor ASKEYEV *,*
*Sergey MONAKHOV * and Tim Hugo SPARKS ***

* Tatarstan Academy of Sciences, Institute of Problems in Ecology and Mineral Wealth, Biomonitoring Laboratory, Daurskaya Street 28, Kazan, Tatarstan Republic, Russia, RU-420087, art.regulus@mail.ru, ORCID: 0000-0002-1214-7355; parus.cyanus@rambler.ru, ORCID: 0000-0001-6589-0479; archaeozoologist@yandex.ru; ORCID: 0000-0002-5304-4985; serega-28@inbox.ru.
** Poznań University of Life Sciences, Department of Zoology, Wojska Polskiego 71c, Poznań, Poland, PL-60-625; Museum of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK, thsparks@btopenworld.com, ORCID: 0000-0003-4382-7051.

KEYWORDS: fish species, environmental factors, probability of occurrence, abundance, east edge of Europe.

ABSTRACT

We studied the probability of occurrence, distribution and abundance of eight small fish species in 512 small rivers at the eastern edge of Europe in a region of high geographical and environmental heterogeneity. Stone loach, common minnow, and gudgeon were recorded in more than 50% of the study sites. Common minnow was the most abundant species, contributing 66% of all captures. Elevation, depth, and width of the river were the main environmental factors influencing the distribution of more than half of the studied fish species. Our research in the eastern edge of Europe shows that fish preferences in terms of probability of occurrence, abundance, and niche breadth for environmental factors can vary greatly, even among closely related species.

ZUSAMMENFASSUNG: Lebensraumpräferenzen von kleinen Fischarten in Flüssen in der Ostgrenze Europas.

Die Untersuchungsregion hat eine hohe geographische Heterogenität, wie auch die Umwelt. Bachschmerle, Erlitze und Gründling wurden in mehr als 50% der Untersuchungsgebieten gefunden. Außerdem hatte Erlitze die höchste Abundanz mit einem Anteil von 66% an den anderen ausgewählten Fischarten. Die Elevation, Tiefe und Breite des Flusses waren die Hauptumgebungsfaktoren, die Verteilung von mehr als Hälfte der ausgewählten Fischarten beeinflusste. Unsere Untersuchung in der Ostgrenze Europas zeigte, dass die Fischpräferenzen für Wahrscheinlichkeiten des Vorkommens, sowie der Abundanz und Nischenbreite für Umgebungsfaktoren sogar zwischen den engen verwandten Fischarten stark variieren können.

REZUMAT: Preferințele de habitat ale speciilor de pești mici în râurile de la limita de est a Europei.

Am studiat probabilitatea apariției, distribuției și abundenței a opt specii de pești mici în 512 râuri mici de la limita de est a Europei într-o regiune cu heterogenitate geografică și de mediu ridicată. Grindelul, boișteanul și porcușorul comun au fost înregistrați în mai mult de 50% din stațiile studiate. Boișteanul a fost cea mai abundentă specie, având o abundență de 66% din toate capturile. Altitudinea, adâncimea și lățimea râului au fost principalii factori de mediu care au influențat distribuția a mai mult de jumătate din speciile de pești studiate. Cercetările noastre de la limita de est a Europei arată că preferințele peștilor în ceea ce privește probabilitatea de apariție, abundența și lățimea de nișă pentru factorii de mediu pot varia foarte mult, chiar și în rândul speciilor strâns înrudite.

INTRODUCTION

Small rivers are the most numerous water bodies within the Republics of Tatarstan and Bashkortostan. They make a significant contribution to biological diversity and are a refuge for many rare and endangered fish species. However, small rivers, due to their accumulative characteristics, are very fragile ecosystems (Pekárik et al., 2009; Biggs et al., 2017; Bănăduc et al., 2021a). The creation of large reservoirs, deforestation, pollution of water bodies with household waste, and grazing in the catchment area have had a strong detrimental effect on river ecosystems and have led to a change in the hydrological balance of small and medium rivers (Kuznetsov, 2005). The occurrence and abundance of many fish species, especially species of the rheophilic complex, have decreased greatly compared to their historical distribution in the Republic of Tatarstan (Askeyev et al., 2016). In addition to anthropogenic activity, the ichthyofauna of small rivers is currently negatively affected by climate warming (Buisson et al., 2013; Comte and Grenouillet, 2013; Bănăduc et al., 2021b). Tatarstan has warmed considerably over the past 30 years (Askeyev et al., 2018, 2020). If these trends continue, then many fish species of boreal origin may struggle to survive (Buisson et al., 2013; Comte and Grenouillet, 2013).

In the current study, we selected the following fish species: stone loach (*Barbatula barbatula* Linnaeus, 1758), common minnow (*Phoxinus phoxinus* Linnaeus, 1758), gudgeon (*Gobio gobio* Linnaeus, 1758), white-finned gudgeon (*Romanogobio albipinnatus* Lukasch, 1933), spined loach (*Cobitis taenia* Linnaeus, 1758), Siberian spined loach (*Cobitis melanoleuca* Nichols, 1925), ruffe (*Gymnocephalus cernua* Linnaeus, 1758) and European bullhead (*Cottus gobio* Linnaeus, 1758) (Red Book of the Republic of Tatarstan, 2016). These species are similar in morphological size, collectively contribute a large proportion of the total numbers of fish in the study region (Askeyev et al., 2015, 2017), and form the basis of the diet of predatory fish species (Askeyev et al., 2021). The study of a large region assures a wide range of environmental conditions, from small streams in upland areas to large flat rivers (Matthews, 2012). The species composition, distribution, occurrence, abundance, and ecology of fish depend largely on current environmental conditions (Matthews, 2012). The values of many environmental factors, such as height above sea level, current velocity, bottom substrate, width, depth and length of the river, affect the frequency and abundance of fish in watercourses. In this regard, it is interesting to know if there are differences between these fish species in preferences for environmental factors in the rivers of the east edge of Europe.

The main aim of the study was to reveal patterns of the distribution of eight fish species on environmental gradients in rivers in the east edge of Europe.

MATERIALS AND METHODS
Study area and fish assemblage data

The study area is located in the extreme east of Europe (in the eastern part of the Ponto-Caspian biogeographic region) (Fig. 1) and covers the Republic of Tatarstan and the mountainous part of the Republic of Bashkortostan. We focused on the fish assemblages of small rivers (length up to 500 km). We excluded rivers strongly impacted by large reservoirs. Fish sampling was conducted at 512 locations. Fieldwork was carried out from May to October in the years 2010-2021 during reduced summer flows. We used three different lengths of nets, depending on the width of the river. Three seining net were 5-15 m in length, 1.2-1.5 m high, 5x5 mm mesh in the wings, 3x3 mm in bags. Dip nets were of 50-70 cm diameter, with 4x4 mm mesh. Lengths of between 200 m (smaller rivers) and 400 m (larger rivers) of the river sites were sampled. For determining the length of the site, we followed the recommendations

for catching fish by Fame Consortium (2004). Caught fish were placed in a plastic basin and identified, counted and measured at the end of each catch session, after which ≥ 90% of fish were returned to the water. Identification of fish was carried out according to Maitland and Linsell (2009) and Makeeva et al. (2011).

The following seven environmental variables were obtained for each site: elevation (altitude) above sea level (from 53 to 720 m, mean 239 m, standard deviation SD 184 m), mean width (from 0.5 to 55 m, mean 7.5 m, SD 15.6 m), mean depth (from 0.11 to 1.8 m, mean 0.63 m, SD 0.36 m), water velocity (from 0 to 1 m/s, mean 0.35 m/s, SD 0.16 m/s), tree/bush cover along banks (from 0 to 100%, mean 55%, SD 27.7%), dominant bottom substrate (1 – mud, 2 – clay or peat, 3 – sand, 4 – gravel, 5 – small pebbles, 6 – large stones up to 150 mm, 7 – large stones 150-300 mm, 8 – boulders > 300 mm) and human impact (as a seven point scale, 0 – no agriculture or forestry, 1 – light agricultural impact – hayfields, limited grazing and forestry at a distance of 0-250 m from the river bank, 2 – moderate agricultural impact – moderate grazing at a distance of 0-250 m from the river bank, the presence of a ford and a watering hole for livestock, 3 – strong agricultural impact – heavy grazing with visible cattle trails, arable land and housing for animals at a distance of 0-250 m from the river bank, 4 – moderate agriculture impact and oil pollution – average grazing and oil and gas extraction at a distance of 0-250 m from the river bank, 5 – urban impact – river site in town or large village, 6 – strong oil and chemical pollution is smelt and visible).
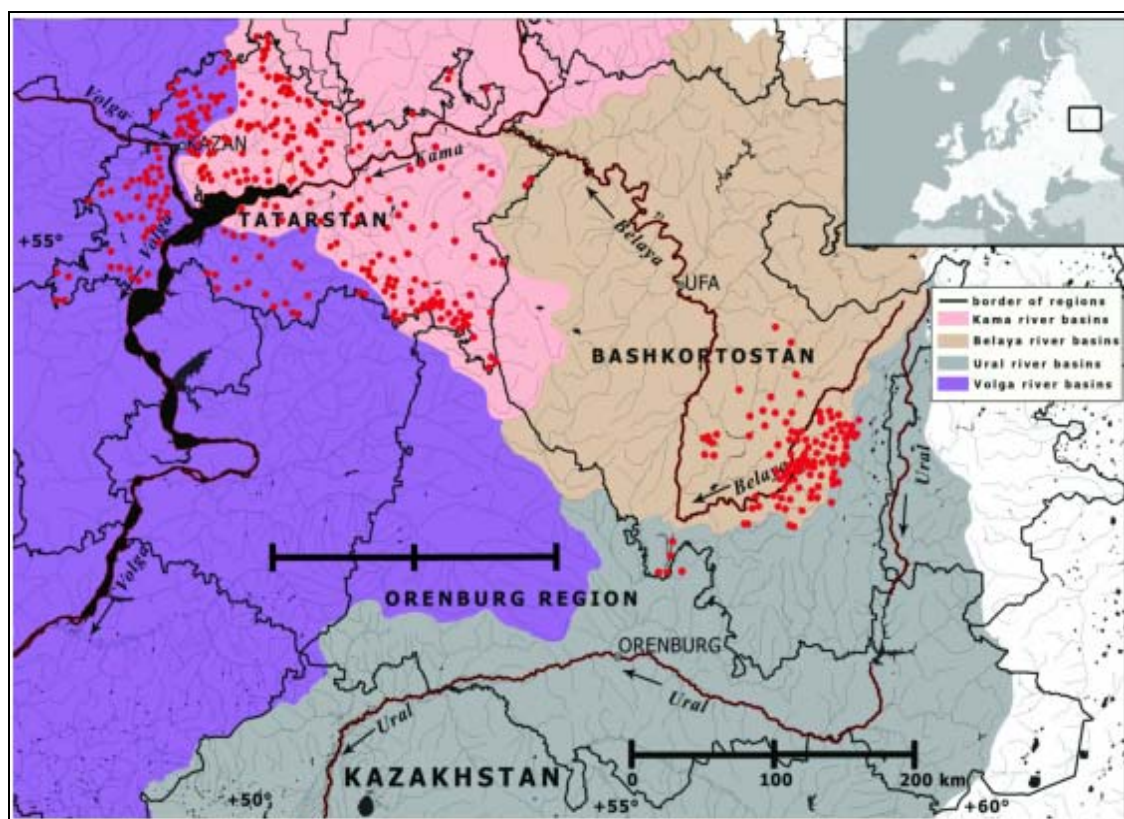

Figure 1: Distribution of the sampling sites
(inset shows location within Europe).

Data analysis

Relationship between fish species and environmental variables

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

Species optimums and niche breadth

Species optima in terms of fish numbers for each environmental variable were calculated in order to rank species by habitat preferences. This model fits Gaussian response models to species abundances along an environmental gradient. The fitted parameters are optimum (i.e. average) and niche breadth/tolerance (i.e. standard deviation). The algorithm is based on weighted averaging (ter Braak and van Dam, 1989).

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

RESULTS AND DISCUSSION

Occurrence and abundance of the fish species

Only stone loach, gudgeon, and common minnow were found in more than half of our studied sites (Tab. 1). Stone loach was the most widespread of the studied fish species (Tab. 1); it occurred at 76% of sites, much higher than in the rivers of Western Europe. For example, it occurred in 66% of French rivers (Maire et al., 2016), 43% of river basins in southwestern France (Santoul et al., 2005), 41% in northeastern Germany (Fieseler and Wolter, 2006), 32% of Czech rivers (Lusk and Pivnicka, 2009), and as juveniles in only 15% of rivers in Lithuania (Stakenas, 2002). The occurrence of gudgeon and common minnow varies strongly in different parts of Europe. For example, common minnow occurred in 64% of rivers in southwestern France (Santoul et al., 2005) and rivers in Latvia (Birzaks, 2012) but was completely absent in the potamal rivers of northeastern Germany (Fieseler and Wolter, 2006). For gudgeon, occurrence ranged from 72% in the Treene River catchment (North Germany) (Radinger et al., 2015) to 43% in France (Maire et al., 2016). These three species are quite common in Romania (Bănărescu, 1964). Common minnow had the highest abundance among the fish species in the current study (Tab. 1); it contributed 66% of the captured individuals. It is also the most abundant species in Great Britain (Pretty et al., 2003).

Table 1: Occurrence and abundance of the studied species. Species arranged in decreasing order of occurrence; mean = mean number of individuals of this species per one study site.

Species	Occurrence % of rivers	Total number caught	Mean ± SD
Stone loach	75.9	9034	17.7 ± 39.2
Gudgeon	67.2	10658	20.9 ± 51.8
Common minnow	59.7	42105	82.6 ± 185.5
Siberian spined loach	24.9	907	1.8 ± 7.3
Spined loach	11.0	226	0.44 ± 1.81
European bullhead	10.6	183	0.36 ± 1.72
Ruffe	6.1	172	0.34 ± 2.43
White-finned gudgeon	5.9	558	1.1 ± 12.3

Environmental factors influencing the distribution of the eight fish species

The presence/absence of each fish species had statistically significant relationships with two or more of the environmental variables (Tab. 2). Seven species were associated with elevation, six with depth and width of the river, four with bottom substrate, three with tree and bush cover, two with human impact and one species with water velocity. All final models had satisfactory (fair to excellent) predictive power (AUC) varying from 0.78 to 0.94 (Tab. 2). For the most abundant and frequently occurring fish species the most influential environmental factors, such as elevation and river morphology, were similar to those in the less environmentally diverse sub area of Tatarstan (Askeyev et al., 2015). Thus, we can say with confidence that these factors are of integral importance for fish species at the eastern edge of Europe.

Models of stone loach and common minnow in relation to environmental factors were quite similar; both species were found mainly in rivers that are small in terms of width and depth (Fig. 2), at a relatively high elevation and with relatively hard bottom substrates (Tab. 2, Fig. 3). Similar results have been reported in many regions of Europe (Bănărescu, 1964; Mastrorillo et al., 1996; Lusk and Pivnicka, 2009; Maire et al., 2016). However, the common minnow in our study, in contrast to the stone loach, had preferences for clear river sites without human impact; a similar relationship with human impact was described in Bănărescu (1964). The negative impact of anthropogenic pressure on common minnow has been described in Finland (Sutela and Vehanen, 2010) and Romania (Bănărescu, 1964). For gudgeon, in contrast to stone loach and common minnow, we observed contrasting preferences for environmental factors. In our study, gudgeon occurred more often in wide and deep rivers (Fig. 2); similar preferences were found in Hungary, Czech Republic, Latvia, and France (Takács et al., 2008; Lusk and Pivnicka, 2009; Birzaks, 2012; Maire et al., 2016). We noted that gudgeon avoided sections of rivers with "hard" substrates that are not suitable for them as food and spawning substrates; similar preferences for this species are described in Lamouroux and Souchon (2002). Another factor (negatively) influencing the occurrence of gudgeon was tree/bush cover. A similar relationship was described in Hungary and France (Takács et al., 2008; Maire et al., 2016). Another possible reason for the gudgeon avoiding high tree/bush cover is a negative relationship with burbot (*Lota lota,* Linnaeus, 1758), which is a predator of gudgeon and prefers shaded areas of rivers (Askeyev et al., 2021). Such strong differences in environmental preferences among the most common and numerous fish species in our study

region emphasize the need for a wide variety of environmental conditions by living organisms. The European bullhead is a typical rheophilic species that prefers rhythral conditions in rivers (Fig. 3), mainly in mountainous areas, avoiding slow moving rivers. The preference of the European bullheads for fast sections of rivers has been previously noted (Blanck et al., 2007; Cismaş et al., 2017). In our study, European bullhead avoided areas with soft substrates such as silt or clay, since they cannot serve as a substrate for reproduction and do not provide a safe refuge from predatory species (Gosselin et al., 2010). The European bullhead can be an indicator of the clarity of watercourses; this species does not occur in areas with a high anthropogenic impact and, moreover, a chemical pressure. A similar reaction of the European bullhead to pollution was noted in Finland and Romania (Sutela and Vehanen, 2010; Curtean-Bănăduc et al., 2017). Unlike the European bullhead, the white-finned gudgeon, and ruffe are potamal species, they occur more often in deep and wide rivers at low elevation (Tab. 2, Fig. 3). The preference of these species for large rivers was noted in the Czech Republic (Lusk and Pivnicka, 2009). The two closely related *Cobitis* species (spined loach, Siberian spined loach) have similar altitude preferences, inhabiting the lower part of the gradient, but the Siberian spined loach prefers deeper water and less tree/bush cover than the spined loach.

Table 2: Coefficients and model summary summarising the relationship between presence/absence of fish and environmental variables. Species arranged in decreasing order of occurrence.

| Species	Constant	Elevation	Width	Depth	Velocity	Tree/bush cover	Substrate	Human impact	AUC
Stone loach	1.64	4.09	−6.25	−0.47			0.34		0.781
Gudgeon	−1.58		0.19	1.56		−0.22	−0.39		0.811
Common minnow	−0.92	2.37	−5.75	−0.51			0.59	−0.31	0.801
Siberian spined loach	−0.49	−2.10		0.95		−1.60			0.778
Spined loach	−1.55	−3.78	1.83						0.763
European bullhead	−5.04	4.39			1.27		0.47	−0.32	0.856
Ruffe	−3.90	−0.21	5.14	2.79					0.931
White-finned Gudgeon	0.57	−5.15	3.61	2.11		−1.54			0.941
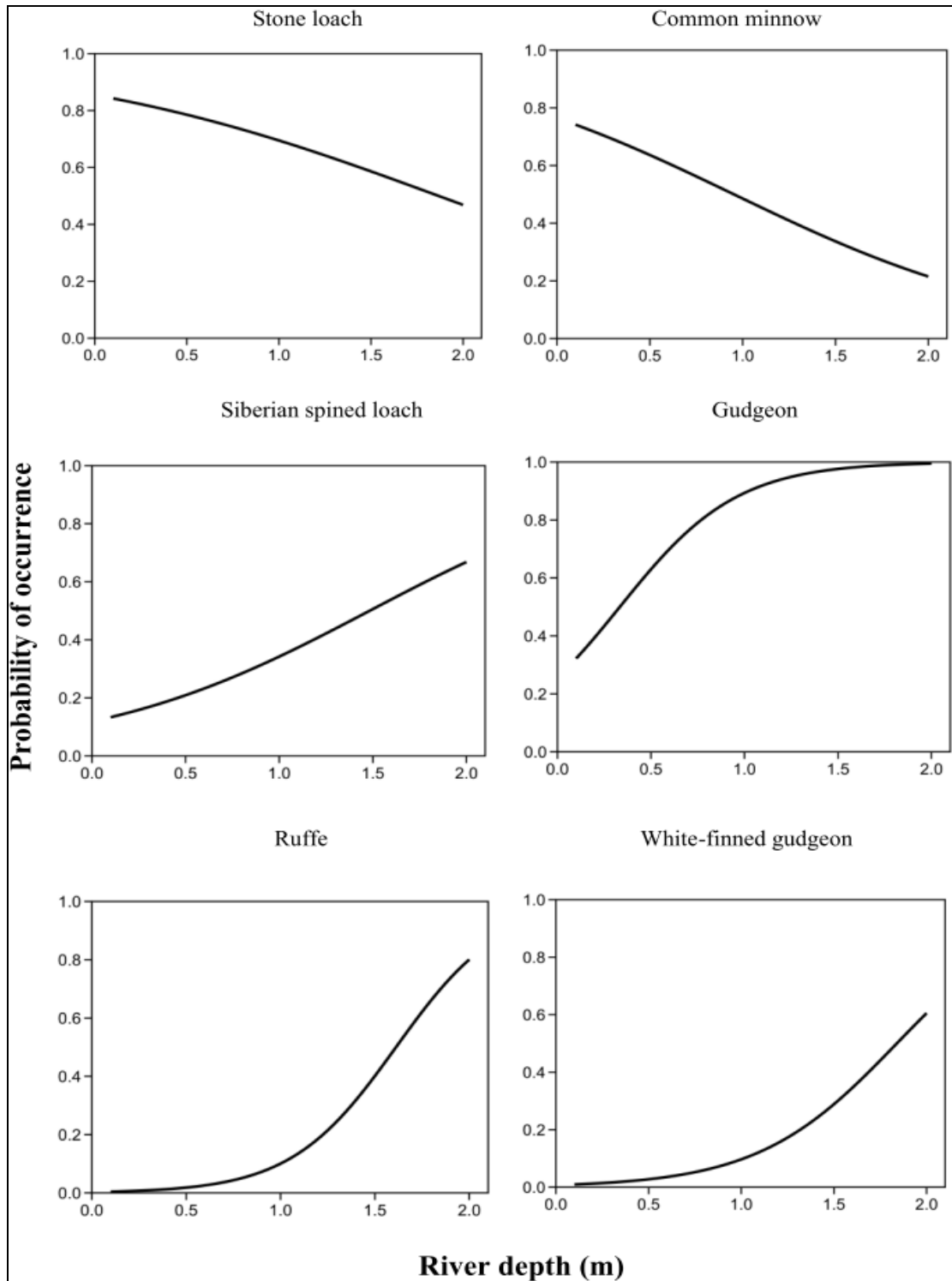
Figure 2: Relationship between the probability of occurrence of fish and depth for six species for which depth was a significant influence. Species arranged in order of increasing depth.
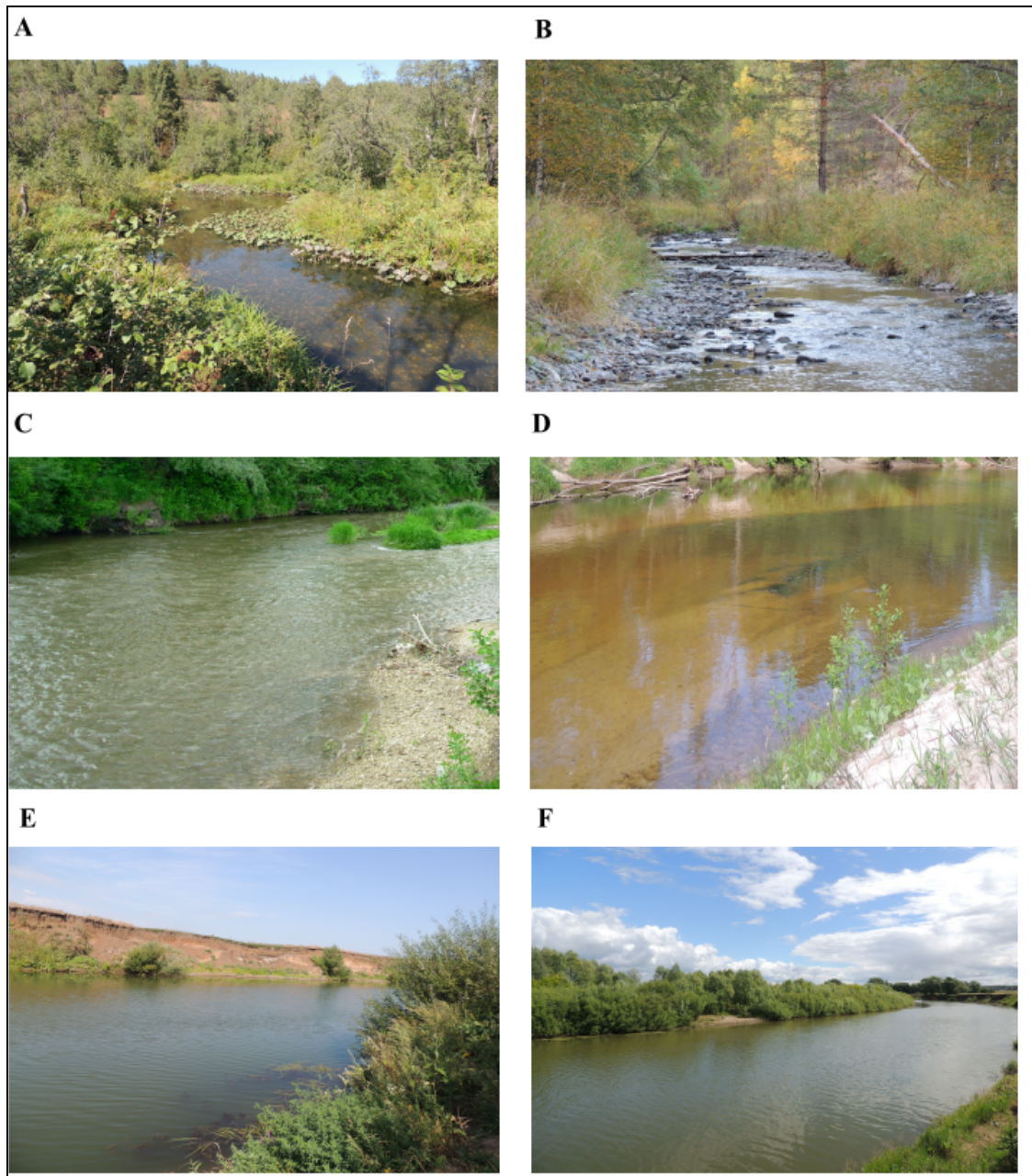
Figure 3: Typical habitats for common minnow and stone loach (A),
for European bullhead (B), for spined loach (C), for gudgeon (D),
for Siberian spined loach (E) for ruffe and white-finned gudgeon (F).

Species optimum and niche breadth

Table 3 shows the optimal points and breadths on four continuous environmental variables; those for elevation have been presented elsewhere (Askeyev et al., 2017). The optimal values varied greatly between fish species for some factors. For example, the difference in optimal river width between the narrowest (stone loach and common minnow) and the widest (ruffe) was 37 m. Ruffe is the only species in our current study that had an optimal depth value of more than one m, while in the common minnow it was < 0.5 m. The white-finned gudgeon had the smallest optimal for tree/bush cover but the highest for velocity. Niche breadth also varied greatly between species, and within the same species to different environmental factors. For example, the ruffe had the widest niche for river width but the narrowest for tree/bush cover. The difference in the niche breadth for river width was more than 24 m. But the niche breadths of all environmental factors did not all differ greatly, for example, the niche breadth for velocity in our study was relatively similar for all fish species. The narrow niche of stone loach for river width and depth was one of the reasons why this species, despite being the most widespread, was not the most abundant species of small rivers at the eastern edge of Europe.

Table 3: Optimum values and tolerance (niche breadth) of the studied fish species against four environmental variables (based on abundance data). Species arranged in decreasing order of occurrence.

Fish species	Width (m)	Depth (m)	Velocity (m/s)	Tree/bush cover (%)
Optimum values				
Stone loach	5.8	0.66	0.35	52.2
Gudgeon	10.6	0.72	0.30	45.3
Common minnow	5.4	0.48	0.39	62.5
Siberian spined loach	9.9	0.79	0.33	35.3
Spined loach	11.3	0.73	0.33	51.6
European Bullhead	8.2	0.53	0.45	65.9
Ruffe	43.3	1.35	0.44	42.3
White-finned gudgeon	27.2	0.93	0.45	34.8
Tolerance (niche breadth)				
Stone loach	5.1	0.26	0.15	26.4
Gudgeon	11.1	0.28	0.14	27.7
Common minnow	15.1	0.47	0.16	22.9
Siberian spined loach	14.8	0.36	0.16	22.5
Spined loach	11.6	0.32	0.14	25.9
European bullhead	9.5	0.29	0.15	20.2
Ruffe	29.7	0.42	0.15	14.9
White-finned gudgeon	11.4	0.29	0.13	15.6

CONCLUSIONS

For rational protection in the era of global climate change, it is important to understand the responses of specific animal species to environmental factors in large and varied landscapes. Our research at the eastern edge of Europe shows that fish preferences for environmental factors can vary greatly, even among closely related species. Thus, we see that the distribution of fish, as well as their protection, can only be satisfactorily explained when using a combination of all the environmental factors involved. Based on the results of our work, the Rychkovskaya Lesostep protected natural area has been created, which includes rivers characterized by a high abundance of the European bullhead which is currently on Tatarstan's Red List.

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IN VITRO AND *IN VIVO* STUDIES OF THE ANTIPARASITIC EFFECT OF ASPIRIN AGAINST *DACTYLOGYRUS EXTENSUS* (MONOGENEA) INVASION IN CARP (*CYPRINUS CARPIO*)

Faik Sertel SECER *[(c.a.)], *Cigdem URKU* **,
Bilgenur HARMANŞA YILMAZ * and *Hijran YAVUZCAN YILDIZ* *

* Ankara University, Faculty of Agriculture, Department of Fisheries and Aquaculture, Ankara, Turkey, sertelsecer@yahoo.com, ORCID: 0000-0002-1940-7949, harmansa@ankara.edu.tr, ORCID: 0000-0003-0679-8327, yavuzcan@ankara.edu.tr, ORCID: 0000-0001-6567-7467.
** Istanbul University, Faculty of Aquatic Sciences, Istanbul, Turkey, curku@istanbul.edu.tr, ORCID: 0000-0003-0381-9321.

KEYWORDS: Aspirin (ASA), *Dactylogyrus*, antiparasitic, gill histopathology.

ABSTRACT

Aspirin is one of the most widely used medications globally as an analgesic and antipyretic drug. Aspirin use as an antiparasitic against fish parasites has never been tested. The parasite *Dactylogyrus* is considered a serious threat to freshwater aquaculture in relation to considerable losses. The present study is aimed to evaluate the antiparasitic effect of aspirin against *Dactylogyrus extensus* on *Cyprinus carpio* and alteration in gill histopathology. *In vitro*, aspirin exhibited antiparasitic activity with median effective concentration (EC50) values at one and six minutes of 8.137 g/L^{-1} and 1.629 g/L^{-1}, were assessed for the first time. *In vivo* antiparasitic efficacy of aspirin to *D. extensus* was found to be 46.49%. Severe lesion profile was observed in histopathological evaluations of the gills.

ZUSAMMENFASSUNG: *In-vitro-* und *in-vivo*-Studien zur antiparasitären Wirkung von Aspirin gegen die Invasion von *Dactylogyrus extensus* (Monogenea) bei Karpfen, (*Cyprinus carpio*).

Aspirin ist eines der weltweit am häufigsten verwendeten Medikamente als Analgetikum und Antipyretikum. Die Verwendung von Aspirin als Antiparasitikum gegen Fischparasiten wurde nie getestet. Der Parasit *Dactylogyrus* gilt aufgrund erheblicher Verluste als ernsthafte Bedrohung für die Süßwasseraquakultur. Die vorliegende Studie zielt darauf ab, die antiparasitäre Wirkung von Aspirin gegen *Dactylogyrus* extensus auf *Cyprinus carpio* und Veränderungen in der Kiemenhistopathologie zu bewerten. In vitro zeigte Aspirin antiparasitäre Aktivität mit medianen Effektivkonzentrationswerten (EC50) von eine und sechs minute von 8,137 g/L-1 und 1,629 g/L-1, was zum ersten Mal bewertet wurde. Die antiparasitäre *In-vivo*-Wirksamkeit von Aspirin gegenüber *D. extensus* betrug 46,49%. Bei histopathologischen Auswertungen der Kiemen wurde ein schweres Läsionsprofil beobachtet.

REZUMAT: Studii *in vitro* și *in vivo* ale efectului antiparazitar al aspirinei împotriva invaziei cu *Dactylogyrus extensus* (Monogenea) la crap (*Cyprinus carpio*).

Aspirina este unul dintre cele mai utilizate medicamente de tip analgezic și antipiretic. Utilizarea aspirinei ca antiparazitar împotriva paraziților peștilor nu a fost niciodată testată. Parazitul *Dactylogyrus* este considerat o amenințare pentru acvacultura de apă dulce ducând la pierderi considerabile. Prezentul studiu își propune să evalueze efectul antiparazitar al aspirinei împotriva lui *Dactylogyrus extensus* de la *Cyprinus carpio* și alterarea histopatologiei branhiale. *In vitro*, aspirina a prezentat activitate antiparazitară cu valori medii ale concentrației efective (EC50) în un și șase minute de 8,137 g/L-1 și 1,629 g/L-1, care a fost evaluată pentru prima dată. Eficacitatea antiparazitară *in vivo* a aspirinei la *D. extensus* a fost de 46,49%. Leziuni severe au fost observate în evaluările histopatologice ale branhiilor.

INTRODUCTION

Monogeneans are members of the parasitic flatworms' class, infecting marine and freshwater fish (Reed et al., 2012). Direct life cycle of the monogenean and their rapid reproduction results in an increase in the incidence of severe diseases in fish cultures. Dactylogrid monogeneans are found on the gills of freshwater fish. The pathogenesis of the Dactylogyrid family is directly related to loss of epithelium in the gills due to feeding by parasites (Jalali and Barzegar, 2005; Andrade-Porto et al., 2017; Hu et al., 2017). Histopathological changes such as telangiectasia and edema as well as hyperplasia and fusion of the secondary lamellae were previously described in fish infected by *Dactylogyrus* species (Fujimoto et al., 2014; Santos et al., 2017). Disrupted respiration function due to the damaged gill tissue can lead to the death of fish (Pimentel-Acosta et al., 2019). Additionally, monogeneans may provide the entry point for secondary infections due to their feeding and attachment form, increasing disease outbreaks (Dove and Ernst, 1998; Zhang et al., 2014).

Aquaculture is a recognized fast-growing food production segment in the overall agri-food sector and is characterized by a dynamic performance over the last 30 years. Today aquaculture meets half of the global fish consumption. However, diseases are a serious threat to sustainable aquaculture, leading to serious economic losses. Discernibly, increased diseases can slow progress of aquaculture. Therefore, control of diseases including parasites in aquaculture is of crucial importance for its future. A wide range of chemicals and drugs have been used to control parasites in order to elevate efficiency of fish culture. These compounds have human and environmental safety concerns, resulting in some limitations of their use. In terms of fish, numerous anti-parasitic chemicals, drugs or herbs were evaluated for their effects on fish (Carraschi et al., 2017; de Oliveira et al., 2017; Guimarães et al., 2007). Aspirin containing an active substance of acetylsalicylic acid (ASA) is commonly used as an analgesic, antipyretic and anti-inflammatory drug for medical treatment. Previous studies on aspirin were to analyse its analgesic effects on fish. Lopez-Luna et al. (2017) reported that immersion in aspirin solutions showed an analgesic effect to noxious chemicals in larval zebrafish. Although the analgesic effects of aspirin were tested in fish, the pathological effects of immersion for aspirin are mostly unknown (Diggles et al., 2017). ASA has been studied for its ecotoxicological effects in relation to pharmaceutical drugs in aquatic ecosystems and histological alterations in fish tissues (Bottoni et al., 2010; Nunes et al., 2015). ASA was tested to evaluate the inhibitory effects on acute stress response of tilapia and related hormones (Van Anholt et al., 2003). ASA was found to control mycelial growth of the fungus *Saprolegnia* which frequently occurs in freshwater fish with negative impact on aquaculture (Sundari et al., 2016). To our knowledge, aspirin has never been tested for its antiparasitic capacity in fish. Thus, considering the advantages of aspirin such as ease of availability and low price, it was worth studying its potential as an antiparasitic for fish *in vitro* and *in vivo*.

The aim of the present study was to assess the median effective concentration (EC50) of aspirin for *D. extensus in vitro* and *in vivo* and antiparasitic efficacy of aspirin in carp (*Cyprinus carpio*) infected with *D. extensus* as well as to analyze the alteration of gill architecture in aspirin-treated carp by histopathology.

MATERIAL AND METHODS

Fish and parasites. Fish were used within ethical framework as approved by the ethics committee of the Ankara University with the reference number 2019-7-72. Carps weighing around 60 g were obtained from laboratory stock maintained in the one-loop aquaponics systems producing carp and mint (*Mentha* spp.) at Ankara University, Department of Fisheries and Aquaculture. Fish were kept in aerated water at 20-22°C at a stocking density of 35 kg/m^3 in fiberglass fish tanks. The dissolved oxygen and the pH were around 5.80 mg/L^{-1}

and 6, respectively. Fish were fed with a standard pellet at a daily rate of 2% body weight. Fish were routinely examined for the presence of parasites. The microscopic examination of mucus on the gill filaments with a light microscope showed the parasite presence. Monogenean gill parasites were confirmed by their morphology and their sclerotised structures using microscopy (Soylu and Emre, 2007; Dzika et al., 2009). The species of the parasite found on the gills of *C. carpio* was identified as *Dactylogyrus extensus.*

For the *in vitro* and *in vivo* experiments, the samples were collected by scraping mucus from the gills and placing into glass microscope slides with 200 µl wells. Mucus scrapes were examined to count the parasites using microscopy for *in vitro* tests. For *in vivo* tests, the mucus scrapes from the gills surface were weighed using analytical balance with 0.0001 g sensitivity. During the procedure required to obtain the mucosa, fish were kept under light anaesthesia.

Aspirin. Antiparasitic capacity of aspirin against *D. extensus* was tested *in vitro* and *in vivo*. Aspirin tablets (each tablet containing 100 mg acetylsalicylic acid) were obtained from a local pharmacy, Ankara, Turkey. Aspirin tablets were dissolved in distilled water to obtain the concentrations of 2.5, 5, 10 and 50 g/L aspirin.

***In vitro* aspirin test against *D. extensus*.** Individual parasite samples were exposed *in vitro* to solutions of aspirin at the following concentrations:2.5, 5, 10 and 50 g/L. Mucus on the gill filaments of carp infected by *D. extensus* were gently scraped to a glass microscope slide by a micro spatula and submerged in the different concentrations of aspirin. Parasite motility was continually checked and dead/live parasites counted every minute. Parasites showing no reaction to disturbance by a thin needle were considered dead. Parasite samples maintained in water were used as control. The same procedure was also applied to control parasites. Each replicate contained five parasites and experiments were run in duplicates for each concentration of aspirin. Mortality rates were analyzed to assess the median effective concentration (EC50) with Probit analysis.

***In vivo* antiparasitic efficacy tests.** *In vivo* tests for aspirin were carried out with carp infected with *D. extensus*. Aspirin was administered by immersion. In *in vivo* tests, aspirin concentration was selected based on *in vitro* EC50 results and aspirin EC50 (2.08 g/L) for five min. was diluted to 1/100, corresponding to 20 mg/L. In preliminary *in vivo* tests, EC50 value at a concentration of 2.08 g/L for *D. extensus* was not tolerated by carp, therefore in immersion tests, EC50 value for five min. was diluted at a ratio of 1:100.

Ten carps invaded by *D. extensus* on the gill filaments were used for immersion treatments. The carps were randomly divided into two groups of fish. Experimental group (N = 10) were immersed in the aspirin solution (20 mg/L for 5 min). The immersion procedure was carried out in a 20 L glass aquaria containing 10 L of aspirin solution. Fish were treated one by one. The scrapes of mucus samples (approximately 0.001 g) from the gills' surface of carp were collected to assess the number of *D. extensus* on the gills for pre- and posttreatment. The antiparasitic efficacy of the aspirin was calculated as percent reduction of parasites after treatment. Following immersion, five fish were separated for gill histopathology.

Histopathological analysis. Histopathological analysis was done for the gills infected by *D. extensus* (positive control) and aspirin-treated fish gills infected by *D. extensus*. The fish gills not exposed to parasites were evaluated as blank control. Gill tissue samples were fixed in 10% buffered formalin. The fixed tissues were washed in tap water and dehydrated with ascending concentrations of ethanol. After dehydration, tissues were cleared in xylene and embedded to paraffin. Histological sections (five µm) were stained with haematoxylin and eosin (H+E), and examined by light microscopy (Bullock, 1978; Culling, 1974).

Statistics. All data from *in vitro* tests of concentrations were used in calculation of median concentration (EC50). EC50 values at the 95% confidence level (95% CL) were evaluated by Probit (Finney, 1971). Other data were compared using variance analysis ANOVA at a significance value of 5%. All statistical analysis was performed with SPSS26.

RESULTS AND DISCUSSION

In vitro **parasite mortality.** Aspirin EC50 for *D. extensus* was 8.137 g/L at one min. exposure; 6.009 g/L at two min.; 3.941 g/L at three min. and 3.379 g/L at four min., and 2.089 g/L at five min (Tab. 1).

In vitro mortality of *D. extensus* showed a concentration- and exposure time-dependent manner for aspirin $p < 0.05$ ($F_{crit} = 2.66130452$). A 100% cumulative mortality was reached in five min. for a concentration of 5 g/L^{-1} aspirin while for the concentration of 50 g/L the cumulative mortality of 100% was observed in one min. (Tab. 1, Fig. 1)

Table 1: Median effective concentrations (EC50) values of aspirin for *D. extensus*.

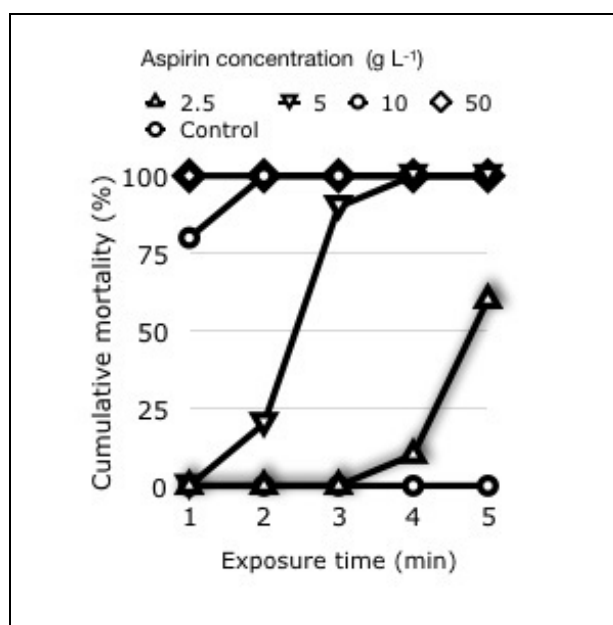| Estimated parameters by Probit	Exposure time (minutes)				
	1	2	3	4	5
EC50 g/L	8.137	6.009	3.941	3.379	2.089
Lower and upper bounds	5.47-10.08	4.89-10.19	2.86-5.25	2.51-4.60	1.62-2.92
Chi-Square	0.21	0.13	1.77	1.50	0.55
P value	0.90	0.93	0.41	0.47	0.75

Figure 1: Cumulative mortality of *D. extensus* (monogenean)
exposed to aspirin solution of different concentrations.

In vivo antiparasitic efficacy. *In vivo D. extensus* found on the gills of carp were significantly decreased by aspirin exposure at a concentration of 20 mg/L for five min. (p < 0.05). The aspirin treatment reduced *D. extensus* on the gills by 46.49%. The mean intensity of *D. extensus* in the mucus of gill (0.001 g) was 45.6 $\pm$ 5.49 before treatment and decreased to 21.2 $\pm$ 2.80 after treatment with aspirin.

Histopathological analysis. In the untreated carp gills, infestation with *D. extensus* was recorded, resulting in: intense hyperplasia in primary and secondary lamellae; occasional edema and necrosis of the secondary lamellae and telangiectasia in secondary lamellae (Fig. 2). In the negative control group, carp without *D. extensus* infection gills tissue showed a normal structure. Treatment with aspirin at 20 mg/L for five min. completely destroyed *D. extensus* in the gills. Severe lesions such as necrosis in secondary lamellae, hyperplasia, and hyperemia in lamellae, intense hyperemia in the blood vessels of the gills, subepithelial edema and intense hyperplasia of the primary lamella ends were obvious in the gills of carp treated with the aspirin solution differently from the gills invaded by *D. extensus*.

In vitro the median effective dose (EC50) of aspirin was 8.137 g/L at one min. for *D. extensus*. The EC50 values of aspirin were time-dependent, decreasing to 2.089 g/L at five min. Data on the EC50 values aspirin for the monogenean parasites of fish do not exist. The data on aspirin EC50 are difficult to compare with other widely used anti-parasitics because of differing time intervals. The maximal period assessed for aspirin EC50 was five min. here as all parasites were dead at six min. in each concentration tested. The incubation time for other widely used anti-parasitics were longer than the maximal period that we detected for EC50 values in this study. For example, Hu et al. (2017) reported that the traditional anthelminthic drug praziquantel EC50 for *D. intermedius* was 2.69 g/L at 24 hours. Formalin EC50 for *D. minutus* at 16 min. was reported as 0.114 mg/mL, indicating rapid effect of aspirin despite higher concentration than formalin (Tancredo et al., 2019). Compared with an aquatic organism, *Daphnia magna* (zooplankton) aspirin LC50 for *D. magna* was found to be 310 mg/L at 48 hours (Bang et al., 2015). Therefore, it can be considered that aspirin affected *D. extensus* at short-term with relatively higher concentrations. *In vitro* the 50 g/L concentration of aspirin had the most rapid efficacy for *D. extensus*, killing all parasites in one min. whereas at the concentration of 2.5 g/L in six min. The effect of aspirin on *D. extensus* was dose-dependent and time-dependent. Similar pattern of time- and concentration dependent was also pointed out for other Monogenean skin parasites (*G. bullatarudis* and *G. turnbulli*) exposed to salt and gill parasite (*D. minutus)* exposed to formalin (Schelkle et al., 2011; Tancredo et al., 2019).

The treatment strategy to remove monogeneans requires first *in vitro* tests (Tavares-Dias and Martins, 2017; Gonzales et al., 2020). These are followed by the *in vivo* experiments. Here, EC50 value determined for five min. *in vitro* tests was applied to *in vivo* tests by diluting at a ratio 1:100. *In vivo* application of EC50 of aspirin for *D. extensus* (as previously assessed *in vitro* EC50 = 2.08 g/L at five min. for *D. extensus*) was found to be 46.49% effective, showing a much higher killing capacity. In the literature, the discrepancy between *in vitro* and *in vivo* results was reported for various antiparasitic applications. Relatively lower efficacy of treatments was attributed to the protective effect of mucus or scales and the location of parasites on the gill tissue in *in vivo* tests (Rintamäki-Kinnunen and Valtonen, 1996; Schelkle et al., 2011; Kumar et al., 2012). *In vivo* aspirin treatment conducted at 1:100 dilution of EC50 eliminated nearly half of *D. extensus* from the gills. Mucus on the gill surface had no protective effect for the parasites in case of aspirin treatment. Important here is that while aspirin treatment was effective in removing parasites, severe destruction of gill tissue occurred.
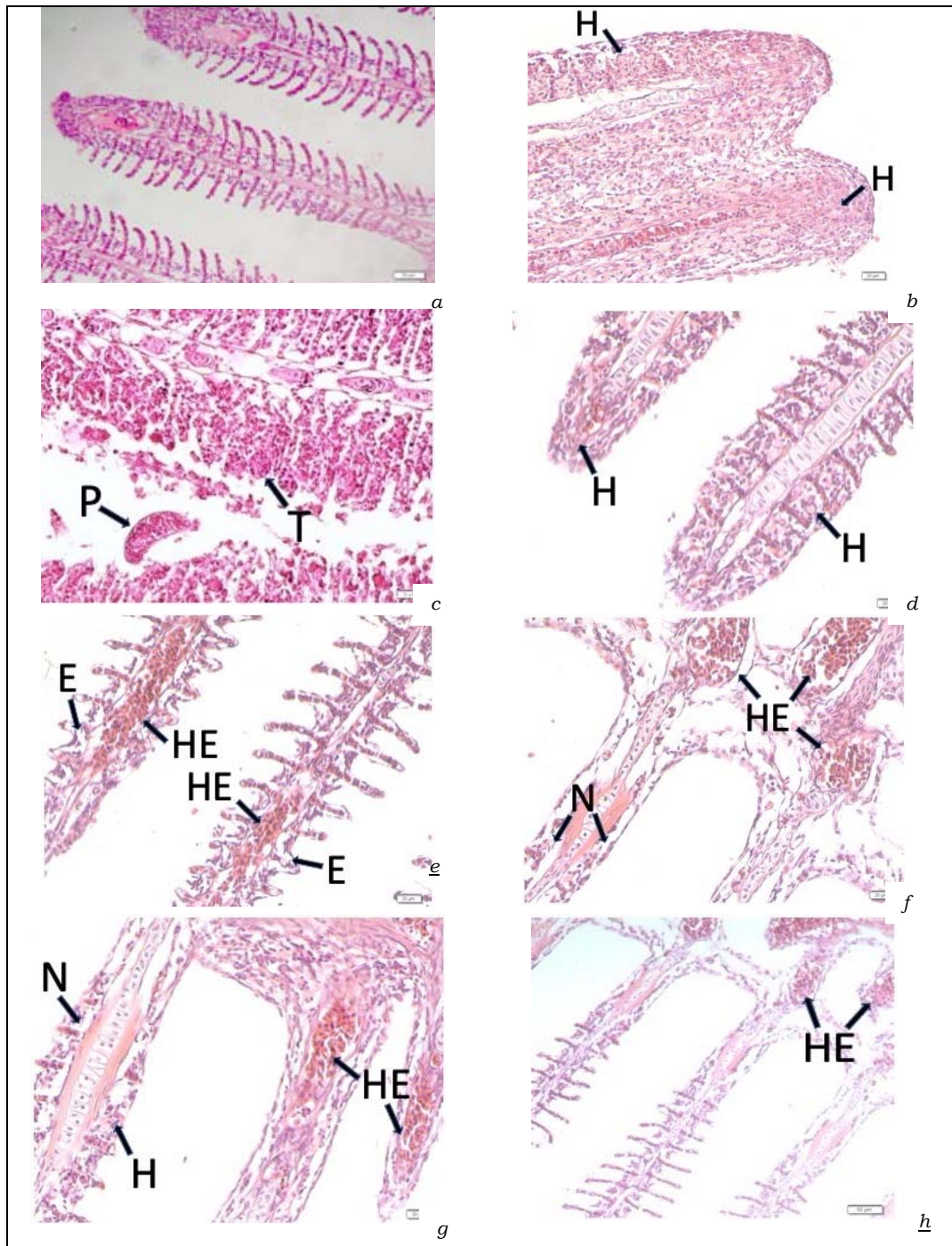
Figure 2. (*a*): Gill structure of the healthy carp (blank control); (*b-c*) Gill structure of carp infected with *D. extensus* (positive control). Dense epithelial cell hyperplasia (H) in primary and secondary lamellae (*b*): Dense epithelial cell hyperplasia (H) in primary and secondary lamellae (*c*): Talengiectasis (T) in secondary lamellae and parasite (P) attached to the lamellae; (*d-h*) Gill structure of aspirin-treated fish. (*d*): Intense hyperplasia (H) of the primary lamella ends and between the second lamella (*e*): Subepithelial edema (E) and hyperemia (HE) in secondary lamellae (*f*): Hyperemia (HE) of blood vessels feeding lamellae and necrosis at the base of the primer and secondary lamellae (*g*): Necrosis (N) in secondary lamellae, hyperplasia (H) and hyperemia (HE) in lamellae (*h*): Intense hyperemia (HE) in the blood vessels of the gills.

Histopathological analysis showed degenerative changes in the gills of aspirin treated fish. The lesion profile involves intense hyperplasia, necrosis, obvious telangiectasia, and edema of some secondary lamellae in gills of carp infected by *D. extensus* was similar to histological alterations reported for carp (*C. carpio*) infected by *Dactylogyrus* genus (Jalali and Barzegar, 2005). The histopathological changes observed in *D. extensus* infected fish in this study are in line with the changes commonly seen in diseased gills expressed by Gjessing et al. (2019) and also similar to the pathology of monogenean infections in fish as studied by Raissy and Ansari (2011) and Santos et al. (2017). The major lesions observed in post-treatment gill samples included intense hyperaemia in blood vessels feeding lamellae and secondary lamella. The other lesions in post treatment gill tissues appear to be specifically associated with the parasite, complying with the study of Strzyżewska-Worotyńska et al. (2017). Nunes et al. (2015) reported that in *Salmo trutta fario* exposed to ASA (100 μg L^{-1} ASA for 28 days), histopathological changes were epithelial lifting, fusion of the secondary lamellae as well as sporadic necrosis, emphasizing non-specific and reversible characters of the lesion. These types of lesions are assumed to form as a barrier to reduce pollutant uptake by minimization of the surface area of the gills without any effect on respiration function (Fernandes and Mazon, 2003). However, in our study, in relation to high concentration of aspirin, post treatment lesions in the gills were degenerative tissue changes, usually irreversible. The appearance of damaged gill tissue can be directly linked to acid properties of aspirin (Nunes et al., 2015). Here the observed gill tissue alterations are considered not reversible. It can be pointed out that osmoregulation capacity of fish can be affected by toxicants in water through altered gill tissue (Bernet et al., 1999). Thus, homeostasis can be challenged by aspirin treatment in relation to disrupted osmoregulation.

CONCLUSIONS

D. extensus is sensitive to aspirin, showing *in vitro* aspirin EC50 of 8.137 g/L at one minute exposure. The mortality of *D. extensus* was distinctly time- and dose-dependent manner *in vitro*, and mortality decreased at lower concentrations of ASA. Antiparasitic efficacy of ASA as applied by exposure of carp at a concentration of 20 mg/L for five minutes can be considered very high. Short term exposure of carp to ASA caused irreversible alterations in gill tissue with severe lesions, making it difficult to recommend as an antiparasitic for gill parasites of fish.

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THE INVASIVE ALIEN SPECIES
IN THE BLACK SEA COAST OF GEORGIA

Madona VARSHANIDZE *, *Marina MGELADZE* *, *Tsiuri GVARISHVILI* *,
Eteri MIKASHAVIDZE *, *Ramaz MIKELADZE* * and *Paata VADACHKORIA* *

* National Environmental Agency, Fisheries, Aquaculture and Water Biodiversity Department, Batumi, 53 Rustaveli Street, Georgia, GE-6000, ORCID: 0000-0001-9592-9098, varshanidzema@gmail.com; ORCID:0000-0001-8174-3188, email:mari.mgeladze@gmail.com; ORCID:0000-0002-5615-4362, email tsiuri.gvarishvili@gmail.com; ORCID: 0000-0001-5631-7902, eteri.mikashavidze@gmail.com; ORCID: ORCID:0000-0003-2971-5811, rmikeladze@gmail.com; ORCID: 0000-0003-4206-0602, vadach@gmail.com.

KEYWORDS: Black Sea coast of Georgia**,** alien species, mezozooplankton, phytoplankton, gelatinous species, fish, bibliometric.

ABSTRACT

The aim of the present paper is to compile a list of the invasive alien species on the Black Sea coast of Georgia. The results have been received from an assessment of data collected from different sources. According to recent data, the greatest number of species (36%) has arrived into the Black Sea from the Mediterranean as a consequence of expanding the natural range or coming through the ballast waters. This proportion has increased with the rising water temperatures of the upper layer. Only a few of these species have become abundant and almost none have reached other seas of the Ponto-Caspian. Among these species are the representatives of micro-, phyto- and zooplankton, demersal plankton, macrophytes, zoobenthos and fish.

RÉSUMÉ: Les espèces exotiques envahissantes de la côte de la mer Noire en Géorgie.

Le but du présent document est de compiler une liste des espèces exotiques envahissantes sur la côte de la mer Noire en Géorgie. Les résultats proviennent d'une évaluation des données agrégées à partir d'un réseau de différentes sources. Selon des données récentes, le plus grand nombre d'espèces (36%) est arrivé dans la mer Noire depuis la Méditerranée à la suite de l'expansion naturelle de l'aire de répartition ou en passant par les eaux de ballast. Cette proportion ne cesse d'augmenter avec la hausse des températures de l'eau de la couche supérieure. Seules quelques-unes de ces espèces sont devenues abondantes et presque aucune n'a atteint les autres mers du Ponto-Caspien. Parmi ces espèces figurent les représentants du micro-, du phyto et du zooplancton, du plancton démersal, des macrophytes, du zoobenthos et des poissons.

REZUMAT: Specii alohtone invazive de pe coasta Mării Negre a Georgiei.

Scopul prezentei lucrări este de a compila o listă a speciilor exotice invazive de pe coasta Mării Negre din Georgia. Rezultatele provin dintr-o evaluare a datelor obţinute dintr-o reţea de surse diferite. Conform datelor recente, cel mai mare număr de specii (36%) a ajuns în Marea Neagră dinspre Mediterană ca urmare a extinderii ariei naturale sau a aducerii lor în apele de balast. Această proporţie continuă să crească odată cu creșterea temperaturii apei din stratul superior al mării. Doar câteva dintre aceste specii au devenit abundente și aproape niciuna nu a ajuns în alte mări Ponto-Caspice. Printre aceste specii se numără reprezentanţii micro, fito și zooplanctonului, planctonului demersal, a macrofitelor, zoobentosului și peștilor.

INTRODUCTION

Invasive alien species (IAS) are one of the great problems of the modern times (Curtean-Bănăduc and Bănăduc, 2008; Skolka and Preda, 2010; Yankova et al., 2013; European Comission, 2016; Anastasiu et al., 2017; Prots and Simpson, 2011; Kohsnud et al., 2013; Popova and Zlatanova, 2017; Radkhah and Eagderi, 2020; Hiz and Aki, 2014; Ghasemi et al., 2014). The increasing number of alien species in the Black Sea since 2000 seems to be related to the climate change as it presumably is connected with a parallel increase in water temperature (Shalovenkov, 2020).

Reliable scientific information and data about the changes in the distribution and abundance of alien species in the Mediterranean and the Black Sea are essential for understanding their ecological and economic impacts, and influence on human health (Öztürk, 2021).

Many alien species are transported daily, around the world (Carlton et al., 1996). The number of alien species in the Black Sea has continually increased (Boltachev and Karpova, 2014). Zaitsev and Mamaev (1997) described 26 alien species from the Black Sea. Subsequently, Zaitsev and Öztürk (2001) recorded that 59 species of alien marine organisms live in the Black Sea, and yet only a few species, such as the sea snail *Rapana venosa* and the comb jelly *Mnemiopsis leidye*, have been well studied in terms of their impact on fisheries (Öztürk, 2021). Other surveys indicated the presence of 156 alien species in the Black Sea, with most of these species coming from the Mediterranean (Shiganova and Öztürk, 2009). The Advisory Group on Conservation of Biological Diversity at the Commission for the Protection of the Black Sea Against Pollution (BSC) has monitored for more than 10 years the appearance of new species in the sea and created a register of alien organisms, which includes 365 marine, brackish, and freshwater species from fungi and unicellular algae to mammals (Aleksandrov et al., 2013). More recently, the intensity of invasion has significantly increased. Especially, since the second half of the twentieth century, a huge number of marine and brackish-water species has been transported daily by ships to the areas of its ports (Shiganova, 2010; Shiganova et al., 2021). According to preliminary estimates, the rate of introduction was about five IAS per year, and registration of at least 85 alien species was predicted by 2020 (Aleksandrov, 2010; Aleksandrov and Shiganova 2018).

Special studies on IAS in the Black Sea region of Georgia have not been conducted so far. The purpose of this article is to compile a list of invasive alien species of the Black Sea coast of Georgia based on the assessment of data collected from various sources.

MATERIAL AND METHODS

The list of alien species occurring over the past few decades on the Black Sea coast of Georgia has been compiled based on a study of original and published data. In total, more than 30 published sources and unpublished data of Georgian experts were reviewed. The taxonomy of marine species is presented according to the World Register of Marine Species (WoRMS).

RESULTS AND DISCUSSION

Over the last few decades, a total of 43 alien species have been recorded in the Black Sea Georgian coast area including phytoplankton (21 species, 49%), zooplankton (two mesozooplankton and two gelatinous/ctenophora species), macrozoobenthos (nine species) and fish (nine species) (Fig. 1, Tabs. 1-4).

Overall, this work provides a comprehensive multidimensional (scientific and bibliometric) view of the invasion of the southeastern Black Sea coast.
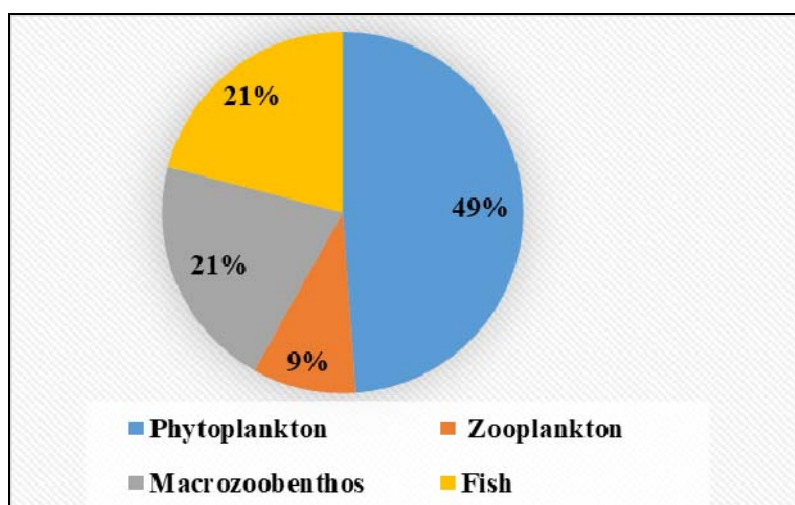
Figure 1: The percentage ratio oft he number of alien species
of different taxonomic groups
on the Black Sea Georgian coast.

The invasive alien phytoplankton species are represented by diatoms and dinophytes in equal numbers. Some of them are quite rare, but many have become quite common species in the Black Sea. The species of the genera *Chaetoceros*, *Gymnodinium*, *Prorocentrum*, *Rhizosolenia*, and *Thalassiosira* are quite common on the Georgian coast (Tab. 1a, b).

Table 1a: Non-native species of phytoplankton in the Black Sea Georgian coast.

	Class	Species
1.	Bacillariophyceae	*Cerataulina bergonii* Ostenfeld, 1903 *Cerataulina pelagica* (Cleve) (Hendey, 1937)
2.		*Chaetoceros danicus* Cleve, 1889
3.		*Chaetoceros diversus* Cleve 1873
4.		*Chaetoceros socialis* H. S. Lauder, 1864
5.		*Lauderia pumila* Castracane, 1886 (*Detonula pumila* Gran, 1900)
6.		*Thalassiothrix mediterranea* var. Pacifica Cupp, 1943 (*Lioloma pacificum* (Cupp) Hasle, 1996)
7.		*Rhizosolenia calcar-avis* (Schultze 1858) B. G. Sundström, 1986 (*Pseudosolenia calcar-avis* (Schultze) Sundström, 1986)
8.		*Rhizosolenia setigera* Brightwell, 1858
9.		*Thalassiosira nordenskioeldii* Cleve, 1873
10.		*Leptocylindrus danicus* Cleve, 1889

Table 1b: Non-native species of phytoplankton in the Black Sea Georgian coast.

	Class	Species
11.	Dinophyceae	*Akashiwo sanguinea* (K. Hirasaka) G. Hansen and Moestrup, 2000 (*Gymnodinium sangineum* Hirasaka, 1924)
12.		*Archaeperidinium minutum* (Kofoid) Jørgensen, 1912 (*Protoperidinium minutum* (Kofoid) Loeblich III, 1970)
13.		*Polykrikos geminatus* (Schütt) D. Qiu and S. Lin, 2013 (*Cochlodinium geminatum* (Schütt) Schütt, 1896)
14.		*Gymnodinium radiatum* Kofoid and Swezy, 1921
15.		*Gymnodinium stellatum* Hul-burt, 1957
16.		*Gymnodinium uberrimum* (G. J. Allman) Kofoid and Swezy, 1921
17.		*Octactis speculum* (Ehrenberg) F. H. Chang, J. M. Grieve and J. E. Sutherland, 2017 (*Distephanus speculum* f. *octonarius* (Ehrenberg) S. Locker and E. Martini)
18.		*Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1976
19.		*Prorocentrum dentatum* Stein, 1883
20.		*Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S. Soehner, Kirsch, Kusber and Gottschling, 2015 (Scrippsiella trochoidea (Stein)
21.	Cryptomonadales	Hillea fusiformis (J. Schiller) J. Schiller, 1925

The presented alien species of zooplankton are distributed throughout the Black Sea. On the Georgian Black Sea coast, they are quite common depending on the season. The thermophilic species of the edible zooplankton *Acartia tonsa* and *Oithona davisae* are dominant in the coastal waters of Georgia in the summer-autumn periods (Kharytonova et al., 2021).

Gelatinous plankton plays an important role in the functioning of the Black Sea marine ecosystems, however in cases of excessive proliferation, this role is negative. Since the 1980s the two invasive alien ctenophore species have invaded the Black Sea. *Mnemiopsis leidyi*, which originates from the Atlantic coast of North and South America, was introduced in 1982 and since then has greatly affected the Black Sea ecosystems (Vinogradov et al., 1989). The invasion by *M. leidyi*, was the most pronounced event that caused cascading effects at higher trophic levels, from a decrease in zooplankton to the collapse of the planktivorous fish and drastic declines among the large pelagic fish and dolphins. Similar effects occurred at lower trophic levels, with a decrease in zooplankton leading to an increase in phytoplankton released from zooplankton grazing pressure, and increases in bacterioplankton, which led to increases in their predators, zooflagellates, and ciliates (Shiganova et al., 2004, 2019, 2021). In 1992, a second ctenophore species, originating from the South Atlantic Ocean, *Beroe ovata* was reported (Konsulov and Kamburska, 1998). Both species have been released with ballast waters into the Black Sea (Ghabooli et al., 2011). The *B. ovata* invasion reduced *M. leidyi* populations and the ecosystem began to recover at all trophic levels (Shiganova et al., 2014, 2018).

Table 2: Invasive alien species of phytoplankton in the Black Sea Georgian coast.

	Class/Order	Species
Mesozooplankton		
1.	Hexanauplia/Calanoida	*Acartia (Acanthacartia) tonsa* Dana, 1849 (*Acartia tonsa* Dana, 1849)
2.	Hexanauplia/Cyclopoida	*Oithona davisae* (Ferrari F. D. and Orsi, 1984)
Macrozooplankton		
3.	Tentaculata/Lobata	*Mnemiopsis leidyi* (Agassiz, 1865)
4.	Nuda/Beroida	*Beroe ovata* Bruguière, 1789

Although, the invasive alien species belong to all the taxon and ecological groups, the benthic IAS prevail in the European seas (Streftaris et al., 2005). In 1978-1979 the bivalve mollusk *A. nadara kagoshimensis* which is native to the west Pacific Ocean was recorded for the first time in Georgia, in the benthos of the Chorokhi River mouth, at depths from five to 20 m. At first, some individuals with sizes of 1-2.5 cm were detected, while afterwards bigger individuals (6-8 cm) were found. These bivalves were particularly abundant at Anaklia Town, where the mussel collectors were installed. Currently, this mussel has been widely distributed in the benthos of the Black Sea of Georgia. According to some authors, the reason of its high proliferation is connected to its massive shell and hermetic hooking, which helps the mussel to survive during oxygen deficiency in water (Gogmachadze and Mikashavidze, 2005; Vadachkoria et al., 2020).

In 1929, the polychaeta *Mercierella enigmatica* (*Ficopomatus enigmaticus*) was first recorded from the brackish Paleostomi Lake in Georgia (Annenkova, 1929). Later, the species was found in Gelendzhik Bay near Novorossisk. It is believed that *Mercierella* (*Ficopomatus*) originates in the brackish coastal lakes of India (Micu and Micu, 2004). Until the mid-sixties, it became established in several other brackish and oligohaline locations along the Black Sea coasts (Zaitsev and Öztür, 2001).

Vodyanitsky and Morozova-Vodyanitskaya in the autumn of 1949 discovered a large number of rapana (*Rapana venosa*) on the Gudauta oyster bank, where, according to their observations, it produced huge devastation. For the first time, the rapana was discovered by the VNIRO expedition in the Anapa region of Tuapse, Sochi, Gudauta. At that time there were known cases of finding a rapana from Batumi to the Kerch Strait (Drapkin, 1953).

Table 3: Alien species of macrozoobentos in the Black Sea Georgian coast.

	Phylum	Species
Macrozoobentos		
1.	Polychaeta	*Dipolydora quadrilobata* (Jacobi, 1883)
2.		*Sigambra tentaculata* (Treadwell, 1941)
3.		*Ficopomatus enigmaticus* (Fauvel, 1923)
4.	Mollusca	*Mya arenaria* Linnaeus, 1758
5.		*Anadara kagoshimensis* (Tokunaga, 1906)
6.		*Rapana venosa* (Valenciennes, 1846)
7.	Arthropoda	*Amphibalanus improvisus* (Darwin, 1854)
8.		*Amphibalanus eburneus* (Gould, 1841)
9.		*Callinectes sapidus* (Rathbun, 1896)

According to the recent revisions, the Black Sea ichthyofauna consists of about 190 species and its diversity increases primarily due to the penetration of fish from the Mediterranean Sea (Yankova et al., 2013, 2014; Boltachev and Karpova 2014). There are nine invasive alien fish species in the Georgian waters of the Black Sea. Some authors described the presence of such species as *Sardinella aurita*, *Sarpa salpa*, and *Sparus aurata* at the beginning of the last century (Mayorova, 1951; Svetovidov, 1964). These three species are Mediterranean immigrants which are not considered alien for the Black Sea – it is considered that they extend their range by natural distribution (*). For the last decade, two species of the family Sparidae, salema *Sarpa salpa* and seabream *Sparus aurata*, have increased considerably their abundance along the Georgian and Ukrainean Crimean coasts and passed from the category "rarely found species" to the category "usual species" (Guchmanidze and Boltachev, 2017).

Liza haematocheila is one of the main invaders established, which have the most dramatic impact on species diversity (Erdogan et al., 2010). The species was intentionally introduced into the Azov Sea and Black Sea for aquaculture purposes from the Amur River estuary to the Sea of Japan in 1972-1982 (Ivanov et al., 2017). It is quite common in Georgian waters and of commercial importance.

The first finding of *Parablennius incognitus* (Bath, 1968) in the Black Sea, according to Bogorodskii (2006), was made off the coast of Abkhazia in 2001. This species was also found off the coasts of Turkey and in the Kerch Strait (Vasil'eva, 2007; Yankova et al., 2013).

In recent years, a new species for the ichthyofauna of Georgia, *Lithognathus mormyrus*, has been described (Guchmanidze and Boltachev, 2017). *Lithognathus mormyrus* is widespread and regularly encountered in the Mediterranean Sea, in the eastern Atlantic Ocean from the Bay of Biscay to the Cape of Good Hope, in the south-west part of the Indian Ocean to the south of Mozambique, and in the Red Sea (Marine Species Identification Portal, 2015). In the Black Sea, a specimen of this species was found on the Romanian coast (Stanciu and Ilie, 1980), on the Bulgarian coast in the Gulf of Varna (Vasil'eva, 2007).

Table 4: Alien fish species on in the Black Sea Georgian coast.

	Order	Species
1.		*Gobius xanthocephalus* Heymer and Zander, 1992
2.	Gobiiformes	*Lithognathus mormyrus* (Linnaeus, 1758)
3.		*Morone saxatilis* (Walbaum, 1792)
4.	Blenniiformes	*Parablennius incognitus* (Bath, 1968)
5.	Mugiliformes	*Planiliza haematocheilus* (Temminck and Schlegel, 1845) *(Liza haematocheila* Temmnick et Schlegel, 1845 = *Mugil soiuy* (Basilewski, 1855)
6.	Gobiiformes	*Pomatoschistus bathi* Miller, 1982
7.		*Pomatoschistus bathi* Miller, 1982
8.	Clupeiformes	*Sarpa salpa* (Linnaeus, 1758)
9.		*Sparus aurata* Linnaeus, 1758

CONCLUSIONS

The list of alien hidrobionts recorded on the Black Sea coast of Georgia until now includes 43 species. The presented information ensures support to future development of prevention and control measures to tackle the issues of the invasive alien species in the region.

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NUTRIENT COMPOSITION AND ORGANOLEPTIC ASSESSMENT OF FISH KILISHI FROM *CLARIAS GLARIEPINUS* AND *MORMYRUS RUME*

Abubakar IBRAHIM * and *Godwin ADAKA* **

* Federal University of Technology, Department of Fisheries and Aquaculture, Nigeria, PBM 1526 Owerri, ibrahimbbkrsdq@gmail.com, revadaka@gmail.com, ORCID: 0000-0001-6584-8289.

KEYWORDS: fish meat, microbial, ingredients, processing, spoilage.

ABSTRACT

Fish samples from fresh fish, cleaned/washed fish; first stage dried, infusion slurry, and second stage dried, finished product and stored kilishi were analysed. Evaluation was carried out on finished products using a 10-point Hedonic scale. Microbial count and moisture content showed significant differences ($\rho < 0.01$). Proximate composition of sun and oven dried kilishi (T_1) record significantly of higher protein (34.13 ± 0.01), higher ether extract (29.45 ± 0.01), higher nitrogen free extract (17.45 ± 0.35), and have moisture content of (10.92 ± 0.29) despite having highest ash content (5.76 ± 0.69) with crude fibre (2.29 ± 0.23). Kilishi (T_2) significantly higher protein (27.13 ± 0.01), higher nitrogen free extract (37.18 ± 0.64), higher ether extract (19.45 ± 0.58), higher ash content (6.09 ± 0.06), despite having higher crude fibre (1.77 ± 0.06) with moisture content of (8.45 ± 0.52); the high proportion of departed groundnut dough mixture resulted in low protein of fish kilishi.

RÉSUMÉ: Composition en nutriments et évaluation organoleptique de kilishi de poisson à partir de *Clarias garepinus* et *Mormyrus rume*.

Des échantillons de poisson frais, de poisson nettoyé/lavé, de poisson séché au premier stade, de boue d'infusion, et de kilishi séché au deuxième stade, de produit fini et stocké ont été analysés. L'évaluation a été réalisée sur les produits finis à l'aide d'une échelle de Hedonic en 10 points. La numération microbienne et la teneur en humidité ont montré une différence significative ($\rho < 0,01$). La composition proximale du kilishi séché au soleil et au four (T_1) présente une teneur en protéines plus élevée ($34,13 \pm 0,01$), un extrait d'éther plus élevé ($29,45 \pm 0,01$), un extrait libre d'azote plus élevé ($17,45 \pm 0,35$) et une teneur en humidité de ($10,92 \pm 0,29$) malgré une teneur en cendres plus élevée ($5,76 \pm 0,69$) et des fibres brutes ($2,29 \pm 0,23$). Kilishi (T_2) significativement plus élevé en protéines ($27,13 \pm 0,01$), plus élevées en extrait libre d'azote ($37,18 \pm 0,64$), plus élevé en extrait d'éther ($19,45+0,58$), plus élevé en cendres ($6,09+0,06$), malgré une teneur plus élevée en fibres brutes ($1,77 \pm 0,06$) et une teneur en humidité de ($8,45 \pm 0,52$); la proportion élevée de mélange de pâte d'arachide perdue a entraîné une faible teneur en protéines du kilishi de poisson.

REZUMAT: Compoziția nutrienților și evaluarea organoleptică a peștilor kilishi din *Clarias gariepinus* și *Mormyrus rume*.

Probe de pește proaspăt, curățat/spălat; au fost analizate în prima etapă suspensia uscată de pastă de infuzie și în a doua etapă produsul uscat, finit și kilishi-ul depozitat. Evaluarea a fost efectuată pe produse finite utilizând o scală de 10 puncte. Numărul de microbi și conținutul de umiditate au arătat o diferență ($\rho < 0.01$). Compoziția proximă a kilishi-ului uscat la soare și la cuptor (T_1) înregistrează semnificativ proteine mai mari (34.13 ± 0.01), extract de eter mai mare (29.45 ± 0.01), extract fără azot mai mare (17.45 ± 0.35) și are un conținut de umiditate de (10.92 ± 0.29), în ciuda faptului că are cel mai mare conținut de cenușă (5.76 ± 0.69) cu fibră brută (2.29 ± 0.23). Kilishi (T_2) proteine semnificativ mai mari (27.13 ± 0.01), extract mai mare fără azot (37.18 ± 0.64), extract de eter mai mare (19.45 ± 0.58), conținut mai mare de cenușă (6.09 ± 0.06), în ciuda faptului că are fibre brute mai mari (1.77 ± 0.06) cu un conținut de umiditate de (8.45 ± 0.52); proporția ridicată a amestecului de aluat de arahide a dus la o proteină scăzută de kilishi.

INTRODUCTION

Fish consumption provides health benefits to the human population. There is strong evidence that fish, in particular oily fish, lowers the risk of coronary heart disease (CHD) mortality by up to 36 percent due to a combination of EPA and DHA (FAO/WHO 2011). In many low-income countries with water and fisheries resources, fish is important for livelihoods, income and as food for the rural poor people who suffer disproportionately from undernutrition, including micronutrient deficiencies (Thompson and Subasinghe, 2011). Fish is by far the most frequently consumed animal-source and thereby makes a valuable contribution to the diversity of everyday diets, dominated by carbohydrate-rich staples. Fish can therefore be considered an irreplaceable animal-source, providing essential nutrients of high bioavailability which are found in limiting amounts in the diet. These nutrients include animal protein, essential fats, minerals, and vitamins. Small fish, eaten whole or as fish products, e.g. dried fish are particularly rich in calcium and other micronutrients (FAO 2014).

Fish is an extremely perishable food with deterioration commencing immediately after harvest (Agbo et al., 2002). Prior to death, the enzymes normally get involved in the digestion of ingested food and all microbial activities are controlled. As soon as fish dies, enzymatic action begins on the flesh and alimentary system leading to soft destructive changes. The process is referred to as autolysis. Post-harvest losses could be minimised by the application of proper preservation and processing methods (Eyo, 1997). Fish is liable to accelerated physiological, chemical, and microbial processes after harvests that invariably lead to deterioration and loss of wholesomeness. It is therefore necessary to come up with some measure of processing such as reduction in moisture content, denaturation of endogenous enzymes and microorganisms or packaging in order to curtail perishability (Eyo, 1997). Processing methods include raising the temperature by canning, boiling, and removal of moisture by natural drying, mechanical drying, smoking and salting, and fish products development such as fish sauces and pastes, marinades (Clucas and Ward, 1996) and of recent fish kilishi (Magawata and Oyelese, 1998).

Kilishi is a Hausa word which refers to slicing, dressing, sun-drying, application of slurry of spices, and roasting. Kilishi is a sliced heat-dried seasoned roasted ready-to-eat meat product known to have originated from the Hausa/Fulani speaking people of Northern Nigeria (Alonge and Hiko, 1981; Igene and Mohammed, 1983; Odusole and Akinyanju, 2003; Olusola, 2006; Okonko et al., 2013). Kilishi production involves dressing, slicing, air drying, application of slurry of ingredients and roasting over glowing coals (Igene et al., 1990) and which was first applied to processing and preservation of fish by Magawata and Oyelese (1998).

Kilishi is a nutrient-dense food that possesses good characteristics for industrial exploitation, contains over 50% protein, 7.5% moisture, 18% lipid, 9.8% ash, and is also very stable to microbial and chemical deterioration (Igene et al., 1990; Igene, 2012; Negbenebor et al., 1990).

The African catfish (*Clarias gariepinus*) is the most sought after species among fish farmers and consumers because it commands good commercial value not only in Nigeria but all over Africa (de Graaf and Janssen, 1996). It can tolerate a large variety of feedstuffs and is very resistant to changing and suboptimal water conditions. It can be farmed in high densities reaching production levels of six to 16 MT/ha on an annual basis when raised in monocultures and fed high quality fish feed (Faturoti, 1989). *Clarias gariepinus* was chosen for kilishi preparation because of its availability locally and higher flesh to bone ratio (Achionye-Nzeh and Omoniyi, 2002). It is commonly known as catfish and a valuable fish in Nigeria, which belongs to the family Clariidae.

Mormyrids or elephant snout fishes are curious looking fish, highly variable in shapes of their head and the extent of their unpaired fins. *Mormyrus rume* belong to the family Mormyridae and are found in freshwaters of tropical Africa (Greenwood et al., 1966).

Little has been documented on fish kilishi: Magawata and Oyelese (2000), Ipinjolu et al. (2004), and Aliyu and Falusi (2006). However Magawata and Oyelese (2000) and Ipinjolu et al. (2004) suggested further work on the appropriate mix of spices being used for production of fish kilishi in order to enhance quality. In view of this and in consideration for value addition, product diversification and reduction of post-harvest fish losses, an evaluation of kilishi processed from *Clarias gariepinus* and *Mormyrus* spp. was conducted.

MATERIAL AND METHODS

The experiment was carried out at the Fish processing unit of the Experimental Farm of the Department of Aquaculture and Fisheries Management of the Faculty of Agriculture, Nasarawa State University Keffi, Shabu-Lafia Campus. Lafia is located on latitude 8°35'N, longitude 8°32'E, altitude 181.53 m above sea level with a mean temperature of 34°C, relative humidity of 40-86% and average day light of 9-12h (NIMET, 2011).

Clarias gariepinus and *Mormyrus rume* were purchased from fish sellers in Lafia fish market and were transported to the experimental location. The ingredients/spices used were the defatted groundnut dough, table salt (sodium chloride), Maggi seasoning and curry powder while spices included dried hot (chilli) pepper (*C. apsicum frutescens*), cloves (*Eugenia caryophyllata*), candle wood (*Fagara zanthoxyloides*), ginger (*Zingiber offinale*), and onion (*Allium cepa*).

The fish were divided into two samples based on their species and were prepared separately using the same spices, ingredients, and quantity. Sample 1 was Kilishi made from catfish while sample 2 was Kilishi made from *Mormyrus rume*.

Slurry was prepared using the same formulation and quantity. The formulations was thick due to high proportion of the principal ingredients (defatted groundnut) used. There was no variation in the spices used. The fish samples were washed thoroughly with saline solution and the scales, bones, fins, heads, and tail removed using dissecting set. This enhanced easy filleting of the fish.

The samples were exposed to air for 10 minutes so as to reduce the moisture content and the freshly prepared fillets were introduced to sun drying raft to reduce its moisture content by evaporation for one hour. The dried fillets were infused in the prepared formulated slurry containing spices and ingredients thoroughly for 10 minutes to ensure absorption of the slurry.

The infused fillet samples were mounted again in a sun drying raft for 21 hours to undergo second drying. This was to ensure a permanent absorption of the slurry on the fillet.

After drying, the dried fillets were roasted for seven minutes in the departmental smoking kiln (to ensure blending of ingredients into the dried fish fillets and to enhance taste of the finished products). The product obtained after roasting is the final prepared kilishi of the samples.

The fish products were packaged in separate clean air tight containers and were labeled according to their respective samples and the fish kilishi was stored at room temperature for the experimental period. The proximate composition of each of the samples were analyzed according to the Association of Official Analytical Chemists (AOAC, 2000).

Samples from the prepared kilishi products were subjected to sensory evaluation immediately to obtain data for the selection of the best out of the two fish species used. The test utilizes the sense of texture, aroma, appearance, taste, and general acceptability for quality assessment of dried kilishi fish. A 40 member's panel was constituted which consisted of staff and students of the faculty. A 10-point hedonic scale ranging from A (Excellent) = 10; B (Good) = 8; C (Fair) = 6; D (Unsatisfactory) = 4, and E (Unacceptable) = 2 was adopted for each quality parameter. Pencils, tissue paper, and water were provided to the judges. Data obtained was subjected to One-way Analysis of Variance (ANOVA) in a completely randomized design using SPSS statistical package. Significant mean differences were separated at 5% significance level.

RESULTS AND DISSCUSION

The results of mean organoleptic qualities of kilishi made from *Clarias gariepinus* and *Mormyrus rume* is presented in table 1. Kilishi made from *Clarias gariepinus* had the highest mean values of 9.50 ± 0.89, 9.10 ± 1.71, 9.90 ± 1.71 and 10.00 ± 0.00 in texture, aroma, taste and general acceptability of the product, while appearance of kilishi made from *Clarias gariepinus* and *Mormyrus rume* had mean values of 9.50 ± 0.89 each. There was difference ($p < 0.05$) in texture, aroma, taste, and general acceptability of the two fish products.

Table 1: Organoleptic of kilishi made from *Clarias gariepinus* and *Mormyrus rume*; [a-b]Means with different superscript along the column differ significantly at $P \leq 0.05$.

Species	Organoleptic parameters				
	Texture	Aroma	Appearance	Taste	General acceptability
Clarias gariepinus	9.50 ± 0.89^a	9.10 ± 1.71^a	9.50 ± 0.89^a	9.90 ± 1.71^a	10.00 ± 0.00^a
Mormyrus rume	8.00 ± 1.45^b	7.80 ± 1.44^b	9.50 ± 0.89^a	9.00 ± 1.03^b	9.40 ± 0.94^a

The results of proximate composition of kilishi made from *Clarias gariepinus* and *Mormyrus rume* is presented in table 2. Kilishi made from *C. gariepinus* had the highest values of 34.13 ± 0.01, 2.29 ± 0.23, 29.45 ± 1.01, and 10.92 ± 0.29 respectively in crude protein, crude fibre, ether extract, and moisture content of the fish product while *Mormyrus rume* had the highest mean values of 6.09 ± 0.01 and 37.11 ± 0.64 in ash and nitrogen free extract of the fish product. From the result, it shows that there was significant difference ($p < 0.05$) in all the nutrient contents assessed.

Table 2: Nutrient composition of kilishi from *Clarias gariepinus* and *Mormyrus rume*; [a-b]Means with different superscript along the column differ significantly at $P \leq 0.05$.

Species	Nutrient composition (%)					
	Crude protein	Crude fibre	Ash	Esther extract	M. content	Nitrogen free extract
Clarias gariepinus	34.13 ± 0.01^a	2.29 ± 0.23^a	5.76 ± 0.69^b	29.45 ± 1.01^a	10.92 ± 0.29^a	17.45 ± 0.35^b
Mormyrus rume	27.13 ± 1.01^b	1.77 ± 0.06^b	6.09 ± 0.01^a	19.45 ± 0.58^b	8.45 ± 0.52^b	37.11 ± 0.64^a

The result of the sensory scores of kilishi prepared from *Clarias gariepinus* and *Mormyrus rume* presented in table 1 above revealed that kilishi made from *Clarias gariepinus* was preferred most in terms of texture, aroma, taste, and general acceptability of the product with mean scores of 9.50 ± 0.89, 9.10 ± 1.71, 9.90 ± 1.71, and 10.00 ± 0.00 respectively. The reason for the preferredness of catfish over *Mormyrus* could be because of its flavour, juiciness and tenderness as reported by Safari et al. (2001). The species of the fish had been influenced by the panellist's decision. According to Moloney (1999), meat texture is an important component of meat tenderness and palatability and it has two major components – the first is the impression of wetness produced by the release of fluids from the meat during the first few chews, while the second is the more sustained juiciness that apparently results from the stimulating effect of fat on the production of saliva and the coating of fat that builds up on the tongue, teeth, and other parts of the mouth. Aroma is a major determinant of consumer acceptability of meat product at the retail counter; therefore one of the most important considerations in meat product merchandising is that of maintaining an optimum aroma or appearance (Hedrick et al., 1994). The sensory rating for aroma in this study was from 7.80 to 9.10. Badau et al. (1997) reported no significant difference for aroma and texture when a standardized kilishi mix powder was compared to traditional kilishi slurry. Igene et al. (1990) on the other hand reported significant differences when aroma and texture were evaluated for, in two prepared products in comparison to a commercial product. The result of this study is in line with the findings of Igene et al. (1990).

The texture of meat can be defined as the sensory manifestation of the structure of meat and the manner in which this structure reacts to the force applied during biting and the specific senses involved in eating (Moloney, 1999). It is hoe meat is felt in the mouth during mastication. Several factors are interwoven which cannot be separated when discussing flavour. It is suspected that many constituents of the tissue become flavour compounds upon being heated. Physiologically, the perception of flavour involves the detection of four basic sensations (including saltiness, sweetness, sourness, and bitterness) by the nerve endings on the surface of the tongue (Hedrick et al., 1994). The result obtained supports the observation of Melton (1990) that as the fat of meat increases so does flavour. The diet that an animal consumes can cause changes in the fatty acid composition and volatile substances in muscle. The sun and oven drying method prevents the kilishi from contamination with dust, flies, egg deposits, which affect the quality of fish kilishi during processing and drying. Therefore, the overall result of mean analysis conducted indicated that kilishi prepared with slurry of treatment one (T_1) and treatment two (T_2) formulation was organoleptically more acceptable. The value addition had transformed the species product and improved its acceptance by consumers who could have rejected it if it were to be presented fresh.

The result on weight loss shows that; the initial weight of fresh fillets has no significance difference ($P > 0.05$) in all the treatments. The result also revealed that treatment two (T_2) appeared relatively low weight, which has more moisture loss than those of treatment one (T_1).

The result of proximate composition of sun and oven dried kilishi prepared with different slurry formulation, kilishi of T_1 formulation dried with sun and oven tent record significantly of higher protein (34.13 ± 0.01), higher ether extract (29.45 ± 0.01), higher nitrogen free extract (17.45 ± 0.35), and have moisture content of (10.92 ± 0.29) despite having highest ash content (5.76 ± 0.69) with crude fibre (2.29 ± 0.23) kilishi of T_2 formulation dried with sun and oven tent record significantly higher protein (27.13 ± 0.01),

higher nitrogen free extract (37.18 ± 0.64), higher ether extract (19.45 ± 0.58), higher ash content (6.09 ± 0.06), despite having higher crude fibre (1.77 ± 0.06) with moisture content of (8.45 ± 0.52); the high proportion of departed groundnut dough mixture resulted in low protein of fish kilishi.

Catfish kilishi recorded the highest crude protein mean of 34.13 ± 0.01 percent; this differed significantly from the mean protein percent of the *Mormyrus rume* 27.13 ± 1.01 (Tab. 2). This indicates that processing fish as in kilishi production improves the percent protein of the product thus making it nutrient dense. Igene et al. (1990) reported a value of 50.02% crude protein for traditional kilishi after roasting. Bello and Tekwata (2015) also reported crude protein between 50.76 and 63.3 for solar tent and sun dried kilishi from bony-tongue (*Heterotis niloticus*). The major part of the protein comes from the groundnut cake which has 55.85% (Badau et al., 1997). All other ingredients contributed their quota of protein too.

The highest crude fibre content obtained in kilishi from *C. gariepinus* (2.29 ± 0.23) was significantly different from that of *M. rume* kilishi (1.77 ± 0.06). Badau et al. (1997) reported crude fibre range of 6.1-25.0% for ingredients used in kilishi production. Though the recipes were constituted from virtually the same ingredients, differences were obtained in the crude fibre values of kilishi products. It can therefore be deduced that the varied rate of absorption and adsorption of the dried raw meat slices in the different spice mixture is responsible for the significant differences observed in the crude fibre content of kilishi produced.

The ash content is an indicator of the mineral content of the fish. The ash content of the kilishi samples ranged from 6.09% in *M. rume* to 5.76% in *C. gariepinus*. The result of ash content is in line with the findings of Bello and Tekwata (2015) who reported ash content values of 5.00 to 5.33 for fish kilishi made from *Heterotis niloticus*. The pattern of ash content observed in this study revealed that the ash content is influenced by the fish species. Also, another reason could be because most of the ingredients in the slurry might have lost their mineral contents into the slurry hence, into the fish product and this agrees with the report of Elizabeth (1995) who observed that the ash content of any processed meat would be the content of the muscle tissue in addition to that of ingredients used.

The ether extract content obtained in this study was highest 29.45 ± 0.01 in kilishi made from *C. gariepinus* and lowest 19.45 ± 0.58 in kilishi made from *Mormyrus rume*. Similar higher values between 17.34 and 19.20 were reported by Iheagwara and Okonkwo (2016). Generally, the fat content of the kilishi samples were high and this can be attributed to the groundnut cake powder which represent a considerable proportion of the product (Igene et al., 1990).

The moisture content of the kilishi samples ranged from 8.45% to 10.09% with *C. gariepinus* having the highest moisture content of 10.09%. The reduction in moisture content of *M. rume* is desirable as this can affect the quality of the sample positively in relation to other kilishi sample (Apata et al., 2013). Generally, the moisture content of the kilishi samples indicates that the kilishi samples were sufficiently dried to minimize microbial growth though moisture values of 6.92%, 9.87%, and 10.00% were recorded by Jones et al. (2001), Apata et al. (2013), and Olusola et al. (2012) respectively.

CONCLUSIONS

It can be concluded that fish kilishi was highly accepted by consumers though the *Clarias* kilishi was mostly preferred. The groundnut paste used significantly affects the product quality of kilishi in terms of nutritional value, but slurry appears to be more acceptable to the consumers.

Based on the experimental result, it recommended that preparation of fish kilishi should be explored as a means of preserving fish to arrest spoilage especially during glut supply and to diversify fish products and this will increase the acceptability of *Clarias gariepinus* and *Mormerus rume* and reduce post-harvest losses.

ACKNOWLEDGEMENTS

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