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CYTOCHROME B MITOCHONDRIAL DNA HAPLOTYPES DIVERSITY OF PIKE-PERCH (*SANDER LUCIOPERCA* L., 1758) FROM DIFFERENT PARTS OF THE RANGE

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Abstract

1141 bp mtDNA fragment containing cytochrome b sequence was analyzed. Samples from the Caspian (53 individuals - marine, 57 - Volga, 6 - Kura and Arax), Baltic (36 - Lake Chudskoye), Azov (9 - Don), Aral (5 - Syr Darya), Aegean (31 - Kardzhali, Pyasachnik, Zhrebchevo) basins were analyzed. All identified haplotypes belonged to haplogroup A. Haplotype A (Slucb2) dominated in all samples except the Aegean ones. Haplotype A1 (differing by one substitution from A), distributed in water bodies of central Europe, was found only in Lake Chudskoye. The less common A2 (Slucb1) differing by 3 substitutions from haplotype A (Slucb2) occurred in most of the Ponto-Caspian samples. In addition, new haplotypes of A haplotype group were described. They differ by single substitutions from the main A and A2 haplotypes: three new haplotypes were found in Caspian Sea samples, one- in the Syr Darya River sample. Another previously undescribed haplotype was found in one individual from the Don and in all studied individuals from the Aegean basin (Bulgaria). This haplotype is transitional between haplogroups A and B. Thus, the absence of the haplotypes dominating in other parts of the range makes the pike-perch from the Aegean water bodies genetically unique.

Keywords: pike-perch, *Sander lucioperca*, mtDNA, genetic diversity, geographic distribution, phylogeography.

INTRODUCTION

The pike-perch *Sander lucioperca* is one of the species with a high level of intraspecies structure heterogeneity. Its variability is related to the complex pattern of fish migrations both within freshwater bodies and between fresh, brackish, and marine waters (Tanasiychuk, 1955, 1974; Belyi, 1965; Nellen, 1965; Klinkhardt and Winkler, 1989; Lehtonen et al., 1996; Kafemann et al., 1998; Diripasko, 2004).

The pike-perch has an extensive range almost throughout Eurasia. In some parts, it was introduced artificially. For instance, it is a native species in the river Danube and rivers and lakes coastal to the Black Sea in Bulgaria.

In the middle of the last century, the massive construction of reservoirs began in Bulgaria. Pike-perch was introduced in different types of reservoirs, where it became a part of the ichthyofauna. Today, pike-perch is a common species in the lowland warm water reservoirs in Bulgaria.

Despite its economic value, the pike-perch is still an underexplored species; genetic studies have referred only to certain parts of its range, mainly Central and Western European ones. Most studies have shown that pike-perch demonstrates a low level of *Cytb* mtDNA polymorphism with a dominance of haplotype A (Slucb2), Genbank № JX025362; nevertheless, various pike-perch genetic lines (A and B) have been identified (Haponski, Stepien, 2013; Kohlmann et al., 2013), making possible to clarify its evolution and colonization history. The mitochondrial B-group was found only in samples from the Western European region (Kohlmann et al., 2013). Earlier study based on usage of 450bp mtDNA *Cytb* sequences did not reveal the mtDNA polymorphism of samples from Aral-Caspian basin and described the presence of the most abundant haplotype A in this area (Barmintseva et al., 2014).

Within the framework of this work, unique samples were collected. This work was aimed to reveal the main phylogeographic groups of previously unexplored Eurasian parts of the pike-perch range.

MATERIALS AND METHODS

In this study, we used the genetic material of the pike-perch collected from 2017 through 2019 in the eastern European part (Bulgaria, Russia) and Asian part of range (Russia, Azerbaijan, Armenia).

The pike-perch samples were obtained through clipping of pectoral fins. Tissue fragments were fixed in 96% ethanol at the sampling sites. A total of 197 individuals of pike-perch were analyzed (sampling locations are listed in Table 1).

DNA from all individuals was isolated and purified using the silica-based method (Ivanova et al., 2006).

Table 1. Samples of pike perch used in the study

Location, year of sampling	No
Volga River, Ahtuba, 2017	11
Lower Volga, 2017	12
Volga River, Rybinskoe Reservoir, 2017	18
Volga River, Rybinskoe reservoir, 2019	10
Middle Volga, 2019	6
North Caspian Sea, 2017	44
Middle Caspian Sea, 2019	9
Kura River, 2019	4
Araks river, Armenia, 2019	2
Don River, Azov basin, 2019	9
Syr-Darya River, Aral basin, 2019	5
Chudskoe Lake, Baltic basin, 2019	36
Kardzhali Reservoir, Aegean basin, Bulgaria, 2019	14
Pyasachnik Reservoir, Aegean basin, Bulgaria, 2019	5
Jrebchevo Reservoir, Aegean basin, Bulgaria, 2019	12

Amplification of the complete *cytochrome b* (1141 bp) of pike-perchs was performed with primers (Kohlmann et al., 2013):
F:5'-GTGACTTGAAAAACCACCGTTG-3',
R:5'-CTCCATCTCCGGTTTACAAGAC-3'.

Amplification was carried out in a total volume of 15 μL (30 mM Tris-HCl (pH 8.6),

16.6 mM (NH4)2SO4, 2.5 mM MgCl2, 0.6 mM dNTP, 3 pmol of each primer, about 100 ng DNA, and 0.5 units of *Taq* polymerase, Evrogen) under the following reaction conditions: the hot start PCR program consisted of an initial denaturation at 95°C for 3 min followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 51.5°C for 1 min, extension at 72°C for 2 min, and a final extension at 72°C for 7 min, using the PCR-primers.

The amplification products were analyzed using agarose gel electrophoresis with subsequent ethidium bromide staining.

Sequencing was performed with an ABI 3500 automated sequencer using the BigDye v.3.1 sequencing kit from PCR flanking primers and backwards using inner primers (Kohlmann et al., 2013):

1R: 5'-GTTTAAGCCAAGGGGGTTGT-3'
2F: 5'-CTCGATTCTTTGCCTTCCAC-3'.

Processing of chromatograms, multiple sequence alignment and tree building was carried out in the Geneious 6.0.5. (Kearse et al., 2012). MST (minimum spanning network) algorithm was used for haplotypes network construction in PopArt (Bandelt et al., 1999).

RESULTS AND DISCUSSION

197 sequences with 1141 bp length of *cytochrome b* mitochondrial gene were obtained. All of them were close or belonged to the A mitochondrial group. In total, we defined 8 haplotypes in our samples. Two sequences from mitochondrial B group were added for further analysis from Genbank: haplotype B – № JX025364 and B1 – № JX025365. Final alignment of 10 haplotypes included 11 polymorphic sites, 6 of them were parsimony informative.

An unrooted neighbor-joining tree of haplotypes were constructed (Fig. 1). The two groups of haplotypes A and B clustered with 100% bootstrap support. The newly found haplotype A-bul, which was possessed by all individuals of the Aegean basin (Bulgaria) and by one individual from the Don River (Azov basin), with high bootstrap support occupies a separate transitional position between groups A and B. Within group A, two subgroups can be conditionally distinguished. The first subgroup includes the most widespread in Eurasia haplotype A, described earlier by Kohlman et al. (2013), identical to the Slucb2 haplotype (Haponski and Stepien, 2013), and close to this one's haplotypes. The second subgroup includes haplotype A2 (identical to haplotype Slucb1 Genbank № KC819823.1 of Haponski and Stepien, 2013), and haplotypes close to this one.

The A-bul haplotype differs from the well-known mass A haplotype by two (A/G) transitions at nucleotide positions 333 and 468 in alignment.

Among small samples of the Kura River (Azerbaijan) – 4 individuals, of the Araks River (Armenia) – 2 individuals, only mass haplotype A was found. In the Don River (Azov basin), in addition to 1 individual with a new haplotype A-bul, 7 individuals of haplotype A and 1 individual with haplotype A2 were identified (Fig. 3).

In the Syr-Darya River (Aral basin), 3 individuals with haplotype A were found, 1 individual was with A2 and 1 individual possessed the new haplotype A-arl (Fig. 3), it indicates requirement for further study of enlarged samples from this area.

As a result of this study, in addition to known haplotypes A and A2, new haplotypes were found in the Caspian Sea samples. The new haplotype A-cas2 differs from the mass haplotype A by the transition (C/T) at nucleotide position 858 in alignment. The new haplotype A2-cas1 differs from the haplotype A2 by one transition (G/A) at nucleotide

position 607 in alignment. The new haplotype A2-cas3 differs from the haplotype A2 by one transition (G/A) at nucleotide position 56. Thus, the sample of the Caspian Sea has the highest haplotype diversity, which may be a sign of one of the historical refugia for this species. The distribution of frequencies in the studied pooled samples is shown in Fig. 2.

The data (Kuzishchin et al., 2018) show the absence of clear genetic isolation in the Lower Volga River for pike-perch. Despite the geographical closeness of the Volga and the Caspian Sea populations, the Volga sample contains only haplotypes A and A2 without other haplotypes, and ratio of A and A2differs from the Caspian Sea samples (A2: Volga – 28%, Kaspian sea – 7%) (Fig. 2, Fig. 3). It might indicate some level of the Volga River pike-perch populations isolation from the Caspian Sea ones.

The sample of the Chudskoe Lake, belonging to the Baltic basin, differs in haplotype diversity from other samples, possesses mass haplotype A, and does not have haplotype A2, which is found in the most of the Ponto-Caspian samples. For one individual from the Chudskoe Lake, we identified haplotype A1 (identical to the sequence from Genbank № JX025363), described earlier in the French population (Kohlmann et al., 2013), which may be a sign of the common origin of individuals from the Baltic basin and individuals from the Western European part of species range.

The connection between the pike-perch from the Don and reservoirs in Bulgaria can be explained by the fact that the individuals living in reservoirs belonging to the Aegean Sea basin originate the areas of the Black Sea basin. Therefore, it will be informative to study pike-perch samples from the Danube River and the Black Sea's coastal water bodies in Bulgaria.

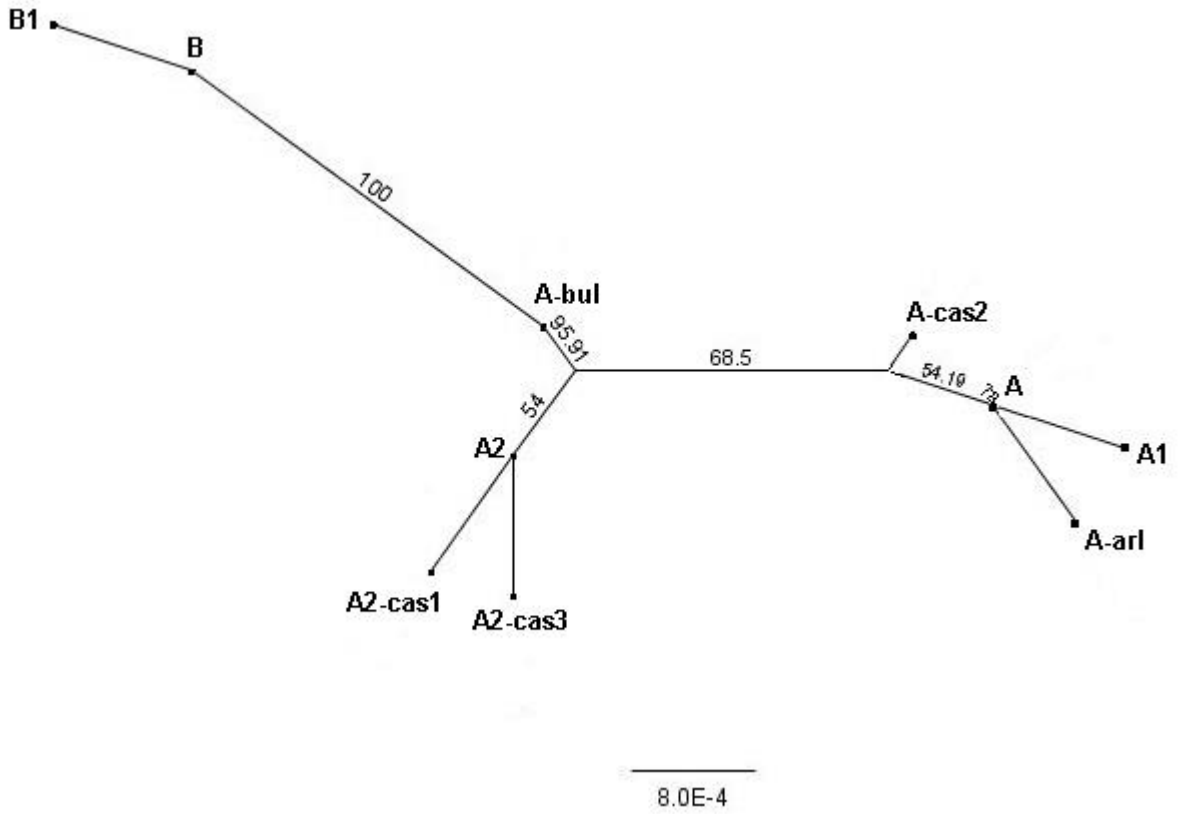

Fig. 1. HKY tree of *Cytb* haplotypes of pike-perch from Eastern European and Asian parts of range, bootstrap– 10000 iterations

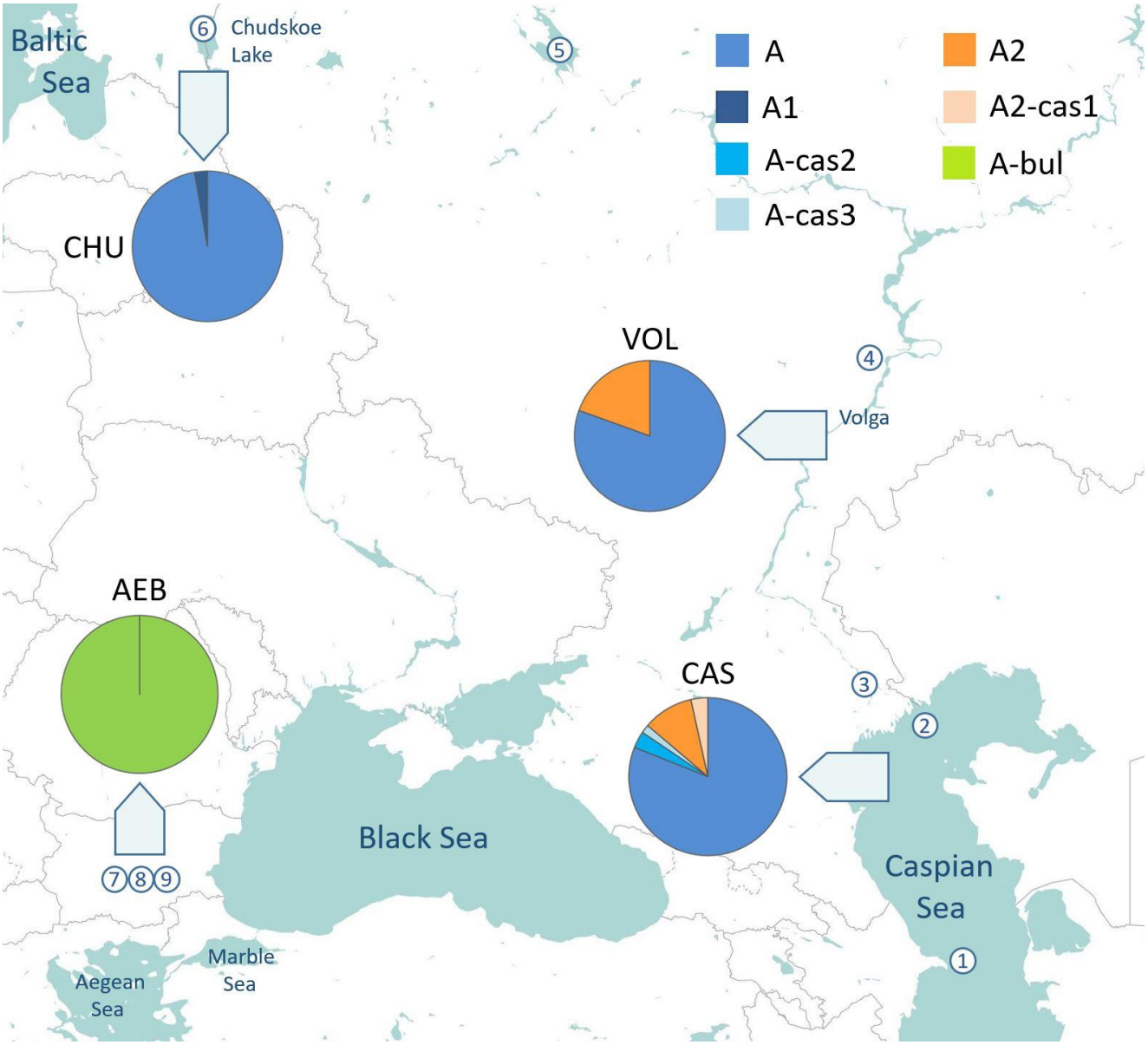

Fig. 2. The map of sample locations and pooled samples haplotype frequencies. **CAS**: **1** – the Middle Caspian Sea, Azerbaijan (n=9), **2** - the Northern Caspian Sea, Russia (n=44); **VOL**: **3** – the Lower Volga (n=23), **4** – the Middle Volga (n=6), **5** – the Rybinsk Reservoir Volga part (n=28); **CHU**: **6** - the Chudskoe Lake (n=36); **AEB: 7 –** Kardzhali Reservoir, the Aegean Basin, Bulgaria (n=14), **8** – Pyasachnik Reservoir, the Aegean Basin, Bulgaria (n=5), **9** – Jrebchevo Reservoir, the Aegean Basin, Bulgaria (n=12).

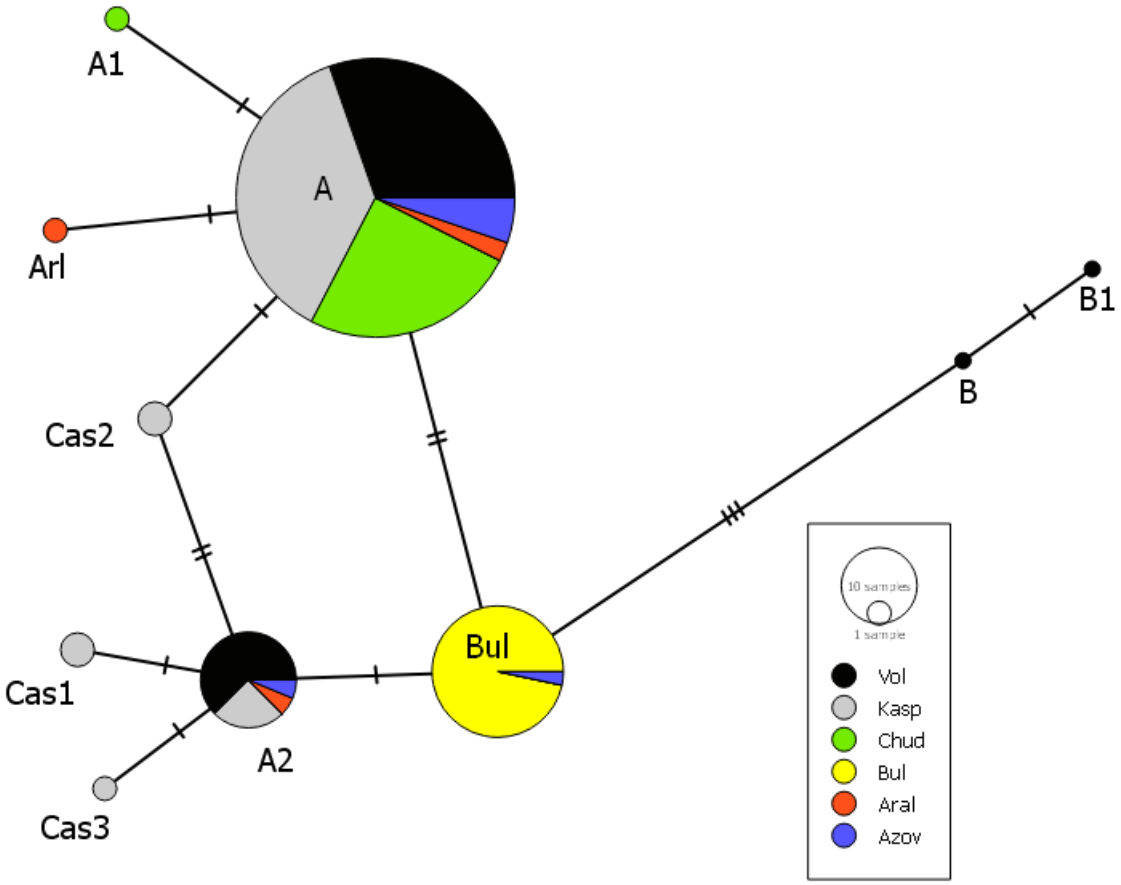

Fig. 3. Pike-perch *Cytb* haplotypes MST network. The size of the circles and the area of the sectors are proportional to the haplotypes frequency in the corresponding samples.

REFERENCES

Bandelt, H., Forster, P., & Röhl, A. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 1999, 16(1), 37–48.

Barmintseva, A.E., Shalgimbayeva, G.M., Koishybayeva, S.K., Sarbakanova, S.T., Asylbekova, S.Z., Isbekov, K.B., and Mugue, N.S., Genetic study of pike perch *Sander lucioperca* (Linnaeus, 1758) and bersh *Sander volgensis* (Gmelin, 1789) from the fishery water-bodies of Kazakhstan, Russ. J. Genet., 2014, vol. 50, no. 7, pp. 749–756.

Belyi, N.D., Biological groups of zander *Lucioperca lucioperca* (L.), Vopr. Ikhtiol., 1965, vol. 5, no. 2 (35), pp. 279–289.

Diripasko, O.A., Phenetic diversity of zander Stizostedion lucioperca from the Sea of Azov, Vopr. Ikhtiol., 2004, vol. 44, no. 2, pp. 249–256.

Haponski, A.E. and Stepien, C.A., Phylogenetic and biogeographic relationships of the Sander pikeperches (Percidae: Perciformes): patterns across North Amer ica and Eurasia, Biol. J. Linn. Soc., 2013, vol. 110, pp. 156–179.

Ivanova, N.V., deWaard, J., and Hebert, P.D.N., An inexpensive, automationfriendly protocol for recover ing highquality DNA, Mol. Ecol. Notes, vol. 6, pp. 998–1002.

Kafemann, R., Finn, J.E., Thiel, R., and Neukamm, R., The role of freshwater habitats for the reproduction of bream in a brackish water system, Pol. Arch. Hydrobiol., 1998, vol. 45, no. 2, pp. 213–233.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28, pp. 1647–1649.

Klinkhardt, M.B. and Winkler, H.M., Einfluss der Salinitat auf die Befruchtungs und Entwicklungsfahigkeit der Eier von viersusswasser fischharten—Plotz (*Rutilus rutilus*), Barsch (*Perca fluviatilis*), Kaulbarsch (*Gymnocephalus cernua*) und Zander (*Stizostedion lucioperca*), Wiss. Z. Univ. Rostock. N-Reihe, 1989, vol. 38, pp. 23–30.

Kohlmann, K., Louati, M., Kersten, P., et al., Detec tion of two major cytochrome b lineages in pikeperch, *Sander lucioperca*, and first data on their distribution in European populations, Environ. Biotechnol., 2013, vol. 9, no. 1, pp. 1–5.

Kuzishchin, KV, Ponomareva, EV, Samoilov, KY, Gruzdeva, MA, Kholodova, MV, Pavlov DS Morphological and genetic traits of pikeperch *Sander lucioperca* of the Volga-Akhtuba Aquatic System: on the spatial structure of a species in the Lower Volga Basin. J Ichthyol, 2018, 58, pp.318–332.

Lehtonen, H., Hansson, S., and Winkler, H., Biology and exploitation of pikeperch, *Stizostedion lucioperca* (L.), in the Baltic Sea area, Ann. Zool. Fen., 1996, vol. 33, pp. 525–535.

Nellen, W., Beitragezur Brackwasserokologie der Fischeim Ostseeraum, Kieler Meeres Forsch., 1965, vol. 11, pp. 192–198.

Tanasiichuk, V.S., Biology of juveniles of the zander of Northern Caspian region, Vopr. Ikhtiol., 1955, no. 3, pp. 87–103.

Tanasiichuk, V.S., Adaptive possibilities of European pikeperch Lucioperca lucioperca (L.), Vopr. Ikhtiol., 1974, vol. 14, no. 5 (88), pp. 806–813.