Трансформация экосистем
Ecosystem Transformation

ISSN 2619-0931 Online
www.ecosysttrans.com

DOI 10.23859/estr-230810
EDN MWCIOQ
UDC 597.2/5; 575.17

Article

Population-genetic structure of the sterlet *Acipenser ruthenus* L., 1758 in the Mologa River during its re-acclimatization

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Abstract. This paper examines the size-age structure, growth and genetic polymorphism of the sterlet population of the Mologa River, Vologda Oblast, formed during reacclimatization measures. The introduction of sterlet into the Mologa River began in 2016, and since 2018 it has been found in research catches. In the channel medial sections of the river, the proportion of sterlet in total catches ranged from 31.4% to 49.3% in numbers and from 26.0% to 64.0% in biomass. In 2022, the catches included fish with a length of 22–51 cm and a weight of 64–958 g of five age groups. The size, weight and age composition of the emerging population is currently determined by the number of juvenile sterlet released in individual years. Mitochondrial DNA analysis revealed 23 different mtDNA haplotypes and low relative haplotype diversity. The level of observed nuclear DNA heterozygosity averaged 0.567 with a range from 0.149 to 0.871. Genetic heterogeneity of individuals in the emerging population of sterlet from the Mologa River is sufficient for the local population of a river.

Key words: size-age structure, linear growth, weight growth, microsatellite loci, alleles, mitochondrial DNA, haplotypes, genetic polymorphism

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To cite this article: Ignashev, A.A. et al., 2023. Population-genetic structure of the sterlet Acipenser ruthenus L., 1758 in the Mologa River during its re-acclimatization. *Ecosystem Transformation* **6** (4), 79–95. https://doi.org/10.23859/estr-230810

Received: 10.08.2023
Accepted: 31.08.2023
Published online: 13.11.2023

DOI 10.23859/estr-230810
EDN MWCIOQ
УДК 597.2/5; 575.17

Научная статья

Популяционно-генетическая структура стерляди *Acipenser ruthenus* L., 1758 реки Молога (Вологодская область) в условиях ее реакклиматизации

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Аннотация. В работе рассмотрены особенности размерно-возрастной структуры, рост и генетический полиморфизм формируемой в ходе реакклиматизационных мероприятий популяции стерляди р. Молога Вологодской области. Вселение стерляди в р. Молога началось в 2016 г., а в научно-исследовательских уловах она стала встречаться с 2018 г. На русловых медиальных участках реки доля стерляди в общих уловах составляла от 31.4% до 49.3% по численности и от 26.0% до 64.0% по биомассе. В 2022 г. в составе уловов отмечены рыбы длиной 22–51 см, массой 64–958 г пяти возрастных групп. Размерный, весовой и возрастной состав формируемой популяции в настоящее время определяется количеством выпускаемой в отдельные годы молоди стерляди. Анализ митохондриальной ДНК выявил 23 различных мтДНК гаплотипа и низкое относительное гаплотипическое разнообразие. Уровень наблюдаемой гетерозиготности ядерной ДНК составил в среднем 0.567 с диапазоном от 0.149 до 0.871. Генетическая разнородность особей в формируемой популяции стерляди р. Мологи достаточна для локальной популяции отдельно взятой реки.

Ключевые слова: размерно-возрастная структура, линейный рост, весовой рост, микросателлитные локусы, аллели, митохондриальная ДНК, гаплотипы, генетический полиморфизм

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Для цитирования: Игнашев, А.А. и др., 2023. Популяционно-генетическая структура стерляди *Acipenser ruthenus* L., 1758 реки Молога (Вологодская область) в условиях ее реакклиматизации. *Трансформация экосистем* **6** (4), 79–95. https://doi.org/10.23859/estr-230810

Поступила в редакцию: 10.08.2023
Принята к печати: 31.08.2023
Опубликована онлайн: 13.11.2023

Introduction

The sterlet *Acipenser ruthenus* L., 1758 is the most commercially valuable fish species in the water bodies of the Vologda Oblast, and the only species of sturgeon to reproduce naturally here (Borisov et al., 2019). The native range of this species within the boundaries of the Vologda Oblast included Sheksna and Mologa (large tributaries of the Volga River), as well as Beloe Ozero. In the 19th century, this species entered the river basin through the North Ekaterininsky Canal, Northern Dvina River, and through the Mariinsky Canal – into Lake Onega (Konovalov and Konovalov, 2016). Currently, small self-reproducing populations of this species have only survived in the Malaya Northern Dvina, Sukhona, Yug and Vaga rivers of the Severodvinsk basin. In the Sheksna and Mologa rivers of the Volga basin, sterlet lost its commercial importance at the beginning of the 20th century, although previously there had been specialized fishing there (Konovalov and Konovalov, 2016). Over the past decades, there has been no reliable information about the existence of a sterlet population in the rivers of the Volga basin within the boundaries of the Vologda Oblast (Ryby Rybinskogo..., 2015; Zelenetsky, 2006).

In water bodies of the European part of Russia, the population of this species is mainly maintained through artificial reproduction (Bykov and Brazhnik, 2022). In the Vologda Oblast, since 2014 measures have been implemented to introduce juvenile sterlet, as part of compensation for damage caused to aquatic biological resources. To date, over 2 million fingerlings of sterlet have been released into the water bodies of the region (the Sukhona, Yug, Vaga, Mologa Rivers and the river part of the Sheksna Reservoir). The most significant positive result from the release of juvenile sterlet was recorded in the Mologa River (Ignashev and Borisov, 2022). Since 2016, over 450 thousand fingerlings of sterlet with an average weight of 5.0–10 g have been released there, including: in 2016 – 13300, in 2017 – 142800, in 2019 – 120600, in 2020 – 170000, in 2021 – 2600 thousand, and in 2022 – 1200 thousand specimens. Juvenile sterlets were released into the lower reaches into the Mologa River approximately 30 km from the mouth in the area of the village of Vanskoe, Ustyuzhensky District, Vologda Oblast. Since 2018, sterlet has been found in research catches, and in 2020, genetic monitoring of the emerging population began.

It is known that the problems of artificial reproduction of animals, including fish, are a decrease in the genetic diversity of populations, inbreeding, disruption of sexual structure and self-reproduction (Altukhov, 2001). In this regard, determining the genetic diversity of the emerging population in a single water body is of particular relevance. The purpose of this work is to assess the population genetic structure of the Mologa River sterlet population during implementation of measures for its re-acclimatization.

Materials and methods

The Mologa River is the largest tributary of the Rybinsk Reservoir; its length is 456 km, and the drainage basin area is almost 30000 km^2 (Shestakova, 2007). It flows through the parts of the Tver, Novgorod and Vologda Oblasts. In research sites, in its lower reaches, the river width is 200–360 m, depth up to 6 m, and flow speed 0.05–0.10 m/sec.

Ichthyological material was collected from fixed gill nets with a mesh pitch of 20–60 mm. Networks in 2018–2022 were installed in the same areas in order in the coastal and medial parts of the river in the area of the villages of Vanskoe and Bugry in the Ustyuzhensky municipal district, Vologda Oblast (Fig. 1). The nets were kept in the watercourse for 24 hours. All fish were subjected to complete biological analysis according to generally accepted methods (Pravdin, 1966). The length of the fish was measured to the roots of the middle rays of the caudal fin. The age of the sterlet was determined in laboratory conditions by cuttings of the marginal rays of the pectoral fins. The total ichthyological material collected included 571 specimens.

Fig. 1. Map of locations of sterlet catching areas on the Mologa River.

Genetic material was collected from freshly caught individuals according to recommended methodology[1]. This included selecting a fragment of the pectoral fin. Before selection, the fin was cleaned of mucus and other external contaminants with a gauze swab, abundantly moistened with a 94–96% ethanol solution. All instruments used to take material (scalpels, scissors, tweezers), when moving from individual to individual, were thoroughly cleaned of tissue remnants from the previous sampling mechanically (with a clean gauze cloth), washed with detergent, distilled water, and disinfected with alcohol. A sample from each individual was placed in an individual test tube and filled with a fixing solution of 94–96% rectified ethyl alcohol of the highest purity; the alcohol : sample ratio was 10:1. The sample was assigned an individual alphanumeric code. The morphometric parameters of the individual were indicated in the statement for the corresponding sample. On average, after three days, secondary fixation of the sample with alcohol was carried out in a ratio of 5:1. Next, the samples were stored in a freezer with a temperature of −10°C. In total, pectoral fin samples from 223 individuals were selected for genetic research.

All samples taken are housed in the Russian National Collection of Reference Genetic Materials (RNCEGM) VNIRO (certificate of official database registration no. 2006620351), each sample is assigned an individual identification number. Isolation and subsequent purification of DNA from sturgeon fins was carried out on PALL 5051 adsorption columns (AcroPrep™ 96 1 ml filter plate with 1.0μm Glass Fiber Media, natural housing) in accordance with the protocol of the Canadian Centre for DNA Barcoding (Ivanova

[1] Methodological recommendations MP4.2.001-2015 “Methods for molecular genetic analysis of aquatic biological resources and aquaculture objects, as well as products from them,” approved by the director of the Federal State Budget Scientific Institution “VNIRO” on January 27, 2015.

et al., 2006). Nuclear DNA samples of juvenile sterlet were analyzed for 14 microsatellite loci (Table 1) with fluorescent tags; PCR conditions were optimized for sturgeon species (Barmintseva and Muge, 2013).

The following conditions were selected for the reactions: the final volume was 15μl, including 16.6 mM $(NH_4)_2SO_4$; 70 mM Tris-HCl (pH 8.6); 200 μM each dNTP; 1.8 mM $MgCl_2$; 1 pM primer modified at the 5' end with the fluorescent dye FAM, HEX or TAMRA; 50–100 ng DNA; 4 pM reverse (unlabeled) primer; 0.8 units of Taq polymerase (Silex, Moscow). The amplification reaction followed the scheme: preliminary DNA denaturation 94 °C – 1 min; 8 cycles: melting – 95 °C – 20 s, primer annealing – 58 °C in the first cycle – 25 s, in each subsequent cycle the annealing temperature decreased by 0.5 °C, DNA synthesis – 65 °C – 40 s; 25 cycles: melting – 95 °C – 20 s, primer annealing – 54 °C – 30 s, DNA synthesis – 65 °C – 40 s; pre-synthesis of DNA at 65 °C – 10 min. The PCR mixture obtained as a result of amplification was diluted with three parts of water (milliQ), then 1 μl of the mixture was transferred to

Table 1. Microsatellite loci for the analysis of polymorphism of sterlet from the Mologa River.

Locus	Primers 5'–3'	Tag
An20	F:AATAACAATCATTACATGAGGCT R:TGGTCAGTTGTTTTTTTATTGAT	HEX
AfuG41	F:TGACGCACAGTAGTATTATTTATG R:TGATGTTTGCTGAGGCTTTTC	FAM
AfuG51	F:ATAATAATGAGCGTGCTTTCTGTT R:ATTCCGCTTGCGACTTATTTA	HEX
AoxD165	F: TTTGACAGCTCCTAAGTGATACC R: AAAGCCCTACAACAAATGTCAC	TAMRA
AoxD161	F: GTTTGAAATGATTGAGAAAATGC R:TGAGACAGACACTCTAGTTAAACAGC	FAM
AfuG63	F:TCCTGGCTAGCGAACGAA R:CTTTTAAATGGGGGACAGACTAT	FAM
AfuG67	F:CAAAGCTAGAACAAGTAAAGAGAA R:GGGGTGTCCTATAATAAAAGTGC	FAM
AfuG112	F:TATTGTTCCTTTATGGTTATG R:TATTTCACTGTCTGTTGTATGTA	HEX
AfuG174	F:CAATGGGGTGGGCAAAAA R:ATTAGGAGTATGGCAGTGTAGAAC	FAM
AoxD234	F:AACTGGCTTTGTGATTGATCC R:TGAAGCAAAGGGTATTATTTGAG	TAMRA
LS-19	F:CATCTTAGCCGTCTGGGTAC R:CAGGTCCCTAATACAATGGC	FAM
LS-39	F:TTCTGAAGTTCACACATTG R:ATGGAGCATTATTGGAAGG	HEX
LS-68	F:TTATTGCATGGTGTAGCTAAAC R:AGCCCAACACAGACAATATC	TAMRA
Aox-45	F:TTGTTCAATAGTTTCCAACGC R:TGTGCTCCTGCTTTTACTGTC	HEX

a new plate and 12 µl of HiDi formamide with a molecular standard was added to determine the size of the amplified DNA fragments. Amplification products were electrophoretically separated in an ABI 3500 Genetic Analyzer capillary electrophoresis system. After this, allele lengths were determined using GeneMarker software (Version 1.2).

Amplification of the control region of mtDNA (D-loops) was carried out with primers: DL651 (ATCTTAACATTCTTCAGTG) and M13AHR3 (TCACACAGGAAAAACAG-CTATGACATACCATAATGTTTCCATCTACC) (Müge et al., 2008). The PCR reaction was carried out in a final volume of 15 µl and contained 100 ng of DNA. Composition of the reaction mixture: 16.6 mM $(NH4)_2SO_4$, 70 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 1.5 pM each primer, 100 µM dNTP, 1 unit of *ColarTaq* polymerase (Silex, Moscow). Amplification was carried out according to the following scheme: preliminary denaturation of DNA 95 °C – 10 min; synthesis of PCR products (30 cycles): melting – 94 °C – 20 s, primer annealing – 52 °C – 40 s, DNA synthesis – 72 °C – 60 s; final completion of circuits: 72 °C – 10 min. Visualization of PCR products was carried out in a 2% agarose gel in 0.5X TBE buffer with SYBR Green I staining. Sequencing of the control region of mtDNA was carried out from one strand with the universal primer M13(−22) – TCACACAGGAACAGCCTATGAC (5 pM) on an ABI 3500 Genetic analyzer, using a set of reagents BigDye™ Terminator Kit v. 3.1 (Applied Biosystems, USA) in a reaction volume of 10 µl and using the 5X reaction buffer supplied with the kit. A reaction mixture was added to a plate with 96 wells, each of which contained a pre-precipitated PCR product: 5X BigDye buffer – 1.7 µl, BigDye – 0.25 µl, primer M13(−22) – 3 pM, water (milliQ) up to 10 µl.

The probability of an individual belonging to an age group was calculated in the Structure 2.3.4 program (Pritchard et al., 2000) with the following parameters: 1000000 MCMC steps, excluding the first 100000. Statistical data (average number of alleles (Na), relative allele frequencies, observed (H_o) and expected heterozygosity (H_e), Wright's fixation index (F), F_{ST}) were obtained in the GenAlex program (Peakall and Smouse, 2006).

The mtDNA sequences were aligned and analyzed using Sequencing Analysis 5.4 (Applied Biosystems, USA) using the bioinformation software package SeqMan® version 12.0 DNASTAR (Madison, WI, USA). The mtDNA haplotype numbers were assigned in accordance with the register of mtDNA haplotypes of sturgeon species of the Federal State Budgetary Scientific Institution "VNIRO". The haplotype network was constructed using PopART (Leigh and Bryant, 2015).

The samples by year of release were very uneven: the bulk were individuals from 2017 (89 specimens) and 2019 (112 specimens), while 2020, 2021 and 2016 were represented by only 3, 5 and 14 individuals, respectively. In this regard, a comparative analysis was only performed among the samples of 2016, 2017 and 2019 releases.

Results and discussion

Growth and size-age structure

During research fishing with fixed nets, sterlet of different ages began to be caught in fixed nets in 2018. It was found both directly at the release site of juveniles in the area of the village of Vanskoe, and 25 km downstream in the area of the village of Bugry. The share of sterlet in the total catches in the riverbed sections of the river varied in different years from 31.4% to 49.3% in numbers and from 26.0% to 64.0% in biomass (Table 2). The highest proportion of sterlet in catches by abundance was observed in 2019, 2020 and 2021, and the highest rates of sterlet catch per net per day were recorded in 2019, 2021 and 2022, amounting to 7.5, 8.3 and 9.4 specimens, respectively. Along with sterlet, the catches included significant quantities of silver bream, white-eye bream, and roach; in smaller quantities, common perch, ruffe, bream, Volga zander, sabrefish, blue bream, and a few specimens of zander, catfish, and common chub. No sterlet were recorded in the catches from nets placed in the coastal sections of the river.

Under conditions of artificial reproduction, the size, weight and age composition of sterlet catches is determined primarily by the volume of juveniles released. Thus, while in 2018 the catches included sterlet of two age groups (1+, 2+) from the releases of 2016 and 2017, in 2022 five age groups were recorded (1+, 2+, 3+, 5 +, 6+) from releases 2016, 2017, 2019, 2020 and 2021 (Table 3). As the number of age groups in the population increases, the size and weight composition of catches becomes more diverse. In the catches of 2018, the length of the fish varied between 24–36 cm, in 2019 – 26–41 cm, in 2020 – 20–44 cm, in 2021 – 20–46 cm, and in 2022 – 22–51 cm, while the weight of fish was 74–224, 82–380, 44–676, 56–736 and 64–958 g, respectively (Table 2, 4, 5).

Table 2. Commercial and biological characteristics of sterlet from the Mologa River based on the results of research fishing with fixed nets in 2018–2022.

Year	Share by number, %	Length, cm			Catch per net per day, specimens	Share by biomass, %	Weight, g			Catch per net per day, kg
		Mean	min	max			Mean	min	max	
2018	33.1	28.9 ± 0.32	24	36	5.0	26.0	135.5 ± 4.55	74	224	0.68
2019	48.1	32.4 ± 0.25	26	41	7.5	56.3	192.0 ± 5.23	82	380	1.63
2020	47.7	36.9 ± 0.92	20	44	3.9	46.2	264.4 ± 20.84	44	676	1.04
2021	49.3	29.0 ± 0.45	20	46	8.3	64.0	191.9 ± 12.36	56	736	2.58
2022	31.4	35.2 ± 0.44	22	51	9.4	57.1	300.7 ± 14.85	64	958	2.83

Table 3. Age composition of sterlet catches from the Mologa River, %.

Year	Age, years						Number of specimens
	1+	2+	3+	4+	5+	6+	
2018	95.6	4.4	–	–	–	–	45
2019	–	97.1	2.9	–	–	–	136
2020	39.4	–	57.8	2.8	–	–	71
2021	2.0	74.5	–	21.5	2.0	–	149
2022	2.9	28.3	43.8	–	23.5	1.8	170

Table 4. Size composition of sterlet catches from the Mologa River, %.

Year	Length, cm											Number of specimens
	20–22	23–25	26–28	29–31	32–34	35–37	38–40	41–43	44–46	47–49	50–52	
2018	–	11.1	35.6	51.1	–	2.2	–	–	–	–	–	45
2019	–	–	7.4	33.1	36.0	19.1	2.9	1.5	–	–	–	136
2020	8.5	23.9	7.0	–	7.0	15.5	22.5	8.5	7.0	–	–	71
2021	1.3	14.8	48.3	12.1	4.0	5.4	8.1	4.7	1.3	–	–	149
2022	0.6	1.8	2.9	18.8	36.5	10.6	5.9	13.5	5.9	1.8	1.8	170

Table 5. Weight composition of sterlet catches from the Mologa River, %.

Year	Weight, g										Number of specimens
	Less than 100	100–200	200–300	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	
2018	11.1	86.7	2.2	–	–	–	–	–			45
2019	0.7	59.6	33.8	5.9	–	–	–	–			136
2020	38.0	4.2	7.0	21.1	23.9	4.2	1.4	–			71
2021	10.7	65.8	3.4	7.4	3.4	6.7	1.3	1.3			149
2022	3.5	36.4	29.4	6.5	7.1	8.2	4.1	1.8	2.4	0.6	170

The first large release (142.8 thousand specimens) of sterlet into the Mologa river took place in 2017, and the following year, fish that had grown to 24–31 cm and 76–198 g were recorded in large numbers in the catches (Tables 3, 4, 6). The total share of fish released in 2017 in the next year's catch (2018) was 95.6%, and 97.1% in 2019 (Table 3). The high proportion of fish released in 2017 in the first two years of observation is associated with both large volumes of introduction in that year, a small number of fish released in 2016 and the absence of artificial reproduction measures in 2018. In subsequent years of research, the number of sterlet of the 2017 generation remained high, and the proportion in catches was 57.8% in 2020, 21.5% in 2021 and 23.5% in 2022. In general, to form a large generation, annual release volumes must be at the level of the receiving capacity, which, in accordance with biological justification when introducing juveniles weighed at least 5 g is identified in the amount of 170 thousand specimens for the Mologa River.

In the Mologa River, the greatest increase in length of the sterlet is observed in the first year of life. A year after release, the length of some sterlet individuals exceeded 30 cm, and the weight was up to 198 g. The most intensively released juveniles grew in the first years of artificial reproduction of this species in the Mologa River. Thus, the average length after a year of sterlets from the 2017 release was 28.3 cm, 2019 release – 23.7 cm, 2020 release – 22.3 cm, and 2021 release – 22.9 cm. Even more significant differences are noted in weight growth. If in 2018 the average weight of yearlings was 133.5 g, then in 2020 it decreased to 72.6 g, in 2021 it was 62.6 g, and in 2022 – 70 (Table 6).

Table 6. Linear and weight growth of Mologa River sterlet.

Year	Age	Length, cm		Weight, g		N
		Mean	min–max	Mean	min–max	
2018	1+	28.3 ± 0.36	24–31	133.5 ± 3.77	76–198	43
	2+	33.3 ± 0.86	31–35	210.0 ± 9.02	196–224	2
2019	2+	32.2 ± 0.23	26–39	187.8 ± 4.78	82–330	132
	3+	40.3 ± 0.67	39–41	376.7 ± 2.41	372–380	4
2020	1+	23.7 ± 0.35	20–27	72.6 ± 2.78	44–104	28
	3+	36.2 ± 0.54	32–42	378.1 ± 14.32	182–584	41
	4+	43.3 ± 1.22	41–44	616.3 ± 56.88	562–676	2
2021	1+	22.3 ± 0.16	20–25	62.6 ± 0.64	56–70	3
	2+	26.8 ± 0.13	24–31	118.3 ± 1.64	70–156	111
	4+	38.5 ± 0.51	33–42	439.2 ± 19.43	240–596	32
	5+	44.3 ± 0.54	44–47	728.0 ± 24.44	656–736	3
2022	1+	22.9 ± 0.48	22–24	70.0 ± 3.56	64–80	5
	2+	29.3 ± 0.27	26–30	142.8 ± 5.34	80–174	48
	3+	33.5 ± 0.19	30–38	221.1 ± 5.28	142–408	74
	5+	42.7 ± 0.30	38–47	550.8 ± 12.19	344–912	40
	6+	49.8 ± 0.30	49–51	928.0 ± 18.79	902–958	3
Average for 2018–2022	1+	26.1 ± 0.33	20–31	103.6 ± 4.08	44–198	79
	2+	29.7 ± 0.20	24–39	155.7 ± 3.19	70–330	293
	3+	35.0 ± 0.28	30–42	269.7 ± 8.21	142–584	119
	4+	38.4 ± 0.27	33–44	426.1 ± 20.33	240–676	34
	5+	42.8 ± 0.29	39–47	561.5 ± 18.52	344–912	43
	6+	49.8 ± 0.30	49–51	928.0 ± 18.79	902–958	3

The identified differences in the linear weight growth of fish in the first year of life are apparently associated with a decrease in their food supply in conditions of population growth. Subsequently, the linear annual growth of fish decreases to 3–6 cm per year. At the same time, an intensive increase in weight is recorded against the background of a slowdown in linear growth. In some years, annual increases in body weight exceed 300–400 g. Even despite the slowdown in sterlet growth in recent years, its overall rate in the Mologa River is high compared to natural populations from the Volga, Kama, and Oka rivers and reservoirs of the Volga cascade (Afanasiev, 1985; Afanasiev and Shurukhin, 1987; Granin and al., 2020; Kuznetsov, 1983; Vasyanin, 1972); it is comparable to the indicators for this species in the middle reaches of the Oka River, where sterlet is also artificially reproduced (Bykov and Palatov, 2019). The high growth rate of "hatchery" populations in younger age groups, and especially in the first year, is due to the fact that fish grown in pools are larger in size and have increased survival rate (Bykov, 2021).

Mitochondrial DNA analysis

Mitochondrial DNA analysis revealed 23 different mtDNA haplotypes in the 223 individuals studied (Table 7). All haplotypes are characteristic of the sterlet of the Volga River basin, for which 161 mtDNA haplotypes have been described to date (Fig. 2) (Shcherbakova et al., 2022). Data from the analysis of larger samples from 2017 and 2019 show a similar result – 0.19 and 0.12, respectively. Data for 2020 and 2021 are unreliable due to the small sample size, but 5 individuals caught from the 2021 release with a single mtDNA haplotype indicate that the juveniles released this year are inbred. In contrast, in 2016, juveniles released were relatively more heterogeneous than releases in other years.

The most frequently observed mtDNA haplotypes in the 2017 sample were no. 77, no. 61, and no. 40, and in the 2019 sample, no. 11, no. 71, and no. 62 (Table 8). At the same time, mtDNA haplotype no. 22 was found only in individuals released in 2021, no. 68 and no. 137 – in 2019, no. 75, no. 83, no. 144 and no. 245 – in 2017, and no. 82 – in 2016. Numerous groups of mtDNA haplotypes represent the offspring of one female (F1 generation) or the offspring of the F1 generation (F2 generation).

Table 7. Characteristics of mtDNA haplotypes of sterlet from the Mologa River released on 2016–2021.

Year of release	2016	2017	2019	2020	2021	Total
Number of individuals studied	14	89	112	3	5	223
Number of haplotypes	8	17	13	3	1	23
Relative haplotype diversity	0.57	0.19	0.12	1.0	0.20	0.10

Table 8. Number of mtDNA haplotypes in Mologa River sterlet from 2016–2021 release.

Year of release	No. mtDNA haplotype																							Total
	11	17	22	23	40	61	62	66	68	71	73	74	75	76	77	78	80	81	82	83	137	144	245	
2016	–	–	–	–	2	–	–	2	–	–	4	2	–	–	1	1	1	–	1	–	–	–	–	14
2017	2	9	–	–	12	14	–	1	–	2	7	2	1	6	16	1	9	2	–	2	–	2	1	89
2019	39	1	–	8	2	–	14	–	5	32	–	3	–	2	1	–	1	1	–	–	3	–	–	112
2020	–	–	–	–	1	–	–	–	–	1	–	–	–	–	1	–	–	–	–	–	–	–	–	3
2021	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Total	41	10	5	8	17	14	14	3	5	35	11	7	1	8	19	2	11	3	1	2	3	2	1	223

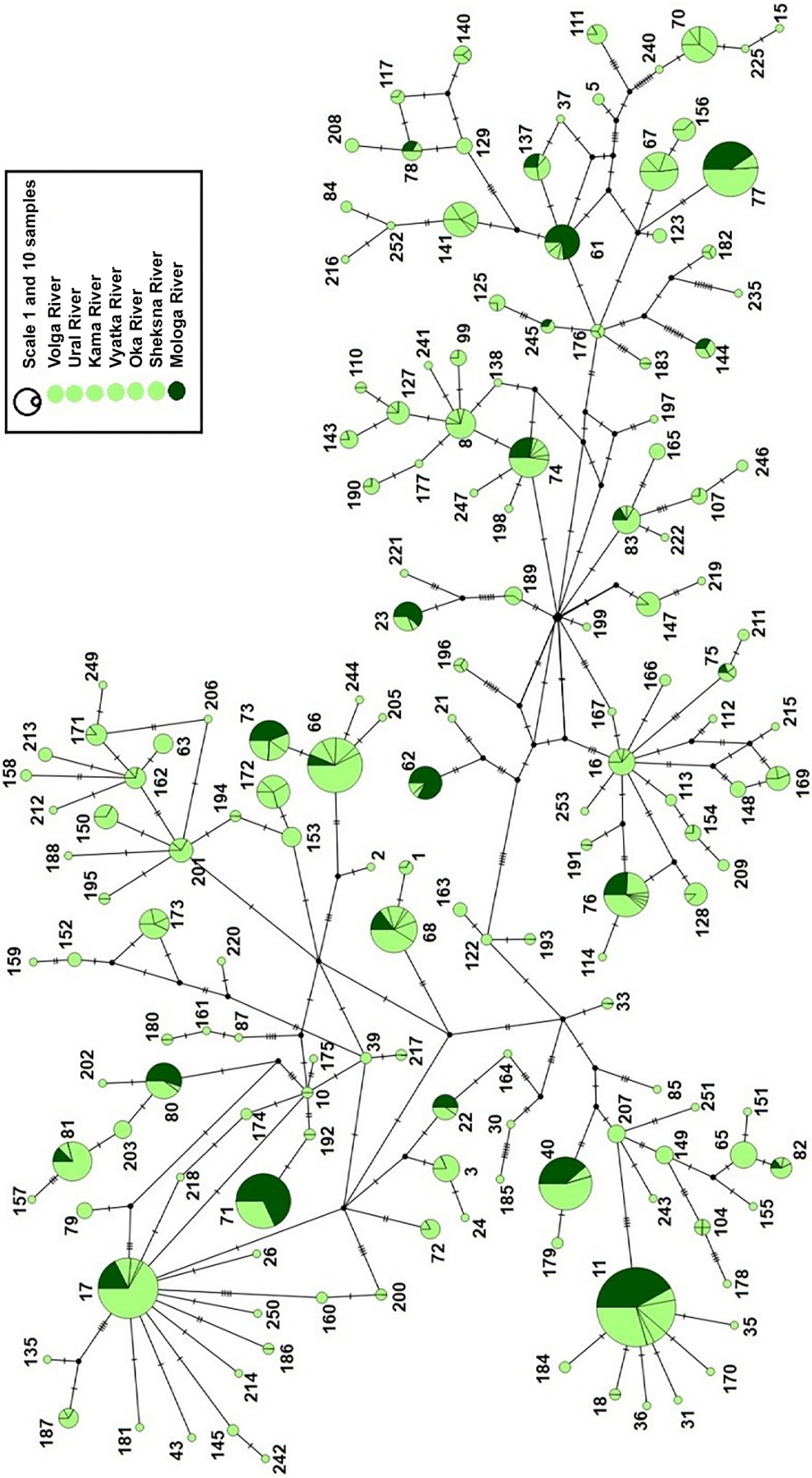

Fig. 2. Scheme of mtDNA haplotypes of sterlet in the Volga-Caspian basin (light green – haplotypes characteristic of sterlet from the Volga, Ural, Kama, Vyatka, Oka, Sheksna rivers; dark green – haplotypes found in the Mologa River),лотипы, обнаруженные в р. Молога).

Nuclear DNA analysis

Analysis of nuclear DNA at 14 microsatellite loci, as expected, did not reveal specific alleles characterizing this sterlet population (Table 9). All 14 loci are amplified, non-monomorphic, have from 4 to 22 alleles per locus and are characterized mainly by high observed heterozygosity (H_o). In more than 60% of loci (9 out of 14), the observed heterozygosity rate is greater than 0.5. This indicator is highest for the An 20 (0.871), AfuG112 (0.867) and Afu G41 (0.845) loci. The lowest heterozygty was detected for loci AoxD234 (0.149), AfuG67 (0.188), LS39 (0.281), AfuG51 (0.333) and AfuG63 (0.363). These same loci are also low-allelic (from 6 to 8 alleles per locus), except for the most multi-allelic locus AoxD234. The level of observed heterozygosity averaged 0.567 with a range from 0.149 (AoxD234) to 0.871 (An20).

Table 9. Characteristics of the studied microsatellite loci of the Mologa River sterlet.

Locus	Number of alleles per locus	Nucleotide pair range	Mass allele, nucleotide pairs (in parentheses – frequency of occurrence)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)
AoxD161	9	102–134	102 (0.52)	0.634	0.612
An20	10	145–181	177 (0.61)	0.871	0.870
AoxD165	13	164–202	176 (0.31)	0.574	0.525
AfuG51	8	232–268	252 (0.81)	0.333	0.321
AfuG41	16	197–257	229(0.20)	0.845	0.773
AoxD234	22	188–296	256(0.17)	0.149	0.157
AfuG112	19	195–291	207 (0.15) 215 (0.15) 223 (0.15)	0.867	0.871
AfuG174	6	139–163	147(0.9)	0.700	0.884
AfuG67	7	180–204	188(0.7)	0.188	0.197
AfuG63	6	104–152	132(0.9)	0.363	0.359
LS39	7	115–139	124(0.4) 127(0.5)	0.281	0.278
LS19	4	134–143	137(0.8)	0.537	0.572
Aox45	17	108–159	129(0.19)	0.796	0.861
LS68	16	177–253	189 (0.13) 201 (0.13) 209 (0.13) 213 (0.13)	0.794	0.868

Table 10. Values of expected and observed heterozygosity of Mologa River sterlet for all loci in different years of juvenile release

Year of release	H_o	H_e	F
2016	0.551	0.544	−0.049
2017	0.561	0.601	0.057
2019	0.587	0.601	0.035
Total	0.567	0.582	0.014

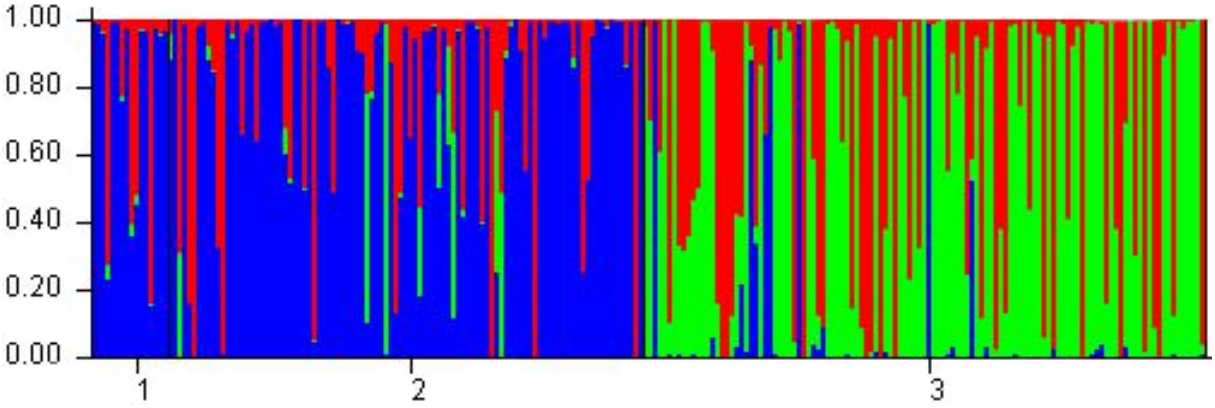

Fig. 3. Posterior probability of the assignment to the Mologa River sterlet clusters from releases of 2016 (1), 2017 (2) and 2019 (3).

The remaining loci show a high level of genetic diversity of the restored sterlet in the Mologa River, which contributes to better adaptation to the environment and ecological plasticity of the entire population.

Comparison of releases of different years according to these indicators demonstrated that in the releases of 2017 and 2019 the observed heterozygosity is less than expected, there is some deficiency of heterozygotes, and the Wright fixation index takes positive values (Table 10). In the 2016 edition, the observed heterozygosity value exceeded the theoretically expected one, and the Wright fixation index had a negative value. As with the results of mtDNA analysis, despite the small number of released individuals, the 2016 release was more strongly polymorphic compared to releases from other years.

Cluster analysis of nuclear DNA parameters to compare the heterogeneity of released sterlet from three different years of release gave a clear division into two groups (Fig. 3). The first group included releases from of 2016 and 2017. Both samples are represented by mixing two clusters (blue and red), and the 2019 sample is represented by mixing the other two clusters (green and red) to form the second group. At the same time, a small proportion of individuals according to their genetic profile corresponds to a different year of release. In the 2017 sample, one individual is a 100% fit, and 4 individuals – more than 50% fit (indicated in green in the “blue cluster”) with the cluster of the 2019 sample. At the same time, six individuals in 2019 (in blue in “green cluster") genetically belong to the sample of 2017. However, according to the size and weight characteristics and the age determined from the cuttings, they belong to a different year of release. In general, the presence of various clusters in the sterlet population of the Mologa River which is being restored shows the presence of genetic heterogeneity, which, of course, is much less than that of the Volga population as a whole, but sufficient for the survival of the local population of an individual river.

Conclusions

As a result of reacclimatization measures, sterlet have began to play a leading role in the ichthyocenosis of the open medial areas of the Mologa River. The proportion of sterlet in total catches in riverbed sections of the river in 2018–2022 varied within a range of 31.4–49.3% in abundance and 26.0–64.0% in biomass. The size, weight and age composition of the emerging population is currently determined by the number of juvenile sterlet released in individual years, and with the increase in the number of age groups in the population, the size and weight composition of the catches becomes more diverse. As in other water bodies, in the Mologa River the most intense linear growth of sterlet is observed in the first year of life, and at older ages the weight growth increases.

Mitochondrial DNA analysis revealed 23 different mtDNA haplotypes characteristic of the sterlet of the Volga basin. The overall relative haplotype diversity of juveniles released into the Mologa River is low (0.10), while in fish released into the watercourse in 2016, this value was noticeably higher (0.57). The mtDNA haplotypes of the developing sterlet population in the Mologa River belong to various haplogroups, which are mainly characteristic and abundant for the Volga River sterlet population. Analysis of nuclear DNA at 14 microsatellite loci in the Mologa River revealed no specific alleles that were not found in sterlet of the Volga River basin. The level of observed heterozygosity averaged 0.567 and

ranged from 0.149 (AoxD234) to 0.871 (An20). Based on the results of nuclear DNA analysis, significant differentiation was noted between the 2016–2017 releases and release in 2019. Consequently, the developing Mologa River sterlet population is characterized by lower genetic heterogeneity of individuals compared to natural populations from the Volga River basin.

To form a multi-age population structure of sterlet with a high number of each age group, annual releases of fingerlings of this species weighing at least 5 g in the amount of 170 thousand specimens annually are necessary. When introducing juvenile fish into a river in order to increase the genetic diversity of the population, to improve adaptation to habitat conditions and to increase ecological plasticity, it is recommended to use more genetically diverse broodstock.

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