

# GENETIC DIVERSITY OF EUROPEAN EEL *ANGUILLA ANGUILLA* (L.) IN LATVIAN LAKES

Jeļena Oreha\*, Linda Bankovska, Muza Kirjušina, Inese Gavarane

Oreha J., Bankovska L., Kirjušina M., Gavarane I. 2024. Genetic diversity of European eel *Anguilla Anguilla* (L.) in Latvian lakes. *Acta Biol. Univ. Daugavp.*, 2024(1): 67-80.

## Abstract

The number of European eel populations have decreased rapidly by over 95% since 1980 throughout its range, due to environmental changes, parasites, bacteria, pollution, ecological changes associated with global warming, loss of habitat, migration barriers, as well as anthropogenic factors – uncontrolled and unsustainable fishing. As a consequence, the European eel has been listed as critically endangered on the IUCN red list. The EU Commission proposed stopping eel fishing for six months in 2023. European eels play an important role in ecosystem dynamics. Eels have the ability to adapt to changing oxygen concentration, and tolerance of different ranges of water salinity. European eels have been used as a bioindicator species worldwide. In addition to a bioindicators species, European eels are a commercially important fish species. The European eel is the only eel species which inhabit in Latvia. Glass eels are mainly used for stocking in lakes and rivers in Latvia. Only four water bodies in Latvia are freely accessible to natural migration of eels. Little is known about the genetic diversity of European eels in Latvian lakes. Research about the genetic diversity of eel populations from waterbodies in Latvia started to develop during the last years and this genetic knowledge is necessary for eel resource management. The present study may provide additional data for further investigation of European eel population in Latvia. In this study eight microsatellite loci were used to investigate the genetic structure within and between samples in five Latvian lakes, namely Liepajas, Usmas, Kisezers, Aluksnes and Sivers. Allelic variation was different in all investigated eel samples; the observed and expected heterozygosity level was quite high. Bayesian-based STRUCTURE analysis suggested that there are three main genetic groups within our study area. The high values of genetic differentiation revealed in the present study are possibly the result of gene pool mixing after multiple restocking events and eel natural migration, where it still is possible.

Keywords: *Anguilla anguilla*, microsatellites, genetic diversity, genetic structure, restocking

\*Corresponding author: Jeļena Oreha. Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Parādes Str. 1A, Daugavpils, LV-5400, Latvia, Email: jelena.oreha@du.lv

Linda Bankovska. State Plant Protection Service of Latvia, National Phytosanitary Laboratory, Lielvārde street 36, Riga, LV-1006, Latvia

Muza Kirjušina. Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Parādes Str. 1A, Daugavpils, LV-5400, Latvia

*Inese Gavarane. Department of Biodiversity, Institute of Life Sciences and Technologies, Parādes Str. 1A, Daugavpils, LV-5400, Latvia*

## **INTRODUCTION**

The number of European eel populations have decreased rapidly by over 95% since 1980 throughout its range, due to environmental changes, parasites, bacteria, pollution, ecological changes associated with global warming, loss of habitat, migration barriers that limit the successful dispersion area of the species, as well as anthropogenic factors – uncontrolled and unsustainable fishing (Moriarty 1990, Dekker 2004, Moriarty 2012). As a consequence, the European eel has been listed as critically endangered on the IUCN red list (Freyhof & Kottelat 2013). A management plan for European eel, was approved in 2007, establishing measures for the recovery of the stock of European eel (Lin et al. 2001). This Regulation (EC) No 1100/2007 establishes a framework for the protection and sustainable use of the stock of European eel in Community waters and in coastal lagoons, in estuaries, and in rivers and communicating inland waters of Member States (Council Regulation 2007 (EC)). Since 2001, the International Council for the Exploration of the Sea (ICES) has recommended that eel catches be “reduced to the lowest possible level”. And in 2021 this was clarified to a recommendation that “there should be zero catches in all habitats in 2022”, including catches of glass eels for restocking and aquaculture. The EU Commission proposed stopping eel fishing for six months in 2023 (ICES Advice 2021).

European eels play an important role in ecosystem dynamics. Eels have the ability to adapt to changing oxygen concentration, and tolerance of different ranges of water salinity (Arleny et al. 2007). They are an essential part of the food chain. Feeding on prey fish eggs, they participate in the biological balance and they are used as food resources for other animals (Deelder 1984). European eels have high potential for bioaccumulation, making them suitable as environmental bioindicators.

According to several studies, for instance Dutil et al. 1985, Castonguay et al. 1989, Bruslé 1991, Robinet & Feunteun 2002, Arleny et al. 2007, European eels have been used as a bioindicator species worldwide to determine the presence of toxic substances that accumulate in the liver and muscles and this species can be used as an indicator of the presence of bacteria in the water (Callol et al. 2015). In addition to a bioindicators species, European eels are a commercially important fish species (Arai 2014).

One of the main aspects of European eel protection is conservation of genetic diversity. Genetic diversity determines the future sustainability, fitness rate and adaptation of populations (Maes & Volckaert 2007). Conservation of the genetic diversity of populations, provides potential to evolve in response to environmental changes. Low genetic diversity results in a number of problems - high susceptibility to parasites and disease, decreased sperm quality, reduced litter size, increasing juvenile mortality - that eventually can lead to extinction of populations (Reed & Frankham 2003, Furlan et al. 2012). One of the most easily applicable and versatile method for investigation of conservation genetics is using microsatellite markers, because they have a number of beneficial properties like high polymorphism, are codominant and, selectively neutral. Those markers provide important information on the genetic variation and genetic structure of investigated populations (Abdul-Muneer 2014).

The number of genetic investigations of European eel have increased in the last 25 years (Ragauskas & Butkauskas 2013, Jacobsen et al. 2014, Ragauskas et al. 2014, Ragauskas et al. 2017, Frankowski et al. 2019, Oreha et al. 2023 and others). Earlier studies supported the hypothesis of panmixia – absence of genetic structure (DeLigny & Pantelouris 1973, Lintas et al. 1998), but recent studies disprove this

theory, and reported evidence for significant structuring of European eel populations (Daemen et al. 2001, Maes & Volckaert 2002, Ragauskas & Butkauskas, 2013, Ragauskas et al. 2017). Ragauskas et al. (2014), investigated European eels from the Curonian Lagoon, Baltic sea and Gulf of Riga, and detected no significant genetic differentiation, however, the population genetic structure could be described as a genetic mosaic.

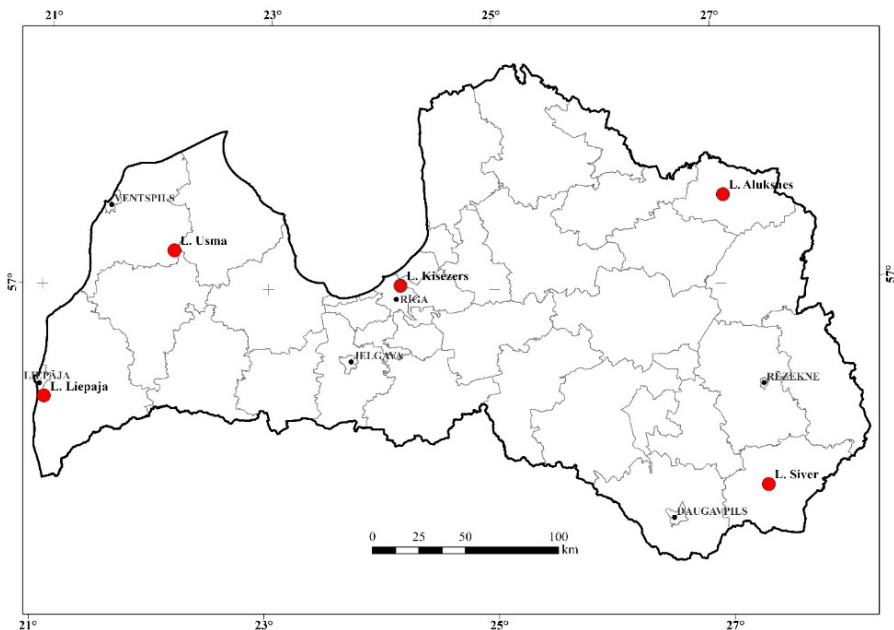
European eel is the only eel species which inhabit in Latvia (Aleksejevs & Birzaks 2011). Nevertheless, there is a lack of information about genetic diversity of European eel in Latvian lakes. Research about genetic diversity of eel population from waterbodies in Latvia has started only recently (Oreha et al. 2023), and this genetic knowledge is necessary for eel resource management. Glass eels are mainly used for stocking of lakes and rivers in Latvia (Shiao et al. 2006). Only four water bodies in Latvia are freely accessible to natural migration of eels (Aleksejevs & Birzaks 2011).

This study assessed the genetic diversity and structure of population of European eel from five lakes in different geographic locations in Latvia using microsatellite markers. This information can contribute to the establishment of a sustainable management plan for this endangered species as well as identifying perspectives for fisheries. The present study may provide additional data for further investigation of populations of European eels in Latvia.

## MATERIALS AND METHODS

### Sample Collection

Samples of fish tissue (skeletal muscles) preserved in 96.6% ethanol from fifty-seven freshwater eels were analysed from five Latvian lakes, namely Lake (hereafter L.) Liepajas, L. Usmas, L. Kisezers, L. Aluksnes and L. Sivers (Fig. 1, Tab. 1). Samples were used from the collection of preserved materials at Laboratory of Parasitology and Histology at Daugavpils University, Latvia. All samples were collected in 2014.



**Figure 1.** Locations of five sampling sites of European eel. Map author: M. Nitcis. Data from <https://data.gov.lv/lv>.

**Table 1.** Sampling sites, location and numbers of individuals of analysed eel *Anguilla anguilla*.

Sampling sites	Location	Number
L. Usma	57°10'49"N, 22°9'27"E	22
L. Liepaja	56°27'37"N, 21°3'14"E.	3
L. Kisezers	57°1'24"N, 24°10'3"E	10
L. Aluksnes	57°25'35.8"N, 27°03'18.7"E	2
L. Siver	56°01'08.0"N 27°19'54.6"E	20

### Microsatellite analysis

DNA was extracted from ethanol-preserved muscle tissue samples and purified using the DNeasy Blood & Tissue Kit (Qiagen, Germany). DNA quantity and quality were assessed by the spectrophotometer NanoDrop-1000 (Thermo-Scientific, USA). The extracted DNA was stored at -20°C until analysis. For the analysis, the DNA was diluted to a concentration of 10 ng/μL.

PCR reaction was carried out using eight microsatellite primer pairs described in Wielgloss et al. (2008) (Tab. 2). Microsatellite amplification was performed using the Veriti 96-Well Thermal Cycler. PCR (polymerase chain reaction) with fluorescently marked primers (the forward primer in each pair being labelled with the fluorescent label HEX or

FAM) was carried out in a final volume of 12 μL, containing 100 ng of DNA sample, 10mM Tris-HCl buffer with 50mM KCl, 1.5 mM MgCl<sub>2</sub>, 2mM dNTPs mix, 0.06 U/μL Taq DNA polymerase, 0.4 μmol/μL of each primer. The PCR thermal cycling program had an initial denaturation at 95 °C for 5 min, followed by 35 cycles with denaturation at 95 °C for 35 s, annealing at 55 °C for 35 s, and extension at 72 °C for 45 s, followed by a 10-min final extension at 72 °C, and cooling at 4 °C. Both positive and negative controls were used during PCR amplification. PCR products were separated on 3130xl Genetic Analyzer (Applied Biosystem, USA) using GeneScan ROX 500 size standard (Applied Biosystem, USA), and alleles were scored with GeneMapper 3.7 software (Applied Biosystem, USA).

**Table 2.** Characterization of microsatellite markers used in research (Wielgloss et al. 2008).

Primer ID	Core motif (N <sub>x</sub> )	Primer sequence (5' - 3')	Label dye
<i>AangCT53</i>	(CT) <sub>17</sub>	F:GGTGACACACAGTCTCTTTGG R: ACAATGCATGTGCCTGAATG	HEX
<i>AangCT59</i>	(CT) <sub>18</sub>	F: GCAACCCTTTCTCACTCCAC R: CTCCTGCGCAAACAAGAAG	HEX
<i>AangCT67</i>	(TG) <sub>6</sub> N <sub>8</sub> (TG) <sub>5</sub> TA(TG) <sub>4</sub> (AG) <sub>2</sub> (TG) <sub>7</sub>	F: GACAGACGGACAGACAATGC R: GGTGGTGAATTTGGTCCTG	HEX
<i>AangCT68</i>	(AG) <sub>22</sub>	F: CCAGGCAATTGCTTTCTCAC R: TCATTGTGTTTGGCACTTCC	FAM
<i>AangCT76</i>	(TC) <sub>17</sub> (AC) <sub>13</sub>	F: CTTGAGCTTGGAGGTGTTCC R: CTGTGCAGGAGTCACGTTTC	FAM
<i>AangCT77</i>	(CT) <sub>46</sub> GT(CT) <sub>3</sub>	F: CCTGATGTTTTAGCGTTTG R: GAAAGTGGGCTCAGTTCTGG	FAM
<i>AangCT89</i>	(CT) <sub>15</sub> (TC) <sub>3</sub> (CT) <sub>4</sub>	F: AACCAGCGAGATGATGATTG R: AGAGCGTGAAGCCTTTTGAC	HEX
<i>AangCA80</i>	(TG) <sub>4</sub> CG(TG) <sub>14</sub>	F: TTCCTCTGGTCTTTCACACG R: AGCTGGAGGACACGGATG	FAM

T<sub>m</sub> melting temperature, T<sub>a</sub> annealing temperature

## Data analysis

The Micro-Checker 2.2.3 software was used to check the data for errors and to identify null alleles and other genotyping errors: short allele dominance (large allele dropout) and scoring of stutter peaks (Van Oosterhout et al. 2004).

The following standard indices of genetic variation were calculated: number and frequency of alleles per locus, occurrence of private alleles in each population, and observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity levels at each locus. The differences and statistical ( $\chi^2$ ) significance between observed and expected heterozygosity values were calculated using POPGENE 1.32 (Yeh et al. 1999) and GenAlEx 6.41 software (Peakall & Smouse 2006). Richness of alleles and private alleles in each population were determined, accounting for differences in the size of samples. The rarefaction procedure was used for the smallest sample size as implemented in the software HP-RARE 1.0 (Kalinowski 2005).

In order to estimate and visualize the genetic structure and differentiation of the studied European eel populations the software STRUCTURE 2.3 (Hubisz et al. 2009) and POPHELPER Structure Web App v1.0.10. (Francis 2017) were used. A model assuming admixture and correlated allele frequencies between  $K$  populations (Burn-ins of 100,000 replications and 300,000 Markov chain Monte Carlo (MCMC) replicates) was used. Sampling locations were used as prior information to assist the structuring (the LOCPRIOR model) as recommended for weak signals of structuring (Hubisz et al. 2009). Values of  $K$  between one and five were tested, running STRUCTURE ten times for each  $K$  and using Evanno's  $\Delta K$  method to determine the most suitable number of clusters (Evanno et al. 2005). The most likely (highest  $\ln \Pr(X|K)$ ) grouping was visualized using POPHELPER Structure Web App v1.0.10. (Francis 2017). The genetic relatedness of the populations was estimated with Nei's index of genetic distance

(Nei et al. 1983) using the software Populations 1.2.32 (Langella 2005). The corresponding dendrogram was created according to the UPGMA method using the software TreeView (Page 1996). Genetic divergence was estimated using pairwise  $F_{ST}$  values (Weir & Cockerham 1984) with GenAlEx 6.41 software (Peakall & Smouse 2006). The  $P$ -values for the pairwise  $F_{ST}$  values were corrected for multiple comparisons using the Bonferroni correction (BFC) following Rice (1989).

## RESULTS

### Genetic variation

The parameters of genetic variation in the studied European eel population in Latvian lakes are shown in Tab 3. A total of 111 alleles from among eight microsatellite loci were determined in five studied samples. Allele number in different samples varied from 21 to 81. Higher allele number were revealed in samples from Lake Sivers and Lake Usmas (81 and 78 accordingly). The mean number of alleles per locus or allelic richness ( $N_{RA}$ ) varied from 2.63 (L. Aluksne) to 3.28 (L. Usmas). The mean number of private alleles ( $N_{RPA}$ ) varied from 0.63 (L. Aluksne) to 1.00 (L. Sivers).

**Table 3.** Summary of genetic statistics of the studied eel samples included in the study.

Samples	$N_A$	$N_{RA}$	$N_{RPA}$	$H_o$	$H_e$
L. Aluksne	21	2.63	0.63	0.563	0.563
L. Kisezers	65	3.16	0.91	0.638	0.792
L. Liepajas	26	2.58	0.81	0.500	0.549
L. Sivers	81	3.24	1	0.625	0.833
L. Usmas	78	3.28	0.93	0.631	0.846

$N_A$  – total number of detected alleles,  $N_{RA}$  – mean allelic richness,  $N_{RPA}$  – private allelic richness,  $H_o$  – observed heterozygosity,  $H_e$  – expected heterozygosity

The observed and expected heterozygosity for all samples over the eight microsatellite loci varied from 0.500 (L. Liepajas) to 0.638 (L. Kisezers) and from 0.549 (L. Liepajas) to 0.846 (L. Usmas), respectively (Tab. 3). The individual locus tests (for each sample) displayed that twelve cases out of 40 had significant deviations of genotype frequencies from Hardy - Weinberg equilibrium (HWE) before BFCs. A significant deviation from HWE was revealed for L. Usmas at five loci, for L. Siviers at four loci and for L. Kisezers for three loci. After BFCs significant deviations of genotype frequencies from HWE were revealed at five loci in samples from two

different lakes. That is, heterozygosity deficits were detected at L. Siviers population at locus *AangCT89* and in L. Usmas at loci *AangCT53*, *AangCT67*, *AangCT76*, *AangCT89*, which was indicated by Micro-Checker as caused by possible presence of null alleles.

Altogether the number of alleles at each microsatellite locus was different. The greatest number of alleles (27) was found at locus *AangCT67*. The minimum numbers of alleles (9) were found at loci *AangCT53*, *AangCT77* and *AangCT89* (data not shown). Tab. 4 shows details of analysed microsatellite loci for investigated samples.

**Table 4.** The standard parameters of genetic variation at eight microsatellite loci in eel population in five Latvian lakes.

Sample	<i>AangCT53</i>	<i>AangCT59</i>	<i>AangCT67</i>	<i>AangCT68</i>	<i>AangCT76</i>	<i>AangCT77</i>	<i>AangCT89</i>	<i>AangCT80</i>	
L. Aluksnes	R	74 - 86	83 - 87	186 - 200	173 - 181	200 - 206	105 - 115	210 - 216	90 - 96
	N	2	2	2	2	2	2	2	2
	N <sub>A</sub>	3	3	2	3	3	2	2	3
	Ho	1.000	0.500	0.000	1.000	0.500	0.000	0.500	1.000
	He	0.625	0.625	0.500	0.625	0.625	0.500	0.375	0.625
L. Kisezers	R	78 - 90	77 - 91	178 - 230	171 - 183	200 - 220	103 - 217	208 - 220	74 - 108
	N	10	10	10	10	10	10	10	10
	N <sub>A</sub>	6	8	12	7	7	8	4	13
	Ho	0.700	0.600	0.800	0.600	0.500	0.800	0.200	0.900
	He	0.695	0.840	0.885	0.815	0.735	0.795	0.665	0.905
L. Liepajas	R	78	83 - 87	158 - 234	185 - 189	206 - 220	105 - 113	208 - 220	86 - 102
	N	3	3	3	3	3	3	3	3
	N <sub>A</sub>	1	2	5	2	4	5	3	4
	Ho	0.000	0.667	0.667	0.000	0.667	1.000	0.333	0.667
	He	0.000	0.444	0.778	0.444	0.667	0.778	0.611	0.667
L. Siviers	R	74 - 94	71 - 91	170 - 214	171 - 193	200 - 222	105 - 117	200 - 218	78 - 110
	N	20	20	20	20	20	20	20	20
	N <sub>A</sub>	8	11	12	10	9	7	8	16
	Ho	0.600	0.550	0.600	0.650	0.600	0.700	0.350	0.950
	He	0.761	0.785	0.874	0.863	0.809	0.854	0.814	0.908
L. Usmas	R	78 - 90	73 - 91	168 - 234	171 - 191	200 - 222	103 - 117	208 - 220	80 - 104
	N	22	22	22	22	22	22	22	22
	N <sub>A</sub>	6	8	17	11	9	8	7	12
	Ho	0.273	0.591	0.591	0.773	0.727	0.955	0.273	0.818
	He	0.761	0.859	0.936	0.863	0.861	0.857	0.793	0.888

N - number of samples, N<sub>A</sub> - number of alleles at each locus, Ho - observed heterozygosity, He - expected heterozygosity.

### Population structure and spatial variation

The pairwise  $F_{ST}$  estimates of genetic differentiation between the studied European eel samples in five Latvian lakes are displayed in Tab. 5. The pair L. Sivers – L. Usmas displayed the smallest differentiation (0.009,  $p \leq 0.05$ ), whereas the pair L. Liepajas – L. Alūksnes had the highest  $F_{ST}$  value (0.140,  $p \leq 0.05$ ). Moderate values of genetic

differentiation were revealed for L. Liepajas – L. Alūksnes, L. Liepajas – L. Kisezers, L. Liepajas – L. Sivers, and L. Liepajas – L. Usmas pairs (0.140, 0.070, 0.075 and 0.078 accordingly). For all other pairs, little genetic differentiation was shown: the  $F_{ST}$  values varied from 0.009 to 0.045 ( $p \leq 0.001$ ) (Tab. 5).

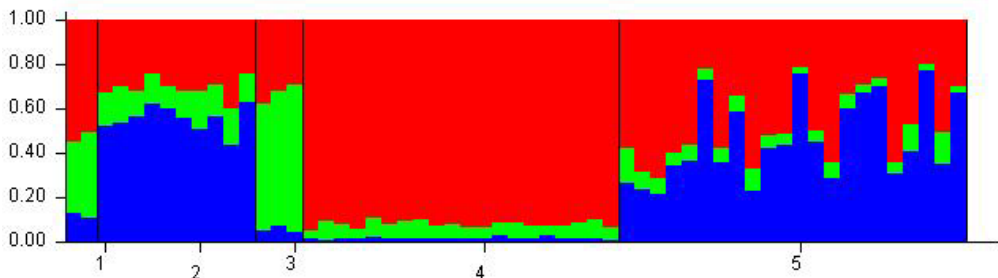
**Table 5.**  $F_{ST}$  values obtained during the pair comparison of European eel samples from the five Latvian lakes.

	L. Aluksnes	L. Kisezers	L. Liepajas	L. Sivers	L. Usmas
L. Aluksnes		*	*	ns	ns
L. Kisezers	0.037		**	**	**
L. Liepajas	0.140	0.070		**	**
L. Sivers	0.035	0.034	0.075		*
L. Usmas	0.045	0.017	0.078	0.009	

ns - not significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ; a value lying in the range between 0 and 0.05 indicates little genetic differentiation; a value between 0.05 and 0.15, moderate differentiation; a value between 0.15 and 0.25 high differentiation; and values above 0.25, very high genetic differentiation (Wright 1978, Hartl & Clark 2007).

Bayesian clustering indicated that the most likely number of genetic clusters was 3. ( $K = 3$ , Fig. 2), placing L. Kisezers and L. Usmas

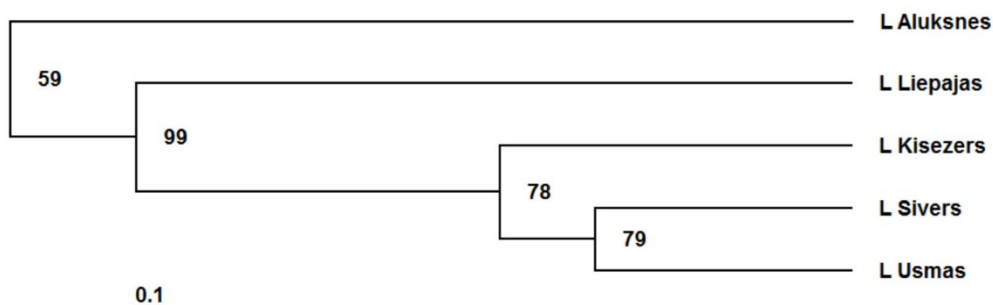
in the first cluster; L. Aluksnes and L. Liepajas in the second cluster; and L. Sivers in the third cluster.



**Figure 2.** Bayesian clustering of all individuals using STRUCTURE (Hubisz et al., 2009) assuming three genetic clusters of individuals ( $K = 3$ ) (1 – L. Aluksnes, 2 – L. Kisezers, 3 – L. Liepajas, 4 – L. Sivers, 5 – L. Usmas). In the STRUCTURE analysis black lines separate individuals from different sampling sites and each individual is represented by a thin vertical line, which is partitioned into K-colored segments representing individual's estimated membership fractions in K clusters.

In order to evaluate genetic distances between eels from investigated lakes a dendrogram based on pairwise Nei's genetic distances (1983) was constructed (Fig. 3). Eels from L. Aluksnes was completely separated from eels in other studied lakes. Eels from L. Liepajas,

Kisezers, Usmas and Sivers grouped into one cluster, and eels of L. Kisezers, Usmas and Sivers formed a sub cluster. of The eels from L. Sivers and Usmas were most closely related.



**Figure 3.** Genetic differentiation of five studied samples from Latvian lakes as revealed by UPGMA tree using Nei et al. (1983) genetic distance (Da). Bootstrap support >50 is shown next to the branching points.

## DISCUSSION

Understanding the genetic diversity of fish populations is critical to protecting rare communities and conserving unique local populations. Estimating the genetic structure of populations and identifying the causes of genetic differentiation and the factors that contribute to variation between and within populations is essential to understanding adaptation and is, therefore, a major goal of population and conservation genetics. The evaluation of genetic structure of the European eel population in waterbodies in Baltic Lakeland as an indicator to the general biodiversity and a tool for species conservation has only recently started (Oreha et al. 2023).

This study used the microsatellite markers *AangCT53*, *AangCT59*, *AangCT67*, *AangCT68*, *AangCT76*, *AangCT77*, *AangCT89* and *AangCA80*, which were developed specifically for *Anguilla anguilla* and showed successful cross-species amplification for nine different anguillid eel species (Wiegloss et al. 2008). Our detected allele sizes across all investigated loci did not

exceed the previously reported ranges (Wiegloss et al. 2008). However, in this study, a narrower size range for some loci was revealed. For instance, in locus *AangCT67* and *AangCT80* the smallest size of alleles in our study were 158bp and 78bp compared with 124bp and 74bp reported by Wiegloss et al. (2008). The allelic size range for locus *AangCT76* in our study was between 200bp and 220bp compared with 196 – 232bp reported by the same authors. This study revealed a higher number of alleles in as the loci *AangCT67* and *AangCT80* (27 and 18 in the present study compared with the previously described 19 and 13). In all other investigated loci, the number of alleles was only one allele more (*AangCT53*, *AangCT68* and *AangCT76*) or less (*AangCT77*, *AangCT89* and *AangCT59*) compared with the previously described results. This indicate that the eels in studied Latvian lakes differ from eel stock from L. Constance in Germany (Wiegloss et al. 2014).

The genetic diversity parameters of eels from L. Kisezers, Sivers and Usmas were similar, and eels from L. Aluksnes and L. Liepajas had smaller values. Most likely this is related to the



size of the analysed samples. The level of heterozygosity, as it is shown in Tab. 4, was quite high for all studied samples and significant deficit of heterozygosity after BFCs was revealed in only five out of 40 cases. This can be explained in different ways. For instance, the reason for that may be the presence of nonamplified (null) alleles. The presence of null alleles in our study was identified in all loci with significant heterozygosity deficit (at L. Sivers population at locus *AangCT89* and in L. Usmas at loci *AangCT53*, *AangCT67*, *AangCT76*, *AangCT89*). Previously, possibility of null allele was described to be in two of in present study chosen markers (*AangCT67* and *AangCT77*). Thus, the presence of null alleles can explain heterozygosity deficit in two loci only in L. Usmas. One more reason could be the use of microsatellite primers developed for a related species can result in non-amplification in the target species, as it was reported earlier for coregonid species (Rogers et al. 2007, Oreha & Škute 2022). However, in the present study the markers used were developed specifically for European eel (*Anguilla anguilla*). Another possibility is consequences of the Wahlund effect. The Wahlund effect is the lack of heterozygous genotypes in large populations as a result of the breakdown of panmixia, due to the fact that large populations consist of a few small subpopulations with insufficient numbers of individuals that are constantly inbreeding (Dharmarajan et al. 2013). Lack of heterozygous genotypes in eel population in this study possibly is a result of the intensive restocking program. The restocking plans were conducted in Latvia for a long time (Bajinskis et al. 2020). In studies of the parasite *Anguillicoloides crassus* in the European eel, Wiegoss et al (2010) investigated sensitivity of parasites to different immigration rates into local *A. anguilla* stocks for two separated river systems, which inferred that under natural recruitment, nematode samples meet Hardy-Weinberg expectations for a single panmictic population. However, studies have shown that

a strong Wahlund effect is most likely due to very recent population mixing under frequent restocking of young *A. anguilla* (Wiegoss et al. 2010).

The Bayesian clustering (Fig. 2) showed that eel individuals could be partitioned into three distinct genetic groups (K=3). Possibly these groups reflect migration likelihood. For instance, native eel migration into and out of Lake Sivers is fully restricted, because of many watermill and HEP dams in the possible migration path. L. Usmas and L. Kisezers are not fully restricted for natural eel migration, because the possible migration path is shorter and there are not so many obstacles (Bajinskis et al. 2020).

The UPGMA tree (Fig. 3) based on Nei's (1983) genetic distances shows a little bit different grouping. For instance, eels from L. Sivers and Usmas were closely related. Eels from L. Aluksnes were completely separated from the eels from other lakes. The likely reason for that is presence in the sample from L. Aluksnes of eel individual, which was identified in previous research as American eel (*Anguilla rostrata*) using mtDNA markers. This was divided as anthropogenic invasion because natural migration for eels in L. Aluksnes is restricted (Oreha et al. 2023). Correlation between genetic and geographic distances was not revealed ( $r = 0.058$ ,  $p = 0.040$ ). However, for natural migrating eels from Mediterranean Sea, Baltic Sea and North Sea, the correlation between genetic and geographic distance was highly significant ( $r = 0.462$  and  $p, 0.007$ ) (Wirth & Bernatchez 2001).

The pairwise  $F_{ST}$  values between eel samples from the five Latvian lakes reflect a quite high, mostly moderate genetic differentiation level (Tab. 5), which is not inherent for naturally migrated eels. In this study revealed  $F_{ST}$  values is much higher than in previous studies, where European eel populations from a number of coastal European countries were, the  $F_{ST}$  varied from 0.0004 to 0.084 (Dannewitz et al.

2005, Palm et al. 2009). Previous studies indicate that migrating eels have weak genetic structure. Comparing of southern eel populations (Mediterranean Sea) versus northern populations (Baltic and North Sea) Wirth and Bernatchez (2001) revealed  $F_{ST}$  values ranging from 0.003 to 0.005. Similar values were described in other studies of eel populations (Daemen et al. 2001). Als et al (2011) shows that one age eels from different spawning sites in Sargasso Sea have  $F_{ST} = 0.0011$ , but  $F_{ST}$  among glass eels from geographical locations ranging from Iceland in the north to Morocco in the south was  $F_{ST} = 0.00024$ . The higher values of genetic differentiation revealed in the present study compared to previous studies are possibly the result of gene pool mixing after multiple restocking events and eel natural migration, where it still is possible.

## CONCLUSIONS

The level of genetic variability differs among studied eel samples. The differences may be caused by such processes as genetic flow and genetic drift, which will influence allele frequencies in various ways. Changes may be observed on the level of population genetic variability and genetic structure as a result of regular, quite intensive restocking until recent time, invasion of parasites and the impact of intensive anthropogenic influence by the fishing. Our present results could be useful in the design and monitoring of conservation programs of eel in Latvian lakes.

## ACKNOWLEDGEMENTS

We are grateful to two anonymous reviewers for their constructive criticism that helped to improve the manuscript.

## REFERENCES

- Abdul-Muneer P.M. 2014. Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies. *Genetics Research International* (6): 1–11. <https://doi.org/10.1155/2014/691759>
- Aleksejevs E., Birzaks J. 2011. Long-term Changes in the Ichthyofauna of Latvia's Inland Waters. *Scientific Journal of Riga Technical University* 7(1): 9–18.
- Als T.D., Hansen M.M., Maes G.E., Castonguay M., Riemann L., Aarestrup K.I.M., Munk P., Sparholt H., Hanel R., Bernatchez L. 2011. All roads lead to home: panmixia of European eel in the Sargasso Sea. *Molecular Ecology* 20(7): 1333–1346. <https://doi.org/10.1111/j.1365-294X.2011.05011.x>
- Arai T. 2014. Do we protect freshwater eels or do we drive them to extinction? *SpringerPlus* 3: 534. <https://doi.org/10.1186/2193-1801-3-534>
- Arleny I., Tabouret H., Rodriguez-Gonzalez P., Bareille G., Donard O.F.X., Amouroux D. 2007. Methylmercury bioconcentration in muscle tissue of the European eel (*Anguilla anguilla*) from the Adour estuary (Bay of Biscay, France). *Marine Pollution Bulletin* 54: 1031–1071. <https://doi.org/10.1016/j.marpolbul.2007.04.004>
- Bajinskis J., Aleksejevs E., Ozoliņa Z., Začs D. 2020. The composition and quality of European eel *Anguilla Anguilla* stock in Lake Rāznas. *Environmental and Experimental Biology* 18: 51–52. <https://doi.org/10.22364/ceb.18.05>
- Bruslé J. 1991. The eel (*Anguilla* sp.) and organic chemical pollutants. *Sci Total Environ* 102: 1–19. [https://doi.org/10.1016/0048-9697\(91\)90305-X](https://doi.org/10.1016/0048-9697(91)90305-X)

- Callol A., Pajuelo D., Ebbesson L., Teles M., MacKenzie S., Amaro C. 2015. Early steps in the European eel (*Anguilla anguilla*) - *Vibrio vulnificus* interaction in the gills: Role of the RtxA13 toxin. *Fish & Shellfish Immunology* 43: 502–509. <https://doi.org/10.1016/j.fsi.2015.01.009>
- Castonguay M., Dutil J.D., Desjardins C. 1989. Distinction between American eels (*Anguilla rostrata*) of different geographic origins on the basis of their organochlorine contaminant levels. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 836–843. <https://doi.org/10.1139/f89-105>
- Council Regulation (EC) No. 1100/2007, (2007.09.18.) European Union regulation. *The Official Journal of the European Union* 248/17. <http://data.europa.eu/eli/reg/2007/1100/oj>
- Daemen E., Cross T., Olleier F., Volckaert F.A.M. 2001. Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. *Marine Biology* 139: 755–764. <https://doi.org/10.1007/s002270100616>
- Dannewitz J., Maes G.E., Johansson L., Wickström H., Volckaert F.A.M., Jarvi T. 2005. Panmixia in European eel: a matter of time. *Proceedings of the Royal Society B* 272: 1129–1137. <https://doi.org/10.1098/rspb.2005.3064>
- Deelder C.L. Food and Agriculture Organization of the United Nations. 1984. Synopsis of biological data on the eel, *Anguilla, anguilla* (Linnaeus, 1758) / prepared by C.L. Deelder. Rome: Food and Agriculture Organization of the United Nations.
- Dekker W. 2004. Slipping through our hands. Population dynamics of the European eel. PhD-thesis, University of Amsterdam 7 - 181.
- DeLigny W., Pantelouris E.M. 1973. Origin of the European eel. *Nature* 246: 518–519. <https://doi.org/10.1038/246518a0>
- Dharmarajan G., Beatty W.S., Rhodes, O.E. 2013. Heterozygote deficiencies caused by a Wahlund effect: Dispelling unfounded expectations. *Journal of Wildlife Management* 77: 226–234. <https://doi.org/10.1002/jwmg.458>
- Dutil J.D., Legare B., Desjardins C. 1985. Discrimination d'un stock de poisson, l'Anguille (*Anguilla rostrata*), basée sur la présence d'un produit chimique de synthèse, le mirex (Discrimination of a fish stock, *Anguilla rostrata*, on the basis of the presence of synthetic chemical product, the mirex). *Canadian Journal of Fisheries and Aquatic Sciences* 42 (3): 455–458.
- Evanno G., Regnaut S., Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology* 14: 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Francis R. M. 2017. “pophelper: an R package and web app to analyse and visualize population structure”. *Molecular Ecology Resources* (17): 27–32. <https://doi.org/10.1111/1755-0998.12509>
- Frankowski J., Hedeholm R., Moeller P.R., Lindner I., Magnussen E. Sanchez M. Bastrop R. 2019. Hybrid ancestry of Greenlandic Nimeriaq (*Anguilla anguilla* x *A. rostrata*). Poster presentation in Náttúruvísindadeildin, Faculty of Science and Technology.
- Freyhof J., Kottelat M. 2013 *Anguilla anguilla*. In IUCN 2013 IUCN Red List of Threatened Species. <http://www.iucnredlist.org>
- Furlan E., Stoklosa J., Griffiths J., Gust N., Ellis R., Huggins R.M., Weeks A.R. 2012.

- Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. *Ecology and Evolution* 195: 844–857. <https://doi.org/10.1002/ece3.195>
- Hartl D.L., Clark A.G. 2007. Principles of Population Genetics. *Écoscience* 14(4): 544–545. [https://doi.org/10.2980/1195-6860\(2007\)14\[544b:POPG\]2.0.CO;2](https://doi.org/10.2980/1195-6860(2007)14[544b:POPG]2.0.CO;2)
- Hubisz M.J., Falush D., Stephens M., Pritchard A.J. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9(5): 1322–1332. <https://doi.org/10.1111/j.1755-0998.2009.02591.x>
- ICES Advice 2021. ICES Advice Publications. 2022 Collection. <https://doi.org/10.17895/ices.pub.c.5796932.v28>
- Jacobsen M.W., Pujolar J.M., Gilbert M.T., Moreno-Mayar J.V., Bernatchez L., Als T.D., Lobon-Cervia J. Hansen M.M. 2014. Speciation and demographic history of Atlantic eels (*Anguilla anguilla* and *A. rostrata*) revealed by mitogenome sequencing. *Heredity* 113: 432–442. <https://doi.org/10.1038/hdy.2014.44>
- Kalinowski S.T. 2005. HP-RARE: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes* 5: 187–189. <https://doi.org/10.1111/j.1471-8286.2004.00845.x>
- Langella O. 2005. Populations 1.2.32: Population genetic software (individuals or populations distances, phylogenetic trees). Accessible online: <http://bioinformatics.org/~tryphon/populations/>
- Lin Y.S., Poh Y.P., Tzeng C.S. 2001. A phylogeny of freshwater eels inferred from mitochondrial genes. *Molecular Phylogenetics and Evolution* 20: 252–261. <https://doi.org/10.1006/mpev.2001.0969>
- Lintas C., Hirano J., Archer S. 1998. Genetic variation of the European eel (*Anguilla anguilla*). *Molecular Marine Biology and Biotechnology* 7: 263–269.
- Maes G.E., Volckaert F.A.M. 2002. Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). *Biological Journal of the Linnean Society* 77: 509–521. <https://doi.org/10.1046/j.1095-8312.2002.00124.x>
- Maes G.E., Volckaert F.A.M. 2007. Challenges for genetic research in European eel management. *Marine Science* 64: 1463–1471. <https://doi.org/10.1093/icesjms/fsm108>
- Moriarty C. 1990. European catches of Elver of 1928–1988. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 75: 701–706. <https://doi.org/10.1002/iroh.19900750603>
- Moriarty C. 2012. The decline in catches of European elver 1980 – 1992. *Fisheries & Aquatic Life* 20: 215–217.
- Nei M., Tajima F., Tateno Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *Journal of Molecular Evolution* 19: 153–170. <https://doi.org/10.1007/BF02300753>
- Oreha J., Morozova A., Garkajs A., Kirjušina M., Škute N. 2023. Genetic Diversity and Population Structure of the European Eel (*Anguilla anguilla*) in Baltic Lakeland. *Environment. Technology. Resources* 1: 161–165. <https://doi.org/10.17770/etr2023vol1.7297>
- Oreha J., Škute N. 2022. Current genetic structure of European vendace *Coregonus albula* (L.) populations in Latvia after multiple past translocations. *Animal*

- Biodiversity and Conservation* 45: 161–173. <https://doi.org/10.32800/abc.2022.45.0161>
- Page R.D. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12(4): 357–358. <https://doi.org/10.1093/bioinformatics/12.4.357>
- Palm S., Dannewitz J., Prestegard T., Wickstrom H. 2009. Panmixia in European eel revisited: no genetic difference between maturing adults from southern and northern Europe. *Heredity* 103: 82–89. <https://doi.org/10.1038/hdy.2009.51>
- Peakall R., Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6(1): 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Ragauskas A., Butkauskas D. 2013. The formation of the population genetic structure of the European eel *Anguilla anguilla* (L.): a short review. *Ekologija* 59(4): 143–154. <http://dx.doi.org/10.6001/ekologija.v59i4.2821>
- Ragauskas A., Butkauskas D., Bianchini M. 2017. Distinct matriline in the panmictic population of the European eel *Anguilla anguilla*? *Aquatic Living Resources* 30: 12. <https://doi.org/10.1051/alr/2017021>
- Ragauskas A., Butkauskas D., Sruoga A., Kesminas V., Rashal I., Tzeng W.N. 2014. Analysis of the genetic structure of the European eel *Anguilla anguilla* using the mtDNA D-loop region molecular marker. *Fisheries Science* 80: 463–474. <https://doi.org/10.1007/s12562-014-0714-1>
- Reed D.H., Frankham R. 2003. Correlation between Fitness and Genetic Diversity. *Conservation Biology* 17(1): 230–237. <http://www.jstor.org/stable/3095289>
- Rice W.R. 1989. Analysing tables of statistical tests. *Evolution* 43: 223–225. <https://doi.org/10.1111/j.1558-5646.1989.tb04220.x>
- Robinet T., Feunteun E. 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* 11: 265–277. <https://doi.org/10.1023/A:1016352305382>
- Rogers S.M., Isabel N., Bernatchez L. 2007. Linkage Maps of the dwarf and Normal Lake Whitefish (*Coregonus clupeaformis*) Species Complex and Their Hybrids Reveal the Genetic Architecture of Population Divergence. *Genetics* 175: 375–398. <https://doi.org/10.1534/genetics.106.061457>
- Shiao J.C., Ložys L., Iizuka Y., Tzeng W.N. 2006. Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios. *Journal of Fish Biology* 69: 749–769. <https://doi.org/10.1111/j.1095-8649.2006.01147.x>
- Van Oosterhout C., Hutchinson W.F., Wills D.P.M., Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4(3): 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- Weir B.S., Cockerham C.C. 1984. Estimating F–Statistics for the Analysis of Population–Structure. *Evolution* 38(6): 1358–1370. <https://doi.org/10.2307/2408641>
- Wielgloss S., Wirth T., Meyer A. 2008. Isolation and characterization of 12 dinucleotide microsatellites in the European eel, *Anguilla anguilla* L., and tests of Amplification in other species of

- eel. *Molecular ecology Resources* (8): 1382–1385. <https://doi.org/10.1111/j.1755-0998.2008.02185.x>
- Wielgoss S., Gilabert A., Meyer A., Wirth T. 2014. Introgressive hybridization and latitudinal admixture clines in North Atlantic eels. *BMC Evolutionary Biology* (14): 61. <https://doi.org/10.1186/1471-2148-14-61>
- Wielgoss S., Hollandt F., Wirth T., Meyer A. 2010. Genetic signatures in an invasive parasite of *Anguilla anguilla* correlate with differential stock management. *Journal of Fish Biology* 77(1): 191–210. <https://doi.org/10.1111/j.1095-8649.2010.02670.x>
- Wirth T., Bernatchez L. 2001. Genetic evidence against panmixia in the European eel. *Nature* 409(6823): 1037–1040. <https://doi.org/10.1038/35059079>
- Wright S. 1978. *Evolution and the Genetics of Population, Variability within and among Natural Populations*. University of Chicago Press, Chicago.
- Yeh F.C., Yang R.C., Boyle T. 1999. POPGENE version 1.31. Microsoft Windows-based Freeware for Population Genetics Software.

Received: 30.06.2024.

Accepted: 01.08.2024.