

INVESTIGATIONS INTO GENETIC DIVERSITY OF THE PERCH INHABITING IGNALINA NUCLEAR POWER PLANT COOLER AND OTHER INLAND WATER BODIES OF LITHUANIA ON THE BASIS OF MtDNA ANALYSIS

Dalius Butkauskas¹, Adomas Ragauskas¹, Aniolas Srunga², Vytautas Kesminas¹, Linas Ložys¹, Isaak Rashal³, Wann-Nian Tzeng⁴, Męcislovas Žalakevičius¹

¹Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania, dalius@ekoi.lt

²Vytautas Magnus University, K. Donelaičio 58, LT-44248 Kaunas, Lithuania

³Institute of Biology, University of Latvia, Miera 3, LV-2169, Salaspils, Latvia

⁴Department of Life Science and Institute of Fisheries Science, National Taiwan University
Taipei, Taiwan 106, ROC

Abstract. In order to collect information about the impact of Nuclear Power Plants (NPP) on the formation of the genetic structure of the population of commercially exploited fish species, our research group initiated an investigation into the genetic diversity of a rapidly changing mtDNA D-loop region of the perch (*Perca fluviatilis*). The perch is a naturally distributed species common in the inland water bodies of Lithuania, so it was chosen as a model species for the current study. The genetic diversity of the perch populations inhabiting the Lake Drūkšiai, the Lake Plateliai, the Lake Metelys and two major Lithuanian rivers Nemunas and Neris was studied on the basis of detection and comparison of 99 sequences of mtDNA D-loop region. Fifteen different haplotypes consisting of 389–400 bp were identified. Eight of them were not observed earlier in other locations in Europe; four new haplotypes were detected in Lake Drūkšiai only. All pairwise comparisons indicated that the perch population inhabiting the Lake Drūkšiai, which is used as cooler of Ignalina Nuclear Power Plant, was significantly ($P < 0.05$) genetically different from other perch populations studied in Lithuania. The data obtained formed background information to be used to investigate the possible impact of NPP on the formation of and changes in the genetic structure of the perch populations.

Keywords: *Perca fluviatilis*, Model species, D-loop, NPP, Lake Drūkšiai, Lithuania.

EŠERIŲ, GYVENANČIŲ IGNALINOS ATOMINĖS ELEKTRINĖS AUŠINTUVE IR KITUOSE LIETUVOS VIDAUS VANDENS TELKINIUOSE, GENETINĖS ĮVAIROVĖS TYRIMAI REMIANTIS mtDNR ANALIZE

Dalius Butkauskas¹, Adomas Ragauskas¹, Aniolas Srunga², Vytautas Kesminas¹, Linas Ložys¹, Isaak Rashal³, Wann-Nian Tzeng⁴, Męcislovas Žalakevičius¹

¹Gamtos tyrimų centras, Akademijos g. 2, LT-08412 Vilnius; el. paštas: dalius@ekoi.lt

²Vytauto Didžiojo universitetas, K. Donelaičio g. 58, LT-44248 Kaunas

³Biologijos institutas, Latvijos universitetas, Miera g. 3, LV-2169, Salaspils, Latvija

⁴Gyvybės mokslų departamentas, Žuvininkystės institutas, Nacionalinis Taivano universitetas
Taipėjus, Taivanas

Santrauka. Siekiant ivertinti žmogaus ūkinės veiklos, susijusios su atominės energetikos vystymu, poveikį verslinių žuvų rūsių populiacinės-genetinės struktūros formavimuisi, buvo inicijuoti ešerių, kaip patogios modelinės rūšies, natūraliai paplitusios Lietuvos vandenye, sparčiai kintančio mtDNR D-kilpos regiono genetinės įvairovės tyrimai Lietuvos vidaus vandens telkiniuose. Ešerių imčių iš Drūkšių, Platelių, Metelių ežerų bei Nemuno žemupio ir Neries ties Vilniumi genetinė įvairovė buvo ivertinta nustatius 99 ešerių 389–400 bp ilgio mtDNR D-kilpos regiono homologines sekas. Identifikuota 15 skirtingų haplotipų, iš kurių net aštuoni anksčiau néra aptiki tiose Europos šalyse. Drūkšių ežere, kurio vandenys naudoti Ignalinos atominės elektrinės aušinimui, rasti keturi nauji ešerių haplotipai, būdingi tik šiai populiacijai. Remiantis D-kilpos sekų analizės metodu nustatyta, kad Drūkšių ežero ešerių imtis patikimai ($p < 0,05$) skyrėsi nuo kitų imčių, surinktų Lietuvoje. Sukaupti duomenys yra pradinė bazinė informacija, kuria remiantis bus atliekami atominių elektrinių poveikio ešerių populiacijų genetinės struktūros formavimuisi tyrimai.

Raktažodžiai: *Perca fluviatilis*, modelinė rūšis, D-kilpa, atominė elektrinė, Drūkšių ežeras, Lietuva.

Introduction. The exploitation of Nuclear Power Plants (NPP) exerts both a direct and indirect environmental impact, not to mention the possibility of large-scale environmental disasters, like the Chernobyl NPP disaster or the events at Fukushima Daiichi Nuclear Power Plant following the March 11 earthquake in Japan. To the best of our knowledge, in the near future there are plans to build several new NPP near the Lithuanian borders, which will surely have a direct effect on some fauna and flora of the inland water bodies of Lithuania. In order to trace and evaluate the probable changes in these

aquatic ecosystems it is necessary to accumulate as many data as possible about the current status of Lithuanian ecosystems. Furthermore, more data about long-term effects of Ignalina Nuclear Power Plant (INPP) in Lithuania must be collected before the construction and exploitation of Visaginas Nuclear Power Plant in the nearby area starts.

Since 1984, the largest Lithuanian lake Drūkšiai has been directly affected by INPP operation: 1) radionuclides (Čepanko *et al.*, 2007) and 2) thermal pollution (Virbickas and Virbickas, 2005). Despite the fact that

decommissioning of the last reactor block of the INPP was started on 31 December 2009, the Lake Drūkšiai is still affected by the anthropogenic activity, such as sewage and chemical water treatment, from Visaginas city (Kesminas and Olechnovičienė, 2008). While technogenic radionuclides, such as ^{137}Cs , ^{60}Co and ^{54}Mn , released from the INPP are treated as harmless (Marčiulionienė *et al.*, 2001) to the Drūkšiai flora and fauna (Čepanko *et al.*, 2006), it is obvious that thermal pollution has drastically changed this ecosystem (Bernotas, 2002; Mažeika *et al.*, 2006). Moreover, hot water seriously affected fish reproduction (Lukšienė *et al.*, 2000).

In general, fish are in the highest positions of the food chain of aquatic ecosystems (Nfon *et al.*, 2009). Besides, fish are a very important resource (Laikre *et al.*, 2005) to human beings. Finally, the NPP impact on the formation of the genetic structure of the population of commercially exploited fish species has been studied insufficiently. Therefore, fish should be studied first.

The Eurasian perch *Perca fluviatilis* (L.) is a freshwater, iteroparous (Blanchard *et al.*, 2005), predatory (Čepanko *et al.*, 2007) commercially exploited (Paulauskas and Ložys, 2001) fish that belongs to the most diverse (Zhaoxia *et al.*, 2010) fish order of Perciformes (Zabotkina *et al.*, 2009). Like almost all species (Leggatt and Iwama, 2003) of this fish order, the perch is diploid, i.e. $2n = 48$ (Virbickas, 2000). Since the perch is quite common (Gyllensten *et al.*, 1985) in Europe, the ecology of this species is well-known, a lot of data have already been accumulated and many new investigations are conducted annually (Linløkken *et al.*, 2008; Overton *et al.*, 2008; Bergek and Olsson, 2009). Due to the above-mentioned reasons, this fish is often chosen as a model species (Behrmann-Godel and Gerlach, 2008; Nurminen *et al.*, 2010).

Thus far the genetic structure of the perch population has been investigated using various molecular markers (Gyllensten *et al.*, 1985; Refseth *et al.*, 1998; Paulauskas and Ložys, 2001; Sruoga *et al.*, 2008). The greatest progress, however, has been made using two types of molecular markers: 1) DNA microsatellites (Bergek and Olsson, 2009; Demandt, 2010) and 2) mtDNA D-loop region (Nesbø *et al.*, 1998b; Nesbø *et al.*, 1999; Sruoga *et al.*, 2007). The perch populations inhabiting the coastal waters of Lithuania (samples from the Baltic Sea near Šventoji and the Curonian Lagoon) have been studied using both DNA microsatellites and mtDNA D-loop (Sruoga *et al.*, 2007; Sruoga *et al.*, 2008). Even so, thus far no attempts have been made to study the genetic diversity of the perch inhabiting the inland water bodies of Lithuania.

During this study, five perch populations inhabiting the inland water bodies of Lithuania were studied using mtDNA D-loop region. The main aims of the study are as follows: 1) to explore the genetic diversity of the perch in the inland water bodies of Lithuania; 2) to find out whether the genetic diversity of the perch populations representing the intact water bodies differs significantly from the genetic diversity of the population that was affected by the INPP operation.

Material and methods. In 2009–2010, a total of 99 perch caught in the Lakes Drūkšiai, Plateliai, Metelys and two major Lithuanian rivers Nemunas and Neris (Fig. 1) were investigated for mtDNA D-loop region polymorphism.

DNA was extracted from frozen or ethanol preserved muscle tissues using the previously described method (Aljanabi and Martinez, 1997) with slight variations. DNA fragments of the perch mtDNA D-loop region were amplified using HV2 and CSB-D primers (Nesbø *et al.*, 1998a). The PCR volume for each sample was 25 μl and consisted of: 1 \times Taq buffer (with 50 mM KCl), 0.2 mM dNTP, 0.2 μM of each primer, 2.5 mM MgCl₂, 0.75 U Taq DNA polymerase (MBI Fermentas), and 0.05 μg of template DNA. Amplification started with an initial denaturation step for 5 min at 96°C followed by 30 cycles of denaturation for 1 min at 96°C, annealing for 1 min at 54°C, elongation for 2 min at 72°C, and ended with a final elongation step for 5 min at 72°C. Agarose Gel Electrophoresis was carried out in the Pharmacia Gel GNA-100® equipment for 40 minutes at 100 V. Purified PCR products and the same primers HV2 and CSB-D were used for DNA sequencing at the Sequencing Centre of the Institute of Biotechnology (Vilnius University, Lithuania) using Big-Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and 3130x/Genetic Analyzer (Applied Biosystems, USA). The newly detected mtDNA D-loop region sequences in 99 perch specimens were aligned together with homologous fragments determined in previous studies (Refseth *et al.*, 1998; Nesbø *et al.*, 1999; Sruoga *et al.*, 2007); and with one sequence from GenBank (accession number EF118012). Both alignment and the construction of UPGMA phenogram were done using the CLC Sequence Viewer 5.1.1 program (free software available from www.clcbio.com). The number of polymorphic sites (S), haplotype diversity (H), average number of nucleotide differences (K), the divergence between groups of sequences as measured by the uncorrected average number of nucleotide substitutions per site between populations (K_{XY}) (Nei, 1987) and fixation index (Φ_{ST}) with the significance level (P) were estimated using the DNAsP 4.50.3 program (Rozas *et al.*, 2003). During these calculations all positions with gaps were included into the analysis. The MEGA4 program (Tamura *et al.*, 2007) was used to represent neighbour-joining (NJ) tree (Saitou and Nei, 1987) based on K_{XY} results.

Results. The amplified fragment was approximately 550 bp, but alignment of DNA sequences was made with 389–400 bp homologous fragments. These fragments of mtDNA comprised partial D-loop sequence and full tRNA-Pro gene. Thus far, 20 (Fig. 2) haplotypes of 47 found in Europe (Refseth *et al.*, 1998; Nesbø *et al.*, 1999; Sruoga *et al.*, 2007) have been detected in the perch from the Lithuanian water bodies (Fig. 3). After the investigation of 33 perch mtDNA sequences, a total of 11 different haplotypes were detected in the samples from the Baltic Sea near Šventoji and the Curonian Lagoon (Sruoga *et al.*, 2007). Two haplotypes (D1 and F8) have not been found in other localities of Europe earlier.

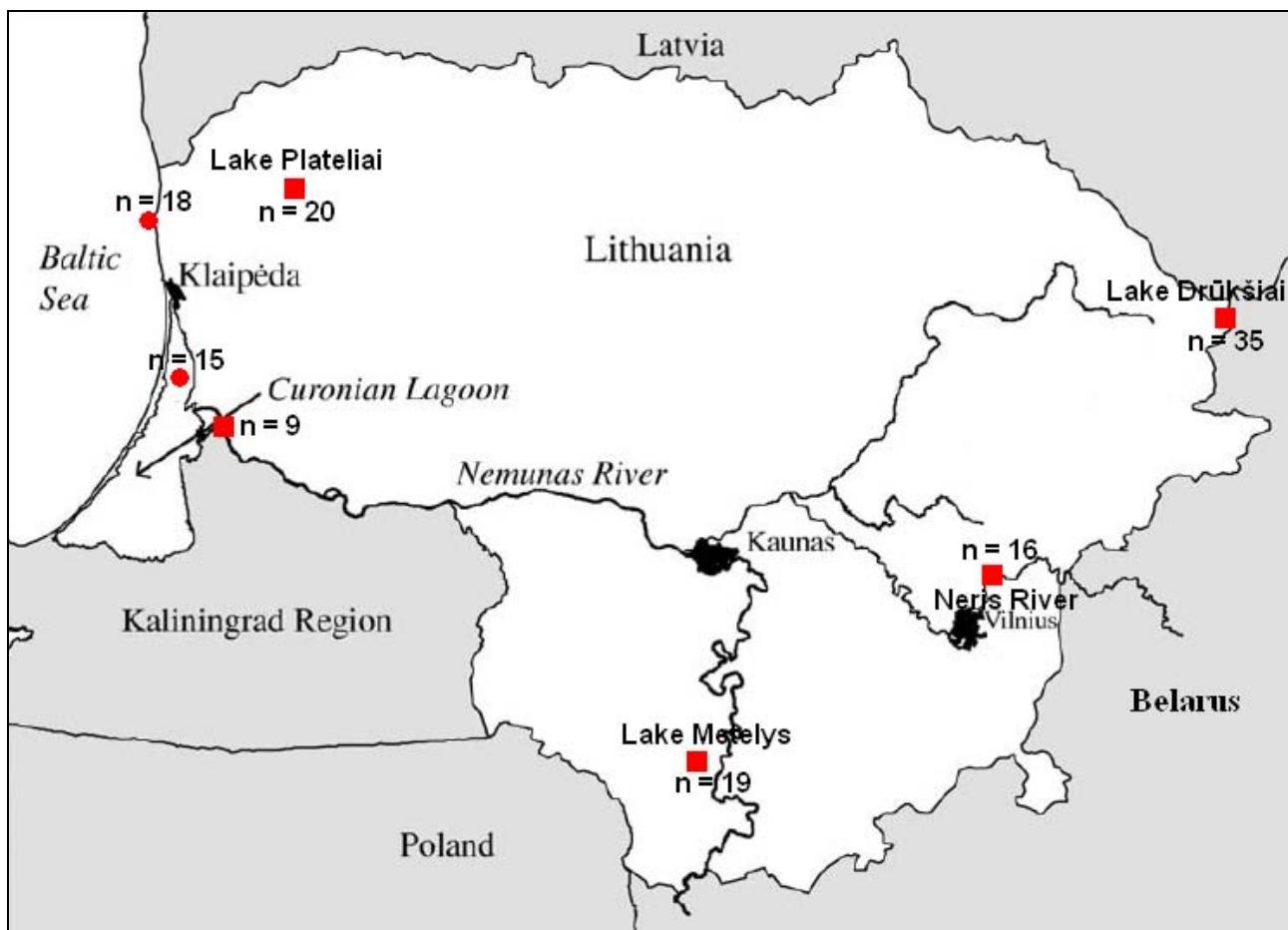


Fig. 1 Perch sampling locations of this (squares) and previous (Sruoga *et al.*, 2007) (dots) studies carried out in Lithuania

No	Haplotype	Prev•	Samples																																
			53	79	80	84	85	86	87	88	89	90	91	92	93	94	96	97	99	211	224	242	259	260	273	277	BS	CL	NDA	NR	LP	LM	LD		
1	A	A7	A	G	C	A	-	-	-	-	-	-	-	-	-	-	A	T	T	A	T	C	C	C	A	G	2	2	1	3	1	5			
2	A2		T	1			
3	A6		T	1	.	1	.	.	.	
4	B		T	T	1	1	1	1	1	9		
5	B4		T	T	3	.	3	3	3	3		
6	B5		T	T	G	1	
7	B6		T	T	T	1
8	B7	C1	T	T	T	1
9	C		T	1	
10	C5		T	14	
11	D		T	T	T	1		
12	D1		T	T	A	T	1			
13	E		T	.	-	A	T	1		
14	F		T	T	1		
15	F8	F1	T	T	14		
16	G		T	T	14		
17	H		T	T	1		
18	J		T	T	1		
19	J3		T	T	T	1		
20	L4		T	T	T	.	G	C	A	C	T	T	G	C	T	A	1				

Fig. 2 Perch mtDNA D-loop region haplotypes found in Lithuania

All sequences are given in relation to the reference sequence (accession number in GenBank is Y14724) ascertained as haplotype A. Due to 10 bp insertion in haplotype L4, other sequences are shown as 400 bp fragments instead of 390 bp. Prev• - Haplotype designation in previous studies (Nesbø *et al.*, 1999; Sruoga *et al.*, 2007). Sampling locations: BS – Baltic Sea, CL – Curonian Lagoon, NDA – Nemunas Delta Area, NR – Neris River, LP – Lake Plateliai, LM – Lake Metelys, LD – Lake Drūkšiai.

During the current study of 99 perch mtDNA, the newly obtained sequences of a total of 15 different haplotypes were observed among the perches sampled in the inland water bodies of Lithuania. Out of these 15 different haplotypes, nine haplotypes have not been detected in Lithuania before. It should be noted that of these nine haplotypes only haplotype J has been found in other localities of Europe earlier. Surprisingly, as many as four haplotypes (B5, B6, B7 and C5) were detected exclusively in the Lake Drūkšiai. Another distinct feature of this lake is that haplotype F, which is really common in Lithuania, was absent there. All other haplotypes (A6, B4, J3 and L4) that have not been detected before were discovered in the Neris River and the Nemunas River delta area.

With the exception of one cluster comprised of L1-L4 haplotypes, the bootstrapping values are low in the UPGMA analysis (Fig. 3). The reason for this is that L1-L4 haplotypes have 10 bp insertions, while only one or two mutational steps separate other haplotypes one from each other. In general, these four haplotypes are heteroplasmic length variants that were described earlier (Nesbø *et al.*, 1998a; Zhaoxia *et al.*, 2010). Because of an unequal time scale, which could be applied to calculating the appearance of single nucleotide mutation in comparison to unknown time passed after insertion of 10 nucleotides that appeared in D-loop sequences it was decided to remove haplotypes L1-L4 with 10 nucleotide insertions from all further analyses.

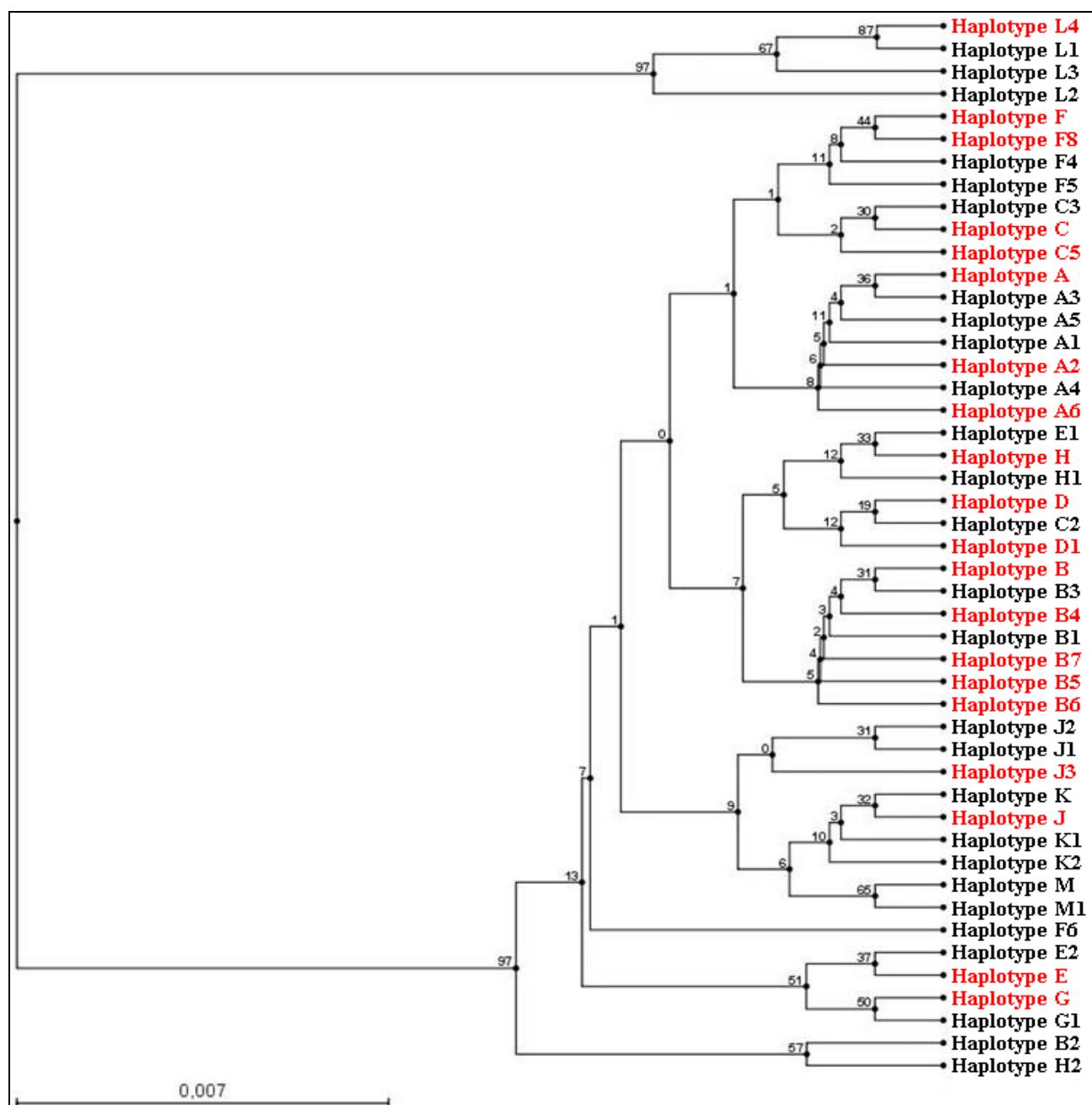


Fig. 3 Groupings of all different perch mtDNA D-loop region haplotypes obtained based on UPGMA phenogram. Haplotypes that are found in Lithuania are marked red.

Pairwise values of Φ_{ST} and P in Lithuania are presented in Table 1. All these comparisons indicate that perch population inhabiting the Lake Drūkšiai is

significantly different genetically from perch populations studied in other locations of Lithuania thus far.

Table 1. Pairwise values of Φ_{ST} and P in Lithuania

SAMPLES	BS	CL	NDA	NR	LP	LM	LD
BS (n = 18)		0.5512	0.5828	0.0381	0.1183	0.3097	0.0001
CL (n = 15)	-0.04514		0.5812	0.0476	0.1220	0.5487	0.0002
NDA (n = 8)	-0.06747	-0.06739		0.0669	0.0527	0.1387	0.0001
NR (n = 16)	0.10434	0.07708	0.04213		0.0001	0.0110	0.0456
LP (n = 20)	0.07386	0.10047	0.07563	0.38056		0.1505	0.0097
LM (n = 19)	0.00270	-0.02401	-0.00229	0.22555	0.04444		0.0000
LD (n = 35)	0.21561	0.20726	0.11326	0.00976	0.49097	0.36198	

Pairwise values of Φ_{ST} are presented below diagonal while P values above diagonal. Statistically significant P values ($P < 0.05$) are shown in bold. Sampling locations: BS – Baltic Sea, CL – Curonian Lagoon, NDA – Nemunas Delta Area, NR – Neris River, LP – Lake Plateliai, LM – Lake Metelys, LD – Lake Drūkšiai. BS and CL data are taken from Sruoga *et al.*, 2007.

NJ tree based on pairwise K_{XY} distances between the perch populations studied in Lithuania is presented in Fig. 4. Two distinct clusters could be revealed. Sampling locations including Lake Metelys and Lake Plateliai together with the Nemunas Delta, the Curonian Lagoon and the Baltic Sea could be identified as representing the western part of Lithuania falling into one cluster in the NJ

tree. Similarly, the Neris River and the Lake Drūkšiai belong to the eastern part of Lithuania. Therefore, main genetic differences between the perch populations from the Lake Drūkšiai and from other sampling locations in Lithuania could be explained not only by isolation by distance but also due to relation of the perch populations to different water basins.

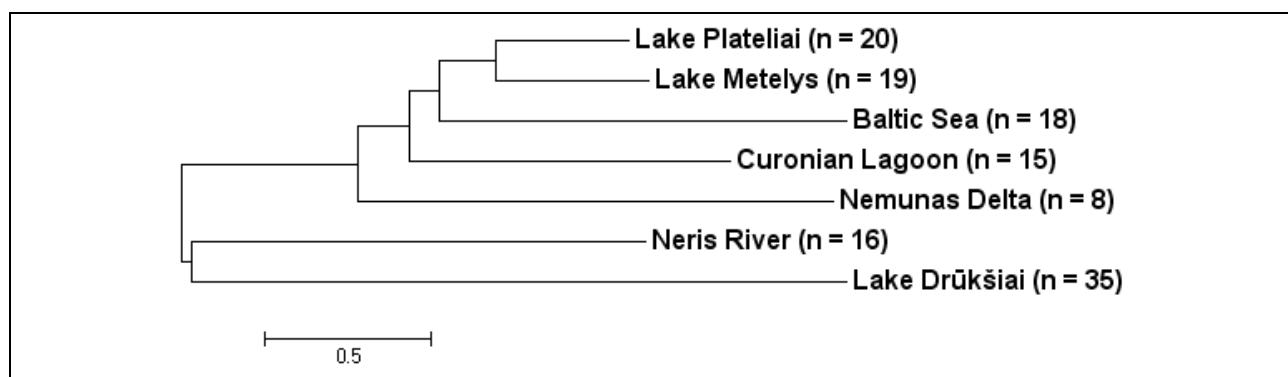


Fig. 4 Neighbour-joining (NJ) tree of the perch populations in Lithuania based on pairwise K_{XY} distances between mtDNA haplotypes. Gaps were considered as the fifth state.

Discussion. There are many reports of various effects of NPP on aquatic ecosystems or their parts (Lukšienė *et al.*, 2000; Virbickas and Virbickas, 2005). However, to the best of our knowledge the NPP impact on the formation of the genetic structure of the population of commercially exploited fish species has not been studied yet. Therefore, it is reasonable to obtain data in this field of research from both the scientific and practical perspective. We chose the Lake Drūkšiai, which had been

used as a water cooling reservoir of INPP for several decades, for this kind of research. Selection of a particular organism as a model species was limited by the fish community of this lake. The latest data (Kesminas and Olechnovičienė, 2008) indicate that the fish community in this lake consists mainly of the silver bream (*Blicca bjoerkna* (L.); 32.9%), the perch (*Perca fluviatilis* (L.); 30.1%) and the roach (*Rutilus rutilus* (L.); 21.7%) species. Other fish species currently living in this lake are

as follows: the bleak (*Alburnus alburnus* (L.)), the bream (*Abramis brama* (L.)), the burbot (*Lota lota* (L.)), the carp (*Cyprinus carpio* (L.)), the crucian carp (*Carassius carassius* (L.)), the gudgeon (*Gymnocephalus cernua* (L.)), the pike (*Esox lucius* (L.)), the rudd (*Scardinius erythrophthalmus* (L.)), the spined loach (*Cobitis taenia* (L.)), the tench (*Tinca tinca* (L.)) and the vendace (*Coregonus albula* (L.)). On the basis of biology of these

14 fish species, the current research status, the importance and applicability of the data obtained, we chose to study the perch. Selection of the Eurasian perch (*Perca fluviatilis* (L.)) as a model species, certain parameters of its genetic diversity and the possibility to use this species for a future research are discussed in detail in the following section.

Table 2. Genetic diversity of mtDNA D-loop region of the perch populations in Lithuania and Europe

Analysis	Population	n / N	Haplotypes	S	H	K	Source
1)	Baltic Sea	18	8	10	0.75163	2.67974	Sruoga <i>et al.</i> , 2007
	Curonian Lagoon	15	6	8	0.64762	2.22857	Sruoga <i>et al.</i> , 2007
	Nemunas Delta	8	5	9	0.78571	3.42857	Current study
	Neris River	16	6	9	0.81667	3.21667	Current study
	Lake Plateliai	20	2	5	0.10000	0.50000	Current study
	Lake Metelys	19	4	7	0.45029	1.13450	Current study
	Lake Drūksiai	35	8	10	0.76471	3.36134	Current study
	Total	131	19	15	0.75760	2.83100	
2)	Latvia	21 / 1	7	7	0.85238	3.17619	Sruoga <i>et al.</i> , 2007
	Estonia	24 / 3	11	12	0.90580	4.41667	Nesbø <i>et al.</i> , 1999
	Finland	10 / 1	4	7	0.77778	2.88889	Nesbø <i>et al.</i> , 1999
	Sweden	81 / 5	8	9	0.70957	2.68086	Nesbø <i>et al.</i> , 1998b; Refseth <i>et al.</i> , 1998
	Norway	98 / 18	9	12	0.77320	2.68146	Refseth <i>et al.</i> , 1998; Nesbø <i>et al.</i> , 1999
	Denmark	10 / 1	1	0	0.00000	0.00000	Nesbø <i>et al.</i> , 1999
	Netherlands	11 / 1	4	6	0.80000	2.94545	Nesbø <i>et al.</i> , 1999
	Germany	10 / 1	3	7	0.73333	3.46667	Nesbø <i>et al.</i> , 1999
	Poland	20 / 3	5	8	0.61579	1.98421	Nesbø <i>et al.</i> , 1999
	Kaliningrad Region*	5 / 1	1	0	0.00000	0.00000	Nesbø <i>et al.</i> , 1999
	Belarus**	10 / 1	1	0	0.00000	0.00000	Nesbø <i>et al.</i> , 1999
	Russia	24 / 2	6	5	0.50000	0.63768	Nesbø <i>et al.</i> , 1999
	Austria	20 / 1	2	4	0.52105	2.08421	Nesbø <i>et al.</i> , 1999
	Slovenia	10 / 1	2	4	0.20000	0.80000	Nesbø <i>et al.</i> , 1999
	Switzerland	20 / 2	3	2	0.63684	0.74737	Nesbø <i>et al.</i> , 1999
	France	57 / 7	2	2	0.06892	0.13784	Nesbø <i>et al.</i> , 1999
	England	10 / 1	1	0	0.00000	0.00000	Nesbø <i>et al.</i> , 1999
	Ireland	7 / 1	1	0	0.00000	0.00000	Nesbø <i>et al.</i> , 1999
	Italy	20 / 2	5	10	0.55789	2.74211	Nesbø <i>et al.</i> , 1999
	Greece	10 / 1	2	2	0.20000	0.40000	Nesbø <i>et al.</i> , 1999
	Romania	21 / 2	5	9	0.70000	1.45714	Nesbø <i>et al.</i> , 1999
	Total	499	34	23	0.83816	2.90555	
3)	Total	630	43	28	0.82414	2.89615	

n is the number of individuals studied, N is the number of locations studied, S is polymorphic sites, H is haplotype diversity, K is the average number of nucleotide differences. • – Represented by the Lake Krylovo, •• – Represented by the Lake Dubrovskoje.

Perch as a model species. Due to practical reasons it was logical to select freshwater fish, whose genetic variability was investigated in the region. However, only the perch has already been studied in Lithuania using direct sequencing of mtDNA D-loop region (Sruoga *et al.*, 2007). Therefore, the selection of a model species was based on additional important criteria. Due to clear disadvantages, such as polyploidy (Leggatt and Iwama, 2003), natural hybridization (Hayden *et al.*, 2010) and

stocking effects (Copp *et al.*, 2005) in fish from the Cyprinidae family it was not reasonable to study cyprinids. Similarly, the pike was not chosen because of its intensive stocking activity in Europe in the past. In fact, the original genetic structure of the pike probably has been changed due to >100 years of artificial transplantations (Nicod *et al.*, 2004). The spined loach was not selected because of uncertainties of its taxonomic position (Bohlen and Ráb, 2001) and the problems

associated with the identification of morphologically similar species (Ludwig *et al.* 2001) in the region. Finally, the burbot is quite rare in Europe and Lithuania (Virbickas, 2000). Contrary to the above-mentioned species, the perch fulfilled all main criteria: abundance and its wide distribution, extended genetic research of perch populations in Europe, diploidy, and no stocking effects. In summing up the afore-said it should be stated that the perch was obviously the most reasonable choice to study the impact of INPP on fish populations for the first time.

The genetic diversity of the perch populations in Lithuania and Europe based on D-loop sequences are presented in Table 2. Before the current study was carried out the most widely studied areas were Norway, Sweden and France. Since two locations have already been studied in Lithuania (Sruoga *et al.*, 2007), similar variability of haplotypes in the inland water bodies of Lithuania was expected to be detected. Major differences of the genetic diversity between the sampling localities in western and eastern Lithuania were brought out in the current study. Besides, differences in the haplotype diversity and the average number of nucleotides are significant in the lakes, rivers and territorial waters. It seems that the greatest haplotype diversity could be found in rivers. The lowest H and K values were detected in the lakes, with the exception of the Lake Drūkšiai, whose genetic diversity was even greater than that of many locations in Europe. Due to the fact that the Lake Drūkšiai harbours unique haplotypes, more extended sampling in and around the Lake Drūkšiai is necessary to enable the possible impact of INPP on the perch population to be evaluated. Judging from the current data, the most likely time of appearance of B5, B6, B7 and C5 haplotypes is the period following the last deglaciation. This idea is supported by the previous studies, the highest values of the haplotype diversity in Estonia and Latvia, detection of new haplotypes in the inland water bodies of Lithuania and detection of haplotype B4 whose frequency was equal to three in the Neris River.

Nevertheless, the hypothesis that INPP had an effect on the perch intraspecific evolution cannot be rejected. The impact of temperature (Lukšienė *et al.*, 2000; Overton *et al.*, 2008) on the perch ecology (Kesminas and Olechnovičienė, 2008; Linlokken *et al.*, 2008) and its genetic background (Rougeot *et al.*, 2003) is obvious. Moreover, recent findings indicate that evolutionary adaptation is not a slow process observable only on geological time scales but also an active process that occurs within a human lifespan (Latta, 2008). Finally, the evolution of ecosystem of the INPP cooler has been intensified up to several hundred times as compared with the natural process (Virbickas and Virbickas, 2005).

To sum up, a high genetic diversity in the Lake Drūkšiai was detected during the first attempt to study a long-term effect of INPP on the perch population using the mtDNA D-loop region sequence analysis. Thus far, the origin of B5, B6, B7 and C5 haplotypes has not been clear. In general, a special program of a long-term ecological research should be developed before starting

exploitation of the Visaginas, Kaliningrad and Astravets NPP in Lithuania, Kaliningrad Region and Belarus. The data obtained from the Lake Drūkšiai, the Nemunas and Neris Rivers should be expanded and used as a background database for genetic monitoring of the perch populations living in these water bodies. Finally, in the later stages of the investigation it would be expedient to choose other fish species.

Conclusions. D-loop sequences analysis revealed that genetic diversity of mtDNA of the perch population inhabiting the INPP cooler is quite different from genetic diversity of perch populations living in other water bodies in Lithuania.

Acknowledgements

Authors are grateful to two anonymous referees for improving the quality of the manuscript. This work was funded by grant No TAP-01/2010 from the Ministry of Education and Science of the Republic of Lithuania and the Mutual Funds for Scientific Co-operation of Lithuania, Latvia and Taiwan (Republic of China). The authors are indebted to C. L. Nesbø and K. S. Jakobsen for corresponding and sharing their data, *i.e.* correct sequences of previously detected different haplotypes. Sincere gratitude goes to the Lithuanian State Studies Foundation and Research Council of Lithuania for providing PhD scholarships in support of this study.

References

1. Aljanabi S. M., Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research.*, 1997. Vol. 25(22). P. 4692–4693.
2. Behrmann-Godel J., Gerlach G. First evidence for postzygotic reproductive isolation between two populations of Eurasian Perch (*Perca fluviatilis* L.) within Lake Constance. *Frontiers in Zoology.*, 2008. Vol. 5. P. 3.
3. Bergek S., Olsson J. Spatiotemporal analysis shows stable genetic differentiation and barriers to dispersal in the Eurasian perch (*Perca fluviatilis* L.). *Evolutionary Ecology Research.*, 2009. Vol. 11. P. 827–840.
4. Bernotas E. Changes in fish biomass under impact of a thermal effluent and eutrophication in Lake Drūkšiai. *Acta Zoologica Lituanica.*, 2002. Vol. 12(3). P. 242–253.
5. Blanchard G., Druart X., Kestemont P. Lipid content and fatty acid composition of target tissues in wild *Perca fluviatilis* females in relation to hepatic status and gonad maturation. *Journal of Fish Biology.*, 2005. Vol. 66(1). P. 73–85.
6. Bohlen J., Ráb P. Species and hybrid richness in spined loaches of the genus *Cobitis* (Teleostei: Cobitidae), with a checklist of European forms and suggestions for conservation. *Journal of Fish Biology.*, 2001. Vol. 59. P. 75–89.
7. Copp G. H., Bianco P. G., Bogutskaya N. G., *et al.*

- To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology., 2005. Vol. 21(4). P. 242–262.
8. Čepanko V., Idzelis R. L., Kesminas V., Ladygienė R. Radiological investigation of roach and perch from some lakes in Lithuania. Journal of Environmental Engineering and Landscape Management., 2006. Vol. 14(4). P. 199–205.
 9. Čepanko V., Idzelis R. L., Kesminas V., Ladygienė R. Accumulation particularities of ⁹⁰Sr and ¹³⁷Cs radionuclides in different fish groups. Ekologija., 2007. Vol. 53(4). P. 59–67.
 10. Demandt M. H. Temporal changes in genetic diversity of isolated populations of perch and roach. Conservation Genetics., 2010. Vol. 11(1). P. 249–255.
 11. Gyllensten U., Ryman N., Ståhl G. Monomorphism of allozymes in perch (*Perca fluviatilis* L.). Hereditas., 1985. Vol. 102(1). P. 57–61.
 12. Hayden B., Pulcini D., Kelly-Quinn M., et al. Hybridisation between two cyprinid fishes in a novel habitat: genetics, morphology and life-history traits. BMC Evolutionary Biology., 2010. Vol. 10. P. 169.
 13. Kesminas V., Olechnovičienė J. Fish community changes in the cooler of the Ignalina nuclear power plant. Ekologija., 2008. Vol. 54(4). P. 124–131.
 14. Laikre L., Palm S., Ryman N. Genetic population structure of fishes: implications for coastal zone management. Ambio., 2005. Vol. 34(2). P. 111–119.
 15. Latta R. G. Conservation genetics as applied evolution: from genetic pattern to evolutionary process. Evolutionary Applications., 2008. Vol. 1(1). P. 84–94.
 16. Leggatt R. A., Iwama G. K. Occurrence of polyploidy in the fishes. Reviews in Fish Biology and Fisheries., 2003. Vol. 13(3). P. 237–246.
 17. Linlokken A., Bergman E., Greenberg L., Seeland PAH. Environmental correlates of population variables of perch (*Perca fluviatilis*) in boreal lakes. Environmental Biology of Fishes., 2008. Vol. 82(4). P. 401–408.
 18. Ludwig A., Bohlen J., Wolter C., Pitra C. Phylogenetic relationships and historical biogeography of spined loaches (Cobitidae, *Cobitis* and *Sabanejewia*) as indicated by variability of mitochondrial DNA. Zoological Journal of the Linnean Society., 2001. Vol. 131(3). P. 381–392.
 19. Lukšienė D., Sandström O., Lounashimo L., Andersson J. The effects of thermal effluent exposure on the gametogenesis of female fish. Journal of Fish Biology., 2000. Vol. 56(1). P. 37–50.
 20. Marčiulionienė D., Kiponas D., Hansen D. Technogeninių radionuklidų akumuliacija Ignalinos AE aplinkoje. /Accumulation of technogenic radionuclides in the environment of the Ignalina NPP. Ekologija., 2001. Vol. 1. P. 52–59 (in Lithuanian).
 21. Mažeika J., Taminskas J., Paškauskas R., et al. Ecohydrological evolution in the catchment of Lake Drūkšiai, Lithuania, under anthropogenic pressure. Ekologija., 2006. Vol. 4. P. 40–50.
 22. Nei M. Molecular Evolutionary Genetics. New York, Columbia University Press, 1987.
 23. Nesbø C. L., Arab M. O., Jakobsen K. S. Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*). Genetics., 1998a. Vol. 148(4). P. 1907–1919.
 24. Nesbø C. L., Magnhagen C., Jakobsen K. S. Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic Sea. Hereditas., 1998b. Vol. 129(3), 241–249.
 25. Nesbø C. L., Fossheim T., Vøllestad L. A., Jakobsen K. S. Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. Molecular Ecology., 1999. Vol. 8(9). P. 1387–1404.
 26. Nfon E., Cousins I. T., Järvinen O., Mukherjee A. B., Verta M., Broman D. Trophodynamics of mercury and other trace elements in a pelagic food chain from the Baltic Sea. Science of the Total Environment., 2009. Vol. 407(24). P. 6267–6274.
 27. Nicod J-C., Wang Y. Z., Excoffier L., Largiadèr C. R. Low levels of mitochondrial DNA variation among central and southern European *Esox lucius* populations. Journal of Fish Biology., 2004. Vol. 64(5). P. 1442–1449.
 28. Nurminen L., Pekcan-Hekim Z., Repka S., Horppila J. Effect of prey type and inorganic turbidity on littoral predator-prey interactions in a shallow lake: an experimental approach. Hydrobiologia., 2010. Vol. 646(1). P. 209–214.
 29. Overton J. L., Bayley M., Paulsen H., Wang T. Salinity tolerance of cultured Eurasian perch, *Perca fluviatilis* L.: effects on growth and on survival as a function of temperature. Aquaculture., 2008. Vol. 277. P. 282–286.
 30. Paulauskas A., Ložys L. Isoenzyme analysis of percids (*Stizostedion lucioperca*, *Perca fluviatilis*, *Gymnocephalus cernua*) in Lithuanian coastal zone of the Baltic Sea, the Curonian Lagoon and Kaunas Water Reservoir. Acta Zoologica Lituanica., 2001. Vol. 11(1). P. 29–38.
 31. Refseth U. H., Nesbø C. L., Stacy J. E., Vøllestad L. A., Fjeld E., Jakobsen K. S. Genetic evidence for different migration routes of freshwater fish into Norway revealed by analysis of current perch (*Perca fluviatilis*) populations in Scandinavia. Molecular Ecology., 1998. Vol. 7(8). P. 1015–1027.

32. Rougeot C., Minet L., Prignon C., Vanderplasschen A., Detry B., Pastoret P-P., Mélard C. Induce triploidy by heat shock in Eurasian perch, *Perca fluviatilis*. Aquatic Living Resources., 2003. Vol. 16(2). P. 90–94.
33. Rozas J., Sánchez-Delbarrio J. C., Messeguer X., Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics., 2003. Vol. 19(18). P. 2496–2497.
34. Saitou N., Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution., 1987. Vol. 4(4). P. 406–425.
35. Sruoga A., Rashal I., Butkauskas D., Ložys L. Variety of mtDNA haplotypes in the populations of the European perch (*Perca fluviatilis*) of the Curonian Lagoon, the coastal zone of the Baltic Sea and the Gulf of Rīga. Proceedings of the Latvian Academy of Sciences., 2007. Vol. 61(3/4). P. 20–30.
36. Sruoga A., Butkauskas D., Rashal I. Evaluation of genetic diversity of perch (*Perca fluviatilis*) and pikeperch (*Sander lucioperca*) populations from Curonian Lagoon and inshore waters of the Baltic Sea. Acta Biologica Universitatis Daugavpiliensis., 2008. Vol. 8(1). P. 81–88.
37. Tamura K., Dudley J., Nei M., Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution., 2007. Vol. 24(8). P. 1596–1599.
38. Virbickas J. Lietuvos žuvys /Fishes of Lithuania. Vilnius, Trys žvaigždutės, 2000. 192 p. (in Lithuanian).
39. Virbickas J., Virbickas T. Results and prospects of the integrated long-term ecological research into Lake Drūkšiai. Acta Zoologica Lituanica., 2005. Vol. 15(2). P. 195–198.
40. Zabotkina E. A., Lapirova T. B., Nazarova E. A. Influence of cadmium ions on some morphofunctional and immune-physiological parameters of perch (*Perca fluviatilis*, Perciformes, Percidae) underyearlings. Journal of Ichthyology., 2009. Vol. 49(1). P. 111–118.
41. Zhaoxia C., Yuan L., Ka Hou C. Broader pattern of tandem repeats in the mitochondrial control region of Perciformes. Chinese Journal of Oceanology and Limnology., 2010. Vol. 28(4). P. 785–794.

Received 4 April 2012

Accepted 21 September 2012