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POPULATION GENETIC STRUCTURE OF SALMON (*SALMO SALAR* L.) AND SEA TROUT (*SALMO TRUTTA* L.) IN LITHUANIAN RIVERS

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KLAIPĖDOS UNIVERSITETAS

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GENETINĖ LAŠIŠŲ (*SALMO SALAR* L.) IR ŠLAKIŲ (*SALMO TRUTTA* L.) POPULIACIJŲ STRUKTŪRA LIETUVOS UPĖSE

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TABLE OF CONTENT

1. INTRODUCTION

Scope of the study. Genetic variation constitutes the basis for biological evolution and consequently influences all levels of biodiversity (Laikre *et al*., 2010)**.** Genetic components of biodiversity are essential for adaptation to environmental changes, sustainable use of recourses and ecosystem recovery (Luck *et al*., 2003; Frankham, 2005; Reusch *et al*., 2005). Species are not genetically homogeneous, but structured into groups of individuals (or populations) that are genetically differentiated (Laikre et al., 2005). Genetic population structure of the species is a pattern of distribution of genetic variation within and between populations and genetic differences between them. For a species like Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.), strong homing instinct (i.e., sexually mature individuals return to spawn to the river in which they were born) provides a possibility to genetically adapt to environmental conditions in their natal rivers and results in the formation of pronounced genetic structure, where each river system contains at least one genetically distinct population (Hansen *et al*., 2002; Koljonen *et al*., 2002; Fraser *et al*., 2007; Apostolidis *et al*., 2008; Ozerov *et al*., 2010). Ignoring or not knowing the genetic population structure may result in loss of genetic diversity, reduced productivity, and ecological damage (Laikre *et al*., 2005).

A large proportion of the natural habitat of the salmon and brown trout has been altered by various human activities (pollution, power plant construction, timber floating) (Parish *et. al*., 1998; Nilsson *et al*., 2005). Subsequently, large part of the intraspecific variability of the salmon and trout has been lost due to environmental degradation and harvesting (Laikre and Ryman, 1996). Furthermore, because of the species economic value, remaining populations are threatened by activities such as releases of translocated or hatchery bred individuals into natural rivers where wild populations of the same species occur (Laikre *et al*., 2010).

Hatchery produced and released salmon constitutes about 70% of the salmon in the Baltic Sea (ICES, 2011). More or less extensive

stocking programs of salmon and brown trout have been carried out for several decades in most of the rivers of the Baltic sea basin, including those which still harbor wild populations (ICES, 2011). It is evident from the many studies that introgression of hatchery reared individuals into the wild populations occurs and results in genetic changes in the wild populations. Moreover, these genetic changes are almost always detrimental to the fitness and survival of individual populations as well as to inter-population genetic variability (Hindar *et al*., 1991; Moran *et al*., 2005; Vasemägi *et al*., 2005b; Apostolidis *et al*., 2008; McGinnity *et al*., 2009; Hansen *et al*., 2010). Therefore, to enable sustainable use and conservation of natural salmon and brown trout populations it is essential to incorporate genetic aspects into the management (Allendorf *et al*., 1987).

Atlantic salmon and brown trout are genetically highly structured species. The brown trout has two alternative life stages: (i) resident form that spend their entire life in a river or a small stream, and often spawn in smaller tributaries of the area and (ii) anadromous form (sea trout) that migrate from the rivers or streams in which they were born to the sea where they forage until reaching sexual maturity and then return to their native rivers to spawn. (Elliott 1994). There is complete reproductive isolation between resident populations which are physically isolated (Moran *et al*., 1995; Bouza *et al*., 1999). Anadromous populations, because of homing behavior and restricted gene flow, are also partly isolated from each other (Hansen *et al*., 2002). Although Atlantic salmon and brown trout are generally well studied species in respect to genetic population structure, very little information on genetic variation and genetic population structure exists for these species in Lithuania. While the distribution, abundance and productivity of salmon and sea trout stocks in Lithuania have regularly been monitored since 1998, only mitochondrial DNA diversity of these species has been studied in Nemunas river basin (Leliūna and Virbickas, 2006; Leliūna, 2010) and the population genetic studies based on more informative nuclear DNA markers have not been carried out so far. This study is the first attempt to gather information on genetic structuring, relationships and dispersal among

Lithuanian salmonid populations based on microsatellite DNA analysis.

Objective and tasks of the study. The objective of this study was to assess genetic diversity of Lithuanian salmon and sea trout populations, to investigate spatio-temporal population structure of sea trout and to provide recommendations for sustainable management of genetic resources.

The main tasks of the study were:

1. to estimate between-river and within-river genetic diversity in wild and enhanced salmon and trout populations;
2. to estimate genetic differences and relationships among populations;
3. to determine hierarchical structure of sea trout populations;
4. to assess temporal stability of the genetic diversity and structure of sea trout populations;
5. to estimate the level and patterns of contemporary gene flow among sea trout populations;
6. to develop guidelines for management of genetic resources.

Novelty of the study. It is the first study of genetic diversity and population genetic structure of salmon and sea trout in Lithuanian rivers based on microsatellite DNA variation. It provides information on the patterns of contemporary natural and human mediated gene flow and the resulting fine scale and temporal population structure of the sea trout in Lithuania. This study provides also information about the genetic impacts of supportive releases in the highly structured population system.

Scientific and practical significance of the results. The results of this study contribute significantly to the general knowledge about the population ecology of salmonid fishes in Lithuanian rivers. This study provides information on spatial and temporal genetic structure that could be used for identifying and analyzing changes caused by

human activities, therefore the results of this study can be important not only in Lithuanian but also throughout Baltic sea region.

Results of this study provide baseline data for monitoring future changes at gene level diversity of salmon and sea trout in Lithuania. This study also provides guidelines for future management of the sea trout populations that should be based on knowledge of genetic structuring, relationships and dispersal among populations. Potentially these guidelines can be incorporated in practical management programs of salmon and trout in Lithuania.

Defensive statements

1. Level of genetic diversity in Lithuanian salmon and sea trout populations is high and similar in all examined river basins as well as between wild and hatchery populations.
2. Lithuanian sea trout populations are hierarchically structured at the level of river basins and at the level of tributaries within the river basins.
3. Genetic structure of sea trout populations fit isolation by distance model and differentiation by drainage are more pronounced than within river basin differentiation.
4. Lithuanian sea trout populations are temporally stable.
5. Contemporary gene flow between Lithuanian sea trout rivers is asymmetric and distance restricted.
6. Genetic diversity and genetic structure of Lithuanian sea trout populations reflects contemporary dispersal and gene flow (both natural and human mediated).

Scientific approval

The results of this study were presented at 5 international conferences:

European Workshop for Doctoral Students on *Salmo salar* and *Salmo trutta* Research (NoWPaS), February 14–17, Roskilde, Denmark

"Genetic risks in relation to compensatory releases of reared salmon in the Baltic Sea". December 7–8, 2010, Älvkarleby, Sweden;

1st , 2nd and 3rd regional student conference on "Biodiversity and functioning of aquatic ecosystems in the Baltic Sea region", Klaipeda, Lithuania, in 2004, 2006 and 2008.

Two papers were published on the dissertation topic:

1. A. Šauklytė, A. Kontautas, A. Paulauskas. 2002. Genetic diversity of farmed and wild populations of Lithuanian stocks of Atlantic Salmon. Proceedings of the Latvian Academy of Sciences. Section B, Vol. 56, No. 3, pp. 20–25.
2. A. Samuiloviene, A. Kontautas, R. Gross. 2009. Genetic diversity and differentiation of sea trout (*Salmo trutta*) populations in Lithuanian rivers assessed by microsatellite DNA variation. Fish Physiology and Biochemistry, Vol. 35, No. 4. pp. 649–659.

Volume and structure of the thesis. The dissertation is presented in the following chapters: Introduction, Literature review, Study Area, Material and Methods, Results, Discussion, Conclusions and References. The volume of the dissertation is 112 pages and references include 144 sources.

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2. LITERATURE REVIEW

2.1 Phylogeography of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*)

2.1.1 Atlantic salmon

The distribution area of Atlantic salmon (*Salmo salar*) is wide and covers north Atlantic river system in North America and Europe. Nowadays its European distribution range extends from northern Portugal to the Pechora River in northwest Russia, including Iceland, the British Isles and the Baltic Sea (Tonteri *et al.*, 2005). In the western Atlantic *Salmo salar* is found in the rivers of USA, Canada and Greenland.

The analysis of various classes of molecular markers showed clear genetic differentiation between western and eastern groups of Atlantic salmon populations (Ståhl 1987; Bermingham *et al*., 1991; McConnell *et al*., 1995a, b; Verspoor *et al*., 1999; King *et al*., 2001) as well as between eastern Atlantic and Baltic salmon groups (Bourke *et al*., 1997; Verspoor *et al*., 1999; Nilsson *et al*., 2001; Consuegra *et al*., 2002; Säisä *et al*., 2005). Consequently, Baltic Sea salmon forms one of the three major groups of Atlantic salmon; the others are groups of western and eastern Atlantic.

For the Baltic Sea salmon, three hypotheses of post-glacial origin have been proposed. One of the hypotheses is that the Baltic Sea could be colonized by salmon from eastern preglacial lakes before the Yoldia sea stage (Kazakov and Titov, 1991, Nilsson *et al*., 2001; Tonteri *et al*., 2005). Other hypothesis proposes a western origin from Atlantic populations via Närke Strait at the beginning of the Yoldia sea stage (Verspoor *et al*. 1999) and third hypothesis suggests combination of both west and east origin of Baltic salmon (Koljonen *et al*., 1999).

The hypothesis that the entire Baltic Sea was colonized from a western refugee (Verspoor *et al*., 1999) was based on the studies of salmon populations from the Gulf of Bothnia only, while other Baltic

Sea areas and populations were not covered by investigations (Säisä *et. al*., 2005). However, knowledge on allozyme and mitochondrial DNA variation provide clear evidence of the genetic differences between southern Baltic populations (Main Basin and Gulf of Finland) and populations that belong to the Gulf of Bothnia (Koljonen *et al*., 1999; Nilsson *et al*., 2001). It was proposed that these differences exist because the postglacial colonization of the Baltic Sea with different lineages from different glacial refuge: present salmon stocks of Estonia, Latvia, Russia and southern Sweden are probably originated from the eastern glacial lakes (the Ice Lake Lineage) and stocks of northern Finland and northern Sweden are Atlantic origin (Atlantic lineage) (Koljonen, 1999).

Later on it was found that based on microsatellite DNA variation, there are genetic differences not only between southern and northern Baltic salmon populations, but also between populations within southern group. Within the Baltic Sea the anadromous salmon populations form three distinct groups, corresponding to the northern (Gulf of Bothnia), eastern (Gulf of Finland and eastern Baltic Main Basin) and southern regions (western Baltic Main Basin) (Säisä *et al*., 2005). These findings indicated that the Baltic Sea was colonized by at least three distinct refuges: the Gulf of Bothnia from an Atlantic refugee, the Gulf of Finland from an eastern ice lake refugee and the southern Main Basin from a southern refugee that was presumably located in the basin of rivers Nemunas, Vistula, Odra and Elbe (Säisä *et al*., 2005).

Colonization hypothesis that entire Baltic Sea has been colonized from eastern preglacial lakes (Kazakov and Titov, 1991, Nilsson *et al*., 2001; Tonteri *et al*., 2005) and uncertainty about the possibility of an immigration from Atlantic were based on the fact that one mtDNA haplotype, which is found in most Atlantic populations, was absent in populations from the Gulf of Bothnia (Nilsson *et al*., 2001). However, Nilsson and co-authors (2001) found that populations from Gulf of Bothnia had several haplotypes that are attributable to Atlantic populations. Studies on allozyme data (Koljonen *et al*., 1999) as well as microsatellite data (Säisä *et al*.,

2005) also showed similarity of the northern Baltic Sea group and Atlantic populations. Consequently, although the original colonization lineage may later have admixed to some extent with other lineages (Säisä *et al*., 2005), theories regarding the origin of the Baltic salmon remain controversial.

2.1.2 Brown trout

Brown trout (*Salmo trutta*) is naturally distributed in Europe, Western Asia and North Africa (Garcia-Marin *et. al*., 1999). Its natural distribution ranges from northern Norway and northeastern Russia, southward to the Atlas Mountains of North Africa. From west to east, its distribution extends from Iceland to the headwaters of Aral Sea in Afghanistan (Apostolidis *et. al*., 1996a; Bernatchez, 2001). Brown trout is one of the genetically most substructured vertebrate species currently known to exist (Allendorf and Leary 1988). A large proportion of the intraspecific biological diversity of the brown trout is represented by genetic differences between populations, and this genetic divergence is often coupled with pronounced phenotypic variation (Apostolidis *et al*., 1997). The analysis of mitochondrial DNA (mtDNA) sequence variation has revealed five major phylogeographic groupings among western and central European populations of brown trout: Atlantic, Adriatic, Danubian, Mediterranean and *marmoratus* (Bernatchez *et al*., 1992; Bernatchez and Osinov 1995; Bernatchez 2001). The Atlantic phylogeographic group is found throughout the Atlantic river systems from Iceland and Norway in the north to Iberia and the Atlas mountains of Morocco and also in the Baltic and White Sea drainages. Danubian lineage is associated with drainages of the Black, Caspian and Aral Sea basins, as well as the Persian Gulf. The distribution of other three lineages – Adriatic, Mediterranean and *marmoratus* – slightly overlap with the other two and differ in distribution pattern within the Mediterranean Sea basin. The *marmoratus* lineage is almost strictly associated with the Adriatic basin. The Mediterranean lineage is predominantly found in tributaries draining in the western basin of Mediterranean Sea,

whereas the Adriatic lineage is distributed in the eastern part of the Mediterranean basin (Bernatchez, 2001).

Very important role in the origin of the five evolutionary lineages of brown trout played isolation of basins that occurred in Europe during Pleistocene glaciations due to climatic and environmental changes. The most ancient fragmentation, which involved Atlantic, Ponto-Caspian and Mediterranean basins, determined separation between Atlantic and Danubian lineages (Apostolodis *et. al*., 1996b). Subsequent and possibly simultaneous fragmentation occurred within the Mediterranean basin, which led to the divergence of the Mediterranean, *marmoratus* and Adriatic lineages (Bernatchez, 2001).

Considering that Atlantic lineage is associated with the Atlantic basin, the center of origin of this lineage is associated with drainages of this system. The northern part of the Atlantic region was ice covered and thus many populations have existed only since postglacial times (Apostolidis *et. al*., 1996a). However, in addition to the brown trout populations being present in unglaciated parts of the Atlantic region, one or more glacial refugia probably existed at the margins of the ice sheets (Ferguson and Fleming 1983; Hamilton *et al*., 1989; Osinov and Bernatchez 1996). This was supported by significant differences in nuclear and mitochondrial DNA markers between Atlantic Iberian populations and more northern Atlantic populations (Moran *et al*., 1995; Antunes *et al*., 1999; Garcia-Marin *et al*., 1999; Weiss *et al*., 2000).

Studies based on variation of allozyme alleles and mitochondrial DNA has proposed that more than one postglacial colonization of northwestern Europe took place. On the basis of variation in allozyme alleles, Ferguson and Fleming (1983) proposed that the northwest Atlantic was colonized independently by two races of brown trout. Hynes *et al*. (1996) analyzed the pattern of distribution of mtDNA and suggested that the post-glacial colonization of northwest Europe was more complex. Garcia-Marin *et al*. (1999) contributed to the hypothesis of multiple colonization and proposed a postglacial recolonization model of the northwest Atlantic, based on

allelic distribution at two enzymatic loci. According this model, colonization occurred from (i) a north-western migration from an eastern Mediterranean-Caspian refuge, (ii) a northern expansion from a refuge in Atlantic drainages of Iberia and southern France, and (iii) a northern and eastern migration from a refuge centered near the English Channel (Garcia-Marin *et al*., 1999a). They suggested that most current populations in the formerly glaciated area are combinations of these lineages. In the subsequent study, this hypothesis was re-evaluated and was argued that distribution of both mtDNA haplotypes and allozyme alleles do not support the contribution of two major glacial refugee (southwest Atlantic and Mediterranean-Caspian Basin) to the postglacial recolonization (Weiss *et al*., 2000). It was also suggested that distribution of mtDNA and nuclear gene markers in previously glaciated areas of northern Europe can be explained by postglacial dispersal from refugee located northwards of the Iberian Peninsula, as well as the Black-Caspian-Aral basins (Weiss *et al*., 2000). Furthermore, Bernatchez (2001) reconciled previous interpretations of the origin and postglacial history of brown trout and supported the existence of northwestern refuge as well as existence of northeastern refuge and also provide evidence for the contribution of a southern refuge. Results of that study implied that northern colonization by this southern group occurred prior to the last glaciations; they also refute a contribution of a Ponto-Caspian lineage. It can be concluded that current genetic diversity in North Atlantic region is the result of independent postglacial colonization by genetically distinct brown trout lineages (Bernatchez, 2001).

2.2 Factors affecting genetic differentiation of populations

Salmonid fishes exhibit complex patterns of genetic differentiation even at microgeographical levels (Garant *et al*., 2000; Spidle *et al*., 2003; Verspoor *et al*., 2005; Dillane *et al*., 2007; Vähä *et al*., 2007; Heggenes *et al*., 2009). The large microgeographical differentiation is mainly associated with reproductive isolation and

homing behaviour (Ferguson, 1989). In some cases genetic differentiation is the result of complete reproductive isolation – distinct populations have been found within the locations that are separated by impassable waterfalls and other geomorphological structures. Otherwise, if there are no physical barriers, strong homing behaviour may be sufficient to maintain genetic differentiation (Ferguson and Mason, 1981; Ståhl 1987). The pattern of substantial microgeographical differentiation may be the result of a combination of mutation, random genetic drift, gene flow and natural selection (Wright, 1931).

Genetic drift. Genetic drift is a random change in allele frequency of population. It occurs if a population size is not infinite. In populations that are not infinitely large, allele frequencies will change over time because to chance. The effects of genetic drift are strongest in small populations: the fewer individuals in the population, the stronger genetic drift affects the population. However, the effect is very small in large populations.

In the short term, over a few generations, a result of genetic drift would be the increasing or decreasing of allele frequencies in a random, unpredictable way. In the longer term, the main result of genetic drift is loss of genetic variation. This occurs because some alleles may not be passed to the next generation and over time the effect of genetic drift will be the loss of alleles by chance. Genetic drift also results in different populations becoming genetically different from each other because different alleles will become more frequent or fixed in different populations.

Natural selection. Natural selection occurs because different genotypes have different fitness. Individuals with some genotypes (those with higher fitness) survive and reproduce more than other individuals. As a result, these genotypes become increasingly more and more common in populations. In different populations, parents of different genotypes pass their genes unequally to the next generation, leading to the genetic differences among isolated populations. So,

genetic drift tends to make different populations genetically different from each other by chance, whereas natural selection tends form genetically different populations due to environmental constraints. Consequently, the traits that have high fitness in one population, and evolve through natural selection, will be different from the traits that have high fitness and evolve through natural selection in another population.

Mutation. Mutation is biochemical change in DNA and assumes changes of one allele into another, what creates new alleles. It is a very unusual process and typical mutation rates are about one mutation in a million genes passed from generation to generation. As a result, evolution through mutation is extremely slow – so slow that it is generally impossible to detect it. However, mutation is important as a source of genetic variation. The process of mutation is the only way in which genetic variability is created, and without mutations there would be no biological diversity.

Another important aspect of genetic mutation is its randomness – it may produce alleles that result in high or low fitness. What happens to those alleles, once they are produced, depends on the natural selection, genetic drift and gene flow.

Gene flow. Gene flow is a change in allele frequency that occurs due to migration of individuals among populations. When individuals move into a population they may bring new alleles which are not present in that population or occur in frequencies that differ from the allele frequencies of that population. Gene flow increases genetic variation within a population. Gene flow tends to make populations genetically similar to each other. The more gene flow occurs, the more similar the populations will become. If less than one individual per generation moves between populations, i.e. the amount of gene flow is very low, then populations will develop complete differences (differences in which alleles are fixed in different populations). In contrast, if migration between populations occur in large numbers of individuals, i.e. the amount of gene flow is very

high, the populations will be like one single population and will have the same alleles in the same frequencies, even if they occur in different environments and differences could otherwise to evolve through natural selection. Large amounts of gene flow will mask the effects of other forms of evolutions and make populations similar.

In between these two situations, movement of at least one individual in each generation from population to population will prevent complete differences – the alleles found in one population will also be found in the other. However, if levels of gene flow are fairly low, the populations may have large differences in allele frequency – an allele that is common in one population may be rare in another.

Generally, selection, genetic drift and gene flow affect genetic variation within populations and genetic differences between populations. Both drift and selection tend to decrease variation within populations and increase differences between populations, whereas gene flow increases variation within populations but makes populations similar.

2.3 Genetic markers

Genetic markers are the genes or fragments of DNA that can be used for population genetics studies. Researchers are very interested in assessing genetic variation of populations and detecting similarities as well as differences of populations in order to optimize conservation strategies. It is difficult to quantify distinctness between populations of the same species using morphological characters, since the most of morphological traits are determined by several genes and are strongly influenced by environmental factors. For these reasons, genetic markers are the most accurate and efficient method to identify discrete populations. Both protein and DNA analysis detect genetic variation that is selectively neutral. However, protein electrophoresis surveys portions of the genome that code the functional biochemical products, so only the functional genes can be detected. Furthermore, a large proportion of genetic variation can arise from silent nucleotide substitutions that are not detectable through protein analysis. Although

allozymes are useful for estimation of genetic variability within and between populations as well as for comparison of populations on both temporal and spatial scales (Koljonen *et al*., 1999; Bouza *et al*., 1999; Cagigas *et al*., 2002), the low variability at allozyme loci in most salmonid species reduces sensitivity of these markers and do not provide the desired resolution (Corujo *et al*., 2004).

In contrast, analysis of DNA detects genetic variation at its most fundamental level, the nucleotide sequence. Thus, DNA analysis allows examination of nucleotide sequences that are not translated into protein products or that have no known function. Because the direct analyses of DNA allow more extensive analysis of the genome and show higher levels of polymorphism, DNA-based markers provide greater ability to resolve slight genetic differences between populations and even to distinguish between individuals (Estoup *et al*., 1998).

DNA-based analyses can be organized into two broad classes that are defined by the type of DNA they detect. One class of studies targets mitochondrial DNA (mtDNA), the other – nuclear DNA (nDNA). Mitochondrial DNA is a small, haploid molecule that is inherited maternally, is composed almost entirely of coding sequence, is free from recombination and have relatively high mutation rate. These features make mtDNA useful for phylogeographical studies (Weiss *et al*., 2000; Asplund *et al*., 2004) and for analysis of spatial and temporal population structure (Laikre et *al*., 2002). The main disadvantage of mtDNA analysis is that this molecule represents a single gene unit where all genes are linked. Analytical techniques for mtDNA include indirect methods such as the analysis of restriction fragment length polymorphism (RFLP), as well as direct analysis of mtDNA sequences.

The analysis of nuclear DNA provides some advantages over the analysis of mtDNA when greater discrimination among populations or individuals is required, primarily because of nDNA`s larger size, greater variability and recombination. nDNA analysis include minisatellites and microsatellites also known as variable number of tandem repeats, or VNTRs. Microsatellites are short, neutral (non-

coding) and highly polymorphic DNA sequences in which variation is expressed by differences in the number of simple sequence (two to six base pairs in length) repeats.

Microsatellite markers are widely used for population genetic studies of salmonid fish. Application of them was successful in phylogeography studies of salmonids (Bernatchez, 2001; Tonteri *et a.l*, 2005; Säisä *et al.*, 2005), in determination of genetic variation in wild and farmed fish populations (Norris *et al*., 1999; Koljonen *et al*., 2002; Was and Wenne, 2002; Machado-Schiaffino *et al*., 2007; Horreo *et al*., 2008) as well as assessing intrapopulation diversity, fine-scale genetic differentiation and relationship of populations (Jensen *et al*., 2005; Campos *et al*., 2007; Sønstebø *et al*., 2007; Apostolidis *et al*., 2008). Microsatellites have been very useful for analysis of hierarchical population structure (Dionne *et al*., 2008), in detection of gene flow (Hansen *et al*., 2007; Palstra *et al*., 2007) as well as in assessment of stocking impact on wild populations (Hansen *et al*., 2000b; Ruzzante *et al*., 2001; Vasemägi *et al*., 2005b; Nilsson *et al*., 2008) and genetic assignment analysis (Hansen *et al*., 2000a; Rengmark *et al*., 2006).

The higher level of allelic variation at microsatellite markers make them useful for addressing questions related to genetic structure, particularly where genetic differentiation may be limited.

The polymerase chain reaction (PCR) enables to amplify DNA sequences up to several million times therefore it provides the possibility of nonlethal sampling. Small amount of tissue (fin clips or scales) may be sufficient for analysis and individuals need not be sacrificed for sampling. This can be an important feature when evaluating genetic change in protected or declining populations and for providing access to DNA of ancient or archived tissue samples. It can provide information about genetic diversity over extensive temporal and spatial scales, especially for populations that no longer exist. Taken together, these characteristics suggest capability to monitoring populations that are small, exploited or declining.

2.4 Genetic management of salmon and trout populations

Large parts of the intraspecific variability of the salmon and trout have been lost due to environmental degradation, harvest and stocking (Parish *et. al*., 1998; Nilsson et al., 2005; Allendorf, 2008). The remaining parts are threatened. Therefore, there is a need for increased conservation efforts on these species. An extensive proportion of the natural habitat of the salmon and brown trout has been affected by various activities (pollution, power plant construction, timber floating) that have altered the natural state of the ecosystem. But it is not enough to consider habitat improvement and to ensure that future manipulations of remaining unexploited areas are avoided as much as possible. To enable sustainable use and conservation of natural salmon and brown trout populations it is essential to incorporate genetic aspects into the management (Allendorf *et al*,. 1987). Furthermore, because of the species economic value, remaining populations are threatened by activities such as releases of translocated or hatchery bred individuals (Laikre *et al*., 2010b). These activities are frequently considered harmless or even beneficial, but may be devastating from a conservation genetic standpoint (Laikre *et al*., 2010b). Therefore, genetic management is an important component of strategies that ensure the conservation and recovery of salmon and trout populations.

Genetic management deals with the genetic factors that affect extinction risk and conservation programes required to minimize these risks. The major issues of genetic management are concerned with monitoring and conserving gene-level biodiversity, resolving spatio-temporal population structure as well as with genetic consequences of stocking practices.

2.4.1. Monitoring genetic diversity

It is recognized that genetic diversity is the basis of evolutionary potential of species. The presence of genetic variation between populations as well as between individuals within populations is essential for their potential to survive and ability to evolve in response to both short-term and long-term environmental

changes (Allendorf *et al*., 2008). The two primary measures of genetic diversity are heterozygosity and allelic diversity. Allelic diversity refers to the number of different alleles at any given locus in the population. Heterozygosity is the percentage of heterozygous loci in a population or individual. Loss of heterozygosity can reduce viability of population by reducing individual fitness, so it is important for immediate adaptation and loss of allelic diversity can affect the ability of populations to evolve in the future (Ryman *et al*., 1995). The population viability strongly depends on the effective population size which determines the rate of loss of genetic diversity in each generation as a result of genetic drift and inbreeding (Frankham *et al*., 2002). Smaller populations tend to lose more genetic variation than large, becoming less able to adapt to a changing environment. It is broadly conceded that an effective population size of at least 500 is required for long-term viability (Laikre *et al*., 2009). The effects of small population size are of major concern because small populations suffer from inbreeding and loss of genetic diversity resulting in elevated extinction risks. Consequently, one of a major objective of genetic management is to minimize inbreeding and loss of genetic diversity.

2.4.2 Resolving spatio-temporal population structure

A large proportion of the intraspecific biological diversity of the salmon and brown trout is represented by genetic differences between populations. Genetic studies help to identify discrete populations and their interactions. It was realized that each river system has at least one genetically distinct population (Ståhl, 1987; Carlsson and Nilsson, 2000; Hansen *et al*., 2002; Koljonen *et al*., 2002; Fraser *et al*., 2007; Apostolidis *et al*., 2008; Ozerov *et al*., 2010) Moreover, often there is a high degree of differentiation among populations even at very small geographical scales therefore it is impossible to detect if a particular water system contains one or more populations without population genetic studies (Carlsson and Nilsson, 2000; Spidle *et al*., 2001; Ruzzante *et al*., 2001). Strong homing of Atlantic salmon and brown trout results in little genetic exchange

between rivers, however occasional straying more likely occur between adjacent rivers. This pattern of gene flow results in association between genetic and geographic distance (or isolation by distance). Many empirical studies in salmonids have revealed significant correlation between geographical and genetic distances (Bouza *et al.*, 1999; Carlsson and Nilsson, 2000; Ruzzante *et al.*, 2001; Campos *et al.*, 2007; Palstra *et al.*, 2007). Thus, even though individuals in each river should be considered as one separate population, genetic diversity in one population can be dependent on other geographically close populations (Vasemägi *et al.*, 2005b). Furthermore, gene flow between local salmon and brown trout populations often are asymmetric and individuals move preferably from large into small populations (Hansen *et al.*, 2007). This pattern of gene flow may be important for maintaining the genetic diversity and viability of the small populations (Consuegra *et al.*, 2005) and providing stability to regional population structure (Palstra *et al.*, 2007). Thus, it is clear that genetic monitoring of contemporary connectivity of populations is necessary for conservation.

Whereas one of the fundamental aims of the conservation genetics is to maintain as much genetic variability within and between populations as possible, it is necessary to study and monitor the amount and distribution of biological diversity over time. Otherwise it would be not impossible to detect negative changes and reductions of this diversity. Generally wild populations of salmon and brown trout are assumed to be genetically temporally stable (Ståhl 1987; Koljonen *et al.*, 1989; Hansen *et al.*, 2002; Verspoor *et al.*, 2005; Campos *et al.*, 2007; Palstra *et al.*, 2007; Vähä *et al,.* 2008). However, some studies have shown temporal variation that includes significant allele frequency differences between temporally separated samples (Laikre *et al.*, 2002; Ostergaard *et al.*, 2003; Jensen *et al.*, 2005; Hansen *et al.*, 2009). Several studies where the original wild population was compared with hatchery stocked populations, indicated clear loss of diversity and decrease of allelic richness in hatchery stocked population (Säisä *et al.*, 2003; Aho *et al.*, 2006). Therefore genetic management must include collecting data of genetic population

structure as well as changes of genetic composition over time in order to identify human mediated loss and change of genetic diversity.

2.4.3 Identifying genetic effects of stocking

Stocking – releasing into the wild fish that were bred in hatcheries or transferred from other location – is very common management practice. It is aimed to enhance the natural population and is generally regarded as beneficial. However, the studies of the efficiency of stocking programmes have showed that genetic introgression of the stocked fish on wild populations is variable and unpredictable. In some cases stocking programmes appear to have been inefficient or introgression is very low (<5%) (Moran *et al*., 1991; Martinez *et al*., 1993; Garcia-Marin *et al*., 1999b; Antunes *et al*., 2001; Aurelle *et al*., 2002; Almodovar *et al*., 2006). Other examples of stocking activities have clearly resulted in survival and reproduction of stocked trout, although the levels of introgression vary very broadly: from less than 25% till more than 70% (Apostolidis *et al*., 1996a, 1997; Berrebi *et al*., 2000; Weiss *et al*., 2001; Jug *et al*., 2005).

Harmful genetic effects of releases on native gene pools and the need of monitoring of such effects where recognized several decades ago (Ryman 1981) and more recent studies also emphasized the deleterious effects of farmed fish on genetic diversity of wild populations (Hindar *et al*., 1991; Moran *et al*., 2005; Vasemagi *et al*., 2005; Apostolidis *et al*., 2008; McGinnity *et al*., 2009; Hansen *et al*, 2010).

Genetic risks associated with releases of hatchery reared fish can be direct and indirect. Indirect genetic changes can result from ecological impacts that arise through competition, introduction of diseases and parasites and increased predation. Stocked trout are often larger than wild, as a result of selection for faster growth together with favorable conditions for growth (such as diet and temperature) in the farm. Because of larger size and more aggressive behaviour that is typical for domesticated fish, stocked fish can competitively displace wild fish (Weber and Fausch, 2003). Introduction of farm-reared

salmonids can increase predation on wild fish through the attraction of predators (Nickleson, 2003). Introduced diseases and parasite can also increase mortality in the wild. Therefore, these ecological impacts can be the reason of lowered effective population size which in turn can cause the loss of genetic variability within populations through increased genetic drift and inbreeding.

Direct genetic impacts emerge when released fish interact and reproduce with the wild fish. Here risks are dependent on the type of stocking. There are recognized two most common types of releases of farmed salmonid fishes: 1) releases of genetically distinct (non-local) populations and 2) releases of local populations from which captive-bred individuals are derived (supportive breeding) (Laikre *et al*., 2010b). In the case of releases of genetically distinct populations, genetic variation of wild populations may be lost and unique gene pools can be destroyed due to strong inflow of genes from non-local population (Moran *et al*., 2005; Apostolidis *et al*., 2008). In addition to genetic differences between wild and reared fish due to different origin, they can differ in their co-adapted gene complexes that are comprised of many genes and are involved in local adaptations. If fish with different co-adapted gene complexes interbreed these gene complexes may be broken down resulting in loss of adaptations, so-called outbreeding depression (Gharrett *et al*., 1999; Muhlfeld *et al*., 2009). Releases of genetically distinct populations can also result in a change of genetic composition of wild population. Several studies had showed that wild local populations can become genetically similar to non-native hatchery stocks (Araguas *et al*., 2004; Vasemägi *at al*., 2005b).

A particular form of stocking – supportive breeding – is a type of breeding-release program where the released fish descend directly from the receiving population (Hansen *et al*., 2000b). A fraction of the wild parental fish is brought into a hatchery for artificial reproduction, and the offspring are released into the natural habitat where they mix with the wild fish. The aim of supportive breeding is to avoid genetic problems of supplemental stocking with farm-reared or non-native brown trout. Although in the case of supportive breeding no

exogenous genes are introduced to the wild population, it may also have strong negative genetic effects. Several studies have shown that even a short period in a hatchery can result in a reduction of subsequent survival and reproductive success, because differential or relaxed selection in hatchery environment alter behavior, physiology and genetics of fish (Glover *et al.*, 2004; Sundström *et al.*, 2004). Moreover, inadvertent artificial mixing of stocks that inhabit the same water system but are spatially or temporally reproductively isolated, can break down the population structure and local adaptations, leading to a loss of productivity and fitness (Stewart *et al*., 2006).

Considering all possible threats, any stocking activity should always be preceded by analysis of potential genetic consequences and organized with aim to eliminate or minimize the negative genetic effects.

2.5 Current status and releases of salmon and brown trout in Lithuanian rivers

There are 12 rivers in Lithuania inhabited by salmon populations of different abundance. The status of these rivers differs. Leaning on historical data and today's situation, salmon rivers can be divided into following groups: 1-inhabited by wild salmon; 2-inhabited by artificially reared salmon; 3-inhabited by mixed salmon population; 4-"potential" rivers, i.e. where salmon occurs occasionally; 5-rivers, where salmon got extinct (Kesminas *et al*., 2003). Purely natural salmon population inhabits Žeimena River and its tributary – Mera, Saria. Mixed, i.e. natural and reared populations are in the rivers Neris, Šventoji, Vilnia, Baltijos Šventoji, Dubysa, Siesartis, Širvinta, Vokė. Populations formed of reared salmon inhabit Virinta, Jūra, Minija rivers and some smaller their tributaries. In the latter rivers artificially reared salmon juveniles are being released for several years already (ICES, 2008).

The observed parr densities in Lithuania are very low in relation to the observed parr densities in most other Baltic rivers (ICES, 2011). There is also remarkable variation in the annual parr

densities, as well as between different rivers. Abundance of salmon parr depends on hydrological conditions, spawning efficiency, protection of spawning grounds and migration ways (ICES, 2011).

Salmon smolt production in Lithuania is affected by remaining pollution, the lack of habitats for salmon and quite high mortality rate caused by predators that is significantly higher compared with typical salmon rivers in north Baltic (ICES, 2011). Total salmon smolt production in Lithuanian rivers in 2000-2011 is presented in Table 1.

Table1: Total salmon smolt production in Lithuanian rivers in 2000-2011 (Kesminas, 2012)

Year	**Total smolt production**
2000	6500
2001	5598
2002	4184
2003	1629
2004	5227
2005	7148
2006	5741
2007	13908
2008	32808
2009	35937
2010	47843
2011	6656

Population of sea trout in Lithuania is greater than that of salmon. Sea trout populations inhabit 76 rivers that belong to 10 major basins: Neris, Žeimena, Šventoji, Minija, Jūra, Dubysa, Bartuva, Akmena-Danė, Šyša, Baltijos Šventoji.

The total annual production of smolts has decreased dramatically since 1999: from 94 500 to 18 000 smolts in 2005, while the potential production was estimated at 323,800 smolts (Kesminas

and Kontautas, 2011). However, smolt production of sea trout increased continually since 2005 and reached 42 300 in 2011 (Table 2). The highest densities of natural sea trout have been reported in western Lithuania – in Minija river, furthermore abundance of sea trout was bigger in small tributaries. (Kesminas and Kontautas, 2011).

Table 2: Smolt production of sea trout in Lithuanian rivers in 2006-2011 (Kesminas, 2012)

River/Year	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**
Neris	5300	6100	12200	4300	3600	3700
Žeimena	1400	4300	2600	2000	2900	1900
Šventoji	3000	3900	4800	5100	4900	5300
Minija	5200	8200	8200	12500	8200	21100
Jūra	1000	900	900	800	800	2500
Dubysa	400	1100	1100	4600	1100	2900
Bartuva	100	100	100	500	400	1000
Akmena-Danė	220	800	800	600	800	500
Šyša	300	500	500	400	500	2500
Baltijos-Šventoji	160	400	400	200	600	900
Total	**17 080**	**26 300**	**31600**	**31 000**	**23900**	**42300**

Salmon and sea trout restocking programme in Lithuania started in 1998. Stocking of salmonids in Lithuanian rivers is presently implemented only for restoring and supporting weak natural populations. Whereas in other countries of Baltic Sea region i.e. Sweden and Finland large scale releases of salmon is proceeded to compensate for the production losses caused by dam construction which prohibit natural migration of spawners to reproduction areas in rivers and migration of smolts to feeding areas in the Baltic Sea. Compared to compensatory releases, supportive releases in order to enhance weak natural populations, constitute only a minor fraction of stocking. The total number of released salmon and trout smolts into the Baltic Sea was about 5.0 millions and about 3.0 millions,

respectively in 2010 (ICES, 2011). In addition to direct smolt releases salmon and trout are released as eggs, alevins, fry and parr. The total number of releases of these younger life stages to the Baltic Sea rivers was 2.6 millions of salmon and 7.8 millions of trout in 2010 (ICES, 2011).

Salmon and trout smolt releases in Lithuanian rivers was 35 500 and 45 000 smolts respectively in 2010, whereas releases of younger life stages was 140 000 and 95 000 of salmon and trout respectively (ICES, 2011). Total releases of salmon and trout individuals in Lithuanian rivers in 2005-2011 are presented in Table 3.

Table 3: Total releases of salmon and trout individuals in Lithuanian rivers in 2005-2011 (ICES, 2011)

Year	**Salmon**	**Trout**
2005	102 000	200 000
2006	30 000	245 000
2007	50 000	185 000
2008	68 000	360 000
2009	108 000	299 000
2010	175 500	140 000
2011	140 200	327 000

Improvement measures in salmon and sea trout rivers include releases of artificially reared individuals, construction of fish ladders, protection of spawning grounds, improvement of migration ways (ICES, 2011; Kesminas and Kontautas 2011). Despite the measures taken, salmon smolt production in Nemunas basin increased very slowly (ICES, 2011) and almost all sea trout stocks remain in a poor state (Kesminas and Kontautas 2011). The importance of genetic studies that help to obtain better understanding of differences between fish populations must be recognized and information of population genetic structure must be incorporated in management and conservation practices.

3. STUDY AREA

The study was based on analysis of 16 populations of salmonid fishes that belong to 7 river basins. Sampling of sea trout populations covered 3 different river basins: Akmena-Danė, Bartuva and Nemunas, whereas wild salmon samples were taken from Žeimena river. The description of study area was based on the study of Gailiušis *et. al*., 2001 and Nemunas river basin district management plan (EPA, 2010).

Nemunas is the longest river in Lithuania. Its total length is 937 km and the basin area constitutes 97 928 km^2. Nemunas flows through the territories of Belarus, Lithuania and Russian Federation (Kaliningrad Region). The Lithuanian part of the basin covers the area of 46 626 km^2, covering 72% of the territory of Lithuania.

The longest and the largest (by their catchment size) tributaries of the Nemunas in Lithuania are Merkys, Neris, Nevėžis, Dubysa, Šešupė, Jūra, and Minija. The names of these rivers are also the names of 7 sub-basins within the Nemunas River basin. The area of this study covered the sub-basins of Dubysa, Jūra, Minija and Šyša.

River Dubysa is a right tributary of the River Nemunas. Its total length is 130.9 km of which 75.5 km accessible for salmonids. The river springs from the Bubiai pond and flows into the Nemunas at 167.5 km from the mouth. The Dubysa basin is narrow (50 km width in its broadest place; its length – about 90 km) because the river bumps into the eastern edge of the Samogitian Upland instead of flowing in the direction of the surface gradient towards the Central Lithuanian Lowland and Karšuva Lowland.

Forests occupy 25% of the area of the basin, the highest forest concentration is in the upper reaches of the river. There are 40 lakes larger than 0.005 km^2 within the area; however, their total area is only 5.5 km^2, that is, the majority of the lakes are small so the lake percentage is only 0.27%. The area of ponds is larger than that of the lakes and totals to about 10 km^2.

The river network in the Dubysa basin contains 774 rivers, of which 154 rivers are longer than 3 km and only 17 tributaries are more than 10 km long. The total length of the rivers is 2 439 km, and the density of the river network is 1.24 km/km^2. The largest tributaries of the River Dubysa are rivers Kražantė (87.4 km), Luknė (25.8 km), Lapišė (21.1 km), Kirkšnovė (24.7 km) and Mūkė (15.8 km).

Previously, there were 11 dams on the River Dubysa but the majority of them have been torn down. There are no major cities or industries in the River Dubysa catchment area. Pollution from households and industrial wastewater is minimal.

River Jūra is a river in western Lithuania and a right tributary of the Nemunas. Its total length is 171.8 km. The springs of the river Jūra are located in Rietavas Plain. In the upper reaches, it flows over the western slopes of the Samogitian Upland, then turns to Karšuva Lowland and crosses the moraine ridge of Vilkyškiai in the very lower reaches. It flows into the Nemunas at 81 km from the mouth.

The wood density is about 27%. The lake percentage is extremely low – only 0.04% (there are 20 lakes larger than 0.005 m^2, with the total area 1.75 km^2). A much larger area, about 16 km^2, is occupied by ponds.

The river network consists of 1 674 rivers, of which 334 are longer than 3 km. The total length of the rivers is 5 724 km. The largest rivers that belong to the river Jūra basin are rivers Šešuvis (114.9 km), Šaltuona (73.2 km), Akmena (70.8 km), Ančia (66.4 km), Ežeruona (36.8 km) and Šunija (35.1 km).

River Minija is the right tributary of Atmata, the northern branch of the Nemunas. It is the eighth river in Lithuania by length (201.8 km) and flows into the Atmata at 3 km from the mouth. The spring of the river is lake Didovo, situated approximately 200 km northeast from the mouth of the Minija River. The major part of the basin is situated in the Coastal Lowland, the upper reaches of the river – in the Samogitian Upland. Near the mouth (18.4 km) the Klaipėda (Vilhelmo) channel connects Minija River with Klaipėda harbour.

The number of lakes is comparatively small (39), the average lake percentage is 0.6%. The basin of the Babrungas, the right tributary of Minija, accounts for the highest lake concentration (lakes occupy 5.5% of the area of the basin), including Lake Plateliai (12 km^2). The wood density is about 32%.

The network of the rivers in the Minija basin consists of 1 359 rivers, of which 269 are longer than 3 km and most of them are less than 30 km length. The total density of the river network is 1.53 km/km^2, the length of the river beds – 4 508 km. The largest tributaries of the river Minija are rivers Tenenys (left tributary, length – 71,9 km), Veiviržas (left tributary, length – 67,9 km), Alantas (left tributary, lenght – 42,9 km), Babrungas (right tributary, lenght – 47,3 km), Salantas (right tributary, lenght 42,1 km). The exceptional feature of Minija River basin is the difference of length of right and left tributaries. Majority of the right tributaries of Minija are short, while the left ones are long, therefore the Minija basin is asymmetric. This asymmetry is caused by relief, which was formed during the glacier period in Western Lithuania and by later postglacial processes.

Agriculture has been prevailing in the rest of the river basin till the middle of 1990s. Since 1995 use of land for agricultural purposes has been decreasing. Currently approximately 53% of land is used for agricultural purposes. The biggest urban areas in Minija River basin are Plunge, Gargždai and Salantai. There are no important industrial branches developed in the Minija River basin, except oil exploration. There are three power stations in Minija River basin.

River Šyša is a river in western Lithuania, a right tributary of the Nemunas. Its total length is 61 km. The springs of the river Jūra are in the neighborhoods of Vainutas. River Šyša flows in to the Atmata, the northern branch of the Nemunas, at 10 km from the mouth. Samples for this study were taken from the right ributary of river – Šustis (42 km).

River Akmena-Danė flows out of the Coastal Lowland and enters the Baltic Sea via Klaipėda Strait. From the springs to the town of Kretinga, the river is called Akmena, and further – Danė.

The wood density in the Akmena-Danė basin is 27% of the area. The river network consists of 60 rivers, half of them are shorter than 5 km and only 3 are longer than 20 km (Akmena-Danė – 62.5 km, Eketė – 23.1 km, Tenžė – 20.7km). The total length of the rivers is 463 km.

River Bartuva is a river in western Lithuania and Latvia. Its total length is 103 km. River Bartuva begins in the Plungė district, 3 km to the north of Lake Plateliai. Bartuva flows through the Coastal Lowland, crosses the Lithuanian–Latvian border at the Apšė mouth and after 46 km enters lagoon Lake Liepaja, which is connected with the Baltic Sea. In the upper courses Bartuva valley is deep and narrow, while in lower courses it becomes much wider.

The wood density of the basin is 3.2%, and the lake percentage is only 0.2%. There are 5 small lakes. The river network in the Bartuva Basin is comprised of 44 rivers longer than 3 km and 144 ones which are shorter than 3 km. The total length of the rivers is 555.8 km. The longest and largest tributaries of the Bartuva according to their catchment areas in Lithuania are the rivers Apšė (40 km), Luoba (52.2 km) and Erla (28 km). Samples for this study were taken from two tributaries of river Luoba – Pragulba (12.8 km) and Guntinas (12.3 km).

River Žeimena is a salmon and sea trout river flowing to the River Neris and its total length is 79.6 km. The formal source of the Žeimena is Lake Žeimenys. The river flows over the sandy plain of Žeimena and the upper reaches of its tributaries drain foots of Aukštaičiai Upland and Švenčionys Upland. The Žeimena basin is notable for a particularly high number of lakes: there are 479 lakes with an area larger than 0.005 km2, their total area is 180 km2 (the lake percentage is 6.4 Meanwhile the density of the river network is rather low – only 0.67 km/km^2.

The river network consists of 524 rivers, of which 104 ones are longer than 3 km and 22 are more than 10 km in length, but the main tributaries are rivers Mera (60 km), Lakaja (29 km), Saria (28 km) and Peršokšna (26 km). The total length of the rivers in the basin is 1 882 km.

The Žeimena basin makes up 11% of the area of the Neris Basin, it accounts for about 25% of the annual flow.

There are no natural or man-made migration obstacles in the river. The river Žeimena is one of the cleanest rivers of Lithuania. This is due to the affluent formation of groundwater and a relatively small anthropogenic impact. According to all the main water-quality criteria the water in Žeimena is very clean.

4. MATERIAL AND METHODS

4.1 Fish samples

Wild Baltic salmon were sampled by electrofishing from the river Žeimena. The River Žeimena is one of the remaining Lithuania's rivers in which natural salmon populations live and reproduce. The salmon stock of the river Žeimena is purely wild since there has been no stocking at all. Farmed salmon samples were taken from the Meškeryne hatchery in 1999, 2000 and 2002 (Table 4). The breeders for hatchery population of 1999 were taken from Daugava river, so the origin of this populations is not local.

Samples of sea trout were caught by electrofishing from 10 wild and five enhanced populations of three river basins in Lithuania: Akmena-Danė, Bartuva and Nemunas (Fig. 1). Within Nemunas basin, we studied sea trout populations from rivers Dubysa, Jūra, Minija and Šyša. These rivers are the main sea trout rivers with largest densities of natural sea trout in Lithuania (Kesminas, 2012). For the following populations temporally replicated samples were obtained: Bonalė (ADB), Pragulba (BP), Dratvinys (NDD), Lapiše (NDLa), Upinikė (NJU), Blendžiava (NMB) and Mišupis (NMM). All sampled populations consisted of the individuals of 0+ age class. Detailed information about samples is provided in Table 4.

Table 4: Information about analyzed salmon and sea trout samples

Sampling location (river basin/tributary/sub-tributary)		Abbreviation	Status of sample	Year of sampling	Sample size
Atlantic salmon					
1.	Nemunas/Neris/Žeimena	NNZ	wild	2000	30
2.	Hatchery	Hat-99/00/02	hatchery	1999/2000/2002	50/30/30
	Total				140
Sea trout					
1.	Akmena-Danė/ Bonalė	ADB-03/07	wild	2003/2007	10/29
2.	Akmena-Danė/ Eketė	ADE	wild	2004	12
3.	Akmena-Danė	ADF	enhanced	2005	30
4.	Bartuva / Guntinas	BG	wild	2004	10
5.	Bartuva / Pragulba	BP-04/07	wild	2004/2007	21/29
6.	Nemunas / Dubysa/Luknė	NDL	wild	2003	17
7.	Nemunas / Dubysa/Dratvinys	NDD-04/07	enhanced	2004/2007	33/30
8.	Nemunas / Dubysa/Lapišė	NDLa-06/07	enhanced	2006/2007	30/31
9.	Nemunas /Jūra /Upynike	NJU-04/06	wild	2004/2006	27/26
10.	Nemunas/Jūra/Eželuona	NJE	wild	2004	17
11.	Nemunas/Jūra/Šunija	NJS	enhanced	2004	30
12.	Nemunas /Minija/Blendžiava	NMB-04/05	wild	2004/2005	28/30
13.	Nemunas/Minija/Mišupis	NMM-05/07	wild	2005/2007	29/21
14.	Nemunas/Minija/Šiūšis	NMTS	enhanced	2005	30
15.	Nemunas/Šyša/Šustis	NSS	wild	2004	37
	Total				557

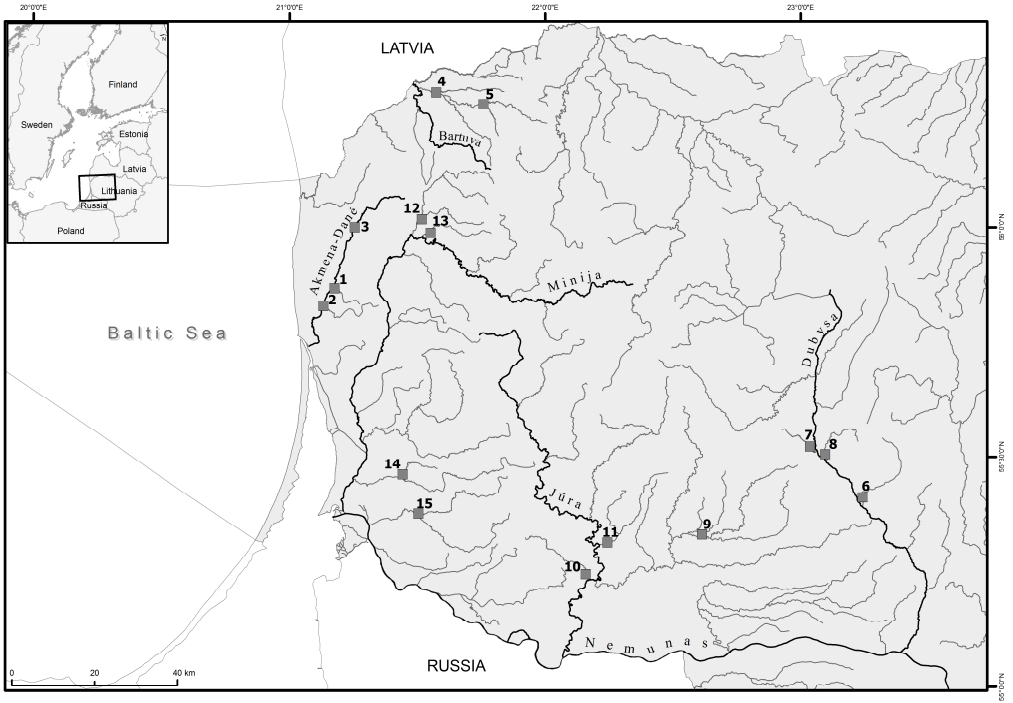


Fig. 1: The origin of analyzed sea trout samples

4.2 DNA isolation and genetic analysis

Genomic DNA was isolated from fin clips or muscle tissue according to the simplified method of Laird *et al*. (1991).

A total of 5 salmon microsatellite loci (*Ssa 197, SSOSL417, Ssa202, SSOSL85 and Ssa171*) as well as 7 sea trout microsatellite loci (*Str60, SSOSL311, Str15, SSOSL483, Ssa197, Ssa85 and SSOSL417*) were analyzed according to protocols outlined in Samuiloviene *et al.* (2009). Briefly, PCR reactions were composed of ca 10 ng DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.1 μM dNTPs, 0.2-0.3 μM of each primer and 0.2 U of *Taq* DNA polymerase (MBI-Fermentas), in a total volume of 10 μl. The forward primers were end-labeled with the fluorescent dye Cy5. For cycling, the following thermal profile was used: initial denaturation at 94 °C for 3 min, 35 cycles of 40 sec at 94 °C, 40 sec at 57 °C, 1 min at 72 °C and final extension at 72 °C for 10 min.

The length of the microsatellite alleles was determined by ALFexpress II DNA analyzer and AlleleLinks v. 1.02 software (Amersham Pharmacia Biotech). A reference sample with known genotype was included on each gel and internal size standards were included in each lane to ensure consistent scoring of genotypes across all gels.

4.3 Statistical analysis

For data analysis, FSTAT v. 2.9.3.2 program package (Goudet 2002) was used for calculating allele frequencies and pair-wise F_{ST} values, for estimating the expected and observed heterozygosities (H_E, H_O) and the allelic richness (A_R), and for testing the significance of differences in average values of A_R, H_E and H_O among the groups of populations (1000 permutations, two-side tests of the null hypothesis of no difference). GENEPOP v. 3.3 (Raymond and Rousset 1995a) was used to test genotypic distributions for conformance to Hardy-Weinberg (HW) expectations and for deficiency or excess of heterozygosity, to test the loci for genotypic disequilibria, and for estimating the significance of allelic differentiation between population pairs. All probability tests were based on the Markov chain

method (Guo and Thompson 1992; Raymond and Rousset 1995b) by using 1000 de-memorization steps, 100 batches and 1000 iterations per batch. The sequential Bonferroni adjustments (Rice 1989) were applied to correct for the effect of multiple tests.

The significance of the differences in pairwise F_{ST} and D_A values observed between temporal replicates and spatial samples within years was assessed by performing nonparametric Mann-Whitney U-test in software package STATISTICA 7. Spatial patterns of differentiation among the populations were tested for their fit to the isolation-by-distance model (Rousset 1997). The significance of the fit was estimated by the Mantel test of the ISOLDE program in the GENEPOP 3.3 software package (Raymond and Rousset 1995a) using 10 000 bootstraps. The populations were tested also for recent reduction of their effective population size by using Wilcoxon sign-rank test as implemented in the BOTTLENECK computer program, assuming the two-phase model of mutation (with 5% multi-step changes and variance of 12) for microsatellite loci (Piry *et al*., 1999).

Contemporary migration rates were estimated using a Bayesian method, implemented in BAYESASS 1.3 (Wilson and Rannala, 2003). Analyses were run for 3 000 000 iterations and sampled every 2000 iterations, with a burn-in of 1 000 000 iterations. The delta values of 0.10, 0.10 and 0.25 for allele frequency, migration rate and the level of inbreeding, respectively were used.

Analysis of molecular variance (AMOVA) incorporated in ARLEQUIN v. 2.00 (Schneider et al. 2000) was used to partition genetic variance hierarchically between river drainages, between populations within river drainages and among individuals within the populations. In order to assess the temporal component of genetic diversity, we defined three hierarchical levels: the first level was associated with variation among sampled populations (geographic component), second level was associated with variation between temporal samples within populations (temporal component), and the third level was associated with variation among individuals within populations. This analysis was performed on the subset of 7 populations for which temporal replicates were available (Table 4).

Genetic distances between the populations were estimated by the D_A distance of Nei *et al*. (1983) and a population tree was constructed with the neighbor joining (NJ) algorithm using DISPAN software (Ota 1993). Bootstrapping 1000 times over loci assessed the strength of the support for each node in the tree.

5. RESULTS

5.1 Sea trout

5.1.1 Hardy-Weinberg equilibrium and genotypic linkage disequilibrium

Exact Hardy–Weinberg tests showed significant deviations from Hardy-Weinberg equilibrium in 16 out of 161 tests at 5% significance level. Two of them remained significant after Bonferroni adjustments, all of them involve different locus in different population (locus SSOSL311 in NDD-04 and locus Ssa197 in NDD-07) (Table 6).

Linkage disequilibrium was not significant for most studied samples and only one to three pairs of loci out of 21 tests per population were in linkage disequilibrium in populations ADB-07, NDD-04, NDD-07, NMB-04, NMB-05, NJS and NSS after applying Bonferroni correction for multiple tests. Significant linkage disequilibrium between loci in these populations can be most probably explained by sampling the siblings (Ohta 1982).

5.1.2 Genetic diversity in wild and enhanced sea trout populations

5.1.2.1 Within locus variability

Within locus genetic diversity was moderate to high with observed heterozygosities ranging from 0.509 (SSOSL438) to 0.899 (SSOSL311) over all samples (Table 5). Individual loci varied in observed heterozygosity among populations, ranging from 0.300 (SSOSL438 in BG04) to 1.000 (SSOSL311 and SSOSL417 in ADB07; SSOSL311 and SSOSL417 in NDL04; SSOSL311 in BG04 and Ssa197 in NMB04) (Table 6). Expected heterozygosities ranged from 0.491 (SSOSL438) to 0.870 (SSOSL311) over all samples (table 5) and from 0.249 (SSOSL438 in NMM05) to 0.911 (SSOSL311 in NJE) for individual loci among populations (Table 6). In total, 67

alleles were observed at the 7 microsatellite loci analysed, ranging from 4 (Str60) to 20 (SSOSL311). Averaged number of alleles detected per population varied from 2.77 (Str60) to 10.36 (SSOSL311) (Table 5).

Table 5: Locus by locus statistics (Aver.A – average number of alleles per locus; Aver.H_E – average expected heterozygosity; Aver.H_O – average observed heterozygosity; SD – standard deviation)

Locus	Aver.A	SD	Aver.H_E	SD	Aver.H_O	SD
Str60	2.772	0.612	0.529	0.073	0.545	0.121
SSOSL311	10.364	2.216	0.870	0.024	0.899	0.070
Str15	4.045	0.722	0.643	0.113	0.678	0.134
SSOSL438	3.455	1.057	0.491	0.111	0.509	0.149
Ssa197	6.091	1.019	0.711	0.100	0.738	0.127
Ssa85	4.318	0.716	0.677	0.064	0.677	0.095
SSOSL417	7.227	1.572	0.788	0.065	0.803	0.111

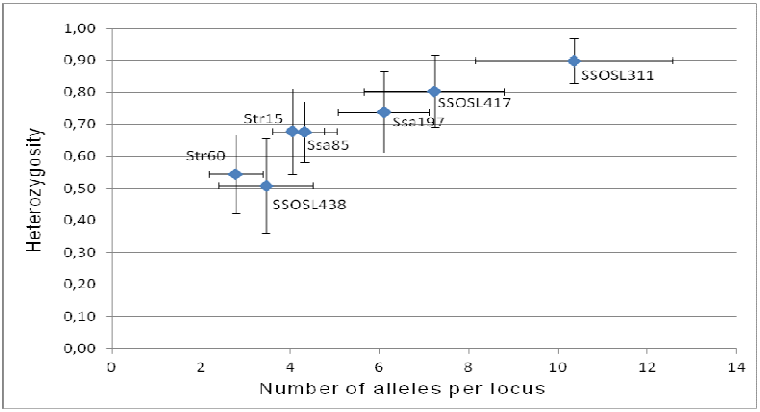


Figure 2: Relationship between the number of alleles per locus and heterozygosity (averages±SD).

Three analysed loci SSOSL311, SSOSL417 and Ssa197 revealed higer level of genetic diversity than Str15, Ssa85, SSOSL438 and Str60. Also it was evident that loci with higher allelic diversity, exhibited higer level of heterosygosity, the only exeption was locus SSOSL438, that has lowest genetic diversity in terms of heterozygosity but not in terms of allele diversity (Fig. 2).

5.1.2.2 Genetic diversity within populations

Genetic diversity within Lithuanian sea trout populations was assessed by heterozygosity and allelic diversity. Allelic diversity refers to the number of different alleles at any given locus in the population. Heterozygosity is the percentage of heterozygous loci in a population.

The total number of alleles over seven loci ranged from 25 in the Guntinas population of the Bartuva river basin (BG) to 45 in the Lapišė population from 2006 that belongs to the Dubysa river of the Nemunas river basin (NDLa-06). As the number of alleles is dependent on the sample size it is more appropriate to characterize the populations based on the corrected parameter, the allelic richness (A_R), which across all populations had a mean value of 4.43 and within populations ranged from 3.53 in Guntinas population of the river Bartuva (BG) to 5.04 Dratvinys population from 2004 that belongs to the Dubysa river (NDD-04) (Table 6).

Average observed heterozygosity across all populations was 0.693 and within populations varied from 0.584 in 2004 sample of Bonalė population of the Akmena-Danė river basin (ADB-04) to 0.797 in Luknė population of the Dubysa river (NDL). Expected gene diversity across all populations was 0.673 and within populations varied from 0.600 in the Eketė population of the Akmena-Danė river basin (ADE) to 0.745 in Dratvinys population from 2004 that belongs to the Dubysa river (NDD-04) (Table 6).

Table6: Micosatellite diversity indices for sea trout populations from Lithuanian rivers. Figures provided are number of alleles (*A*), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosity and P-value for deviation from expected Hardy-Weinberg proportions (P_{HW}; significant deviations indicated in bold).

Basin/Population	**Str60**	**SSOSL311**	**Str15**	**SSOSL438**	**Ssa197**	**Ssa85**	**SSOSL417**	**All loci**
Akmena-Danė								
Bonalė 2003								
A	2	9	4	5	5	3	6	4.86
Ar	2	8.542	3.993	4.4	4.568	2.996	5.733	4.605
He	0.442	0.908	0.742	0.558	0.616	0.616	0.811	0.670
Ho	0.400	0.889	0.700	0.500	0.500	0.500	0.600	0.584
P_{HW}	1.0000	0.4180	0.7340	0.8044	0.3905	0.3198	0.1694	0.7240
Bonalė 2007								
A	2	11	5	6	7	4	9	6.29
Ar	1.999	8.184	4.344	3.631	4.819	3.238	5.83	4.578
He	0.422	0.899	0.726	0.474	0.626	0.574	0.816	0.648
Ho	0.448	1.000	0.621	0.586	0.759	0.655	1.000	0.724
P_{HW}	1.0000	0.5276	0.2742	0.8535	0.4349	0.8444	0.4943	0.9093
Eketė								
A	2	7	4	3	6	3	5	4.29
Ar	2	6.667	3.538	2.639	5.503	2.667	4.89	3.986
He	0.526	0.863	0.431	0.301	0.754	0.518	0.808	0.600
Ho	0.800	0.778	0.417	0.333	0.667	0.667	0.833	0.642
P_{HW}	0.1998	0.0101	0.5606	1.0000	0.0931	0.2914	0.2631	0.0533

	Str60	**SSOSL311**	**Str15**	**SSOSL438**	**Ssa197**	**Ssa85**	**SSOSL417**	**All loci**
Upper reaches								
A	3	12	5	3	7	5	8	6.14
Ar	2.859	7.206	4.102	2.266	5.834	4.377	6.246	4.699
He	0.581	0.862	0.723	0.484	0.821	0.677	0.844	0.713
Ho	0.533	0.867	0.929	0.500	0.833	0.700	0.852	0.745
P_{HW}	0.0191	0.9834	0.0980	0.5198	0.2121	0.6752	0.7325	0.1871
Bartuva								
Guntinas								
A	2	6	4	3	3	4	3	3.57
Ar	2	5.778	3.993	2.996	2.968	4	3	3.534
He	0.505	0.837	0.699	0.647	0.611	0.642	0.626	0.652
Ho	0.800	1.000	0.778	0.300	0.800	0.625	0.700	0.715
P_{HW}	0.1731	0.9568	0.7789	0.0524	0.3307	0.1497	0.1998	0.1565
Pragulba 2004								
A	2	9	4	4	5	4	5	4.71
Ar	2	6.435	3.627	3.219	4.151	3.842	3.777	3.864
He	0.508	0.838	0.668	0.422	0.645	0.700	0.639	0.631
Ho	0.429	0.947	0.800	0.350	0.650	0.810	0.714	0.671
P_{HW}	0.6605	0.3162	0.1292	0.4116	0.1673	0.3071	0.7219	0.3390
Pragulba 2007								
A	2	13	4	4	5	4	10	6.00
Ar	2	8.038	3.806	3.773	3.657	3.997	6.07	4.477
He	0.508	0.873	0.716	0.676	0.479	0.766	0.794	0.688
Ho	0.679	0.793	0.724	0.536	0.448	0.636	0.759	0.654
P_{HW}	0.1268	0.1182	0.9726	0.2668	0.0966	0.6059	0.0818	0.0835

	Str60	**SSOSL311**	**Str15**	**SSOSL438**	**Ssa197**	**Ssa85**	**SSOSL417**	**All loci**
Nemunas/Dubysa								
Luknė								
A	3	10	5	2	7	5	7	5.57
Ar	2.727	7.606	4.328	2	5.644	4.38	6.328	4.716
He	0.569	0.867	0.738	0.508	0.790	0.726	0.845	0.720
Ho	0.647	1.000	0.706	0.647	0.765	0.813	1.000	0.797
P_{HW}	0.2928	0.9698	0.6041	0.3428	0.3188	0.0163	0.9939	0.3016
Dratvinys 2004								
A	3	12	5	4	6	4	9	6.14
Ar	2.872	7.725	4.814	3.396	5.037	3.935	7.467	5.035
He	0.589	0.870	0.784	0.612	0.773	0.733	0.881	0.749
Ho	0.606	0.879	0.774	0.758	0.719	0.667	0.935	0.763
P_{HW}	0.1049	**0.0030**	0.0154	0.5528	0.3404	0.4377	0.3421	0.0046
Dratvinys 2007								
A	3	10	4	5	6	3	7	5.43
Ar	2.465	6.979	3.76	3.222	4.798	2.968	6.184	4.339
He	0.505	0.869	0.632	0.437	0.753	0.611	0.831	0.663
Ho	0.633	0.931	0.724	0.500	0.767	0.600	0.833	0.713
P_{HW}	0.2744	0.0349	0.6471	0.8004	**0.0014**	0.5732	0.5301	0.0246
Lapišė 2006								
A	3	15	5	4	6	4	8	6.43
Ar	2.951	8.292	4.663	3.335	4.803	3.916	5.734	4.813
He	0.595	0.879	0.776	0.606	0.700	0.725	0.810	0.727
Ho	0.567	0.833	0.767	0.667	0.633	0.633	0.833	0.705
P_{HW}	0.5267	0.0433	0.0985	0.6732	0.3058	0.7821	0.9006	0.3099

	Str60	**SSOSL311**	**Str15**	**SSOSL438**	**Ssa197**	**Ssa85**	**SSOSL417**	**All loci**
Lapišė 2007								
A	4	12	4	4	7	5	8	6.29
Ar	3.241	8.141	3.967	3.1	5.782	4.336	5.787	4.908
He	0.609	0.879	0.751	0.524	0.798	0.751	0.795	0.730
Ho	0.484	0.786	0.742	0.484	0.839	0.742	0.871	0.707
P_{HW}	0.3040	0.2602	0.7209	0.7731	0.9906	0.6226	0.0555	0.5271
Nemunas/Jūra								
Upynikė 2004								
A	3	12	4	3	7	5	7	5.86
Ar	2.924	7.943	3.881	2.293	4.801	4.314	5.832	4.570
He	0.539	0.880	0.713	0.419	0.680	0.703	0.817	0.679
Ho	0.444	0.960	0.741	0.333	0.741	0.704	0.852	0.682
P_{HW}	0.4432	0.4349	0.9697	0.4981	0.2911	0.6590	0.7662	0.8556
Upynikė 2006								
A	3	11	4	2	6	5	9	5.71
Ar	2.959	7.92	3.663	2	4.223	3.938	5.891	4.371
He	0.594	0.883	0.671	0.449	0.544	0.642	0.687	0.639
Ho	0.615	0.923	0.846	0.577	0.680	0.692	0.615	0.707
P_{HW}	1.0000	0.1319	0.2128	0.1956	0.8953	0.4395	0.0391	0.1744
Eželuona								
A	3	11	4	4	6	5	8	5.86
Ar	2.727	8.675	3.449	3.655	4.979	4.332	7.145	4.995
He	0.563	0.911	0.623	0.576	0.604	0.749	0.874	0.700
Ho	0.412	0.938	0.625	0.706	0.647	0.882	0.923	0.733
P_{HW}	0.5335	0.9822	0.3498	0.9265	0.5056	0.1539	0.9758	0.8461

	Str60	**SSOSL311**	**Str15**	**SSOSL438**	**Ssa197**	**Ssa85**	**SSOSL417**	**All loci**
Šunija								
A	3	9	4	3	7	4	8	5.43
Ar	2.893	7.407	3.275	2.952	5.782	3.45	5.334	4.442
He	0.586	0.883	0.658	0.540	0.830	0.676	0.794	0.710
Ho	0.593	0.867	0.571	0.633	0.900	0.733	0.852	0.736
P_{HW}	0.1326	0.1694	0.4904	0.6149	0.7313	0.0282	0.0640	0.0563
Nemunas/Minija								
Blendžiava 2004								
A	2	10	4	2	5	4	7	4.86
Ar	1.998	7.08	3.417	2	4.579	3.938	5.509	4.074
He	0.409	0.850	0.467	0.456	0.771	0.742	0.808	0.643
Ho	0.481	0.875	0.536	0.536	1.000	0.679	0.679	0.684
P_{HW}	0.6298	0.2358	1.0000	0.4249	0.1542	0.0107	0.0128	0.0190
Blendžiava 2005								
A	3	8	4	2	6	4	8	5.00
Ar	2.244	5.982	3.288	2	4.789	3.772	5.91	3.997
He	0.332	0.818	0.535	0.503	0.730	0.666	0.764	0.621
Ho	0.400	0.933	0.621	0.700	0.690	0.733	0.800	0.697
P_{HW}	0.6336	0.5332	0.5998	0.0611	0.8457	0.9757	0.4051	0.6853
Mišupis 2005								
A	3	10	3	3	7	5	7	5.43
Ar	2.767	7.492	2.867	2.35	5.877	3.748	5.404	4.358
He	0.570	0.873	0.525	0.249	0.795	0.626	0.763	0.629
Ho	0.444	0.786	0.621	0.207	0.741	0.586	0.741	0.589
P_{HW}	0.4156	0.3280	0.7288	0.0410	0.4246	0.3483	0.1981	0.2038

	Str60	**SSOSL311**	**Str15**	**SSOSL438**	**Ssa197**	**Ssa85**	**SSOSL417**	**All loci**
Mišupis 2007								
A	3	8	3	3	6	5	7	5.00
Ar	2.421	6.259	2.976	2.337	4.9	4.389	5.372	4.093
He	0.522	0.832	0.640	0.292	0.667	0.733	0.760	0.635
Ho	0.421	0.950	0.762	0.333	0.714	0.714	0.762	0.665
P_{HW}	0.6305	0.4157	0.4616	1.0000	0.1315	0.6582	0.2377	0.6070
Šiūšis								
A	4	9	2	3	7	5	7	5.29
Ar	3.324	7.678	1.998	2.465	6.103	3.632	5.72	4.417
He	0.619	0.891	0.399	0.495	0.837	0.628	0.795	0.666
Ho	0.533	0.897	0.393	0.467	0.900	0.467	0.833	0.641
P_{HW}	0.7448	0.7194	1.0000	0.7241	0.3316	0.0344	0.4512	0.5716
Nemunas/Šyša								
Šustis								
A	3	14	4	4	7	5	6	6.143
Ar	2.832	7.912	3.215	3.411	5.774	3.597	5.071	4.545
He	0.548	0.873	0.535	0.580	0.813	0.690	0.775	0.688
Ho	0.622	0.939	0.514	0.541	0.853	0.649	0.676	0.685
P_{HW}	0.7485	0.3938	0.935	0.2199	0.9063	0.9834	0.0404	0.5860

The Wilcoxon's sign-rank test detected significant excess of heterozygosity in all populations of the Nemunas basin, in the Guntinas and Pragulba-2007 populations of the Bartuva basin, and also in the enhanced population of Akmena-Danė, i.e. the H_E values were significantly larger than the heterozygosity expected at mutation drift equilibrium ($p<0.05$), indicating that many Lithuanian sea trout populations have recently experienced severe reduction in their effective population size.

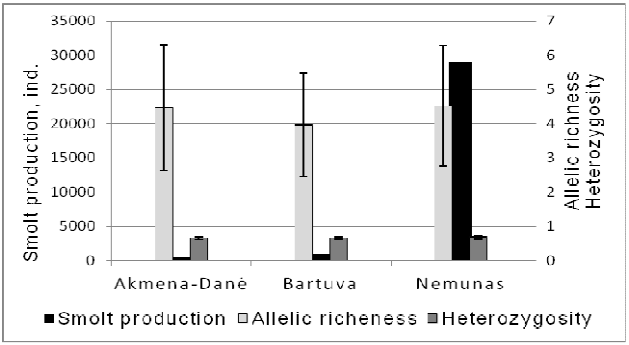
5.1.2.3 Genetic diversity among populations

For comparison of genetic diversity between different river basins, populations from the same river basin were grouped together. We compared allelic richness (*A*r) and expected as well as observed heterozygosities (H_E and H_O).

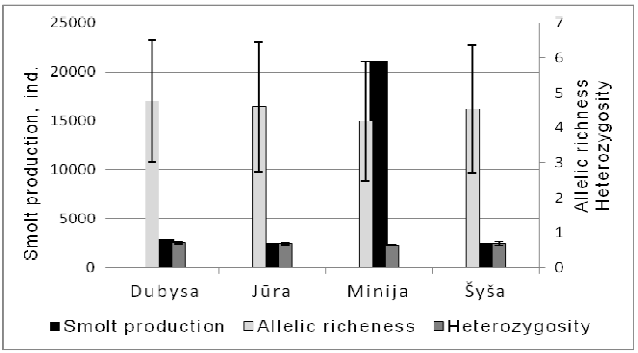
At first we compared three major river basins: Akmena-Danė, Bartuva and Nemunas and found no significant differences neither in average allelic richness nor in average heterozygosities ($p>0.05$). Further we compared the level of genetic diversity between major tributaries of the Nemunas river: Dubysa, Jūra, Minija and Šyša. Here we detected that populations from Dubysa river sub-basin exhibited higher level of average allelic richness as well as average heterozygosity than populations from Minija river sub-basin ($p<0.05$).

At the same time the averaged diversity indices were compared with smolt production of analyzed rivers (Table 2, Kesminas, 2012). It became clear that even if analyzed rivers differ strongly in their smolt production, the level of genetic diversity in these rivers is generally the same (Fig. 3).

Comparison of wild and enhanced populations as well as comparison of temporal samples revealed that there were no significant differences in genetic diversity between the wild and enhanced populations ($p>0.05$). Similarly, no significant differences were observed in average allelic richness and gene diversity between temporal samples of the same population ($p>0.05$).



(a)



(b)

Figure 3: Comparison of the average diversity indices and smolt production of analyzed river basins (a) and sub-basins of Nemunas (b)

5.1.2.4 Allele frequencies

Range of allele lengths in each locus are given in the Table 7. Allele frequencies of each population at each locus are presented in Table 8.

Table 7: Range of allele lengths in each locus

Locus	Range of allele lengths (bps)
Str60	95 – 105
SSOSL311	121 – 169
Str15	214 – 230
SSOSL438	95 – 113
Ssa197	126 – 162
Ssa85	106 – 120
SSOSL417	171 – 197

Populations of the river Bartuva basin shared almost the same number of alleles over all loci with populations of river Akmena-Danė basin and with populations of river Nemunas basin (42 alleles (70%) and 45 alleles (70%), respectively). Whereas a total of 14 and 4 private alleles were observed in the Akmena-Danė and Bartuva populations, respectively and 2 and 18 private alleles were found in the Bartuva and Nemunas populations, respectively. The proportion of shared alleles between populations of Akmena-Danė basin and Nemunas river basin was 76%, but considering only wild sea trout populations, it became lower (72%) and comparable with other values of between basin comparisons.

Within the Nemunas river the highest proportion of alleles over all loci shared populations from Dubysa and Jūra (82%), whereas the most divergent population that shared the least proportion of alleles with other populations was population from Šyša river sub-basin: the proportion of shared alleles was 73%, 74% and 76% with populations of Dubysa, Minija and Jūra, respectively.

Considering all populations separately, a total of 6 private alleles were found in 5 populations and most of them (5 out of 6) were observed in populations that belong to the Nemunas basin: Ežeruona population and Šunija population of Jūra river sub-basin (NJE and NJS) had 2 and 1 private alleles, respectively and Dratvinys population (NDD-07) and Lapišė population (NDLa-06) of Dubysa

river sub-basin had one private allele each. Only a single private allele was found in the Akmena-Danė basin (ADB-07) while there were no private alleles in the Bartuva basin. The frequency of private alleles in the populations of Nemunas basin did not exceed 0.05, whereas the frequency of the private allele in Akmena-Danė basin was 0.07.

There was at least one very rear allele in each locus across all populations. Two loci exhibited high proportion of rare alleles: in the locus SSOSL438, three out of seven loci (43% of loci) were very rare (frequency ranged from 0.02 to 0.05); similarly, in the locus SSOSL417, four out of thirteen loci (31% of loci) were very rare (frequency ranged from 0.02 to 0.07). The majority of rare alleles were attributable to wild sea trout populations, however five very rare alleles were found only in enhanced populations.

We also found several very frequent alleles that frequency in some populations exceeded 0.70. For example, the frequency of allele 134 of locus Ssa197 was 0.71 in BP-07 population and the frequency of allele 99 of locus Str60 was 0.80 in NMB-05 population. Similarly, there were several alleles that were very frequent in a particular river basin: the average frequency of allele 103 of locus SSOSL417 was 0.71 in populations of Akmena-Danė basin and in wild populations of Minija river sub-basin. Also the average frequency of allele 114 of locus Ssa85 and allele 222 of locus Str15 was 0.57 and 0.63 in populations of Akmena-Danė river basin and populations of Minija river sub-basin, respectively.

We found five alleles that are characteristic only for populations of Nemunas river basin but were found in enhanced populations of Akmena-Danė basin.

Table 8: Allele frequencies of each Lithuanian sea trout population at each locus

Locus	Populations										
Ssa197	**ADB03**	**ADB07**	**ADE04**	**ADF05**	**BG04**	**BP04**	**BP07**	**NDL04**	**NDD04**	**NDD07**	**NDLa06**
126		1,72	4,17								
130	20,00	13,79	45,83	28,33	50,00	15,00	13,79	5,88	20,31	10,00	11,67
134	60,00	58,62	12,50	18,33	40,00	55,00	70,69	20,59	29,69	33,33	48,33
138	10,00	12,07		21,67		7,50		35,29	7,81	6,67	3,33
142			16,67	3,33			1,72	5,88	31,25	33,33	23,33
146				13,33		20,00	8,62	23,53	4,69	1,67	6,67
150		3,45		5,00	10,00		5,17	5,88			
154	5,00	6,90		10,00		2,50		2,94	6,25	15,00	6,67
158		3,45	12,50								
162	5,00		8,33								
Ssa85	**ADB03**	**ADB07**	**ADE04**	**ADF05**	**BG04**	**BP04**	**BP07**	**NDL04**	**NDD04**	**NDD07**	**NDLa06**
106		1,72		10,00	12,50	9,52	22,73	9,38	15,15		13,33
112	30,00	18,97	4,17	25,00	56,25	16,67	18,18	43,75	21,21	40,00	21,67
114	55,00	60,34	62,50	50,00	25,00	42,86	22,73	21,88	37,88	10,00	40,00
116	15,00	18,97	33,33	8,33	6,25	30,95	36,36	21,88	25,76	50,00	25,00
118				6,67				3,13			
120											

Str15	ADB03	ADB07	ADE04	ADF05	BG04	BP04	BP07	NDL04	NDD04	NDD07	NDLa06
214		6,90									
220	15,00	5,17	12,50	8,93	22,22	5,00	8,62	8,82	17,74	15,52	31,67
222	30,00	39,66	75,00	39,29	11,11	17,50	22,41	29,41	33,87	55,17	26,67
224	40,00	29,31	8,33	28,57	16,67	47,50	32,76	38,24	16,13	20,69	18,33
226	15,00	18,97	4,17	21,43	50,00	30,00	36,21	20,59	20,97	8,62	16,67
228				1,79				2,94	11,29		6,67
230											
SSOSL438	**ADB03**	**ADB07**	**ADE04**	**ADF05**	**BG04**	**BP04**	**BP07**	**NDL04**	**NDD04**	**NDD07**	**NDLa06**
95	5,00	5,17									
103	65,00	70,69	83,33	63,33	40,00	12,50	30,36	55,88	51,52	73,33	50,00
105	5,00	1,72	4,17	1,67		2,50	8,93		9,09	6,67	5,00
107	5,00	3,45			15,00	10,00	14,29		4,55	1,67	6,67
109		1,72									
111	20,00	17,24	12,50	35,00	45,00	75,00	46,43	44,12	34,85	16,67	38,33
113										1,67	
Str60	**ADB03**	**ADB07**	**ADE04**	**ADF05**	**BG04**	**BP04**	**BP07**	**NDL04**	**NDD04**	**NDD07**	**NDLa06**
95	70,00	70,69	50,00	36,67	40,00	54,76	51,79	50,00	37,88	61,67	30,00
97											
99	30,00	29,31	50,00	53,33	60,00	45,24	48,21	44,12	51,52	35,00	55,00
105				10,00				5,88	10,61	3,33	15,00

SSOSL311	ADB03	ADB07	ADE04	ADF05	BG04	BP04	BP07	NDL04	NDD04	NDD07	NDLa06
121				1,67					1,52	13,79	6,67
125				1,67	22,22	13,16	25,86	3,33			
129	16,67	13,79			5,56	26,32	12,07	6,67	1,52	1,72	1,67
131	11,11	15,52		15,00		21,05	1,72	10,00	9,09	17,24	8,33
133	5,56	10,34	22,22	1,67			3,45		12,12	18,97	1,67
135	16,67	8,62		8,33	27,78	21,05	18,97	26,67			6,67
137	5,56	3,45	5,56	15,00			1,72	16,67			
139	11,11	15,52	22,22	1,67	16,67		3,45			3,45	1,67
141	22,22	6,90	22,22	21,67		2,63	3,45	20,00	27,27	13,79	18,33
143	5,56		5,56				1,72		1,52		
145				6,67					1,52	1,72	1,67
147					5,56				13,64	1,72	5,00
149						2,63					1,67
151		3,45							7,58	17,24	3,33
153		13,79	16,67	21,67		2,63	5,17	3,33	9,09		13,33
155		6,90	5,56	3,33			5,17	6,67	6,06	10,34	3,33
157		1,72		1,67				3,33			1,67
159						7,89	8,62	3,33	9,09		25,00
161	5,56				22,22	2,63	8,62				
169											

SSOSL417	ADB03	ADB07	ADE04	ADF05	BG04	BP04	BP07	NDL04	NDD04	NDD07	NDLa06
171		1,72		18,52			1,72	12,50	8,06	10,00	3,33
173	35,00	13,79		14,81			10,34	15,63	9,68	6,67	3,33
175	15,00	27,59	20,83	12,96	55,00	40,48	25,86	6,25	9,68	18,33	18,33
177									3,23		
181	25,00	24,14	29,17	14,81		7,14	13,79	25,00	20,97	16,67	25,00
183				1,85			1,72	9,38	14,52	30,00	28,33
185	5,00	1,72	25,00						16,13	8,33	
187											1,67
189	10,00	18,97	8,33	25,93	20,00	45,24	34,48	25,00	9,68	10,00	15,00
191		1,72	16,67	1,85		4,76	1,72	6,25	8,06		5,00
193							3,45				
195	10,00	8,62		9,26	25,00	2,38	3,45				
197		1,72					3,45				

Table 8 (continued)

Locus	Populations										
Ssa197	**NDLa07**	**NJU04**	**NJU06**	**NJE**	**NJS**	**NMB04**	**NMB05**	**NMM05**	**NMM07**	**NMTS05**	**NSS04**
126											
130	9,68	1,85	10,00	11,76	15,00	23,21	43,10	16,67	7,14	5,00	7,35
134	29,03	51,85	66,00	61,76	21,67	32,14	25,86	37,04	54,76	16,67	26,47
138	6,45	14,81	2,00	5,88	20,00	5,36	12,07	18,52	2,38	26,67	23,53
142	30,65	1,85	2,00	8,82	23,33		1,72	5,56	11,90	6,67	
146	12,90	7,41	8,00					5,56		16,67	8,82
150		3,70		5,88	3,33	25,00	6,90	9,26	14,29	10,00	5,88
154	6,45	18,52	12,00	5,88	3,33	14,29	10,34	7,41	9,52	18,33	4,41
158	4,84				13,33						23,53
162											
Ssa85	**NDLa07**	**NJU04**	**NJU06**	**NJE**	**NJS**	**NMB04**	**NMB05**	**NMM05**	**NMM07**	**NMTS05**	**NSS04**
106	3,23	3,70	1,92					1,72	4,76	1,67	2,70
112	32,26	46,30	15,38	32,35	20,00	23,21	23,33	32,76	23,81	51,67	32,43
114	24,19	24,07	53,85	32,35	40,00	33,93	50,00	51,72	40,48	31,67	39,19
116	29,03	16,67	23,08	23,53	36,67	28,57	18,33	6,90	23,81	11,67	24,32
118	11,29	9,26	5,77	8,82	3,33	14,29	8,33	6,90	7,14	3,33	1,35

Str15	NDLa07	NJU04	NJU06	NJE	NJS	NMB04	NMB05	NMM05	NMM07	NMTS05	NSS04
214											
220	27,42	12,96	5,77	12,50		5,36	3,45				2,70
222	32,26	35,19	48,08	53,13	46,43	71,43	63,79	63,79	40,48	73,21	63,51
224	20,97	37,04	21,15	3,13	30,36	14,29	24,14	25,86	42,86		24,32
226	19,35	14,81	25,00	31,25	21,43	8,93	8,62	10,34	16,67	26,79	9,46
228											
230					1,79						
SSOSL438	**NDLa07**	**NJU04**	**NJU06**	**NJE**	**NJS**	**NMB04**	**NMB05**	**NMM05**	**NMM07**	**NMTS05**	**NSS04**
95											5,41
103	64,52	72,22	67,31	61,76	63,33	66,07	45,00	86,21	83,33	33,33	58,11
105	9,68	1,85		11,76	16,67					3,33	8,11
107	1,61			5,88				3,45			
109									2,38		
111	24,19	25,93	32,69	20,59	20,00	33,93	55,00	10,34	14,29	63,33	28,38
113											
Str60	**NDLa07**	**NJU04**	**NJU06**	**NJE**	**NJS**	**NMB04**	**NMB05**	**NMM05**	**NMM07**	**NMTS05**	**NSS04**
95	45,16	24,07	28,85	52,94	35,19	27,78	18,33	40,74	39,47	38,33	59,46
97	3,23									3,33	
99	43,55	62,96	55,77	41,18	53,70	72,22	80,00	51,85	57,89	48,33	31,08
105	8,06	12,96	15,38	5,88	11,11		1,67	7,41	2,63	10,00	9,46

SSOSL311	NDLa07	NJU04	NJU06	NJE	NJS	NMB04	NMB05	NMM05	NMM07	NMTS05	NSS04
121	3,57	6,00		9,38	16,67	20,83	28,33	17,86	27,50	10,34	1,52
125											
129	5,36	2,00	15,38		13,33	25,00	23,33	16,07	20,00	15,52	1,52
131	3,57	10,00	5,77	15,63	3,33	8,33	1,67	7,14			1,52
133	8,93	4,00	9,62	12,50	6,67	20,83	8,33	10,71	10,00	15,52	24,24
135		8,00	23,08	9,38	16,67	2,08	5,00	5,36	7,50	17,24	7,58
137	1,79								2,50		
139	3,57	12,00	5,77	15,63		6,25	10,00	3,57		6,90	4,55
141	23,21	24,00	1,92	15,63	10,00	4,17	20,00	23,21	22,50	10,34	18,18
143					5,00					5,17	3,03
145	21,43							7,14			4,55
147	5,36					4,17					
149					15,00						
151	10,71		13,46	3,13							1,52
153	5,36	2,00		6,25	13,33	4,17	3,33			8,62	4,55
155	7,14	18,00	13,46	6,25		4,17		7,14	2,50		15,15
157		2,00	3,85	3,13				1,79	7,50		1,52
159		10,00	3,85								10,61
161		2,00	3,85							10,34	

SSOSL417	NDLa07	NJU04	NJU06	NJE	NJS	NMB04	NMB05	NMM05	NMM07	NMTS05	NSS04
171	3,23	27,78	5,77		1,85	3,57	13,33	7,41	4,76		5,41
173	1,61		3,85	7,69	14,81			5,56	4,76	10,00	5,41
175	35,48	9,26	7,69	19,23	29,63	12,50	6,67	9,26	16,67	13,33	31,08
177					1,85						
181	20,97	20,37	5,77	19,23	25,93	21,43	13,33	37,04	23,81	23,33	29,73
183	14,52	9,26	13,46	7,69	1,85	8,93	13,33	9,26		5,00	9,46
185	4,84		5,77	7,69			3,33				
187											
189	14,52	24,07	53,85	23,08	20,37	28,57	43,33	29,63	40,48	35,00	18,92
191	4,84	5,56	1,92	11,54	3,70	23,21	3,33	1,85	2,38	10,00	
193									7,14		
195		3,70	1,92	3,85		1,79	3,33			3,33	
197											

5.1.3 Genetic differentiation and relationships among sea trout populations

Significant differences in allele frequencies were detected between 225 out of 231 population/sample pairs. One pair of populations with homogenous allele frequencies belonged to the Dubysa river sub-basin (NDD-04 and NDLa-06) and two pairs involved populations from two different basins, Akmena-Danė and Nemunas: ADF and NDL, and ADB-04 and NJE. Three other pairs with homogenous allele frequencies represented temporal samples of the same population: ADB-04 and ADB-07, BP-04 and BP-07, and NMM-05 and NMM-07. Interestingly, all other temporal samples (NDD-04/07, NDLa-06/07, NJU-04/06, NMB-04/05) showed statistically highly significant differences in allele frequency distribution. Differences in NDD and NJU temporal samples mostly depended on allele frequency shifts in locus Ssa85 – the difference of allele 114 and allele 116 frequencies between temporal samples of NDD was 28% and 24%, respectively; similarly the difference of allele 112 and allele 114 frequencies between temporal samples of NDD was 31% and 30%, respectively. The largest allele frequency shifts in NDLa and NMB temporal samples were observed in locus SSOSL311 and SSOSL438, respectively (Table 8).

The level of differentiation between the major river basins was significantly higher than between populations from the same river basin ($p<0.001$, Mann-Whitney U-test). The most distinguished were the populations from Bartuva river basin which displayed average F_{ST} values of 0.111 and 0.103 in pair-wise comparisons with Akmena-Danė and Nemunas populations, respectively, whereas the average F_{ST} between the Akmena-Danė and Nemunas populations was 0.063 (Table 9). The level of population differentiation within the three river basins was similar: average F_{ST} ranged from 0.049 (Akmena-Danė) to 0.056 (Nemunas). Within the Nemunas basin, the average differentiation between tributaries of Minija, Jūra and Dubysa ranged from 0.046 to 0.069 (Table 9).

Table 9: Pairwise F_{ST} values (above diagonal) and D_A distances (below diagonal) between Lithuanian sea trout populations

	ADB-04	**ADB-07**	**ADE**	**ADF**	**BG**	**BP-04**	**BP-07**	**NDL**	**NDD-04**	**NDD-07**	**NDLa-06**
ADB-04		-0.010	0.083	0.037	0.092	0.093	0.050	0.037	0.042	0.069	0.049
ADB-07	0.065		0.074	0.049	0.116	0.102	0.068	0.068	0.061	0.076	0.066
ADE	0.199	0.168		0.066	0.181	0.208	0.159	0.123	0.054	0.087	0.092
ADF	0.148	0.114	0.220		0.084	0.098	0.079	0.017	0.026	0.084	0.042
BG	0.203	0.216	0.345	0.245		0.085	0.049	0.093	0.089	0.150	0.084
BP-04	0.167	0.156	0.326	0.178	0.155		0.019	0.079	0.100	0.162	0.085
BP-07	0.144	0.132	0.265	0.160	0.123	0.071		0.059	0.063	0.107	0.049
NDL	0.160	0.147	0.285	0.061	0.273	0.148	0.138		0.036	0.066	0.046
NDD-04	0.188	0.169	0.179	0.123	0.311	0.217	0.172	0.138		0.040	0.005
NDD-07	0.181	0.163	0.205	0.175	0.337	0.271	0.225	0.177	0.090		0.059
NDLa-06	0.193	0.166	0.226	0.119	0.265	0.166	0.140	0.117	0.048	0.117	

	NDLa-07	**NJU-04**	**NJU-06**	**NJE**	**NJS**	**NMB-04**	**NMB-05**	**NMM-05**	**NMM-07**	**NMTS**	**NSS**
NDLa-07		0.041	0.073	0.022	0.028	0.068	0.099	0.053	0.048	0.082	0.037
NJU-04	0.117		0.046	0.034	0.054	0.058	0.077	0.037	0.030	0.086	0.059
NJU-06	0.136	0.084		0.032	0.061	0.056	0.067	0.052	0.035	0.088	0.075
NJE	0.102	0.106	0.108		0.036	0.042	0.086	0.031	0.033	0.059	0.030
NJS	0.137	0.159	0.167	0.125		0.045	0.067	0.035	0.030	0.068	0.031
NMB-04	0.155	0.109	0.128	0.107	0.140		0.027	0.034	0.041	0.058	0.063
NMB-05	0.158	0.105	0.116	0.127	0.127	0.047		0.064	0.071	0.063	0.095
NMM-05	0.115	0.080	0.105	0.104	0.109	0.082	0.077		0.013	0.080	0.036
NMM-07	0.157	0.115	0.125	0.134	0.106	0.099	0.100	0.060		0.106	0.060
NMTS	0.172	0.149	0.148	0.127	0.127	0.126	0.121	0.120	0.157		0.055
NSS	0.107	0.097	0.126	0.123	0.110	0.146	0.151	0.091	0.143	0.134	

F_{ST} values between temporal replicates of the populations were significantly lower than between spatial samples within years (P<0.001, Mann-Whitney U-test).

Pair-wise genetic distances (D_A) were calculated between all populations pairs (Table 9). Estimation of the genetic distance between three major river basins showed that populations of Bartuva river basin were mostly genetically distant – average D_A values between Bartuva river and Akmena-Danė river basins as well as between Bartuva and Nemunas basins were 0.21 and 0.22, respectively and were significantly higher than between Akmena-Danė and Nemunas river basins (D_A = 0.16). Within Nemunas river basin, the largest genetic distance was between Dubysa and Minija river sub-basins and the smallest – between Jūra and Šyša river sub-basins (0.16 and 0.11, respectively).

Genetic distances between the major river basins was significantly higher than between populations from the same basin (p<0.05, Mann-Whitney U-test). Similarly, genetic distances between the different river sub-basins within Nemunas river basin was significantly higher than between populations from the same river sub-basin (p<0.05, Mann-Whitney U-test).

The Mantel test for isolation by distance over all populations revealed non-significant (p>0.05) association between the geographical and genetic distances. The same was true in more regional scale, within the Nemunas basin (p>0.05). However, when the enhanced populations were excluded from the analysis, association between geographical and genetic distance became highly significant both for all studied populations and for populations from the Nemunas basin (P<0.01).

The Neighbor-joining dendrogram of the Lithuanian sea trout populations based on D_A genetic distances illustrates the grouping of populations into two moderately supported (bootstrap value 57) major clusters (fig. 4).

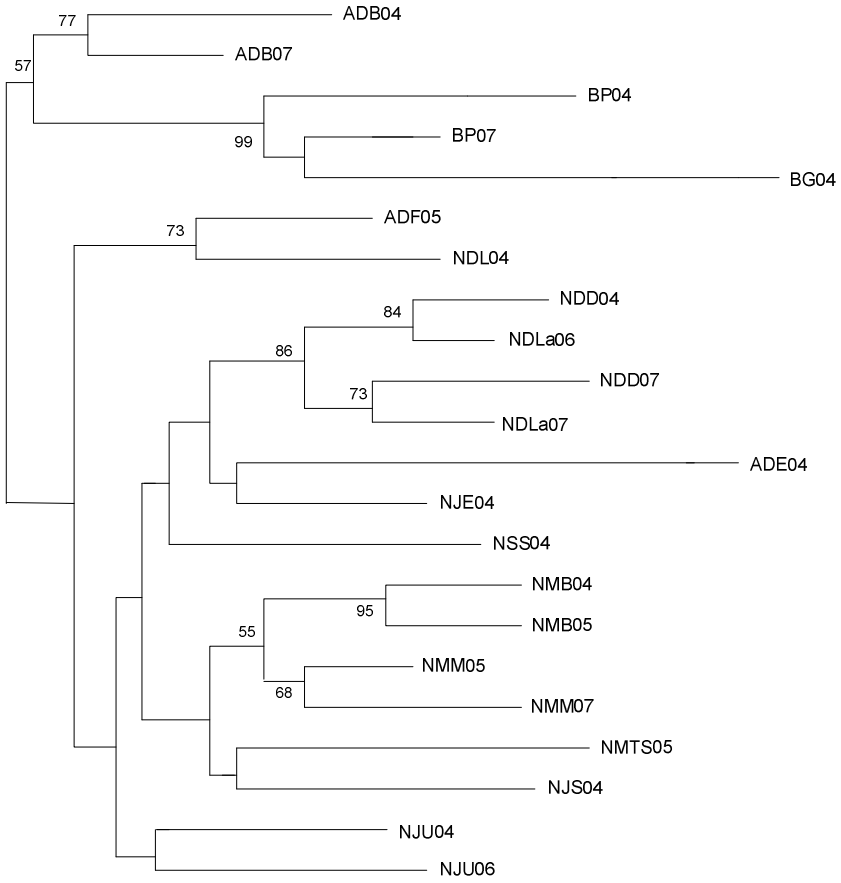


Figure 4: NJ dendrogram of the Lithuanian sea trout populations based on D_A distances.

One cluster consisted mainly of the populations from the Nemunas basin (with the exception of ADF and ADE-04 populations from the Akmena-Danė basin) while the other consisted of two well-supported subclusters that corresponded to the river basins of Akmena-Danė and Bartuva, respectively (Figure 4). Within the Nemunas cluster, the populations from Dubysa and Minija rivers formed two well supported sub-clusters (with the exception of NDL and NMTS) while the Jūra populations did not cluster together (Figure 4).

5.1.4 Hierarchical analysis of genetic structure

Analysis of molecular variance (AMOVA) was used to partition genetic variation into hierarchical levels. For the first analysis, the populations were grouped according to three major river basins (Nemunas, Akmena-Danė and Bartuva) that formed the highest level of hierarchy. The second level consisted of populations within basins and the third level consisted of individuals within the populations. The AMOVA analysis revealed that most of total variation of microsatellite loci (91.63%) was due to variation within the populations, whereas 3.04% was distributed among river basins and 5.33% was distributed among populations within river basins (Table 6). The percentage of variation due to differences between population groups increased to 4.66% after excluding enhanced populations (Table 10).

For the second analysis, the highest level of hierarchy consisted of four tributaries of the Nemunas (Minija, Jūra, Dubysa and Šyša), second level consisted of populations within tributaries and the third level consisted of individuals within the populations. The AMOVA analysis revealed that 93.86% of total genetic diversity within the Nemunas basin was due to variation within the populations; 4.34% was distributed among populations, and only 1.80% was explained by differences among the tributaries.

Table 10: Hierarchical gene diversity analysis of Lithuanian sea trout populations

Population groups for the highest level of hierarchy	Percentage of variation		
	Among groups	Among populations within groups	Within populations
Main river basins (Nemunas, Akmena-Dane, Bartuva)	3.04	5.33	91.63
Major tributaries within the Nemunas basin (Dubysa, Jura, Minija, Sysa)	1.80	4.34	93.86
Major tributaries within the Nemunas basin (Dubysa, Jura, Minija, Sysa) with hatchery stocks excluded	2.32	3.99	93.68
Main river basins (Nemunas, Akmena-Dane, Bartuva) with hatchery stocks excluded	4.66	5.06	90.28
Populations with temporal replicates (Bonalė, Pragulba, Dratvinys, Lapišė, Upinikė, Blendžiava, Mišupis)	5.40	2.01	92.59

After excluding the enhanced stocks from the analysis, 3.99% of the total genetic diversity was distributed among populations, and 2.32% was distributed among the tributaries (Table 10).

For the third analysis, only populations with temporal replicates were included in order to assess the relative magnitude of temporal *versus* spatial variation. AMOVA analysis revealed that the temporal variation (2.01% of the total variation) was almost three times smaller than the spatial variation (5.40%) but still significant (P<0.001), indicating that significant differences of allele frequencies existed among the temporal replicates within populations (Table10).

5.1.5 Contemporary gene flow

Based on the results of BAYESASS analysis, the most isolated populations (with the proportion of non-migrants 0.90 or higher) were Bonalė (ADB) and Eketė (ADE) from Akmena-Danė river basin, Pragulba from Bartuva river basin (BP), and Blendžiava (NMB), Šiūšis (NMTS) and Šustis (NSS) from Nemunas river basin, whereas the least isolated populations (with the proportion of non-migrants 0.80 or less) were enhanced population of Akmena-Danė river basin (ADF), Guntinas population from Bartuva river basin (BG), and Luknė (NDL), Ežeruona (NJE), Šunija (NJS) and Mišupis (NMM) from Nemunas river basin (Table11). The recent migration rates between populations belonging to different river basins (Akmena-Danė, Bartuva, Nemunas) were quite low: typically 0.00 or 0.01, rarely 0.02 or 0.03 (Table 11). The highest between river basin migration rates were observed from Bonalė population of Akmena-Danė river basin (ADB) to Ežeruona population of Jūra river sub-basin (NJE), from enhanced population of Akmena-Danė river (ADF) to Luknė population of Dubysa river sub-basin (NDL) and from Mišupis population of Minija river sub-basin (NMM) to enhanced population of Akmena-Danė river basin (ADF) (m = 0.05, 0.04 and 0.04, respectively).

Table 11: Bayesian assessment of migration proportions by populations (BAYESASS, Wilson and Ranalla 2003). Bolded terms along the diagonal represent proportion of nonmigrants within a population; values in rows represent migrants received from other sites; values in columns represent migrants donated to other populations.

	ADB	ADE	ADF	BG	BP	NDL	NDD	NDLa	NJU	NJE	NJS	NMB	NMM	NMTS	NSS
ADB	**0.98**	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ADE	0.01	**0.94**	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
ADF	***0.04***	0.01	**0.76**	0.00	0.01	0.01	0.01	0.02	0.03	0.00	0.01	0.01	***0.04***	0.03	0.01
BG	0.02	0.01	0.02	**0.74**	***0.08***	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
BP	0.00	0.00	0.00	0.00	**0.98**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NDL	0.02	0.01	***0.04***	0.00	0.03	**0.69**	0.02	0.03	***0.04***	0.00	0.01	0.02	0.02	0.03	0.02
NDD	0.02	0.00	0.00	0.00	0.00	0.00	**0.84**	***0.10***	0.01	0.00	0.00	0.01	0.00	0.00	0.00
NDLa	0.00	0.00	0.00	0.00	0.00	0.00	***0.08***	**0.82**	0.01	0.00	0.01	0.00	***0.04***	0.01	0.01
NJU	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.88**	0.00	0.00	***0.05***	0.03	0.00	0.00
NJE	***0.05***	0.01	0.01	0.01	0.01	0.01	0.03	0.02	***0.06***	**0.68**	0.01	***0.05***	0.02	0.02	0.01
NJS	0.02	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	**0.78**	***0.08***	0.01	0.01	***0.05***
NMB	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	**0.99**	0.00	0.00	0.00
NMM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	***0.18***	**0.77**	0.01	0.01
NMTS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.98**	0.00
NSS	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.01	**0.90**

Immigration rates to the Nemunas river basin were also quite low and mostly associated with Luknė population of Dubysa river (NDL) and Ežeruona population of Jūra river (NJE). Other populations from Nemunas river basin possessed negligible proportions of immigrants from Akmena-Danė and Bartuva river basins (Table 11).

Considering migration between tributaries of the Nemunas river (Dubysa, Jura, Minija and Šyša), populations of Minija and Šyša were mostly isolated, whereas Jūra and Dubysa received immigrants from populations that belonged to different sub-basins. For example, Šunija population of Jūra river (NJS) received relatively high proportion of immigrants from Blendžiava population of Minija river (NMB) and from Šustis population of Šyša river (NSS) (m = 0.08 and 0.05, respectively), while Ežeruona (NJE) and Upinikė (NJU) populations of Jūra river received immigrants from Blendžiava population of Minija (NMB; m = 0.05). Populations of the Dubysa river, Luknė (NDL) and Lapiše (NDLa), displayed relatively high proportions of immigrants (m = 0.04) from Upinikė population of Jūra river (NJU) and from Mišupis population of Minija river (NMM), respectively (Table 11).

Within the major tributaries of the Nemunas, the highest migration rates were observed in Minija and Dubysa river sub-basins. Within Minija sub-basin, Mišupis (NMM) population received high proportion of immigrants from Blendžiava (NMB; m = 0.18) and within Dubysa sub-basin, relatively high reciprocal migration (m = 0.08 and 0.10) between Dratvinys (NDD) and Lapiše (NDLa) was observed (Table 11). Relatively high migration rates we also found within the Jūra river sub-basin: in Ežeruona (NJE), the proportion of immigrants from Upinikė (NJU) was 0.06 (Table 11).

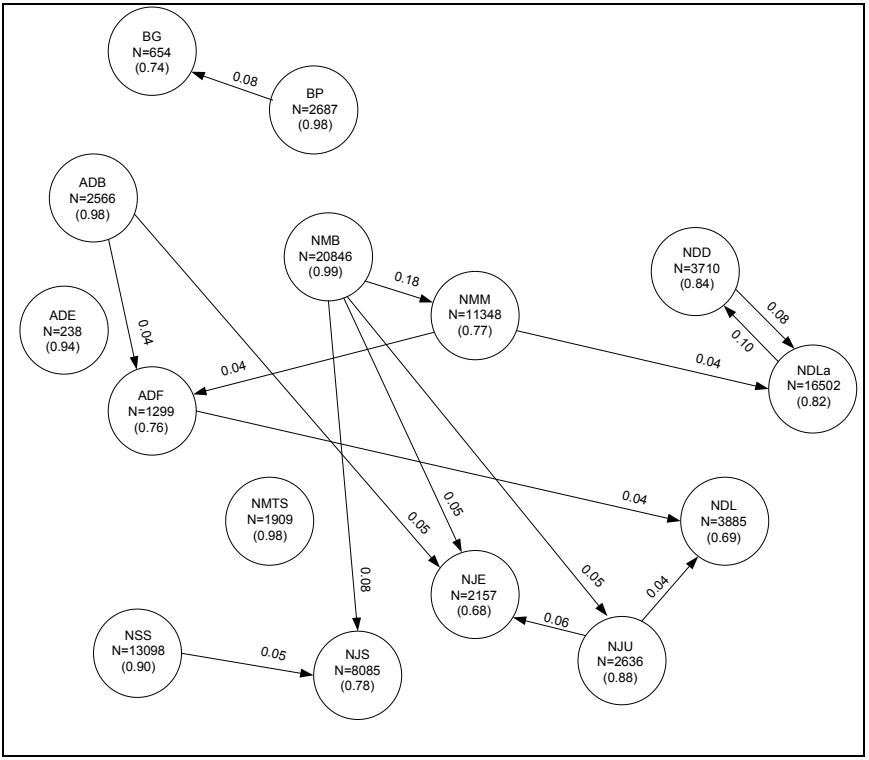


Fig. 5: Gene flow between Lithuanian sea trout populations based on contemporary migration estimates. Arrows indicate direction of gene flow; N denotes estimated numbers of sea trout parr and numbers in brackets indicate proportion of nonmigrants within population. Numbers on arrows represent proportion of migrants. Only migration rates larger than 0.03 are displayed.

Movement of sea trout between analyzed rivers was generally asymmetric. Rivers characterized by large numbers of sea trout parr, frequently functioned as sources of migrants. In Minija river basin, Blendžiava (NMB) and Mišupis (NMM) provided migrants not only to the neighboring rivers but also to the rivers that belong to other basins (Table 11, Fig. 5). Similarly, Pragulba population of Bartuva river basin (BP), Bonalė population of Akmena-Danė river basin (ADB) as well as Šustis population of Šyša river sub-basin (NSS) functioned as a source of migrants to smaller populations. Ežeruona population of Jūra river sub-basin (NJE) that is characterized by one of the smallest number of sea trout parr received immigrants from the largest number of populations (Table 11, Fig. 5). Large proportion of immigrants (from 0.16 to 0.24) were also observed in most enhanced populations of sea trout (ADF, NDD, NDLa and NJS). The only exception was NMTS where the proportion of non-migrants was very high (0.98).

Genetically effective migration rate depended also on geographical distance. In majority of cases, migration was more frequent to geographically closer populations. For example, Mišupis (NMM) received high proportion of immigrants from nearby population (26.9 km) Blendžiava (NMB) within the Minija sub-basin. Migration between geographically remote rivers was rare and the proportion of migrants was smaller.

5.2 Atlantic salmon

5.2.1 Genetic diversity in wild and farmed salmon populations

Exact Hardy-Weinberg tests showed significant deviations from Hardy-Weinberg equilibrium in 3 out of 25 tests at 5% significance level after Bonferroni adjustments. Two of them were in Hatchery-2000 population (SSOSL417 and Ssa202) and one was in Hatchery-1999 population (locus Ssa197 (Table 13)).

Linkage disequilibrium was not significant for most studied samples and only one and two pairs of loci were in linkage disequilibrium in populations Hatchery-2000 and Hatchery-2002 after applying Bonferroni correction for multiple tests. Significant linkage disequilibrium between loci in these populations can be most probably explained by sampling the siblings (Ohta 1982).

In total, 53 alleles were observed at the 5 microsatellite loci analysed, ranging from 6 (SSOSL85) to 20 (Ssa197). Average number of alleles detected per population varied from 3.25 (SSOSL85) to 9.75 (Ssa197) (Table 12).

Within locus genetic diversity was moderate to high with observed heterozygosities ranging from 0.591 (SSOSL85) to 0.808 (Ssa197) over all samples (Table 12). Individual loci varied in observed heterozygosity among populations, ranging from 0.388 (Ssa171 in Hat99) to 10.96 (SSOSL417 in Hat00) (Table 13). Expected heterozygosities ranged from 0.528 (SSOSL85) to 0.816 (Ssa197) over all samples (Table 12) and from 0.424 (Ssa171 in Hat99) to 0.881 (Ssa197 in Hat99) for individual loci among populations (Table 13).

Table 12: Locus by locus statistics (Aver.A – average number of alleles per locus; Aver.H_E – average expected heterozygosity; Aver.H_O – average observed haterozygosity; SD – standard deviation)

Locus	**Aver.A**	**SD**	**Aver.H_E**	**SD**	**Aver.H_O**	**SD**
Ssa197	9.750	4.500	0.816	0.059	0.808	0.125
SSOSL417	5.500	1.915	0.652	0.037	0.754	0.161
Ssa202	5.750	0.500	0.736	0.098	0.730	0.102
SSOSL85	3.250	1.258	0.528	0.042	0.591	0.126
Ssa171	5.750	2.217	0.613	0.142	0.637	0.185

The total number of alleles over seven loci ranged from 23 in the Hatchery2000 population to 43 in the Hatchery-1999 population. Allelic richness across all populations had a mean value of 5.46 and

within populations ranged from 4.51 in Hatchery-2000 population to 6.89 in Hatchery-1999 population (Table 13).

Table13: Micosatellite diversity indices for salmon populations from Lithuanian rivers. Figures provided are number of alleles (*A*), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosity and P-value for deviation from expected Hardy-Weinberg proportions (P_{HW}; significant deviations indicated in bold).

Population	**Ssa197**	**SSOSL417**	**Ssa202**	**SSOSL85**	**Ssa171**	**All loci**
Žeimena						
A	10	6	6	3	5	6.00
Ar	9.250	5.517	5.992	3.000	5.000	5.75
He	0.836	0.667	0.773	0.583	0.739	0.719
Ho	0.931	0.655	0.821	0.759	0.759	0.785
P_{HW}	0.355	0.434	0.519	0.146	0.363	
Hat-1999						
A	16	8	5	5	9	8.60
Ar	12.437	6.543	4.129	4.319	7.022	6.89
He	0.881	0.689	0.593	0.493	0.424	0.616
Ho	0.840	0.800	0.600	0.460	0.388	0.618
P_{HW}	**0.010**	0.084	0.657	0.149	0.075	
Hat-2000						
A	6	4	6	3	4	4.60
Ar	5.729	4.000	5.880	3.000	3.957	4.51
He	0.740	0.649	0.816	0.537	0.584	0.665
Ho	0.633	0.960	0.800	0.546	0.607	0.709
P_{HW}	0.1607	**0.0002**	**0.0005**	0.3272	0.5872	
Hat-2002						
A	7	4	6	2	5	4.80
Ar	6.965	3.984	5.733	2.000	4.756	4.69
He	0.806	0.602	0.762	0.497	0.704	0.674
Ho	0.828	0.600	0.700	0.600	0.793	0.704
P_{HW}	0.656	0.299	0.370	0.414	0.452	

Average observed heterozygosity across all populations was 0.704 and within populations varied from 0.618 in Hatchery-1999 population to 0.785 in wild Žeimena population. Expected gene diversity across all populations was 0.669 and within populations

varied from 0.618 in Hatchery1999 population to 0.719 in wild Žeimena population (Table 13).

The Wilcoxon's sign-rank test detected significant excess of heterozygosity in all studied salmon populations ($p<0.05$), except Hatchery-1999. It indicates that other hatchery populations as well as wild Žeimena population have recently experienced severe reduction in their effective population size.

Tests for comparison of genetic diversity indices showed that Hatchery-1999 population exhibited higher allelic richness than other two hatchery populations ($p<0.05$), but detected no significant differences in observed and expected heterozygosities ($p>0.05$). Similarly, comparison of genetic diversity of wild and hatchery populations found no significant differences neither in average allelic richness nor in average heterozygosities ($p>0.05$).

5.2.2 Allele frequencies of salmon populations

Range of allele lengths in each locus are given in the Table 14 and allele frequencies of each population at each locus are presented in Table 15.

Table 14: Range of allele lengths in each locus

Locus	Range of allele lengths (bps)
Ssa197	164-224
SSOSL417	159-203
Ssa202	240-268
SSOSL85	183-201
Ssa171	208-240

The proportion of shared alleles between populations of Žeimena and hatchery populations of local origin (Hatchery-2000 and Hatchery-2002) was 76%, whereas only 42% of common alleles were observed between Žeimena population and Hatchery-1999 population.

Table 15: Allele frequencies of each Lithuanian salmon population at each locus

Lokus	Populations			
Ssa197	**Žeimena**	**Hat-1999**	**Hat-2000**	**Hat-2002**
164	6,67	13,79		1,72
168	21,67	5,17		24,14
172	20,00	27,59		27,59
176				1,72
188	8,33	13,79	11,00	5,17
192			6,00	
194			1,00	
196			12,00	
198			1,00	
204	1,67		10,00	1,72
206			2,00	
208	41,67	29,31	4,00	13,79
210			1,00	
212			23,00	5,17
214			3,00	
216		5,17	4,00	12,07
218			2,00	
220			17,00	
222			2,00	
224		5,17	1,00	6,90
SSOSL417	**Žeimena**	**Hat-1999**	**Hat-2000**	**Hat-2002**
159	52,00	5,00	1,00	
161	8,00	11,67	8,00	22,41
163			2,00	1,72
183			7,00	8,62
185	14,00	56,67	50,00	51,72
187			9,00	
191			1,00	
193	26,00	26,67	22,00	13,79
203				1,72

Ssa202	Žeimena	Hat-1999	Hat-2000	Hat-2002
240	2,00			5,36
244	20,00	38,33	18,00	30,36
248	16,00	11,67	21,00	8,93
252			58,00	
256		1,67	2,00	
260	24,00	25,00	1,00	33,93
264	24,00	10,00		10,71
268	14,00	13,33		10,71
SSOSL85	**Žeimena**	**Hat-1999**	**Hat-2000**	**Hat-2002**
183	13,64			10,34
187			4,00	
195			1,00	
197	63,64	42,00	21,00	36,21
199	22,73	58,00	68,00	53,45
201			6,00	
Ssa171	**Žeimena**	**Hat-1999**	**Hat-2000**	**Hat-2002**
208	3,57		2,04	
212	12,50	6,90	1,02	12,07
216		25,86	7,14	10,34
220	25,00	43,10	75,51	41,38
224			2,04	
228			2,04	
232			5,10	10,34
236	58,93	22,41	3,06	25,86
238			2,04	

Considering all populations separately, a total of 22 private alleles were found in 4 populations and most of them (19 out of 22) were observed in Hatchery-1999 population. Two private alleles were found in the wild Žeimena population and only a single private allele was found in the Hatchery-2002 population. The frequency of private alleles in the populations of Žeimena and Hatchery-2002 did not exceed 0.02, whereas some private alleles of Hatchery-1999 population dominated over all others. For example, the frequency of

allele 252 in locus Ssa202 was 0.58, similarly the frequencies of alleles 196 and 220 in locus 197 were 0.12 and 0.17 (Table 15).

We found some very rare alleles, that frequency did not exceed 0.05, for example the frequency of allele 163 in locus SSOSL417 and allele 256 in locus Ssa202 did not exceed 0.02. We also found some alleles that dominate over others, for example the frequency of alleles 197 and 199 in locus SSOSL85 ranged from 0.86 to 1.00 across all populations, similarly the frequency of alleles 220 and 236 in locus Ssa174 ranged from 0.66 to 0.84.

5.2.3 Genetic differentiation and relationships among salmon populations

Significant differences in allele frequencies were detected between all studies salmon populations. Pair-wise F_{ST} values were calculated between all populations pairs (Table 16). The most distinguished were the Hatchery-1999 population which displayed F_{ST} values of 0.126, 0.127 and 0.252 in pair-wise comparisons with Žeimena, Hatchery-2002 and Hatchery-2000 populations, respectively, whereas the lowest F_{ST} value was between Žeimena and Hatchery-2002 populations (F_{ST} = 0.013) (Table 16).

Pair-wise genetic distances (D_A) were also calculated between all populations pairs (Table 16). The Hatchery-1999 population was mostly genetically distant – average D_A values between Hatchery-1999 population and wild Žeimena population as well as between Hatchery-1999 population and Hatchery-2000 was 0.308 and 0.453, respectively. Whereas the smallest genetic distance was found between wild Žeimena population and Hatchery-2002 population (D_A = 0.085).

Table 16: Pairwise F_{ST} values (above diagonal) and D_A distances (below diagonal) between Lithuanian salmon populations

	NNZ	**Hat-1999**	**Hat-2000**	**Hat-2002**
NNZ		0.126	0.107	0.013
Hat-1999	0.308		0.252	0.127

Hat-2000	0.168	0.453		0.111
Hat-2002	0.085	0.316	0.138	

6. DISCUSSION

6.1 Genetic diversity of sea trout and salmon populations

Analysis of microsatellite DNA variation revealed high level of polymorphism in Lithuanian sea trout populations. Comparisons of mean expected heterozygosity and mean allelic richness between river basins revealed that all examined river basins exhibited similar levels of genetic diversity in spite of significant differences in the estimates of their smolt production. However, comparison of genetic diversity of populations that belong to Nemunas river basin revealed significant differences between populations that belong to sub-basins of the tributaries Minija and Dubysa. The reason of increased genetic variation in the Dubysa river sub-basin can be natural or human mediated gene flow, as according to estimates of recent migration rates, the most isolated populations in terms of immigrants, belong to the Minija sub-basin, whereas populations from Dubysa river sub-basin receive immigrants not only from Nemunas basin but also from populations that belong to Akmena-Danė basin (Table 11, Fig. 5).

The levels of polymorphism and genetic diversity found in this study are comparable to those reported in studies from other regions of Atlantic sea trout. Average number of alleles per locus in Lithuanian populations ranged from 3.57 to 6.29, whereas the range of this measure was from 5.33 to 7.33 in Danish anadromous brown trout populations (6 common loci with our study; Hansen *et al*., 2002), from 5.33 to 6.67 in Polish sea trout populations (three common loci; Was and Wenne, 2003) and from 3.68 to 6.17 in Norwegian brown trout populations (four common loci; Sønstebø *et all*. 2007). Similarly, expected heterozygosity in Lithuanian populations ranged from 0.60 to 0.72, whereas the range of this measure was from 0.62 to 0.70 in Danish anadromous brown trout populations, from 5.33 to 6.67 in Polish sea trout populations and from 3.68 to 6.17 in Norwegian brown trout populations. However, in Spain it was found higher levels

of genetic diversity than seen in our study: number of alleles per locus varied from 11.67 to 17.00 and expected heterozygosity varied from 0.83 to 0.90 (Campos *et al.*, 2007).

It was found no evidence of recent bottleneck in Spanish sea trout populations (Campos *et al.*, 2007), whereas our results showed that most Lithuanian sea trout populations have recently experienced severe reduction in their effective population size. It cannot be excluded, however, that the Danish, Polish and Norwegian populations have also suffered from the reduced effective population size.

The level of polymorphism was also high in studied Atlantic salmon populations. Genetic diversity of Lithuanian salmon populations was comparable with other salmon populations from Baltic Sea region. Allelic richness (*Ar* = 5.46) and mean heterozygosity (*He* = 0.67) of Lithuanian populations was consistent with that reported for Estonia and Latvia (Estonia: *Ar* = 5.13; *He* = 0.60; Vasemägi *et al*. 2005a and Latvia: *Ar* = 6.90; *He* = 0.69; Säisä *et al.*, 2005). Therefore, genetic diversity of Lithuanian salmon populations was lower than reported in Gulf of Bothnia (*Ar* = 8.3; *He* = 0.72; Säisä *et al*., 2005) and in southern populations of Baltic sea (*Ar* = 8.4; *He* = 0.73; Säisä *et al*., 2005). The level of genetic diversity of Barent and White Sea populations as well as of populations of Eastern Atlantic and Spain was even more higher (Barent and White Sea: *Ar* = 10.6; *He* = 0.78; Säisä et all. 2005; Eastern Atlantic: *Ar* = 12.1; *He* = 0.81; Säisä *et al*., 2005; Spain: *Ar* = 9.5; *He* = 0.76; Horreo *et al*., 2008).

Analysis of genetic distances (D_A) and *Fst* values revealed that, because of non-local origin, Hatchery-1999 population was very distinct from other analyzed populations. Moreover, average genetic distance between this population and other analyzed populations was 0.36 and was similar to that reported between eastern Baltic sea and Gulf of Bothnia populations (D_A = 0.35; Säisä *et al*., 2005). At the same time, average *Fst* value between wild Žeimena population and other hatchery populations was 0.08 and was similar to that reported

between wild Estonian salmon populations (*Fst* = 0.10; Vasemägi *et al*., 2005a).

The level of genetic diversity in wild, enhanced and farmed populations of salmon and sea trout in Lithuania was similar. Population size bottlenecks were detected not only in enhanced or hatchery populations but also in majority of wild populations, where releases of artificially reared trout have never been carried out. Furthermore, the genetic composition of enhanced populations was more similar to the wild populations of Nemunas basin than to the other analyzed populations. These results are consistent with the Lithuanian stocking program that is based on wild spawners of local origin (Virbickas and Kesminas 2002).

6.2 Genetic structure of sea trout populations

Significant population differentiation was found both between river systems and between most population pairs within the same river system. Even geographically adjacent tributaries exhibited significant genetic differences. This indicates that Lithuanian sea trout populations are structured into distinct breeding units.

Quantitative analysis of genetic differentiation showed that genetic differences between populations from Akmena-Danė river basin and other analyzed populations as well as between populations from Bartuva river basin and other analyzed populations were larger than differences between populations from Minija, Jūra, Dubysa and Šyša sub-basins. The same situation was obvious from comparison of genetic distances which showed that populations from Akmena-Danė river basin as well as populations from Bartuva river basin are genetically more distinct from other studied populations whereas populations from Minija, Jūra, Dubysa and Šyša sub-basins are genetically more similar. This pattern could be explained by different geographical situation of analyzed rivers: Akmena-Danė drains to the Curonian Lagoon, Bartuva drains to the Liepaja Lake which is connected with the Baltic Sea, whereas Dubysa, Jūra, Minija and Šyša are tributaries of the Nemunas river, providing more possibilities for gene flow among the populations from the same river basin.

However, regardless clear importance of geographic region to genetic differences, we found no statistically significant differences of allele frequencies and no statistically significant *Fst* values between enhanced population of Akmena-Danė (ADF) and Luknė population from Dubysa river sub-basin (NDL) as well as between Bonalė-2004 population of Akmena-Danė (ADB-04) and Ežeruona population from Jūra river sub-basin (NJE). This finding could reflect relatively high level of gene flow mediated by stocking practices that translocate fish from one river basin to other.

Analysis of proportions of shared alleles between three major river drainages showed that this proportion between populations of Akmena-Danė river basin and populations of Nemunas river basin was higher than between populations of Bartuva and Akmena-Danė river basin and between populations Bartuva and Nemunas river basins, therefore populations of Akmena-Danė and Nemunas river basins are more similar in allele composition in comparison with populations of Bartuva river basin. However, when we considered only wild sea trout populations of Akmena-Danė and Nemunas river basins, proportion of shared alleles became lower and comparable with other values of between basin comparisons.

These similarities were also reflected in the relatively weak inter-regional structuring of populations – the AMOVA analysis showed that regardless of population grouping strategy, within-region variation was higher than the between-region variation and the genetic distance based analysis clearly showed only two main population groups with high bootstrap support. These results indicate that for the Lithuanian sea trout, the population structure at the level of within river basins is more pronounced than at the level of between river basins. It also shows the impact of both natural gene flow and translocations of fish from different tributaries of Nemunas and also from different river basins during stocking practices. The latter became obvious after excluding enhanced populations from the AMOVA analysis since the percentage of among-basin differentiation increased markedly. AMOVA results also indicated that although structuring between three regions is not strong when all populations

are considered, there is evidence that wild populations exhibit much stronger differentiation at this spatial scale (Table 10).

6.3 Temporal stability of sea trout populations

Analysis of temporal stability of populations revealed statistically significant differences in allele frequency distribution and significant F_{ST} values between temporal replicates taken from Dratvinys and Lapiše populations from Dubysa river sub-basin (NDD and NDLa), Upinikė population from Jūra river sub-basin (NJU) and Blendžiava population from Minija river sub-basin (NMB). Temporal AMOVA also showed significant changes in allele frequencies among temporal replicates within populations. This indicates that these populations are not temporally stable over short time scales of two to four years. Unstable population genetic composition and structure has been reported also in several other studies of salmonids (e.g. Laikre *et al*., 2002; Ostergaard *et al*., 2003; Jensen *et al*., 2005; Hansen *et al*., 2009) though there were also studies that demonstrated stability of genetic population structure, for instance in Atlantic salmon (Nielsen *et al*., 1999; Tessier and Bernatchez 1999; Palstra *et al*., 2007; Vähä *et al*., 2008) and also brown trout (Hansen *et al*., 2002; Campos *et al*., 2007). Main reasons of significant temporal genetic changes include random genetic drift in populations that have very small effective population size (Laikre *et al*., 2002; Palm *et al*., 2003), population size bottlenecks or extinction–recolonization events determined by unfavorable environmental conditions (Østergaard *et al*., 2003; Jensen *et al*., 2005) and effects of captive breeding as well as introgression from other populations by stocking activity (Säisä *et al*., 2003; Hansen *et al*., 2009). In our study we found evidence of recent population bottleneck in many populations. However, reduction of effective population size was detected not only in populations with unstable allele frequency distribution, but also in other populations from Nemunas and Bartuva basins in which allele frequencies were stable over time.

Computer simulations have provided evidence that in organisms with overlapping generations the total population consists of several age classes (cohorts) that may have different allele frequencies and such population will display considerably larger allele frequency shifts than the population with discrete generations (Ryman 1997). Moreover, it was shown that if sampled population consists of individuals of only single year class (cohort), changes in allele frequencies can be much more pronounced than in sample that consists of different cohorts and that the possibility to find significant differences in allele frequencies is higher when samples do not consist of even representation of a cohort (Ryman 1997; Palm *et al*., 2003). In our study, sampled populations consisted of the individuals of the 0+ age class, therefore allele frequency differences that were found between temporal samples of the same river were likely due to restricted number of age classes. Moreover, the AMOVA analysis showed that the genetic variation attributable to temporal variation within populations is three times lower (2.0%) than the variation attributable to spatial variation among sampling sites (5.4%). This indicates that temporal variation within sampling sites was relatively low compared to the spatial differences between them. The higher variation attributable to spatial variation compared to temporal variation was also reflected in the pairwise F_{ST} and D_A values, which were significantly lower between temporal replicates of the populations than between spatial samples within the years ($p<0.05$; Mann-Whitney U-test). Moreover, temporal samples grouped together according to the site of origin in the neighbor-joining dendrogram. These results provide additional evidence that spatial diversity is a more important constituent of total genetic diversity of Lithuanian sea trout populations and allows treating them as temporally stable. Several previous studies have also reported temporal variation within populations that was several times lower than the spatial component of variation and did not change the spatial differentiation pattern and accordingly, allowed to infer temporal stability of the analyzed populations (Campos *et al*., 2007; Heggenes *et al*., 2009; Ozerov *et al*., 2010).

6.4 Impact of contemporary gene flow on fine scale genetic structure of sea trout populations

Analysis of contemporary migration rates showed that two of the currently largest populations (NMB, NMM) were the main donors of migrants and that the direction of gene flow was predominantly from large into small populations. These findings indicate asymmetric pattern of contemporary gene flow where larger populations act as sources of migrants and smaller populations act as recipients. This result is in good accordance with the other recent studies of brown trout and salmon where asymmetric gene flow from large into small populations was observed (Hansen *et al.*, 2007, Palstra *et al*., 2007). It is evident that asymmetric gene flow can be helpful in maintaining genetic diversity in small populations (Palm *et al*., 2003; Consuegra *et al*., 2005). For instance, the Ežeruona population from Jūra river sub-basin (NJE) has one of the smallest parr densities among Lithuanian sea trout populations and nevertheless exhibits a relatively high level of genetic diversity, similar or even higher than those found in much larger populations. This can be explained by naturally occurring gene flow as we found that this population receives the largest number of migrants from other rivers. The gene flow is of such magnitude that prevents loss of genetic diversity but preserves genetic differences. Therefore, magnitude and asymmetry of natural gene flow could be very important for the preservation of genetic variability in the Lithuanian sea trout.

It is obvious that geographic distance between populations can be important contributor to genetic structure but, in contrast to many other studies of salmonids (Bouza *et al.*, 1999; Carlsson and Nilsson, 2000; Ruzzante *et al.*, 2001; Campos *et al.*, 2007; Palstra *et al.*, 2007), we did not detect significant correlation between geographical and genetic distances when all populations were considered. However, when the enhanced populations were excluded from the analysis the correlation became highly significant. Consequently, natural patterns of isolation-by-distance could be modified by human impact through transplantations of trout between populations. Indeed, we found a relatively high migration rates between wild and enhanced sea trout

populations that belong to different river basins. For example, migration rate from ADF to NDL and from NMB to NJS was 0.04 and 0.08, respectively, whereas the waterway distance between these populations was 294 km and 248 km, respectively. This suggests the possibility of transplantation of juveniles between wild and enhanced populations.

Altogether, our results showed relationships between genetic diversity, gene flow and population structure of Lithuanian sea trout. It is obvious that genetic diversity and population genetic structure of Lithuanian sea trout reflects contemporary dispersal and gene flow, both natural and human mediated. The larger portion of the genetic diversity revealed in this study is distributed among populations within geographical regions than between regions. The proportion of variance attributable to differences between regions was highest (4.13%) when only wild populations were considered in analysis. Furthermore, the genetic structure of wild sea trout populations fit isolation by distance model where geographical distances between populations are important, whereas no evidence that limited gene flow between remote populations is an important factor for the observed genetic structure was apparent when all studied populations were considered. Consequently, human mediated gene flow from stocked to wild populations alters hierarchical as well as spatial population structure of Lithuanian sea trout. It is also evident, that asymmetrical and distance restricted natural gene flow between wild populations is one of the fundamental contributor to the genetic structure of Lithuanian sea trout. Asymmetrical and quite extensive gene flow may also determine relatively small genetic differences among rivers in this region as well as maintenance of genetic diversity and counteraction inbreeding in small populations by immigration from larger ones.

6.5 Implications for management of salmon and sea trout populations

This study provided the detailed information on the amount of genetic diversity and distribution of genetic variation between and within populations, i.e. information on the spatial genetic structure, of

salmon and sea trout in Lithuania. This knowledge can be useful and must be incorporated into management actions of salmon and sea trout recourses in Lithuania.

This study reported high levels of genetic diversity both in wild and enhanced populations. It was also found an importance of geographic region to genetic differences. These findings were ascribed to proper Lithuanian stocking program that is based on wild spawners of local origin. There are a number of studies showing that releases of populations of non-local origin have affected wild populations. It was demonstrated that releases of non-native hatchery reared salmon caused genetic homogenization between wild populations and hatchery stocks (Vasemägi *et al*., 2005b) as well as altered the genetic integrity of unique gene pools of wild trout since stocked and wild trout interbreed (Apostolidis *et al*., 2008). It was also evident that stocking with non-indigenous trout has disturbed the genetic patterns of wild populations (Moran *et al*., 2005) and caused introgression of exotic alleles and even domination of them in intensively stocked populations (Jug *et al*., 2005). All these threats, consequently, can disrupt local adaptations and reduce fitness of wild populations (Utter, 2001; Alendorf *et al*., 2001).

Also there is empirical evidence that maintaining populations in hatcheries for several generations may cause negative genetic effects. For example, Säisä et al. (2003) assessed genetic changes in two Atlantic salmon stocks in Finland by comparing genetic parameters of these stocks before and after long-term (40 and 33 years respectively) captive breeding and found that hatchery broodstocks experienced significant decrease in allelic richness and mean heterozygosity. Similarly, Aho *et al*. (2006) found that in hatchery broodstocks of sea trout in Finland genetic diversity decreased over time since founding and that the size of founding population is an important factor for the level of genetic variability over time. Similarly, positive correlation was found in Atlantic salmon between mean heterozygosity and number of founder individuals (Verspoor, 1998).

Genetic changes in hatchery stocks that have been reared in captivity for several generations can result in severe negative effects on wild populations they interact with. A 37-years study of Atlantic salmon in Ireland showed that release of captive bred salmon into the wild can substantially disrupt the capacity of natural populations to adapt to higher winter water temperatures associated with climate variability (McGinnity *et al*., 2009). Similarly, Hansen *et al*., (2010) examined Danish populations of brown trout that were supplemented by hatchery releases for 60 years and found evidence of contemporary selection in wild populations against alleles introduced by hatchery strain trout.

Supportive breeding in Lithuania is based only on wild breeders. All produced offspring are released in to the rivers, thus the brood-stock is obtained from the wild every year. This type of stocking is referred as supportive breeding (Hansen *et al*., 2000b) to differentiate it from other forms of stocking. Results of this study indicated that this breeding practice is adequate for maintaining genetic diversity levels similar to the wild populations.

Even though supportive breeding has advantages over stocking with non-local or farm-reared domesticated fish, it is not without its problems. Several studies showed that even a short period in a hatchery can result in reduction of survival and reproductive success because hatchery environment alter behaviour and physiology and also because genetic changes that arise due to differential or relaxed selection (Glover *et al*., 2004; Sundström *et al*., 2004). Consequently, the period in the hatchery should be as short as possible.

It was evident that genetic diversity can decrease in hatcheries because of breeding related individuals or using a small numbers of parents (Norris *et al*., 1999). The fish brought into captivity have more offspring than the wild fish if the breeding program is successful. Therefore, if a limited number of parents is used, inbreeding and loss of genetic variability in the naturally spawning population can occur because higher reproductive rates of reared fish affects the overall effective population size (Ryman and Laikre, 1991; Ryman 1994;

Ryman *et al*., 1995; Waples and Do 1994; Nomura 1999). It is recognized that the number of unrelated individuals founding the new population should be as large as possible and comprise at least 50 genetically effective founders (Allendorf and Ryman 1987). The reason of reduced effective population size also can be the unequal sex ratio; therefore an equal number of each sex should be used, with at least 25 of each sex. Therefore, to preserve as much genetic variation as possible, it is also essential to optimize breeding programmes with regard to number of parents, relatedness of breeders and sex ratio of breeders.

Genetic effects of stocked individuals also depend on population genetic structure of wild populations and the degree of genetic divergence between the released individuals and the wild population. This study revealed that Lithuanian sea trout populations form three main population groups that correspond to three main river basins: Akmena-Danė, Bartuva and Nemunas. It was also obvious that there is genetic structuring at the level of tributaries. Therefore, management strategies of Lithuanian sea trout should take into account that populations inhabiting different rivers and different tributaries of the same river are genetically differentiated and should be regarded as a separate distinct populations. Consequently, adults used for stocking should be taken from the same tributary and the progeny should be released in the same tributary in order to maximize their chances of survival and recruitment. Existing evidence that relocation of fish from one neighbouring geographical group to another during stocking practices was not very successful (as influence of stocked fish to wild populations was very little) (Sønstebø *et al*., 2007) and low survival of even moderately differentiated hatchery fish compared to the local wild population (Hansen *et al*., 2000a) provide empirical support that conservation strategies must be based on local populations.

This study revealed close genetic similarity between enhanced population of Akmena-Danė (ADF) and Luknė population from Dubysa river sub-basin that belong to the Nemunas basin (NDL). The genetic relationship between populations that inhabit different river

basins may be attributable to enhancement of Akmena-Danė with hatchery reared individuals originated from Nemunas basin. This study also suggested that human mediated gene flow from stocked to wild populations alters hierarchical as well as spatial population structure of Lithuanian sea trout. Fish translocations between different river basins, particularly between Nemunas and Akmena-Danė and also between different rivers basins within Nemunas basin, could result in greater within-region variation in comparison with between-region variation and no detection of isolation-by-distance effect. According these findings, translocations between rivers that belong to different river basins, as in the case of Akmena-Danė and Dubysa, should be strictly avoided and concerning rivers of Nemunas river basin, appropriate procedures ensuring that hatchery reared individuals originated from one river sub-basin will not be released in other, should be developed.

Some recent empirical studies provided evidence for asymmetric gene flow from large to small populations in salmonid fishes and predicted that it can be important for maintaining genetic diversity and countering inbreeding depression in small populations and that possible future population recoveries will be mediated primarily by the remaining large populations (Consuegra *et al*., 2005; Palstra *et al*., 2007; Fraser *et al*,. 2007; Hansen *et al*., 2007). This study also indicated asymmetric pattern of contemporary gene flow in Lithuanian sea trout populations and suggested that it prevents loss of genetic diversity in relatively small natural populations (as in the case of Ežeruona population). It was also evident that presently largest wild populations (NMB and NMM in Nemunas river basin, ADB and BP in Akmena-Danė and Bartuva river basin, respectively) are the main donors of migrants in corresponding region; therefore it is very important to these populations should be managed very carefully. Even if contemporary dispersal occurs predominantly from current large census populations into smaller populations, long-term patterns can be quite different, with small populations functioning as sources of gene flow (Palstra *et al*., 2007). Therefore it is important to continue focusing conservation efforts on small rivers. Special

attention must be paid to Ežeruona from Jūra river sub-basin and Eketė from Akmena-Danė river basin because these populations have several very rare alleles and Ežeruona has two private alleles that can be easily lost.

Another very important result of this study is that due to relatively extensive contemporary gene flow within river basins, inappropriate genetic manipulation of fish in one population may negatively affect the whole river system. One of the most important issues in conservation programmes is re-introduction of former salmon or trout rivers. According recent studies, the best donor populations for such reintroductions are geographically proximate wild populations (Vasemägi *et al*., 2005a).

The important concept in the management of threatened salmonid species is effective population size (*Ne*). *Ne* is a measure of the rate of genetic drift and is directly related to the rate of loss of genetic diversity and the rate of increase in inbreeding within a population. The rate of loss of genetic diversity due to genetic drift is grater in populations with small *Ne* and this rate is expected to increase as *Ne* decreases (Frankham *et al*., 2002). Therefore in order to understand how genetic diversity in the stocks of Atlantic salmon and sea trout in Lithuania may be affected in the future, it would be useful to know *Ne* of each population.

This study provides basic information regarding current genetic composition and population genetic structure of Atlantic salmon and sea trout. This information can constitute important prerequisite for genetic monitoring that is needed for identifying and preserving gene level diversity.

Based on the results of this study the following recommendations for management are suggested:

1. Breeding practice that is based on wild breeders only is adequate for maintaining genetic diversity levels similar to the wild populations and for avoidance of negative genetic changes in wild populations. Maintaining a hatchery broodstock will result in the problems of domestication.

2. To preserve as much genetic variation as possible, it is also essential to optimize breeding programmes with regard to number of parents, relatedness of breeders and sex ratio of breeders.
3. Management strategies of Lithuanian sea trout should take into account that populations inhabiting different rivers and different tributaries of the same river are genetically differentiated and should be regarded as a separate distinct populations. Consequently, adults used for stocking should be taken from the same tributary and the progeny should be released in the same tributary in order to maximize chances of survival and recruitment.
4. Translocations between rivers of different river basins, as in the case of Akmena-Danė and Dubysa, should be strictly avoided. Appropriate procedures preventing releases of hatchery reared individuals into other river basin than their origin, should be developed for Nemunas river basin.
5. Indicated asymmetric pattern of contemporary gene flow in Lithuanian sea trout populations can be important for maintaining genetic diversity and countering inbreeding depression in small populations. Therefore, presently largest wild populations (NMB and NMM in Nemunas river basin, ADB and BP in Akmena-Danė and Bartuva river basin, respectively) should be managed with special regard.
6. Special attention must be paid to conservation efforts on small rivers such as Ežeruona from Jūra river basin and Eketė from Akmena-Danė river basin. These populations have several very rare alleles and Ežeruona has two private alleles that can be easily lost.
7. Due to relatively extensive contemporary gene flow within river basins, inappropriate genetic manipulation of fish in one population may negatively affect the whole river system. Therefore, the best donor populations for re-introductions of former salmon or trout rivers are geographically proximate wild populations.

8. It is necessary to implement genetic monitoring program that is needed for identification of diversity changes at gene level and preserving existing genetic recourses.

7. CONCLUSIONS

1. Level of genetic diversity in Lithuanian salmon and sea trout populations was high despite recent population size bottlenecks in many of them. All examined river basins exhibited similar levels of genetic diversity in spite of significant differences in the estimates of their smolt production.
2. Most of the tributaries of the Nemunas river also displayed similar levels of genetic diversity, the only exceptions were the tributaries Dubysa and Minija. Higher rates of genetically effective immigrants even from different river basin could be the reason of increased genetic variation in Dubysa river in comparison to Minija river.
3. Level of genetic diversity in wild, enhanced and farmed populations of salmon and sea trout in Lithuania was quite similar that is consistent with the Lithuanian supportive breeding program that is based only on wild spawners of local origin.
4. Populations inhabiting different rivers and different tributaries of the same river were genetically differentiated. Population structure of analyzed sea trout populations corresponded to three main river basins: Akmena-Danė, Bartuva and Nemunas. Structuring within Nemunas basin was significantly weaker.
5. Genetic structure of wild sea trout populations fitted isolation by distance model, whereas it was not relevant when all studied populations were considered. Similarly, proportion of variance attributable to differences between regions was highest when only wild populations were considered in analysis. Consequently, human mediated gene flow from stocked to wild populations alters hierarchical as well as spatial population structure of Lithuanian sea trout.
6. Spatial diversity of Lithuanian sea trout populations was more important constituent of total genetic diversity than temporal

variation therefore Lithuanian sea trout populations were temporally stable.

7. Lithuanian sea trout was characterized as a population system with asymmetric and distance restricted contemporary gene flow where larger populations acted as sources of migrants and smaller populations acted as recipients and where gene flow between different river basins was more restricted than within river basins.
8. Future management strategies should consider maintaining of individual populations even at tributary level and ensuring the natural levels of gene flow among populations.

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