Genetic diversity of a Daugava basin brown trout (*Salmo trutta*) brood stock

Thomas Schmidt1,2,* , Matiss Zagars3, Armands Roze3 and Ralf Schulz1,2

1 Institute for Environmental Sciences, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany
2 Eusserthal Ecosystem Research Station, University of Koblenz-Landau, Birkenthalstrasse13, 76857 Eusserthal, Germany
3 Institute for Environmental Solutions, Lidlauks, 4101 Priekulu Parish, Priekulu County, Latvia

Abstract – Genetics play an increasingly important role in the conservation of threatened fish populations. We have examined twelve microsatellite markers to determine the genetic diversity of a brood stock of brown trout from the Latvian Daugava river basin, used in a local supportive breeding program and compared diversity values to other Baltic populations. Allelic data was further inspected for indications of increased inbreeding. Additionally, we have analyzed the mitochondrial control region to classify the population within a broader phylogenetic framework. We found that the genetic diversity was comparatively low, but there was no strong evidence of high inbreeding. A newly detected mitochondrial haplotype indicates unnoticed genetic diversity of “Atlantic lineage” brown trout in the Daugava basin region. Our study provides first genetic details on resident brown trout from the Baltic Daugava river basin to improve the regional conservation management of this valuable genetic resource and contributes phylogeographically useful information.

Keywords: *ex situ* conservation / individual inbreeding coefficient $F$ / D-loop / effective population size $N_e$ / salmonids

**Résumé** – Diversité génétique d’un stock de géniteurs de truite brune (*Salmo trutta*) du bassin de la Daugava. La génétique joue un rôle de plus en plus important dans la conservation des populations de poissons menacées. Nous avons examiné douze marqueurs microsatellites pour déterminer la diversité génétique d’un stock de géniteurs de truite du bassin letton de la Daugava, utilisé dans le cadre d’un programme de reproduction locale et comparé les valeurs de diversité à celles d’autres populations baltes. Les données sur les allèles ont été analysées plus à fond afin de déceler des signes d’augmentation de la consanguinité. De plus, nous avons analysé la région de contrôle mitochondrial pour classifier la population dans un cadre phylogénétique plus large. Nous avons constaté que la diversité génétique était comparativement faible, mais qu’il n’y avait pas de preuves solides de consanguinité élevée. Un haplotype mitochondrial nouvellement détecté indique une diversité génétique inaperçue de la truite de “lignée atlantique” dans la région du bassin de la Daugava. Notre étude fournit les premiers détails génétiques sur la truite résidente du bassin de la Daugava balte afin d’améliorer la gestion régionale de la conservation de cette ressource génétique précieuse et fournit des informations utiles sur le plan phylogéographique.

**Mots-clés** : conservation *ex situ* / coefficient de consanguinité individuelle $F$ / boucle D / taille effective de la population $N_e$ / salmonidés

1 Introduction

Numerous fish populations are threatened by different environmental factors and human activities (e.g. Freyhof and Brooks, 2011), and diverse measures are taken to conserve such populations. Populations involved in conservation programs should be genetically characterized and compared to other populations of the species to set up appropriate conservation strategies (e.g. Attard et al., 2016; Luck et al., 2003; Palsbøll et al., 2007). Such information may help to prioritize conservation strategies (Araguas et al., 2007; Fraser and Bernatchez, 2001; Luck et al., 2003; Nunney and Campbell, 1993).

Supportive breeding is a common *ex situ* conservation strategy, often used after or along with *in situ* strategies like...
directly increasing population size. In the ideal case, specimen effective population size breeding programs is often restricted. Thus, typically the number of spawners in breeding programs is often restricted. Thus, typically the effective population size \( N_e \) of brood stocks is low, which may lead to adverse genetic effects, such as inbreeding depressions (e.g. Fraser, 2008; Naish et al., 2013). Genetic analyses of brood stocks (spawners or descendants) may indicate such issues early (e.g. Naish et al., 2013).

Brown trout (Salmo trutta) is a species with a high level of genetic diversity and complicated spatial patterns of genetic variability indicate a complex evolutionary history (e.g. Bernatchez, 2001; Cortey et al., 2009; Laikre, 1999; Lerceteau-Kohler et al., 2013; McKeown et al., 2010). Based on analyses of mitochondrial DNA (mtDNA) at least six major genetic lineages have been detected in different regions of the Eurasian native range of the species (Bernatchez, 2001; Cortey et al., 2009; Suarez et al., 2001; Susnik et al., 2005). The “(Northern-) Atlantic lineage” is the most widespread lineage and natively distributed from western to northern Europe. Beside this large scale variability, brown trout may show considerable regional or even local differentiation (e.g. Lehtonen et al., 2009; Palmé et al., 2013), which may indicate local adaption (Meier et al., 2011), and plays an increasingly important role in conservation (e.g. Fruciano et al., 2014; Vilas et al., 2010). Additionally, natural patterns of diversity have been altered by various human activities (Kohout et al., 2012 and citations therein) more recently, which adds further complexity. Nevertheless, in several regions and certain populations human influence on the genetic diversity of brown trout may be still absent or negligible (Lerceteau-Kohler et al., 2013; Van Houdt et al., 2005). Although genetic diversity of brown trout has been studied frequently, genetic data on this species are still lacking for important regions within its natural distribution range, like the Baltic Daugava river basin. Against this background, we have analyzed genetically a brood stock of brown trout to enhance a local conservation initiative in Latvia. This brood stock is used to restock and support a threatened population of resident brown trout in Virgulica creek in the Daugava river basin. Virgulica creek brown trout are mainly threatened by the loss of suitable spawning grounds caused by extensively increased dam buildings of beavers (Castor fiber). This had led to a considerable decline in population size, and several stretches of the creek have been totally without trout. To protect the fish population of Virgulica creek, spawning grounds have been restored and offspring of a locally derived brood stock of brown trout was used to repopulate the creek afterwards. These efforts were undertaken by local, private initiatives, which may be considered a common situation for conservation efforts of single, specific fish stocks. Typically, comprehensive genetic analyses, including populations outside the focal area, are far beyond the capabilities of such initiatives. However, local breeding programs may benefit from genetic analyses of the population under consideration itself and further comparisons with data from other regions – if available (cp. George et al., 2009).

To improve the Virgulica creek trout restoration efforts, we have used nuclear and mitochondrial markers and compared the genetic diversity of the brood stock to other Baltic populations. Further, we have examined the allelic data for indications of inbreeding. Finally, our study provides first genetic details on resident brown trout from the Baltic Daugava river basin and contributes new phylogeographically relevant information on brown trout from an understudied region. Overall, this study may improve the regional management of the valuable genetic diversity of brown trout in the Daugava river basin.

2 Materials and methods

2.1 Location and brood stock

Virgulica creek is a small (length ca. 20 km) tributary of River Pededze, in the Daugava river basin in Latvia (57.44°N, 27.33°E) and a typical salmonid creek of the region (Fig. 1). The dominant land use around Virgulica creek is forestry, but also some agriculture. In the 1960/70s the creek was partly straightened for agricultural land reclamation. In recent years the main threat to brown trout in Virgulica creek was loss of spawning grounds and habitat fragmentation caused by beaver dams. Licensed recreational angling takes place at the creek, but fishing pressure is generally low. However, illegal poaching has been observed.

Virgulica creek has a historic watermill dam 150 m before joining River Pededze, which presumably prevents fish upstream migrations. No stocking with foreign brown trout happened within the last 20 years and there is no indication for earlier introductions. Thus, we consider the Virgulica creek brown trout as autochthonous.

The Virgulica creek brood stock was derived from 50 wild brown trout, caught in the most downstream, largely unmodified stretch of the creek in 2009. Eggs of several randomly selected females were fertilized with the milt of the respective number of likewise randomly selected males. Thereby, all available specimen (50), regardless of phenotypic properties (e.g. size, early/late maturity), were used to conserve genetic diversity and avoid artificial selection.

2.2 Sampling and genetic analysis

In November 2011 adipose fin tissue of 25 specimens of the first generation offspring of the breeding program was clipped and tissue samples were preserved in 96% ethanol and stored at −20°C in the lab. DNA was extracted using a modified (Wetjen et al., 2017) salt protocol (Aljanabi and Martinez, 1997).

Nuclear DNA of all 25 specimens was examined at twelve microsatellite loci in two multiplex-PCRs (Type-it Microsatellite PCR Kit, QIAGEN) and one single PCR (Tab. 1). The 5μl reaction volumes contained different volumes of the primers (Tab. 1), 1x Type-it Multiplex PCR Master Mix, 0.5x Q-Solution (QIAGEN) and 10 ng DNA. Cycling parameters were: initial denaturation (95°C, 5 min), 30 cycles at 94°C (30 s), 57°C (90 s), 72°C (60 s), and final extension at 60°C (30 min). The loci were analyzed on an automated sequencer (CEQ 8000, Beckman Coulter) using the GenomeLab DNA Size Standard Kit (400 and 600 respectively, Beckman Coulter).
Further, the control region (CR) of the mtDNA of 11 specimens was amplified with primers Str-L19 (5' - CCAC-TAGCTCCAAAGCTA-3') and Str-H17 (5' - ACTTTC-TAGGGTCCATC-3') (Bernatchez et al., 1992), as detailed in Wetjen et al. (2017). Bidirectional sequencing was done by SeqIT GmbH & Co KG.

2.3 Data analysis

Prior to further analyses we checked completeness of allelic data and determined polymorphism of loci to reject uncomplete and monomorphic loci. MICRO-CHECKER Version 2.2.3 (Van Oosterhout et al., 2004) was used to test for null alleles. We determined the size range of alleles [base pairs (bp)], the number of alleles and genotypes, the allele frequencies (Supplemental Information Tab. S1), the allelic richness (AR) and observed and expected heterozygosity ($H_{obs}$ and $H_{exp}$) for each locus and calculated the difference $H_{exp} - H_{obs}$ and the fixation index $F_{IS}$. Further, we tested conformity to the Hardy–Weinberg equilibrium (HWE) per locus. At population level we calculated the expected heterozygosity $H_e$ and the means of

<table>
<thead>
<tr>
<th>Locus</th>
<th>Concentration [µM]</th>
<th>PCR</th>
<th>Size range [bp] (Ref.)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MST 15</td>
<td>0.05</td>
<td>multiplex A</td>
<td>214–224</td>
<td>Presa and Guyomard (1996)</td>
</tr>
<tr>
<td>Sco 216</td>
<td>1.0</td>
<td>single</td>
<td>329–347</td>
<td>Dehaan and Ardren (2005)</td>
</tr>
<tr>
<td>Ssa 85</td>
<td>0.0125</td>
<td>multiplex A</td>
<td>110–138</td>
<td>O'Reilly et al. (1996)</td>
</tr>
<tr>
<td>SsaA 86</td>
<td>0.025</td>
<td>multiplex A</td>
<td>169–241</td>
<td>King et al. (2005)</td>
</tr>
<tr>
<td>SSOSL 85</td>
<td>0.15</td>
<td>multiplex A</td>
<td>194</td>
<td>Slettan et al. (1995)</td>
</tr>
<tr>
<td>MST 60</td>
<td>0.05</td>
<td>multiplex B</td>
<td>97–111</td>
<td>Presa and Guyomard (1996)</td>
</tr>
<tr>
<td>MST 73</td>
<td>0.075</td>
<td>multiplex B</td>
<td>140–158</td>
<td>Presa and Guyomard (1996)</td>
</tr>
<tr>
<td>OMM 1310</td>
<td>0.5</td>
<td>multiplex B</td>
<td>175–289</td>
<td>Palti et al. (2002)</td>
</tr>
<tr>
<td>Sco 204</td>
<td>0.035</td>
<td>multiplex B</td>
<td>107–173</td>
<td>Dehaan and Ardren (2005)</td>
</tr>
<tr>
<td>Ssa 410 UOS</td>
<td>0.5</td>
<td>multiplex B</td>
<td>198–324</td>
<td>Cairney et al. (2000)</td>
</tr>
<tr>
<td>OMM 1323</td>
<td>0.05</td>
<td>multiplex A</td>
<td>101–206</td>
<td>Palti et al. (2002)</td>
</tr>
<tr>
<td>Ssa 417 OUS</td>
<td>0.15</td>
<td>multiplex B</td>
<td>265–424</td>
<td>Cairney et al. (2000)</td>
</tr>
</tbody>
</table>

Fig. 1. Location of the Virgulica creek (red cross) in Latvia and approximate locations of non-hatchery samples from Carlsson and Carlsson (2002), Carlsson and Nilsson (2000), Lehtonen et al. (2009), Nilsson et al. (2008), Östergren et al. (2015), Samuiloviene et al. (2009), Was and Bemas (2016), and Was and Wenne (2002) (Tab. 2). Locations for Östergren et al. (2015) are indicated at the respective river outlets.
the above genetic diversity values per locus and specimen respectively with standard errors (SEM). For comparison of our results on \( n = 25 \) specimens from Virgulica creek we acquired genetic diversity values for 77 Baltic population samples from Carlsson and Carlsson (2002) Carlsson and Nilsson (2000) Lehtonen et al. (2009) Nilsson et al. (2008) Östergren et al. (2015) Samuiloviene et al. (2009) and Was and Wenne (2002) (Fig. 1, Tab. 2). We obtained the mean number of alleles (\( n = 34 \)), the mean AR (\( n = 55 \)), \( H_{\text{obs}} \) (\( n = 35 \)) and \( H_{\text{exp}} \) (\( n = 77 \)) per population.

For each specimen we derived a likelihood function of the individual inbreeding coefficient \( F \) and estimated a mean \( F \) by randomly sampling 1000 \( F \)-values from the distribution of the probability density from this function. Further, we estimated the pairwise relatedness over all loci \( M_{xy} \) (Blouin et al., 1996) between all specimens. We used ‘adegenet’ v. 1.3-9.2 (Jombart, 2008), ‘hierfstat’ v. 0.04-10 (Goudet, 2013) and ‘Demerelate’ v. 0.9-3 (Kraemer and Gerlach, 2017) in R v. 3.0.2 (R Core Team, 2013).

MitDNA CR sequences were aligned and assigned to previously published haplotypes (Bernatchez, 2001; Bernatchez et al., 1992; Cortey and Garcia-Marin, 2002; Duftner et al., 2003; Kohout et al., 2012; Weiss et al., 2001) and major mtDNA lineages (Bernatchez, 2001; Bernatchez et al., 1992) using the Geneious 6.0 software (Biomatters). Haplotype diversity \( h \) was estimated as \( h=n(n-1)/(1-\sum x_i^2) \), with sample size \( n \) and frequency of haplotype \( x_i \) (Nei and Tajima, 1981). For comparison we obtained or calculated haplotype diversities from Cortey and Garcia-Marin (2002; \( n = 10 \)), Duftner et al. (2003; \( n = 5 \), and Kohout et al. (2012; \( n = 29 \) for 44 populations with at least 10 specimens genotyped.

3 Results

The locus Ssa 417 UOS could not be amplified in 17 samples (68%), while for all other loci percentage of missing data was within an acceptable range (\( \leq 12\% \)). Loci Ssa 417 UOS and OMM 1323 were 100% monomorphic, and thus rejected, so that further analyses included allelic data from 25 individuals at 10 polymorphic loci. No evidence for null alleles was found.

For the Virgulica stock the mean number of alleles was 3.40 (SEM 0.43) and the mean AR was 3.80 (SEM 0.04). The mean \( H_{\text{obs}} \) was 0.52 (SEM 0.02) and the mean \( H_{\text{exp}} \) was 0.53 (SEM 0.05). Table 3 shows detailed characteristics per locus. All four diversity values were in the lower quartile of the respective values obtained from other studies (Fig. 2). The mean number of alleles in the reference samples ranged from 3.2 to 8.0 (median 4.72, mean 4.75, SEM 0.23) and the mean AR from 3.26 to 8.57 (median 4.61, mean 5.28, SEM 0.20). The range of \( H_{\text{obs}} \) was 0.39 to 0.80 (median 0.64, mean 0.62, SEM 0.02) and 0.47 to 0.75 (median 0.66, mean 0.65, SEM 0.01) for \( H_{\text{exp}} \).

The mean difference of \( H_{\text{obs}} - H_{\text{exp}} \) was 0.01 (SEM 0.43), mean \( F_{IS} \) was 0.04 (SEM 0.56) and significant deviation from HWE was observed at locus Ssa410UOS (Tab. 3). The genetic diversity within the population \( H_s \) was 0.53.

Estimates of mean \( F \) ranged from 0.147 (sample ID: F1459) to 0.53 (F1464). The mean of \( F \) of 20 specimens were below 0.33, slightly exceeded 0.4 for two specimens (0.41; F1456, F1461) and were higher than 0.5 for another three (0.53; F1464, F1467, F1473) (Supplemental Information Tab. S2). Figure 3 shows a graphical representation of the likelihood functions of \( F \). At population level, the mean of individual \( F \)-values was 0.26 (SEM 0.03). Pairwise relatedness \( M_{xy} \) ranged from 0.25 (F1461–F1468) to 0.83 (F1464–F1470) with a mean of 0.53 (SEM 0.0065) (Fig. 4).

Based on a comparison of 247 bp of haplotype At-s1 (310 bp; GenBank accession number M97969; Bernatchez, 2001; Bernatchez et al., 1992) and based on 401 bp to haplotype Atl (464 bp; AF321990; Weiss et al., 2001) all mtDNA CR sequences were identical. A comparison of the full 946 bp segments assigned eight specimens to haplotype H2 (1012 bp; AF273087) and two specimens to haplotype H3 (1012 bp; AF274574) in Cortey and Garcia-Marin (2002). The haplotypes H2 and H3 are identical to the haplotypes At1b and

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**Table 2.** Number of population samples, mean number of specimens per sample, total number of specimens, number of analyzed microsatellites (\( N_{MS} \)), river and sea basin of sample origin, and years of sampling from eight studies from which genetic diversity data were acquired for comparison with our results on the Virgulica creek brood stock sample. Comparisons of diversity values per sample are shown in Figure 2.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Specimens</th>
<th>( N_{MS} )</th>
<th>River basin(s)</th>
<th>Sea basin(s)</th>
<th>Year(s) of sampling</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>49 8</td>
<td>Sävarån</td>
<td>Bothnian Bay</td>
<td>2005-2006</td>
<td>Nilsson et al. (2008)</td>
</tr>
<tr>
<td>13</td>
<td>21.77 (2.19)</td>
<td>283 8</td>
<td>Akmena-Dane, Bartuva, Dubyra, Jura, Minija</td>
<td>Southern Baltic Sea</td>
<td>2003-2005</td>
<td>Samuiloviene et al. (2009)</td>
</tr>
<tr>
<td>9</td>
<td>101.00 (8.72)</td>
<td>909 7, 12 Vistula</td>
<td>Southern Baltic Sea</td>
<td>1971, 2003, 2011</td>
<td>Was and Bernas (2016)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Genetic diversity values of the \( n = 25 \) specimens determined per locus. Bold numbers indicate significant \( (p < 0.0001) \) deviations from Hardy–Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles</th>
<th>Size range [bp]</th>
<th>Genotypes</th>
<th>AR</th>
<th>( H_{\text{obs}} )</th>
<th>( H_{\text{exp}} )</th>
<th>( H_{\text{exp}} - H_{\text{obs}} )</th>
<th>( F_{\text{IS}} )</th>
<th>( \chi^2 )</th>
<th>d.f.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MST 15</td>
<td>3</td>
<td>221–227</td>
<td>5</td>
<td>3.00</td>
<td>0.40</td>
<td>0.52</td>
<td>0.12</td>
<td>0.24</td>
<td>5.8164</td>
<td>3</td>
<td>0.1209</td>
</tr>
<tr>
<td>Sco 216</td>
<td>3</td>
<td>151–179</td>
<td>5</td>
<td>3.00</td>
<td>0.50</td>
<td>0.57</td>
<td>0.07</td>
<td>0.13</td>
<td>2.5972</td>
<td>3</td>
<td>0.4580</td>
</tr>
<tr>
<td>Ssa 85</td>
<td>3</td>
<td>111–115</td>
<td>6</td>
<td>3.00</td>
<td>0.64</td>
<td>0.62</td>
<td>-0.02</td>
<td>-0.02</td>
<td>1.8441</td>
<td>3</td>
<td>0.6054</td>
</tr>
<tr>
<td>SsaAa 86</td>
<td>2</td>
<td>174–182</td>
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<td>2.00</td>
<td>0.32</td>
<td>0.36</td>
<td>0.04</td>
<td>0.12</td>
<td>0.3770</td>
<td>1</td>
<td>0.5392</td>
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<tr>
<td>SSOSL 85</td>
<td>3</td>
<td>178–188</td>
<td>4</td>
<td>2.99</td>
<td>0.32</td>
<td>0.42</td>
<td>0.10</td>
<td>0.24</td>
<td>3.3179</td>
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<td>0.3452</td>
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<td>93–97</td>
<td>3</td>
<td>4.00</td>
<td>0.64</td>
<td>0.49</td>
<td>-0.15</td>
<td>-0.30</td>
<td>2.2306</td>
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<tr>
<td>MST 73</td>
<td>2</td>
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<td>2.00</td>
<td>0.48</td>
<td>0.48</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0000</td>
<td>1</td>
<td>1.0000</td>
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<tr>
<td>OMM 1310</td>
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<td>182–192</td>
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<td>3.92</td>
<td>0.58</td>
<td>0.61</td>
<td>0.03</td>
<td>0.05</td>
<td>2.1724</td>
<td>6</td>
<td>0.9032</td>
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<tr>
<td>Sco 204</td>
<td>6</td>
<td>98–172</td>
<td>9</td>
<td>8.49</td>
<td>0.64</td>
<td>0.62</td>
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<td>4.9649</td>
<td>15</td>
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<tr>
<td>Ssa 410 UOS</td>
<td>6</td>
<td>202–254</td>
<td>7</td>
<td>5.64</td>
<td>0.64</td>
<td>0.59</td>
<td>-0.05</td>
<td>-0.08</td>
<td>\textbf{54.5449}</td>
<td>\textbf{15}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OMM 1323</td>
<td>1</td>
<td>153</td>
<td>1</td>
<td>172</td>
<td>9</td>
<td>8.49</td>
<td>0.64</td>
<td>0.59</td>
<td>54.5449</td>
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<td>&lt;0.0001</td>
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<tr>
<td>Ssa 417 OUS</td>
<td>1</td>
<td>369</td>
<td>1</td>
<td>115</td>
<td>3</td>
<td>2.00</td>
<td>0.32</td>
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<td>0.3770</td>
<td>1</td>
<td>0.5392</td>
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</table>

Mean (SEM) 3.40 (0.43) 5.10 (0.03) 3.80 (0.04) 0.52 (0.02) 0.53 (0.05) 0.01 (0.43) 0.04 (0.56)

AR: allelic richness, \( H_{\text{obs}} \) observed heterozygosity, \( H_{\text{exp}} \) expected heterozygosity, \( F_{\text{IS}} \) fixation index, HWE deviation from Hardy–Weinberg equilibrium.

At1d in Duftner et al. (2003). A third haplotype, represented by one specimen, was not found in any previous study. It differs from haplotypes At1b and At1d by one mutation at nucleotide position 527 (Tab. 4). This sequence was named At1q, following the designation of Duftner et al. (2003) to standardize haplotype nomenclature, and deposited in GenBank (KT360957).

Additionally, we compared haplotypes At1b, At1d, and At1q to a 285 bp segment at the 3' end of the CR associated to haplotype At1 (328 bp; Bernatchez et al., 1992). Haplotype At1d differs at three positions from At1, while haplotypes At1b and At1q, which are identical in this segment, differ at four positions. Differences include two insertions/deletions in either of these cases (Tab. 4).

The estimated haplotype diversity \( h \) was 0.47, and thus within a medium range: It is between the lower quartile and the median of haplotype diversities found in populations from earlier studies \((n = 44\), range 0.00 to 0.90, mean 0.53, SEM 0.04; Fig. 5).

4 Discussion

The comparison of genetic diversity values of Baltic brown trout populations based on neutral nuclear markers overall revealed that the diversity of the Virgulica creek brood stock is rather low. Low genetic diversity is often regarded as a warning signal that a population might be or become threatened by increased inbreeding or deleterious genetic drift (e.g. Naish et al., 2013 and citations therein). Thus, maintenance or establishment of high levels of genetic diversity is a common aim of conservation efforts (Saura and Faria, 2011). Nevertheless, low genetic diversity may occur in small, wild salmonid populations without preventing survival and adaption (Pujolar et al., 2016 and citations therein), so that the comparatively low genetic diversity of the Virgulica creek brood stock itself is not necessarily a major concern.

Direct comparisons of genetic diversity between micro-satellite based studies might be affected e.g. by the selection of different markers (Ryman et al., 2006). However, this effect should be reduced at population level by averaging over a number of loci with different levels of polymorphism. The number of loci in the studies used here for comparison ranged from 5 to 14 (mean 8.1, SEM 0.25; Tab. 2). However, the necessary number of loci is disputable (Selkoe and Toonen, 2006). Further, rare alleles might be missed because of low sampling sizes, which also makes comparisons between studies difficult. Our sample of the Virgulica creek brood stock is at the lower range of sampling sizes per population used for comparison (Tab. 2). This might partly explain the comparatively low diversity found here. However, measured by the low number of 50 specimens in the parental generation, we believe that our sample is representative for the brood stock. The range of genetic diversity values compared here, might in part reflect the ecological range of Baltic brown trout (e.g. effective population sizes, life history traits, isolation, or population history, like bottlenecks), so that overall, despite methodological difficulties, we believe that this approach is helpful in providing a context for the further assessment of the Virgulica creek population.

The mean difference of \( H_{\text{exp}} - H_{\text{obs}} \) the mean \( F_{\text{IS}} \), and deviations from HWE, provide better evidence for inbreeding than above genetic diversity values. Both, mean \( H_{\text{exp}} - H_{\text{obs}} \) and \( F_{\text{IS}} \), were nearly zero. Significant deviation from HWE was found only at a single locus. Thus, all three values did not indicate significant inbreeding at the population level. In contrast we have found certain indications for inbreeding at the individual level, i.e. mean \( F > 0.4 \), in 5 specimens (20%). We consider this indication as weak \((0.4 < F \leq 0.5) \) for two (8%) specimens and as reasonable \((F > 0.5) \) for three (12%) specimens. Higher values of \( F \) in first generation offspring may be explained by kinship within the sample or the parental generation. The mean, range and frequency distribution of \( M_{xy} \) in the brood stock sample match the expectations for full
sibs (or parent–offspring pairs). Our results come very close to the findings of Blouin et al. (1996) for a breed of full siblings from wild parents in mice (*Mus musculus*). Thus, our sample apparently includes mostly closely related specimens. The spawners of the brood stock were mainly sampled from just several stretches of a rather small section of Virgulica creek. Thus, already this sample may have contained (half) siblings. By mixing sperm and eggs of several spawners the risk of producing offspring exclusively from one pair of siblings was minimized. However, mating of (half) siblings may also occur in the wild, especially in headwaters where effective population sizes are typically low (Hansen and Jensen, 2005). Also, at the population level, mean $F$ appears uncritically low. Thus, we believe that the proportion of specimens with indications of reasonable inbreeding of less than 15% is acceptable in a local breeding program (cp. Ruzzante et al., 2001). However, in a future perspective the Virgulica creek breeding program could benefit from conducting sib-avoidance matings to further delay inbreeding. Overall, our analyses of microsatellite data confirm that low genetic diversity itself is a rather insufficient indicator of inbreeding. Thus, aiming at increasing genetic diversity in conservation programs may not be simply justifiable by avoidance of inbreeding depressions.

Our analyses of the mtDNA CR have demonstrated that the brown trout population of Virgulica creek shows /C0 as expected for the Daugava river basin (northern) "Atlantic" haplotypes. Haplotypes At1b and At1d are the most common haplotypes in central and northern Europe. This is well reflected in our results: 91% of the specimens were examined in the present study (a: 3.40, b: 3.80, c: 0.52, d: 0.53).
were assigned to either of them. One out of just eleven analyzed specimens, however, revealed a previously undescribed haplotype. This suggests that the Daugava river basin may hold undetected genetic diversity of brown trout. This assumption is supported by the variation at the 3’ end of the CR compared to haplotype At1 (sensu Bernatchez et al., 1992). Such previously undetected diversity in understudied regions is potentially phylogeographically relevant and lastly important to establish appropriate conservation strategies at a larger scale (e.g. Cortey and Garcia-Marin, 2002; Schenekar et al., 2014). Our study provides a first basis towards future conservation strategies for brown trout in the Daugava river basin.

From an applied perspective, our study shows the importance of comparing genetic diversity data between studies to better evaluate values measured in a single population of interest. This comparison revealed, that the genetic diversity of the Virgulica creek brood stock is relatively low. Analyses of inbreeding showed, that – despite overall low diversity – the supportive breeding procedures applied in the Virgulica creek program appear appropriate to conserve the valuable genetic diversity of brown trout at a local scale.

Table 4. Variable base positions among the three haplotypes of the n=11 specimens based upon 946 bp of the mtDNA CR and additionally a 285 bp segment of the 3’ end of haplotype At1 (328 bp; acc. no. M97968; Bernatchez et al., 1992). Nucleotide positions are numbered according to the reference sequence ‘haplotype 2’ (AF273087; Cortey and Garcia-Marin, 2002). Identity with the reference sequence is indicated with . and indels are marked with /. Number (N) and relative frequency (Freq.) of each haplotype is given.

<table>
<thead>
<tr>
<th>Haplotype name †</th>
<th>Variable sites</th>
<th>N</th>
<th>Freq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT-s1</td>
<td>At1</td>
<td>H2</td>
<td>At1b</td>
</tr>
<tr>
<td>AT-s1</td>
<td>At1</td>
<td>H3</td>
<td>At1d</td>
</tr>
<tr>
<td>AT-s1</td>
<td>At1</td>
<td></td>
<td>At1q</td>
</tr>
</tbody>
</table>

†The haplotype names are composed of the haplotype names of the reference sequences and separated by |, as follows: Bernatchez et al. (1992) and Bernatchez (2001) | Weiss et al. (2001) | Cortey and Garcia-Marín (2002) | Dufner et al. (2003) and this study.
‡Position 731 is followed by another three cytosine nucleotides in haplotypes At1b, At1d and At1q.
§Position 750 is followed by two more adenine nucleotides in haplotypes At1b, At1d and At1q.
**Fig. 5.** Frequency (bars) and distribution (boxplot) of haplotype diversity $h$ of 44 samples of brown trout from Cortey and Garcia-Marin (2002; $n=10$), Duftner et al. (2003; $n=5$) and Kohout et al. (2012; $n=29$). Asterisks and dashed line mark the haplotype diversity of the Virgulica creek brood stock (0.47).

**Supplementary Material**

Supplementary tables.

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**References**


