Supporting Information for:

Genomic dynamics of brown trout populations released to a novel environment

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Table of Contents:

| Appendix/S1 | Detailed material and methods | Page 2 |
|-------------|------------------------------|--------|
| Table S1    | Mapping statistics           | Page 6 |
| Table S2    | Number of variants and windows used for diversity metrics | Page 6 |
| Table S3    | Pooled heterozygosity score ($H_P$) for each population pool | Page 6 |
| Table S4    | Statistical tests for amount $H_P$ between pairwise pools | Page 7 |
| Table S5    | Statistical tests for $\pi$ between pairwise pools | Page 7 |
| Table S6    | Statistical tests for Tajima’s D between pairwise pools | Page 7 |
| Table S7    | Distributions of SNP counts in the different delta allele frequency bins for different functional impacts | Page 8-9 |
| Table S8    | Functional categories of SNPs fixed between introduced populations | Page 10 |
| Table S9    | Read depth for non-synonymous SNPs identified as candidates for adaptive divergence between introduced populations A and B, and candidates for novel selection in the new lake system | Page 10 |
| Table S10   | Genes of adaptive importance for introduced populations | Page 11-12 |
| Table S11   | Genes putatively shaped by relaxed selection in the new environments on chromosome 28 | Page 13 |
| Table S12   | Genes putatively under directional selection in the new environments on chromosome 7 | Page 14-15 |
| Table S13   | Glossary for some of the terminology used | Page 17 |

| Figure S1   | Genome wide heterozygosity per pool ($H_P$) in 5 kb windows | Page 18 |
| Figure S2   | Genome-wide nucleotide diversity ($\pi$) and Tajima’s D ($T_D$) | Page 19 |
| Figure S3   | Boxplots of difference in allele frequency ($\Delta AF$) between each of the introduced populations A and B and established populations LB and HV for putatively selective regions | Page 20 |
| Figure S4   | Potentially adaptive differences between the two introduced populations A and B | Page 21 |
Appendix S1: Detailed material and methods

Study system
The introduced populations A and B exhibited significant allele frequency differences at multiple allozyme loci, particularly at AGP-2, where the population from Lake Kallsjön was close to fixation for one of the alleles at this locus, while in Lakes Fälpfjälltjärnarna two alleles occurred at about equal frequencies (Palm & Ryman 1999). Spawners from each population were chosen to be homozygous for different alleles at this locus, thereby enabling discrepancy of lineages after release to the same environment (Palm & Ryman 1999). Brown trout established rapidly in the lakes Bävervattnen area after the release and have been sampled continuously with tissues stored in -80°C freezers. The first documented reproduction occurred only a few years after the release (Palm & Ryman 1999) and ten years later both lakes downstream of the release site harbored strong populations from which sampling was easy. During the period 2003-2007 several lakes below the release site were sampled and brown trout establishment confirmed in all of those lakes (Figure 1; unpublished data).

Table. Sampling information for the material used in this study (cf. Figure 1).

| Lake                          | Geographic location (coordinates in WGS84 dec) | Area (km²) |
|-------------------------------|-----------------------------------------------|------------|
| Lilla Bävervattnet (established population LB) | 64.048928 14.633335 | 0.132      |
| Haravattnet (established population HV)       | 64.027934 14.715422 | 0.121      |
| Kallsjön (source population introduced population A) | 63.6167 13.0000 | 158        |
| Fälpfjälltjärn p 645, northern (source population introduced population B) | 64.181465 14.739145 | 0.1281     |
| Fälpfjälltjärn p 671, southern (source population introduced populations B) | 64.173004 14.737597 | 0.0929     |

DNA extraction
Genomic DNA was extracted and eluted in 100 µl elution buffer. DNA fragmentation was assessed on 2% agarose gels stained with GelRed and absorbance at 260/280. High molecular weight DNA from each individual was quantified using fluorometry (Qubit; Thermo Scientific) and combined at equal concentrations for each population to achieve 3 μg pooled genomic DNA in a volume within the range of 65–120 µl.

Library construction and sequencing
PCR-free paired-end libraries had an average insert size of 350 bp, sequencing used read length 150 bp and was performed by NGI across 3 (introduced populations A and B) or 4 (established populations LB and HV) lanes per pool.

Mapping and variant calling
The quality of sequenced reads from each pool were assessed using FastQC v.0.11.5 (Leggett et al. 2013) and results from different pools jointly evaluated using MultiQC v.1.5 (Ewels et al. 2016). Quality assessments of bam files were obtained from SAMtools flagstat and Qualimap v.2.2.1 (García-Alcalde et al. 2012), and summarized in MultiQC v.1.5 (Ewels et al. 2016). Variant calling was conducted in SAMtools using minimum base and mapping quality scores of 20 and the parameter “base alignment quality” (BAQ; “-B”) to reduce false SNPs caused by misalignments, resulting in one mpileup file for all four pools. The mpileup file was then converted to synchronized format using the ‘mpileup2sync.jar’ script of PoPoolation2 v.1.201 and used for downstream analyses of allele frequencies and FST in PoPoolation2 v.1.201. The mpileup file was also split for estimation of within-population metrics in PoPoolation v.2.2.
(Kofler et al. 2011a), which requires pool-based pileup files. Estimates of within-population metrics from Pool-seq data are sensitive to sequencing errors and variation in coverage (Kofler et al. 2011a). The mpileups were therefore subsampled without replacement to uniform depths (20-150X) using the ‘subsample-pileup.pl’ script implemented in PoPoolation v.2.2 (Kofler et al. 2011a). Depth thresholds were chosen based on the mode of the read depth histogram for each pool (using a minimum depth of 0.5xmode and a maximum depth of mode+0.5xmode for subsampling; see Kurland et al. 2019).

VCF files were created from bam files using BCFools v.1.10 (Li et al. 2009) by calling and genotyping raw variants in the software’s mpileup and call algorithms, using default settings. Variants were compared with those called in the estimation of allele frequencies in Popoolation2 v1.201 (Kofler et al. 2011b), only keeping sites found in both files. These were then controlled for mapping quality, number of high-quality bases, read positional bias, base quality bias, and mapping quality versus strand bias, before filtering for mapping quality 100. The final set (7.4x10⁶ variants) were annotated in SnpEff v.5.0 (Cingolani et al. 2012).

**Patterns of genomic variation and divergence**

Nucleotide diversity (π; Tajima, 1983) and Tajima’s D (TD; Tajima, 1989) were estimated using the ‘variance-sliding.pl’ script of PoPoolation v.2.2 (Kofler et al. 2011a) in 5 kilo base pair (kb) non-overlapping windows, with a minor allele count of 2 for a SNP to be called and applying the same depth thresholds as for the subsampling (20-150X). Windows were retained for subsequent analysis if at least 80% of the window was covered with data within these depth thresholds.

We primarily used the default $F_{ST}$ (Nei 1973) but also computed the alternative option in PoPoolation2 which is the approach of Karlsson et al. (2007) since these two approaches have different merits (Saha et al. 2021, their Supporting Appendix S4). A minor allele count of 3 was applied for SNP calling. Windows were only retained if the fraction of the number of sites within a window covered with data exceeded 80%. Genetic relationships among populations were examined by creating a dendrogram in TreeMix (Pickrell & Pritchard 2012), which constructs a maximum likelihood phylogeny based on the genomic data and compares the covariance structure calculated for the estimated dendrogram to the observed covariance between populations. TreeMix was run with default settings and the results visualized in MEGA X (Kumar et al. 2018).

**Genomic distribution of SNPs**

SnpEff v.5.0 (Cingolani et al. 2012) was used to annotate the genomic distribution of variants and to classify them into functional elements (non-synonymous, synonymous, untranslated region (UTR), 5 kb upstream, 5 kb downstream, intragenic, and intergenic). Functional elements from less confidant annotations, e.g. missing start and stop codons from the transcript (Barrio et al. 2016), were omitted and some functional elements were combined in order to avoid too small classes at high ΔAF (e.g. 3’ UTR and 5’ UTR combined to functional class “UTR”). For each of these functional categories, the allele frequency differences between introduced populations were sorted into bins (10 equally large bins of ΔAF=0-0.1, ΔAF=0.1-0.2 etc.). Log2 fold change was retrieved by comparing the observed and expected number of SNPs per category and bin. The expected number of SNPs in each category and bin was estimated as p(category) x n(bin), where p(category) equals the proportion of a given SNP category in the full genome and n(bin) the number of SNPs in an allele frequency bin. Statistical significances of deviations of observed from expected SNP counts were tested with standard chi-square tests of independence.

**Screening for indications of selection in the new environment**
We screened for signatures of selection in the new environments by considering local reductions of heterozygosity score within each pool ($H_P$) relative to its pool-based genome-wide average (cf. Rubin et al. 2010; Kardos et al. 2015; Kjærner-Semb et al. 2016; Willoughby et al. 2018). Normalized $H_P$ ($ZH_P$) was used in this approach $ZH_P = (H_P - \mu H_P) / \sigma H_P$ as calculated per population pool (following Rubin et al. 2010).

In order to identify putatively selected genes in the new environment, we selected windows (5 kb) of particularly low $ZH_P$ out of the windows with $ZH_P < 0$ in descendant populations (below 5th percentile of $ZH_P$ in LB and HV, respectively). Precedence was given to the windows with lowest levels of $H_P$ in both descendant populations and regions with multiple adjacent windows. Patterns of population divergence ($F_{ST}$) surrounding these windows was then studied to corroborate assumptions of selection. The same approach was given to windows of markedly low diversity within introduced populations. The functional annotation of SNPs within windows of low $H_P$ in introduced populations and established populations respectively were extracted from the VCF annotated in SnpEff v.5.0 (Cingolani et al. 2012).

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Table S1. BAM file statistics from QualiMap for Pool-seq data from 4 populations pools mapped to the S. trutta assembly (size 2.4 Gb), using bwa mem and default settings. Results are presented for properly paired-end reads with minimum base quality 20. Average coverage and its standard deviation (SD), and mean mapping quality is also included. Down-stream analyses were additionally limited to reads with read depth 20-150X.

| Statistic                                          | Introduced population A | Introduced population B | Established population LB | Established population HV |
|----------------------------------------------------|-------------------------|-------------------------|----------------------------|---------------------------|
| Number of sequenced bases (x 10⁹)                  | 194                     | 209                     | 166                        | 192                       |
| Number of reads mapped as pairs (x 10⁹)            | 1.45                    | 1.54                    | 1.42                       | 1.22                      |
| Percentage of reads mapped as pairs (%)            | 92.5                    | 93.0                    | 93.2                       | 93.4                      |
| Average depth of coverage                          | 82                      | 88                      | 81                         | 70                        |
| Mean mapping quality                               | 32.9                    | 33.1                    | 32.9                       | 33.1                      |
| General error rate (%)                             | 0.61                    | 0.61                    | 0.59                       | 0.61                      |

Table S2. Summary of number of variants and windows (5 kb in size) supporting each diversity metric. The mpileup file used 1.8x10⁹ variant loci of which only biallelic SNPs, mapped to chromosomes (no orphans), and within coverage 20-150X were used for estimating population genomic parameters. Minimum coverage of 80% (min. cov. 0.8) implies whether windows were retained for subsequent analysis if at least 80% of the window was covered with data within these depth thresholds (marked “Yes”), otherwise (marked “-“) all windows were used. Allele frequency was only estimated per variant site and not within windows.

| Diversity measure                                      | No. SNPs | No. 5 kb windows | Read depth | Min. cov. 0.8 |
|--------------------------------------------------------|----------|------------------|------------|---------------|
| Allele frequency and pooled heterozygosity score (Hs)  | 10,651,540 | 174,763         | 20-150X    | -             |
| Nucleotide diversity and Tajima’s D                    | 5,740,787 | 331,179         | 20-150X    | Yes           |
| F_{ST}                                                 | 11,007,131 | 329,853         | 20-150X    | Yes           |

Table S3. Genome-wide pooled heterozygosity score for each of the population pools (H_{F}; Rubin et al. 2010) along with 95% confidence intervals estimated within 5 kb windows.

| Population                      | Mean \(H_F\) | 95% CI       |
|---------------------------------|---------------|--------------|
| Introduced population A         | 0.180         | 0.1798-0.1806|
| Introduced population B         | 0.162         | 0.1613-0.1621|
| Established population LB       | 0.189         | 0.1885-0.1892|
| Established population HV       | 0.195         | 0.1943-0.1951|
Table S4. Pairwise comparisons of genome wide heterozygosity per pool (\(H_P\)) in Wilcoxon rank sum test. Significance threshold \(p<2.5\times10^{-11}\) (\(\alpha=0.05\) corrected for genome size of 2.4 Gb).

| Comparison                      | W (x10^{12}) | p (W)       |
|---------------------------------|--------------|-------------|
| Introduced population A: B      | 117          | <2.2 x 10^{-16} |
| Introduced population A: LB     | 104          | <2.2 x 10^{-16} |
| Introduced population A: HV     | 104          | <2.2 x 10^{-16} |
| Introduced population B: LB     | 97           | <2.2 x 10^{-16} |
| Introduced population B: HV     | 97           | <2.2 x 10^{-16} |

Table S5. Pairwise comparisons of genome wide nucleotide diversity (\(\pi\)) within population pools in Wilcoxon rank sum test. Significance threshold \(p<2.5\times10^{-11}\) (corresponding to \(\alpha=0.05\) corrected for multiple testing across a genome size of 2 Gb).

| Comparison                      | W      | p (W)       |
|---------------------------------|--------|-------------|
| Introduced population A: B      | 606    | <2.2 x 10^{-16} |
| Introduced population A: LB     | 531    | <2.2 x 10^{-16} |
| Introduced population A: HV     | 510    | <2.2 x 10^{-16} |
| Introduced population B: LB     | 473    | <2.2 x 10^{-16} |
| Introduced population B: HV     | 452    | <2.2 x 10^{-16} |
| Established population LB: HV   | 526    | <2.2 x 10^{-16} |

Table S6. Pairwise comparisons of genome wide Tajima’s D (\(T_D\)) within population pools in Wilcoxon rank sum test. Significance threshold \(p<2.5\times10^{-11}\) (corresponding to \(\alpha=0.05\) corrected for multiple testing across a genome size of 2 Gb).

| Comparison                      | W (x10^9) | p (W)       |
|---------------------------------|----------|-------------|
| Introduced population A: B      | 62       | <2.2 x 10^{-16} |
| Introduced population A: LB     | 61       | <2.2 x 10^{-16} |
| Introduced population A: HV     | 57       | <2.2 x 10^{-16} |
| Introduced population B: LB     | 54       | <2.2 x 10^{-16} |
| Introduced population B: HV     | 50       | <2.2 x 10^{-16} |
| Established population LB: HV   | 59       | <2.2 x 10^{-16} |
Table S7. Distributions of SNP counts in the delta allele frequency (ΔAF) bins for different functional categories (non-synonymous and synonymous coding sequences, untranslated region (UTR), 5 kb upstream, 5 kb downstream, intragenic, and intergenic). Binned ΔAF are made for the contrast between introduced populations A and B and established populations LB and HV, respectively. Observed and expected number of SNPs of each category and bin were used to estimate M-values of log2fold change in observed vs. expected SNP count. Statistical significance of deviations from expected values are tested with stander χ²-analysis (df=1). P-values in bold indicate those below significance threshold p<2.5x10⁻⁴ (α=0.05 corrected for multiple testing across a genome size of 2 Gb).

| ΔAF bin | Functional category | Observed | Expected | M     | p       | Observed | Expected | M     | p       |
|---------|---------------------|----------|----------|-------|---------|----------|----------|-------|---------|
|         | Coding (non-synonymous) | 17566 | 14584.07 | 0.26839 | 1.2999E-134 | 38373 | 35799.8 | 0.10014 | 3.9991E-42 |
|         | Coding (synonymous) | 20775 | 16955.91 | 0.29306 | 4.4063E-189 | 44287 | 41621.9 | 0.08954 | 5.3513E-39 |
|         | Downstream | 58130 | 60302.27 | -0.0529 | 9.07246E-19 | 148852 | 140825 | 0.00542 | 0.1476925 |
|         | Intergenic | 428279 | 426427 | 0.00625 | 0.004567152 | 1041704 | 1046757 | -0.007 | 7.8582E-07 |
|         | Intragenic | 603032 | 599013.1 | 0.00965 | 2.07255E-07 | 1471678 | 1470407 | 0.00125 | 0.2945658 |
|         | Upstream | 1989812 | 207777.4 | -0.0636 | 4.02371E-86 | 508312 | 510035 | -0.0049 | 0.0158696 |
|         | UTR | 28174 | 29708.34 | -0.0765 | 5.49073E-19 | 72635 | 72925.5 | -0.0058 | 0.28197841 |
|         | Coding (non-synonymous) | 16156 | 15857.34 | 0.02692 | 0.01770667 | 22702 | 23277.4 | -0.0361 | 0.00016242 |
|         | Coding (synonymous) | 17880 | 18436.26 | -0.0466 | 1.5768E-05 | 26487 | 27063 | -0.031 | 0.00046261 |
|         | Downstream | 66930 | 65567 | 0.02968 | 1.0209E-07 | 96750 | 96247.4 | 0.00751 | 0.10522883 |
|         | Intergenic | 464348 | 463656.5 | 0.00215 | 0.309866565 | 677267 | 680613 | -0.0071 | 5.0016E-05 |
|         | Intragenic | 648940 | 651310.3 | -0.0053 | 0.00331336 | 955524 | 956075 | -0.0008 | 0.57343218 |
|         | Upstream | 226344 | 225917.5 | 0.00272 | 0.369569743 | 335157 | 331630 | 0.01526 | 9.0805E-10 |
|         | UTR | 32479 | 32302.05 | 0.00788 | 0.32483396 | 48435 | 47417 | 0.03065 | 2.9377E-06 |
|         | Coding (non-synonymous) | 14339 | 15185.22 | -0.0827 | 6.55283E-12 | 10563 | 11698.3 | -0.1473 | 8.9617E-26 |
|         | Coding (synonymous) | 16672 | 17654.82 | -0.0826 | 1.3951E-13 | 12499 | 13600.8 | -0.1219 | 3.4676E-21 |
|         | Downstream | 63155 | 62787.9 | 0.00841 | 0.142913928 | 47831 | 48370.1 | -0.0162 | 0.01423404 |
|         | Intergenic | 445054 | 444004.2 | 0.00341 | 0.115129984 | 345266 | 342049 | 0.01351 | 3.7822E-08 |
|         | Intragenic | 621407 | 623704.1 | -0.0053 | 0.003629483 | 479153 | 480485 | -0.004 | 0.0546537 |
|         | Upstream | 218363 | 216341.9 | 0.01342 | 1.39051E-05 | 167088 | 166664 | 0.00367 | 0.29899455 |
|         | UTR | 31621 | 30932.9 | 0.03174 | 9.13968E-05 | 24297 | 23829.9 | 0.02801 | 0.0024782 |
|         | Coding (non-synonymous) | 9983 | 10623.07 | -0.0897 | 5.29387E-10 | 4048 | 4826.22 | -0.2537 | 3.9849E-29 |
|         | Coding (synonymous) | 11610 | 12350.72 | -2.0892 | 2.6445E-11 | 4957 | 56111.1 | -0.1788 | 2.4953E-18 |
|         | Downstream | 44163 | 43924.3 | 0.00782 | 0.254735099 | 19420 | 19955.5 | -0.0392 | 0.00015035 |
|         | Intergenic | 308559 | 310610.4 | -0.0096 | 0.0000232551 | 144499 | 141115 | 0.03419 | 2.0873E-19 |
|         | Intragenic | 436016 | 436322.4 | -0.001 | 0.64271699 | 198041 | 198228 | -0.0014 | 0.67497059 |
|         | Upstream | 154242 | 151345.5 | 0.02735 | 9.66228E-14 | 67664 | 68758.5 | -0.0231 | 2.994E-05 |
|         | UTR | 22243 | 21639.62 | 0.03968 | 4.10077E-05 | 9696 | 9831.2 | -0.02 | 0.17271952 |
|         | Coding (non-synonymous) | 6881 | 7633.92 | -0.1498 | 6.8506E-18 | 1372 | 1555.96 | -0.1815 | 3.1064E-06 |
|         | Coding (synonymous) | 8455 | 8875.441 | -0.7 | 8.08852E-06 | 1659 | 1809.01 | -0.1249 | 0.00042033 |
|         | Downstream | 31941 | 31564.76 | 0.01709 | 0.034201739 | 6089 | 6433.6 | -0.0794 | 1.7377E-05 |
|         | Intergenic | 222073 | 223210 | -0.0074 | 0.016104654 | 47131 | 45495.1 | 0.05096 | 1.7262E-14 |
|         | Intragenic | 312630 | 313548.8 | -0.0042 | 0.100819666 | 64017 | 63908.2 | 0.00245 | 0.66683393 |
| Type               | Chr        | Start | End   | Length | Gene | Intragenic | Intergenic | Upstream | Downstream | Coding (non-synonymous) | Coding (synonymous) |
|--------------------|------------|-------|-------|--------|------|------------|------------|----------|------------|-------------------------|--------------------|
| UTR                | 0.5-0.6    |       |       |        |      |            |            |          |            |                         |                    |
|                    |            |       |       |        |      | 15579      | 0.03912    | 0.00178  | 0.00969    | 5229                    | 6191               |
| Coding (non-synonymous) | 0.5-0.6    |       |       |        |      | 15579      | 0.03912    | 0.00178  | 0.00969    | 5229                    | 6191               |
| Coding (synonymous) | 0.5-0.6    |       |       |        |      | 15579      | 0.03912    | 0.00178  | 0.00969    | 5229                    | 6191               |
| Downstream         | 0.5-0.6    |       |       |        |      | 22826      | 0.00965    | 0.31225  | 0.1843     | 159730                  | 159730             |
| Intergenic         | 0.5-0.6    |       |       |        |      | 159730     | 0.00555    | 0.12899  | 0.13153     | 159730                  | 159730             |
| Intragenic         | 0.5-0.6    |       |       |        |      | 225268     | 0.00024    | 0.93744  | 19066      | 18581                   | 18581              |
| Upstream           | 0.5-0.6    |       |       |        |      | 78839      | 0.01313    | 0.01066  | 6067       | 78124.94                | 78124.94           |
| UTR                | 0.5-0.6    |       |       |        |      | 11314      | 0.01842    | 0.17430  | 811        | 11710.43                | 11710.43           |
| Coding (non-synonymous) | 0.6-0.7    |       |       |        |      | 3452       | 0.01302    | 1.13246  | 233        | 3778.018                | 3778.018           |
| Coding (synonymous) | 0.6-0.7    |       |       |        |      | 4105       | 0.00976    | 1.44361  | 147        | 439.445                 | 439.445            |
| Downstream         | 0.6-0.7    |       |       |        |      | 15638      | 0.00154    | 0.89409  | 929        | 15621.36                | 15621.36           |
| Intergenic         | 0.6-0.7    |       |       |        |      | 110658     | 0.0025     | 0.56417  | 5797       | 110466.3                | 110466.3           |
| Intragenic         | 0.6-0.7    |       |       |        |      | 154976     | 0.00019    | 0.61356  | 8210       | 155174.9                | 155174.9           |
| Upstream           | 0.6-0.7    |       |       |        |      | 54575      | 0.00197    | 0.00122  | 2764       | 53824.93                | 53824.93           |
| UTR                | 0.6-0.7    |       |       |        |      | 7550       | 0.00276    | 0.09619  | 207        | 7695.974                | 7695.974           |
| Coding (non-synonymous) | 0.7-0.8    |       |       |        |      | 2341       | 0.00836    | 0.00505  | 155        | 2480.642                | 2480.642           |
| Coding (synonymous) | 0.7-0.8    |       |       |        |      | 2673       | 0.01096    | 8.48213  | 94         | 2884.074                | 2884.074           |
| Downstream         | 0.7-0.8    |       |       |        |      | 10213      | 0.00662    | 0.66418  | 765        | 10256.97                | 10256.97           |
| Intergenic         | 0.7-0.8    |       |       |        |      | 73115      | 0.01155    | 0.03042  | 4541       | 72532.07                | 72532.07           |
| Intragenic         | 0.7-0.8    |       |       |        |      | 101568     | 0.00445    | 0.31657  | 6383       | 101887.7                | 101887.7           |
| Upstream           | 0.7-0.8    |       |       |        |      | 35579      | 0.00967    | 0.20625  | 2178       | 35341.39                | 35341.39           |
| UTR                | 0.7-0.8    |       |       |        |      | 4947       | 0.0306     | 0.13500  | 128        | 5035.168                | 5035.168           |
| Coding (non-synonymous) | 0.8-0.9    |       |       |        |      | 1350       | 0.01633    | 3.16646  | 134        | 1511.793                | 1511.793           |
| Coding (synonymous) | 0.8-0.9    |       |       |        |      | 1542       | 0.1889     | 2.68961  | 90         | 1757.659                | 1757.659           |
| Downstream         | 0.8-0.9    |       |       |        |      | 6159       | 0.0214     | 0.2447    | 615        | 6250.966                | 6250.966           |
| Intergenic         | 0.8-0.9    |       |       |        |      | 44350      | 0.00477    | 0.48640  | 3505       | 44203.66                | 44203.66           |
| Intragenic         | 0.8-0.9    |       |       |        |      | 62614      | 0.01203    | 0.03691  | 5057       | 62094.02                | 62094.02           |
| Upstream           | 0.8-0.9    |       |       |        |      | 21319      | 0.0148     | 0.13507  | 1716       | 21358.32                | 21358.32           |
| UTR                | 0.8-0.9    |       |       |        |      | 3102       | 0.01046    | 0.68624  | 135        | 3079.583                | 3079.583           |
| Coding (non-synonymous) | 0.9-1      |       |       |        |      | 861        | 0.2449     | 6.15177  | 81         | 1020.274                | 1020.274           |
| Coding (synonymous) | 0.9-1      |       |       |        |      | 996        | 0.2521     | 3.34158  | 62         | 1186.203                | 1186.203           |
| Downstream         | 0.9-1      |       |       |        |      | 4013       | 0.0721     | 0.00155  | 344        | 4218.632                | 4218.632           |
| Intergenic         | 0.9-1      |       |       |        |      | 29114      | 0.0351     | 3.22217  | 2417       | 29832.02                | 29832.02           |
| Intragenic         | 0.9-1      |       |       |        |      | 43741      | 0.06184    | 3.10755  | 2063       | 41905.82                | 41905.82           |
| Upstream           | 0.9-1      |       |       |        |      | 14249      | 0.0287     | 0.01740  | 1085       | 14535.71                | 14535.71           |
| UTR                | 0.9-1      |       |       |        |      | 1803       | 0.205      | 1.54479  | 72         | 2078.339                | 2078.339           |
Table S8. Functional categories of SNPs exhibiting marked divergence between introduced populations A and B. SNPs included are within the 95th percentile of difference in allele frequency between the two introduced populations A and B (ΔAF > 0.73, 319,274 SNPs) that also exhibit significant allele frequency difference between introduced populations by Fisher’s exact test (significance threshold p<2.5x10^{-11}, corresponding to α=0.05 corrected for multiple testing across a genome size of 2 Gb).

| SNP category           | Number of SNPs |
|------------------------|----------------|
| Coding (non-synonymous)| 2,905          |
| Coding (synonymous)    | 3,676          |
| Downstream             | 13,491         |
| Intergenic             | 99,867         |
| Intragenic             | 2,37           |
| Upstream               | 143,623        |
| UTR                    | 48,355         |

Table S9. Testing deviation in read depth for non-synonymous SNPs identified as candidates for selection: adaptive divergence between introduced populations A and B, and candidates for novel selection in the new lake system (directional and relaxed selection). Read depth for each category of SNPs is compared to an equally large sample taken at random from the genome-wide biallelic SNPs used in estimation of population genetic metrics in paired t-tests. Errors were normally distributed in all samples of SNPs (Shapiro test, p>0.05). The fold change (M) between candidate SNPs and the random sample was estimated as of log2fold change in observed (candidate SNPs) vs. expected (randomly sampled SNPs) read depths.

|                          | n  | read depth | read depth (random sample) | t       | df | p       | M   |
|--------------------------|----|------------|----------------------------|---------|----|---------|-----|
| Candidate SNPs for adaptive difference between introduced population A and B | 22 | 74         | 63                         | 2.73    | 21 | 0.01    | 0.24|
| Candidate SNPs for directional selection on chr 7 | 14 | 65         | 59                         | 0.11    | 13 | 0.92    | 0.15|
| Candidate SNPs for relaxed selection on chr 28 | 7  | 64         | 59                         | 0.79    | 6  | 0.46    | 0.1 |
Table S10. Genes putatively of adaptive importance for introduced populations found by considering windows (5 kb) of high differentiation between the two introduced populations simultaneously showing low diversity within each introduced population \((F_{ST} > 95^{th}\text{ percentile and diversity within each introduced population below } 5^{th}\text{ percentile of } \pi)\). Gene model predictions are given for non-synonymous SNPs found within these windows that exhibit significant allele frequency difference between introduced populations, tested using Fisher’s exact test in Popoolation2 (significance threshold \(p < 2.5\times10^{-11}\), corresponding to \(\alpha = 0.05\) corrected for multiple testing across a genome size of 2 Gb). SNP coordinate and the frequency of the major allele in each of the four populations is given. Predicted gene models (trout gene) are described as found in the brown trout genome. Gene model predictions (gene) and the general description of the biological function (function) found in the nearest related species (species) is also given for each gene model.

| Chr | Position | Frequency of major allele | Introduced population A | Introduced population B | Established population LB | Established population HV | p (-log10) | Brown trout | Gene description | Gene | Function | Other species |
|-----|----------|--------------------------|-------------------------|-------------------------|---------------------------|---------------------------|-----------|--------------|----------------|------|-----------|--------------|
| 1   | 70548609 | 0.07                     | 1.00                    | 0.68                    | 0.21                      | 37                        | LOC115206622 | cytochrome P450 2K1 | CYP2U1 | Arachidonic metabolism | Rainbow trout (Oncorhynchus mykiss) (Katchamart et al. 2002) |
| 1   | 70551453 | 0.03                     | 0.99                    | 0.71                    | 0.29                      | 42                        | LOC115162910 | cadherin-23-like | CHD23 | Hearing | Zebrafish (Danio rerio) (Söllner et al. 2004) |
| 1   | 70551474 | 0.99                     | 0.01                    | 0.33                    | 0.73                      | 42                        | LOC115208429 | integrin, beta 7 | ITGB7 | Immunity | Vertebrates (Takada et al. 2007) |
| 2   | 74904357 | 0.00                     | 1.00                    | 0.70                    | 0.49                      | 45                        | LOC115208416 | RNA-binding protein 12-like | RBM12 | RNA binding | Vertebrates (Yang et al. 2008) |
| 14  | 31522273 | 0.29                     | 0.91                    | 0.63                    | 0.38                      | 15                        | LOC115208429 | chromodomain helicase DNA binding protein 6 | CHD6 | Immunity | Atlantic salmon (Salmo salar) (Dettleff et al. 2017) |
| 14  | 56699003 | 0.06                     | 0.97                    | 0.76                    | 0.47                      | 41                        | LOC115208416 | synaptotagmin-like 2a | SYTL2 | Immunity | Atlantic cod (Gadus morhua) (Kleppe et al. 2013) |
| 14  | 57378213 | 0.09                     | 0.98                    | 0.66                    | 0.35                      | 41                        | LOC115208416 | RNA-binding protein 12-like | RBM12 | RNA binding | Mammals (Verhoeven et al. 1998) |
| 19  | 14605425 | 0.23                     | 0.99                    | 0.81                    | 0.59                      | 25                        | LOC115154100 | alpha-tectorin-like | TECTA | Hearing | Atlantic salmon (Salmo salar), brown trout (S trutta) (Isorna et al. 2017) |
| 19  | 18820277 | 0.01                     | 1.00                    | 0.75                    | 0.54                      | 52                        | LOC115158534 | circadian locomoter output cycles protein kaput-like | CLOCK | Circadian rhythm | Atlantic salmon (Salmo salar), brown trout (S trutta) (Isorna et al. 2017) |
| 22  | 28858862 | 0.04                     | 0.99                    | 0.71                    | 0.38                      | 39                        | LOC115158539 | NA | unchar. | NA |
| Position | Chromosome | Score (Score2) | Score (Score3) | Score (Score4) | Score (Score5) | Description | Transcript | Function | Organism | Reference |
|----------|-------------|----------------|----------------|----------------|----------------|-------------|------------|----------|----------|-----------|
| 22       | 29058276    | 0.00           | 0.98           | 0.71           | 0.42           | 35          | LOC115158545 multifunctional protein ADE2-like | PAICS | Purine metabolism | Zebrafish (Ng et al. 2009) |
| 22       | 29058298    | 0.00           | 0.98           | 0.70           | 0.37           | 34          | LOC115158562 regulator of G-protein signaling 5-like | RGS5 | Immunity | Atlantic salmon (Salmo salar) (Tacchi et al. 2011) |
| 22       | 29707140    | 0.01           | 1.00           | 0.90           | 0.31           | 45          | LOC115160992 titin-like | TELT | Muscle protein | Atlantic salmon (Salmo salar) (Ørnholt-Johansson et al. 2017) |
| 24       | 26309434    | 0.99           | 0.00           | 0.26           | 0.77           | 52          | LOC115160992 titin-like | TELT | Muscle protein | Atlantic salmon (Salmo salar) (Ørnholt-Johansson et al. 2017) |
| 24       | 26310645    | 0.97           | 0.31           | 0.62           | 0.79           | 23          | LOC115171775 adenylate cyclase type 9-like | ADCY9 | cAMP production (intracellular signal transduction) | Mammals (Hacker et al. 1998) |
Table S11. Genes putatively shaped by relaxed selection in the new environments on chromosome 28. Gene model predictions are given for non-synonymous SNPs found within c. 1 Mb region flanking a window (5 kb) with reduced heterozygosity ($H_P$) in introduced populations compared to established populations ($ZH_P$>0 in both descendant populations and $ZH_P$<4 in both introduced populations). The coordinate is given for each SNP along with the frequency of the major allele in each of the four populations. Predicted gene models (gene) are described as found in brown trout, three of which overlap (LOC115165612, LOC115165613, and LOC115165615). Gene name (gene) and the general description of the biological function (function) found in the nearest related species (species) is also given for each gene model.

| Position | Introduced population A | Introduced population B | Established population LB | Established population HV | Gene | Gene description | Gene | Function | Species & reference |
|----------|-------------------------|-------------------------|---------------------------|--------------------------|------|------------------|------|----------|-------------------|
| 16103241 | 0.01                    | 0.99                    | 0.67                      | 0.36                     | LOC115165610 | adipolin-like     | LOC115165610 | signaling receptor binding |
| 16123301 | 1                       | 0.02                    | 0.32                      | 0.72                     | LOC115165611 | 45 kDa calcium-binding protein | LOC115165611 | calcium ion binding, immunity | Rainbow trout (Oncorhynchus mykiss) (Porteros et al. 1997) |
| 16158101 | 0.13                    | 1                       | 0.75                      | 0.53                     | LOC115165612 | homeodomain-interacting protein kinase 1-like | LOC115165612 | ATP binding, immunity | Atlantic salmon (Salmo salar) (Zueva et al. 2018) |
|          |                         |                         | LOC115165613              |                           | LOC115165613 | arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 3-like | LOC115165613 | GTPase activator activity |
|          |                         |                         | LOC115165615              |                           | LOC115165615 | uncharacterized   | LOC115165615 | NA |
| 16159716 | 0.11                    | 1                       | 0.74                      | 0.53                     | LOC115165612 | homeodomain-interacting protein kinase 1-like | LOC115165612 | ATP binding |
|          |                         |                         | LOC115165613              |                           | LOC115165613 | arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 3-like | LOC115165613 | GTPase activator activity |
|          |                         |                         | LOC115165615              |                           | LOC115165615 | uncharacterized   | LOC115165615 | NA |
| 16160196 | 0.89                    | 0.99                    | 0.91                      | 0.84                     | LOC115165612 | homeodomain-interacting protein kinase 1-like | LOC115165612 | ATP binding |
|          |                         |                         | LOC115165613              |                           | LOC115165613 | arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 3-like | LOC115165613 | GTPase activator activity |
|          |                         |                         | LOC115165615              |                           | LOC115165615 | uncharacterized   | LOC115165615 | NA |
| 16160295 | 0.03                    | 1                       | 0.71                      | 0.39                     | LOC115165612 | homeodomain-interacting protein kinase 1-like | LOC115165612 | ATP binding |
|          |                         |                         | LOC115165613              |                           | LOC115165613 | arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 3-like | LOC115165613 | GTPase activator activity |
|          |                         |                         | LOC115165615              |                           | LOC115165615 | uncharacterized   | LOC115165615 | NA |
| 16171500 | 0.97                    | 0                       | 0.33                      | 0.68                     | LOC115165613 | arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 3-like | LOC115165613 | GTPase activator activity |
Table S12. Genes putatively under directional selection in the new environments found on chromosome 7. Gene model predictions are given for non-synonymous SNPs found within regions of reduced variation on chromosome 7 in established populations compared to introduced populations ($ZH_P<2$ in both established populations and $ZH_P>0$ in both introduced populations; see Appendix 2 for details). The coordinate is given for each SNP along with the frequency of the major allele in each of the four populations. Predicted gene models (gene) are described as found in brown trout. Gene name (gene) and the general description of the biological function (function) found in the nearest related species (species) is also given for each gene model.

| Chr | Position | Introduced population A | Introduced population B | Established population LB | Established population HV | Gene      | Gene description                          | Gene | Function | Species & reference                  |
|-----|----------|-------------------------|-------------------------|---------------------------|---------------------------|-----------|------------------------------------------|-------|----------|--------------------------------------|
| 7   | 30024542 | 0.80                    | 1.00                    | 0.89                      | 0.86                      | LOC115197214 | junctional adhesion molecule A-like | JAML  | Immunity | Grass carp (Ctenopharyngodon idellus), red drum (Sciaenops ocellatus) (Zhang et al. 2014; Du et al. 2015) |
|     | 30161516 | 1.00                    | 0.92                    | 0.93                      | 0.99                      | LOC115197215 | LHFPL tetraspan subfamily member 6 protein-like | LHFPL6 | uncharacterized | Rainbow trout (Oncorhynchus mykiss) (Conde-Sieira et al. 2018) |
|     | 30280884 | 0.66                    | 1.00                    | 0.89                      | 0.95                      | LOC115197216 | forkhead box protein O1-A-like | FOXO1 | Metabolism | Arctic charr (Salvelinus alpinus) (Striberny et al. 2019) |
|     | 30417516 | 1.00                    | 0.72                    | 0.59                      | 0.90                      | LOC106602895 | transcription factor Sox-19a-like | LOC106602895 | Metabolism | Arctic charr (Salvelinus alpinus) (Striberny et al. 2019) |
|     | 30417713 | 1.00                    | 0.71                    | 0.71                      | 0.87                      | LOC115197223 | calcium signal-modulating cyclophilin ligand-like | CAMLG | Calcium mobilization | Human (Homo sapiens) (Bram et al. 1996) |
|     | 30609653 | 0.68                    | 0.69                    | 0.57                      | 0.83                      | LOC115197223 | fatty acid hydroxylase domain containing 2 | FAXDC2 | Metabolism | Atlantic salmon (Salmo salar) (Caballero-Solares et al. 2018) |
|     | 30886239 | 0.78                    | 0.78                    | 0.60                      | 0.86                      | faxdc2     | La ribonucleoprotein domain family member 1 | LARP1 | Metabolism | Human (Homo sapiens) (Burrows et al. 2010) |
| 31084864  | 1.00   | 0.69  | 0.57  | 0.85  | RNA binding motif protein 41 | rbm41  | mRNA splicing | Vertebrates (Yang et al. 2008) |
| 31084992  | 0.77   | 0.74  | 0.66  | 0.80  | RNA binding motif protein 41 | RBM41  | mRNA splicing | Vertebrates (Yang et al. 2008) |
| 31090307  | 0.70   | 0.71  | 0.56  | 0.78  | RNA binding motif protein 41 | LOC115197235 | histone H4 transcription factor-like | Zebrafish (Danio rerio) (Swartz et al. 2014) |
| 31418246  | 0.71   | 0.99  | 0.94  | 0.81  | LOC115197236 | nuclear cap-binding protein subunit 3-like | NCBP3 | Immunity | Vertebrates (Yang et al. 2020) |
| 31464927  | 0.69   | 1.00  | 0.90  | 0.97  | LOC115197236 | LOC115197236 | histone H4 transcription factor-like | Zebrafish (Danio rerio) (Swartz et al. 2014) |

**References for tables S10-S12**

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Table S13. Glossary for some of the terminology used.

| Concept     | Description                                                                                                                                                                                                 |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AF          | Allele frequency within a population pool of the globally major allele per variant site (SNP), i.e., the allele that is most common across all four population pools analyzed in the present study. |
| ΔAF         | Pairwise difference in allele frequency of major allele between population pools                                                                                                                             |
| Major allele | Most common allele found across all population pools                                                                                                                                                        |
| Minor allele | Least common allele found across all population pools                                                                                                                                                       |
| Hp          | Heterozygosity score per population pool calculated as: $H_p = 2 \frac{\sum_{n}^{\text{MAJ}} \sum_{n}^{\text{MIN}}}{(\sum_{n}^{\text{MAJ}} + \sum_{n}^{\text{MIN}})^2}$, where $\sum_{n}^{\text{MAJ}}$ and $\sum_{n}^{\text{MIN}}$ are the sums of the major and minor allele counts across all pools, respectively (Rubin et al. 2010) |
| ZH_p        | $H_p$ normalized relative its mean and standard deviation as follows: $Z_{H_p} = \frac{(H_p - \mu_{H_p})}{\sigma_{H_p}}$, where $\mu_{H_p}$ and $\sigma_{H_p}$ are the mean and standard deviation of $H_p$, respectively (Rubin et al. 2010). The distribution of $Z_{H_p}$ is characterized by $\mu_{H_p} = 0$ and $\sigma_{H_p} = 1$ |

Gene model A region of the genome predicted to be translated into a protein.

Non-synonymous Substitutions within coding regions (regions of the genome predicted to encode proteins) that change the encoded amino acid, i.e. a missense mutation

Synonymous Substitutions within coding regions (regions of the genome predicted to encode proteins) that do not change the encoded amino acid

Downstream Substitutions within a 5 kb long nucleotide sequence downstream of a gene

Intergenic Substitutions within the nucleotide sequence located between genes

Intragenic Substitutions within the non-coding region of the nucleotide sequence within a gene (e.g. introns)

Upstream Substitutions within a 5 kb long nucleotide sequence upstream of a gene

UTR Either of two untranslated regions (5' and 3') that flank the coding sequence of a strand of mRNA

bp base pair

kb $10^3$ base pair

Mb $10^6$ base pair

Gb $10^9$ base pair

M-value M-values equal the log2fold change of the observed number of SNPs in a given annotation category for a specific interval of ΔAF against the expected SNP count. M-value shows relative abundance of SNPs in a given AF with different functional annotation. Positive values show observed frequency is more than expected while negative shows observed frequencies is less than expected
Figure S1. Genome wide heterozygosity per pool ($H_P$) in 5 kb windows. (A) Distributions of $H_P$ and $Z$-transformed $H_P$ ($ZH_P$) with median values depicted by black vertical lines and (B) Manhattan plot of $H_P$ along chromosomal coordinates for each pool.
Figure S2. Distribution of genome wide nucleotide diversity ($\pi$) and Tajima’s D ($T_D$) within population pools. Tables include the population mean for each measure, their 95% confidence intervals (CI), and range. $\pi$ and $T_D$ were estimated across 331,179 windows corresponding to 5,740,787 variants. Window size = 5,000 bp (5 kb) and fraction depth covered ≥ 0.8 (i.e. a window was only retained if at least 80% of its SNPs had a read depth between 20X and 150X).
Figure S3. Boxplots of difference in allele frequency ($\Delta AF$) between each of the introduced populations A and B and established populations LB and HV for regions putatively under selection in introduced populations (A) and established populations (B), respectively. (A) shows $\Delta AF$ for 2,106 windows (5 kb) with low genetic variation in the introduced populations as compared to the established (Z-transformed $HP$ ($ZH_P$) $>0$ in both established populations and $ZH_P <0$ in both introduced populations) potentially indicating relaxed selection in the new environments. Each established population is compared to introduced population A (blue) and B (orange). (B) shows $\Delta AF$ for 1,642 windows of reduced variation in established populations as compared to introduced populations ($ZH_P<0$ in both established populations and $ZH_P>0$ in both introduced populations) potentially indicating regions of directional selection in the new environment. Each established population is compared to Introduced populations A (blue) and B (orange). Stars above brackets indicate significance levels of two-sample Wilcoxon test.
Figure S4. Potentially adaptive differences between the two introduced populations A and B. (A) Windows (5 kb) showing low diversity within each of introduced population A and B (diversity within each introduced population below 5th percentile of π; blue and yellow circles for introduced population A and B, respectively) and of marked divergence between introduced populations ($F_{ST}$ above 95th percentile, red circle), and their overlap. Candidates for adaptive divergence are defined as windows exhibiting marked differentiation between introduced populations with simultaneously low diversity within each introduced population; 403 such windows are found and genes predicted for non-synonymous SNPs within these windows are listed (Table S8). (B) Diversity and divergence surrounding candidate for adaptive divergence; a c. 2 M base pair (Mb) region on chromosome 2 containing a swarm of fixed SNPs $F_{ST}$ between introduced populations A and B. $F_{ST}$ between released introduced population A and B and established populations LB and HV, and π and $T_D$ for each population is included. Arrows refer to gene models predicted for non-synonymous SNPs within these regions (Table S8). Windows where differentiation between the released introduced populations is above the 95th percentile of $F_{ST}$ and diversity within each introduced population below the 5th percentile of π are marked in black. $F_{ST}$ and π were estimated within 5 kb windows.