

1 **Exploring a Pool-seq only approach for gaining population genomic insights in**
2 **non-model species**

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6 **Appendix S1: Background on sympatric brown trout population pairs used in the present**
7 **study**

8 We study four populations (two population pairs) that inhabit small freshwater lakes in a remote,
9 mountainous area within the Hotagen Nature Reserve, located at c. 700 m elevation in Jämtland
10 County, central Sweden (Fig. 1). These lakes belong to a long-term monitoring project during
11 which c. 100 individuals per lake have been collected annually since the mid-1980s and
12 onwards (Jorde & Ryman, 1996; Laikre, Jorde, & Ryman, 1998; Palm & Ryman, 1999; Palm,
13 Laikre, Jorde, & Ryman, 2003; Charlier, Palmé, Laikre, Andersson, & Ryman, 2011;
14 Charlier, Laikre, & Ryman, 2012; Palmé, Laikre, & Ryman, 2013, Andersson et al. 2017a; b).
15 Typically, 100 fish have been collected annually from each lake (including from Lakes Lilla
16 Bävervattnet, Stora Bävervattnet, Västra Trollsvattnet and Östra Trollsvattnet that were used for
17 the present study; Fig 1). All samples have been stored in -80°C freezers since the time of
18 collection.

19 **Introduced sympatric populations**

20 The introduced pair of populations was released into the Lakes Bävervattnen system, two
21 interconnected lakes of < 1 km² at near 700 m elevation (Fig. 1). A waterfall separates these
22 lakes from downstream waters inhabited by brown trout, and the lakes were inhabited only by
23 Arctic charr (*Salvelinus alpinus*) prior to the introduction (Fig. S1). The released individuals
24 originated from two separate populations showing diverse ecological features and that had been
25 isolated from each other since the last glaciation (c. 5,000 - 9,000 years ago; Ryman et al.,
26 1986; Palm & Ryman, 1999). One of the introduced populations (I) is from a hatchery population
27 frequently used to stock waters in Jämtland County (Bergman, Halvarson, & Larsson, 1989).
28 This hatchery strain originates from a wild population from Lake Kallsjön, a large lake of c. 155
29 km² at 380 m elevation that belongs to the River Indalsälven catchment, draining into the Baltic
30 Sea. Fish in the Lake Kallsjön population were large, slow-growing and piscivorous, down-
31 stream spawners with a presumed capacity for long distance migration (Ryman et al., 1986).
32 Construction of hydro power plants in the 1940s obstructed spawning and ultimately led to the
33 wild populations' extinction in the 1980s. Population decline was noticed in the 1940s, and a
34 hatchery population was created to compensate this loss. This hatchery population was initially
35 maintained by contribution from wild spawners from River Kallströmmen, but as the wild
36 population diminished this contribution dwindled, and since the 1950s the hatchery population is
37 mainly upheld by hatchery reared fish. The other introduced population (II) originates from a
38 landlocked population inhabiting Lakes Fälpfjälltjärnarna, two tiny lakes of < 1 km² at 645 m
39 elevation. The fish here exhibit traits typical of small-mountain-lake populations; small body size,
40 maturation at an earlier age and an insect based diet (Ryman et al., 1986).

41 Previous analyses have shown that the Lake Kallsjön and Lakes Fälpfjälltjärnarna populations
42 exhibit significant allele frequency differences at multiple allozyme loci, particularly so at AGP-2,
43 where the Lake Kallsjön population was close to fixation for one of the alleles at this locus (the
44 so-called 100 allele) while in the Lakes Fälpfjälltjärnarna the 100 allele and another allele (called
45 50) occurred at equal frequencies (Palm & Ryman, 1999). Using the AGP-2 locus, spawners
46 from each population were chosen to be homozygous for different alleles (100/100 vs. 50/50),
47 thereby enabling discrimination of lineages after introduction (Palm & Ryman, 1999). According
48 to hatchery records more than 100 potential breeders of both sexes of the Lake Kallsjön
49 population were accessible for mating in the fall of 1978, and over 100,000 eggs were
50 produced. For the Lakes Fälpfjälltjärnarna population, wild spawners were caught during the
51 spawning season of 1978. The fish were transported to the hatchery, where they were
52 genotyped and stripped. Of the 102 spawners genotyped, 6 females and 11 males were
53 identified as homozygous for the 50 allele and used to produce offspring.

54 The introduction of the juveniles was conducted in late July in 1979 by releasing 1,000 fry from
55 each population to two locations in the Lakes Bävsvattnen system (Fig. S1; Ståhl & Ryman,
56 1982). The fish in these lakes have been monitored since 1988 primarily using allozymes but
57 with subsets analyzed for mtDNA, microsatellites, and SNPs (Laikre et al. 1998; Palmé et al.
58 2013; Andersson et al. 2017a). After the release, 41 individuals could be classified based on
59 their age and *AGP-2* genotype to either of the two released populations, all of which were used
60 in the present study. The additional 59 fish used here were “pure” F1 (offspring from matings
61 within populations as clarified from *AGP-2* genotype) randomly collected out of c. 700 available
62 fish.

63 **Naturally occurring sympatric populations**

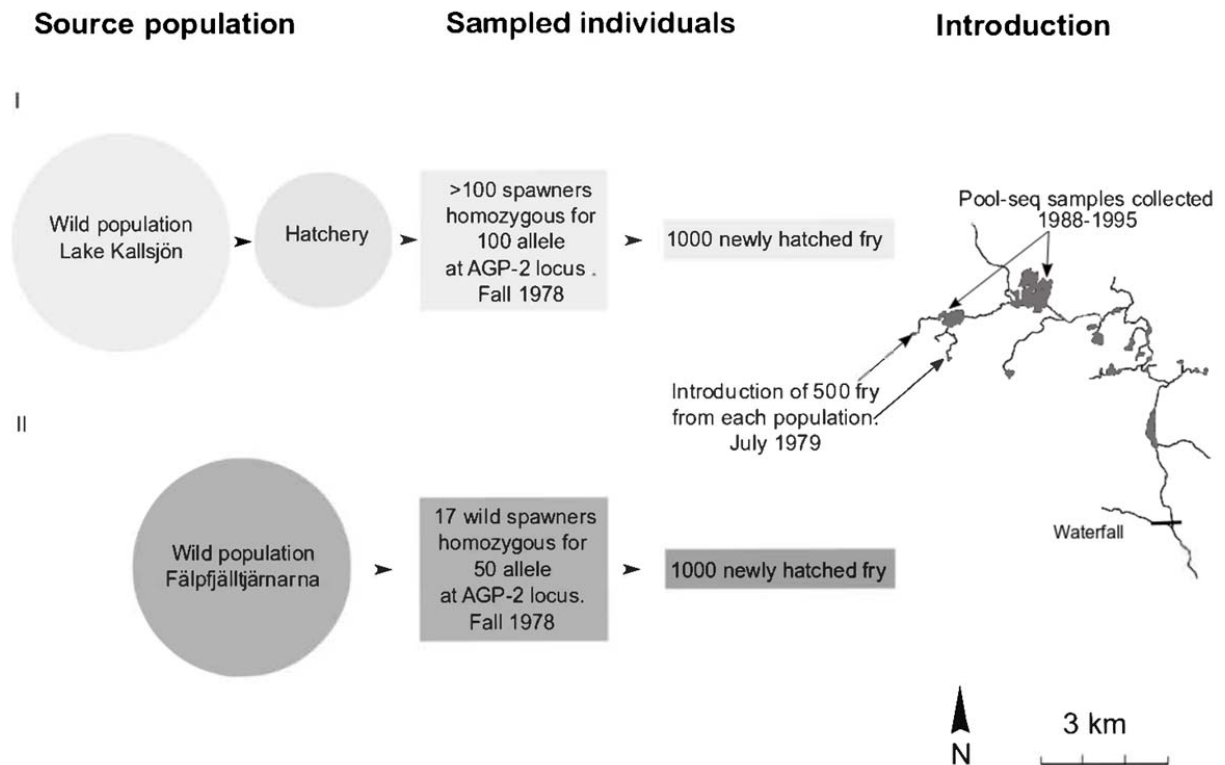
64 The Lakes Trollsvattnen system is comprised of a series of tiny interconnected mountain lakes
65 and tributaries at the northernmost part of the River Indalsälven drainage system. The two main
66 lakes (Östra and Västra Trollsvattnen) are oligotrophic, c. 0.1-0.17 km² in size and with a depth
67 of mostly 1-2 m.

68 These lakes have been sampled since the 1980s and an initial analysis using 14 polymorphic
69 allozyme loci revealed significant heterozygote deficiencies in both of the lakes (Jorde &
70 Ryman, 1996). Further analyses confirmed population genetic structuring in the pooled material
71 from both lakes ($F_{IT}=0.042$; Palmé et al., 2013), as well as within each of the two lakes
72 separately ($F_{IS}=0.038$ and 0.044 for Östra and Västra Trollsvattnen, respectively). STRUCTURE
73 analysis suggested two populations in the total material as well as within each lake regardless
74 of assumptions of ancestry and allele-frequency models (Palmé et al., 2013). Considering
75 individuals assigned to the clusters suggested by STRUCTURE at cut-offs for assignment
76 between 0.5-0.9 yielded F_{ST} between 0.1- 0.2 (Palmé et al., 2013; Andersson et al., 2017a).
77 However, in an analysis of 3,093 SNPs from a subset of the initially studied individuals, genetic
78 differentiation is a fraction of that detected by allozymes ($F_{ST} = 0.03$; Andersson et al., 2017a).
79 Based on analyses of phenotypic traits and diet, the populations in Lakes Trollsvattnen give no
80 indication of trophic polymorphism or ecological niche separation (Andersson et al., 2017b). The
81 fish used in the present study were a randomly chosen sample from c. 2,000 individuals with an

82 assignment probability of ≥ 0.8 to either of the two genetic clusters based on allozyme genotype
83 (see Andersson et al., 2017a for more details).

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85 **Figure S1** Schematic illustration of sympatric populations I (hatchery source) and II (lake
86 resident source), and their introduction to Lakes Bävervattnen.



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