

# Mapping and monitoring genetic diversity of an alpine freshwater top predator by applying newly proposed indicators

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## Abstract

Genetic diversity is the basis for population adaptation and long-term survival, yet rarely considered in biodiversity monitoring. One key issue is the need for useful and straightforward indicators of genetic diversity. To test newly proposed indicators, we monitored genetic diversity over 40 years (1970-2010) in metapopulations of brown trout inhabiting 27 small mountain lakes representing 10 water systems in central Sweden. Three of the indicators were previously proposed for broad, international use for the Convention on Biological Diversity (CBD) context, while three others were recently elaborated for national use by a Swedish science-management effort and applied for the first time here. The Swedish indicators use molecular genetic data to monitor genetic diversity within and between populations and assess the effective population size ( $N_e$ ). We used a panel of 96 SNPs and identified 29 discrete populations retained over time. Over 40 percent of the lakes harbored more than one population indicating that brown trout biodiversity hidden as cryptic, sympatric populations are more common than recognized. The  $N_e$  indicator showed values below the threshold ( $N_e \geq 500$ ) in 20 populations with five showing  $N_e < 100$ . Although statistically significant genetic diversity reductions occurred in several populations, they were mostly within proposed threshold limits. Metapopulation structure appears to buffer against diversity loss; when applying the indicators to metapopulations most indicators suggest an acceptable genetic status in all but one system. The CBD indicators agreed with the national ones but provided less detail. We propose that all indicators applied here are appropriate for monitoring genetic diversity within species.

## 1. INTRODUCTION

Genetic diversity is the basis for species evolution, and high genetic diversity is vital for adaptation to changing climate, habitats, and diseases (Bitter et al., 2019; Lai et al., 2019). Genetic diversity plays a substantial role for ecosystem function, and can affect ecosystem resilience, stability, and services in a similar manner as species diversity (Cook-Patton et al., 2011; Yang et al., 2015). Low genetic diversity increases the risk of extinction (Spielman et al., 2004; Hellmair & Kinziger, 2014).

International policy, including the UN Convention on Biological Diversity (CBD; [www.cbd.int](http://www.cbd.int)), identifies intraspecific diversity (=genetic diversity within species) as one of the three pillars of biodiversity that should be identified, monitored, conserved, and sustainably used. However, the implementation of this policy has long lagged behind, and particularly so for genetic diversity (Laikre et al., 2010; Hoban et al., 2013; Bruford et al., 2017).

The CBD Strategic Plan for 2011-2020 had a goal to safeguard genetic diversity ([www.cbd.int/sp](http://www.cbd.int/sp); Goal C); the Target associated with this goal focusses on cultivated species, their wild relatives and socio-economically important species. The main indicator to monitor progress towards this target follows number and threat status of local animal breeds (Tittensor et al., 2014). So far, strategic targets and indicators for genetic diversity of wild species have been missing, but proposals for such measures that can be applied globally have recently been presented for the CBD “post-2020” global biodiversity framework (Díaz et al., 2020; Laikre

et al., 2020; Hoban et al., 2020, 2021a). The three pragmatic indicators for genetic diversity proposed for global use include 1) the proportion of populations within species with an effective population size  $N_e$  [?] 500, 2) the proportion of genetically distinct populations maintained within species, and 3) the number of species and populations in which genetic diversity is being monitored using DNA-based methods (Laikre et al., 2020; Hoban et al., 2020). Several countries are starting to apply these indicators (Drs. Jessica da Silva, Alicia Mastretta-Yanes, Henrik Thurfjell, pers.comm.).

Also, several countries have moved forward with respect to monitoring genetic diversity using DNA-based techniques (i.e., applying Indicator 3 of Laikre et al., 2020/Hoban et al., 2020). Countries in the forefront include Switzerland where five key species were recently identified for an ambitious pilot project involving sampling over full species ranges and using whole genome resequencing (Martin Fischer pers. comm.; [www.gendiv.ethz.ch](http://www.gendiv.ethz.ch)). In Scotland, a scorecard method using published information on genetic diversity and knowledge of experts has been adopted and applied to 26 species identified as of particular concern (Hollingsworth et al., 2020). In Sweden, the Swedish Environmental Protection Agency (SEPA) has prioritized species for monitoring (Posledovich et al., 2020a, b) and have initiated work on a few of these species. The Swedish Agency for Marine and Water Management (SwAM) has run a science-management collaboration to develop a pilot program for monitoring genetic diversity over contemporary time frames using DNA-based techniques and three new DNA-based indicators (Johannesson & Laikre, 2020). Here, we present and apply these indicators for the first time.

Specifically, we map and monitor genetic diversity within and between populations over time using brown trout in alpine lake systems in protected areas in central Sweden as a model. The brown trout was selected due to the availability of temporally separate samples (from the 1970s and from the 2010s). The species is suitable also because of its tendency to form genetically distinct populations over even restricted areas (Bekkevold et al., 2020), thus enabling monitoring of the between population diversity component. We were particularly interested in mapping the potential occurrence of multiple, genetically distinct populations in the same small lake (so-called cryptic sympatry; Andersson, 2021). Such hidden biodiversity has only been documented in two cases for brown trout (Ryman et al., 1979; Andersson et al., 2017a; Saha et al., 2021) but may be more common than currently recognized because of limited statistical power in detecting them using typically applied sample sizes (Jorde et al., 2018). Finally, the brown trout carries a key ecological role in these lakes where it is a top predator and often the only fish species; its cultural and socio-economic value is also high (Frank et al., 2011; Marco-Rius et al., 2013).

## 2. MATERIALS AND METHODS

### 2.1 Study area and sampling

The study area is located in the mountainous range of Jamtland County, central Sweden, and includes 27 lakes/tarns located in five protected areas (Figure 1; Table S1). Several of the localities are connected to each other via creeks, and there is a total of seven such “metapopulations” with 2-7 lakes/tarns per system included in this study. Two of these metapopulations (with a total of 7 lakes) are located above the tree line (>700 meters above sea level), while the remaining four are set below the tree line. The samples also include three “independent” lakes (all below the tree line) which are not closely connected to any of the other sampling localities. All sampling sites represent the uppermost parts of water systems draining into either River Angermanalven or River Indalsalven, two major rivers that drain into the Baltic Sea c. 400 km from the sampling sites (Figure 1).

The sampling was performed in collaboration with the Jamtland County Administrative Board at two points in time, the 1970-80s and 2010s (Table S1), and was often coordinated with their test fishing activities within the regional environmental management. Fish collected in the 1970-80s were from the first studies of genetic variation in natural populations in Sweden (Allendorf et al., 1976; Ryman et al., 1979; Ryman & Stahl, 1980; Ryman, 1981, 1983), and we included  $n = 1,263$  fish from those collections that have been stored in a frozen tissue bank at the Department of Zoology, Stockholm University. Sampling localities to be included in the present study were selected based on possibilities to obtain additional samples in collaboration with

local authorities, sport fishing clubs and/or Sami communities. One of the study areas (system Hotagen 4; Figure 1) is part of a long-term genetic monitoring research effort that we (N.R., L.L.) have run since the 1970s (Jorde & Ryman, 1996; Laikre et al., 1998; Charlier et al., 2012; Palme et al., 2013; Andersson et al., 2017b). Present day samples (2010s) included  $n = 1,319$  fish collected in 2012, 2014, 2017, and 2018.

## 2.2 Genotyping

In total,  $n = 2,582$  brown trout were genotyped for the present study. Genomic DNA was extracted from c. 50 mg muscle tissue using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s instructions and eluted in 100  $\mu$ l elution buffer. DNA quality was assessed by electrophoresing an aliquot through a 1% agarose gel and subjectively assessing the proportion of high-molecular weight DNA relative to degraded DNA. Double-stranded DNA was quantified using a Qubit fluorometer (ThermoScientific) and normalised to 30–50 ng/ $\mu$ l.

Genotyping was carried out using an EP1™ 96.96 Dynamic array IFCs genotyping platform (Fluidigm) comprising 96 SNPs. Our present 96 SNPs were selected from 3,782 SNPs variable in Danish brown trout (Bekkevold et al., 2019). The 96 SNPs are distributed across the genome, with 1-3 SNPs on each of the 40 chromosomes (Saha et al., 2021).

## 2.3 Population genetic analysis

Individual fish with genotype call rates below 0.7 ( $n = 10$ ) were excluded, resulting in a total of 2,572 individuals used in further analyses. We quantified genetic diversity in several ways described here and these measures were used for the indicators (section 2.4). We assessed the most likely number of populations ( $K$ ) using structure (v.2.3.4; Pritchard et al., 2000; Falush et al., 2003). For this, we pooled the material from time points and localities within metapopulation, in order to investigate whether the same genetic populations appear in multiple lakes and/or are stable over time. In total, seven metapopulations and three separate lakes were analyzed (Table S1) like this with structure. We used the default model allowing population admixture and correlated allele frequencies, applying the alternative (population-specific) ancestry prior, with ALPHA=1/number of samples (i.e. the number of lake and time point combinations; Wang, 2019). No *a priori* information was used. The burn-in length was 250,000 and the number of Markov chains (MCMC) 500,000. Estimations of  $Q$  (assignment probability; the mean individual probability of belonging to a certain genetic cluster) and the most likely value of  $K$  (simulated  $K = 1-15$ ) was repeated over 20 runs, with the output analysed using kfinder (v.1.0; Wang, 2019) and structure harvester (v.0.6.94; Earl & vonHoldt, 2012). Mean individual  $Q$  over the 20 runs was derived from the clumpp software (v.1.1.2; Jakobsson & Rosenberg, 2007). The most likely number of  $K$  was based on the parsimony index ( $PI$ ) recommended by Wang (2019). Individuals were assigned to the cluster for which they had the highest  $Q$ .

We defined the clusters identified by structure as populations and all further analyses are based on these populations. We use “population” and “cluster” synonymously from here on, and if such populations occur within the same metapopulation we also use term “subpopulation” for such populations/clusters). We measured genetic diversity at two points in time for each population by estimating observed and expected heterozygosity ( $H_O; H_E$ ), the average number of alleles per locus ( $N_A$ ) using genalex v.6.5 (Peakall & Smouse, 2006, 2012), allelic richness ( $A_R$ ) using fstat (v.2.9.4; Goudet, 2003), and the proportion of polymorphic loci ( $P_L$ ). Confidence intervals for diversity measures, as well as tests for normality of data were calculated in statistica (v.7.1; StatSoft, Inc., 2005). To test for changes in the genetic diversity measures over time we performed non-parametric Wilcoxon matched pairs test as well as Student’s t test for paired samples (heterozygosity, average number of alleles per locus, allelic richness), and  $\chi^2$  tests (proportion of polymorphic loci) using statistica and Microsoft excel. Statistical analyses investigating relationships between genetic diversity and the physical parameters of the localities were carried out using statistica.

We estimated effective population size ( $N_e$ ) for individual populations (cluster) with the temporal method using tempofs (sampling plan II; Jorde & Ryman, 2007), as well as with the linkage disequilibrium method (Hill, 1981; Waples, 2006; Waples & Do, 2010) as implemented in neestimator v.2.1 (Do et al., 2014). Confidence intervals for  $N_e$  were obtained from the respective software.  $N_e$  for metapopulations were

estimated using the temporal method only.  $N_e$  estimation in substructured (non-isolated) populations is complex and we follow suggestions from Ryman et al. (2014, 2019) when interpreting the estimations (below).

$F_{ST}$  (Weir & Cockerham, 1984) quantifying temporal genetic heterogeneity within populations and genetic heterogeneity among subpopulations within metapopulations were obtained using genepop (v.4.3; Raymond & Rousset, 1995; Rousset, 2008). chifish (v.5.0; Ryman, 2006) was used for significance testing of allele frequency change over time, while testing for spatial genetic differentiation was performed in genepop and statistica. The relationship among genetic clusters and metapopulations was illustrated with a phylogenetic tree from Poptree2 (Takezaki et al., 2010).

## 2.4 Indicators

In general, by mapping genetic diversity within and between populations we can identify populations with particularly low levels of genetic diversity in relation to other populations of the same species, and/or populations that appears isolated from other population at one point in time. Mapping data can thus tell us something about the genetic status, but monitoring over time is needed to understand if the observations are temporally stable.

To quantify changes in genetic diversity over time we used three indicators suggested for national monitoring and management of genetic resources in aquatic environments in Sweden (Johannesson & Laikre, 2020). We also applied the three indicators proposed for the CBD context by Laikre et al. (2020) and elaborated by Hoban et al. (2020, 2021b) and Laikre et al. (2021; Figure 2). These indicators are pragmatic and designed to be applicable at a global scale by all nations with indicators 1 and 2 possible to apply using proxies in the absence of genetic data (Figure 2; Hoban et al., 2020, 2021b; Laikre et al., 2021).

The three indicators suggested by Johannesson & Laikre (2020) all represent application of proposed CBD indicator 3 (Figure 2) – i.e. they are based on genetic data. They have only been presented in Swedish (a peer-reviewed report to SwAM; Johannesson & Laikre, 2020), so we present those indicators in more detail here. Indicator 1, denoted  $\Delta H$ , reflects changes of within population genetic diversity, measured as changes in expected heterozygosity ( $H_E$ ) between two points in time (Figure 3). Other measures of intrapopulation genetic diversity are also considered in this indicator such as potential changes in observed heterozygosity ( $H_O$ ), allelic richness ( $A_R$ ), number of alleles per locus ( $N_A$ ), and proportion of polymorphic loci ( $P_L$ ).

Indicator 2 concerns the effective population size,  $N_e$ , and is quantified for single isolated populations, subpopulations within metapopulations, full metapopulations, or for species with a continuous distribution for subareas over the distribution range (cf. Hoban et al., 2021b). Indicator 3 is used to monitor between population genetic diversity. We call this indicator  $\Delta \Phi_{ST}$  and it quantifies the change of genetic differentiation among populations between points in time. It applies to systems of more or less genetically connected populations. However,  $\Delta \Phi_{ST}$  also addresses the degree of population retention over time (Figure 3).

Clearly, there is overlap and close connection between the CBD indicators and the Swedish

national indicators (Figure 2). All national indicators represent application of CBD indicator 3 and all national indicators provide information that can be used feed into CBD indicators 1 and 2 (Figure 2). It should be noted that none of these indicators explain the underlying causes of potential changes of within and between population genetic diversity. They only inform about change, and depending on the degree of detected change (below; Figure 3) they call for more or less rapid measures to investigate what the causes are.

### 2.4.1 Threshold values for indicators

Threshold values for indicators are regarded as helpful in management to evaluate rates of change (Maria Jansson, SwAM, pers. comm.). Proposed threshold values for the  $\Delta H$  indicator relate to the recommendation that in 100 years, a population should retain at least 95% of its heterozygosity (Allendorf & Ryman, 2002). The proposed thresholds values for this indicator are: less than 0.05% reduction per year, assuming constant

rate of change (reflecting retention of c. 95% heterozygosity after 100 years); this rate of reduction is suggested to reflect status of color green for “Good” (Figure 3). A rate of reduction between 0.06-0.3% per year (reflecting expected retention of c. 75-94% heterozygosity over 100 years) is proposed to reflect status “Warning”/yellow where further investigation of the reason for reduction is warranted. Finally, a reduction rate of more than 0.3% per year (resulting in [?] 75% of genetic variation expected to be retained after 100 years) reflects status red alert where prompt measures are called for to understand the reason for decline and thereafter taking immediate steps to halt the reduction and restore genetically safe conditions.

Here, we apply indicator  $\Delta H$  in each of the identified populations (that occur in samples at both points in time) as well as to the metapopulations that they belong to. Genetic diversity was measured as expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), allelic richness ( $A_R$ ), number of alleles per locus ( $N_A$ ), and proportion of polymorphic loci ( $P_L$ ), and testing for potential changes was done by t-tests and non-parametric Wilcoxon matched pairs tests. In cases with statistically significant change, we translate the difference between the two points in time approximately 40 years apart (details on time span between samples in Table S1) into an annual change. Depending on the observed rate of change we translate it into either of the three indicator signals – green, yellow, or red (i.e., “Good”, “Warning”, or “Alarm”). If genetic diversity within sampling localities does not change (no statistically significant change) or with a statistically significant increase over time we consider the  $\Delta H$  indicator as green/“Good”. We apply the same threshold values for annual genetic reduction (i.e., [?]0.05%; 0.06-0.3%; [?]0.3%; Figure 3) for not only  $H_E$  but also for the other measures of genetic diversity ( $H_O$ ,  $A_R$ ,  $N_A$ ,  $P_L$ ).

Suggested thresholds for the  $N_e$  indicator are based on the conservation genetic rule of thumb that  $N_e$  [?]50 and  $N_e$  [?]500 is necessary for a population’s short term and long-term survival, respectively (Franklin, 1980; Jamieson & Allendorf, 2012). The proposed thresholds are:  $N_e$  [?]500 (“Good”),  $50 < N_e < 500$  (“Warning”), and  $N_e < 50$  (“Alarm”), and should apply to single isolated populations as well as to metapopulations. The  $N_e$  of local subpopulations of metapopulation cannot be ignored, however. Rather, it is important that gene flow is of a magnitude that assures that sufficient levels of genetic diversity reaches the population so that the adaptive potential is maintained. Laikre et al. (2016) suggested that the realized, local effective sizes of metapopulations should also reflect inbreeding rates that are so low that realized local  $N_e$  [?]500 for long term viability to be attained.

In practice, however, it is not straightforward to estimate the  $N_e$  that reflect the actual rate of inbreeding ( $N_{eI}$ ) and/or loss of additive genetic variance ( $N_{eAV}$ ) in substructured populations (Hössjer et al., 2016; Ryman et al., 2014, 2019). Here, we use  $N_e$  estimates from both the temporal ( $N_{eV}$ ) and linkage disequilibrium methods ( $N_{eLD}$ ; Section 3.2) and base classifications on the estimate of these two that generally is the largest, in line with recommendations for non-isolated populations (Ryman et al., 2019). We apply this indicator to metapopulations as well as to separate subpopulations.

For the  $\Delta F_{ST}$  indicator we are aware of no previously suggested guideline or rule of thumb. We apply and extend the proposal from Johannesson and Laikre (2020) that without detectable change of  $F_{ST}$  among populations over time, the status of this indicator is classified as “Good” (Figure 3). With an increase of  $F_{ST}$  between the two points in time that reflect a c. 25% decrease of genetic exchange between populations (number of migrants is reduced by 25%) this is classified as “Warning”. A decrease of  $F_{ST}$  is expected with increase of gene flow. Such increase can be warranted following management activities to connect previously fragmented populations. However, decrease of  $F_{ST}$  can also be an effect of homogenization following e.g. release activities. Such activities are not expected to be carried out in the present case since all monitored lakes are located in protected areas. However, large scale release activities resulting in genetic homogenization have been documented in e.g. Baltic salmon populations (Östergren et al., 2021). Thus, because decreased divergence can also reflect a genetic threat, we propose (in line with Johannesson & Laikre, 2020) that an  $F_{ST}$  reduction representing c. 50% increase of gene flow should classify as a “Warning” in cases where unwanted gene flow can have occurred. With a  $\Delta F_{ST}$  reflecting a 50% decrease of the number of migrants or a 100% increase in genetic exchange (number of migrants) this indicator is classified as “Alarm”. Further, if one or more local population goes extinct over the monitoring period this indicator is also classified as

“Alarm”. We note that these proposed limits are highly subjective and “forgiving” with respect to changes of connectivity.

We apply the following approach (described in detail in Appendix S1) for converting a statistically significant  $\Delta F_{ST}$  into an indicator of change in genetic exchange (migration) between subpopulations. Thus, we translate the observed  $F_{ST}$  among subpopulations at the first point in time (here denoted “past” and referring to the 1970-80s samples) to the expected number of migrants by assuming an island model in migration-drift equilibrium. This hypothetical island model has the same number of subpopulations as the metapopulation considered, and the subpopulation  $N_e$  is set to the harmonic average  $N_e$  over those subpopulations. In the next step we calculate limiting values for change of migration rates. Here we consider a reduction of migration by 25% or 50% to correspond to yellow/warning or red/alarm, respectively. Similarly, we consider an increased migration rate of 50% or 100% to reflect yellow/warning or red/alarm. Finally, we translate the limiting values of migration rates into  $F_{ST}$  values and compare them to the empirically observed  $F_{ST}$  at the second time point (here denoted “present” and referring to the 2010 samples).

### 3. RESULTS

We first report findings of the measures of genetic diversity applied and then translate these observations into indicator values (section 3.5).

#### 3.1 Genetic structuring and occurrence of sympatric populations

Altogether, structure analyses identified 31 separate clusters (populations) in the 27 lakes included in the present study, with 13-199 fish assigned to each cluster (Table S2; Figure 4). 29 of these 31 clusters occurred at both points in time and we refer to them synonymously as populations, clusters, and subpopulations (if they occur within the same metapopulation). Two populations were not present in the 1970-80s (past) sample but appeared in the recent 2010s (present) sample (in Skåarnja 1 and Hotagen 1; Figure 4). No population was lost over the c. 40 years between sampling, although the relative frequencies at which populations occurred in the samples differed significantly between time points in five metapopulations (Figure S1a-d, f;  $\chi^2=6.16-47.60$ ;  $p=0.000-0.046$ ).

Although all 29 populations identified in the 1970-80s samples remained over time, allele frequency changes occurred in many of them.  $F_{ST}$  between time points within populations varied between 0 and 3 percent (Table S3a). In 15 of the 29 populations, genetic divergence between time points was significantly different from zero ( $p=0-0.02$ ; Table S3a).

Sympatric populations (i.e. genetically divergent clusters coexisting within the same lake) were observed at both time points in 12 lakes (Figure 4; Table S2; Figure S1). These lakes were distributed across five metapopulations, and the “independent” lake Ännsjön. In the remaining two metapopulations and the two independent lakes, we observed only one population per lake. The occurrence of sympatric populations does not appear to correlate with the number of creeks/streams connected to the lake (i.e. potential spawning sites;  $r = 0.29$ ;  $p=0.14$ ), lake area ( $r = 0.27$ ;  $p=0.18$ ), or average lake depth ( $r = -0.31$ ;  $p=0.20$ ).

The genetic relationships among the identified populations reflect the geographic location of the lakes and systems. Overall, the branching of the phylogenetic tree (Figure S2) corresponds to the main river drainages (Figure 1, 4). Lake Ännsjön was found to harbor three subpopulations and is thus viewed as a metapopulation. The other two separate lakes (Saxvattnet and Rörvattnet) only harbored one population per lake. Thus, we have a total of eight metapopulations (Figure 4).

#### 3.2 Genetic diversity within populations and metapopulations

Levels of genetic diversity within populations varied among areas but were lower in the two metapopulations located in areas above the tree-line (Skåarnja 1 and 2) as compared to those in areas below the tree-line (Table S4, Figure 5, Figure S3-S7). In the above tree-line systems, heterozygosities (expected as well as observed) were always below 20 percent, while the corresponding estimates for below tree-line areas were at or above 20 percent (Table S4). The same trend was observed in the allelic diversity measures ( $A_R, N_A$ ,

and  $P_L$ ) where lower estimates were observed in the above tree-line systems. This difference was significant for all measures at both points in time, except for allelic richness, differing only in present samples (p-values varying between  $<0.001$  and  $0.012$ ; Table S4).

We observe temporal fluctuations in levels of diversity within populations (clusters) and metapopulations but with no general, overall trend of increase or decrease over time for any of the five diversity measures among the 29 populations that occur at both points in time (all  $p > 0.05$ ; Sign test; Figure 5, Figures S3-S5; Table S5). Most striking is the pronounced decrease of genetic diversity in three out of six populations in the above tree-line metapopulations Skåarnja 1 and 2 (Figure 5, 7; Figure S3-S5; Table S5). The opposite trend is observed in e.g. metapopulation Hotagen 4 where genetic diversity is increasing in all subpopulations and significantly so in three out of seven (Figure 5, 7).

### 3.3 Genetic divergence between populations

Genetic divergence among populations measured as  $F_{ST}$  are summarized in Table 1 and Table S6. Average  $F_{ST}$  among populations within metapopulations varied between 0.06 and 0.16 in the 1970-80 samples (past) and from 0.04 to 0.18 in the present samples and were significantly larger than zero in all lake systems at both points in time (all  $p < 0.001$ ; Table 1). Among non-sympatric populations,  $F_{ST}$  ranged between 0.07-0.16 (past) and between 0.08-0.18 (present) while among sympatric populations (i.e., populations coexisting in the same lake)  $F_{ST}$  spanned 0.05-0.10 (past) and 0.04-0.09 (present; Table 1). A significant change of  $F_{ST}$  among populations over time was observed in three of the eight systems where we have more than one population per system (Table 1). One case shows significant increase of  $F_{ST}$  (Skåarnja 2;  $p = 0.006$ ; Wilcoxon matched pairs test; Table 1). In contrast, there was a statistically significant decrease of  $F_{ST}$  in two of the below tree-line metapopulations (Jougdadalen and Hotagen 4;  $p = 0.011$  and  $0.006$ , respectively; Wilcoxon matched pairs test; Table 1).

### 3.4 Effective population size

Average estimates of variance effective size ( $N_{eV}$ , per generation) per population (cluster) varied between 47 and infinity, and was below 500 in 20 of the 29 populations that were observed at both points in time and thus allowed assessment of  $N_{eV}$  (Figure 4; Table S7). These estimates are expected to coincide with the inbreeding effective size of a subpopulation under isolation. In the face of migration, inbreeding rates are expected to be lower than what these estimates indicate (i.e., local  $N_{eI}$  is expected to be larger than local  $N_{eV}$ , cf. Ryman et al., 2019). Metapopulation  $N_{eV}$  was below 500 in five of the eight metapopulations and in one of the two single lakes. In three metapopulations and in one of the single lakes  $N_{eV}$  was above 500 (Figure 4; Table S7).

There was a significant positive correlation between  $N_{eV}$  and expected heterozygosity ( $H_E$ ) ( $r = 0.48$  and  $p = 0.008$  for present estimates; Figure 6a). A similar relationship between  $N_{eV}$  and the other diversity measures was also observed at both time points (Figure S8; Table S8).

There was no apparent link between  $N_{eV}$  and predicted retention of expected heterozygosity ( $H_E$ ; section 2.4.1) over 100 years ( $r = 0.39$ ;  $p = 0.16$ ; Figure 6b).

$N_e$  estimates based on the linkage disequilibrium method ( $N_{eLD}$ ) where consistently lower than estimates based on the temporal method ( $N_{eV}$ ) (Table S7). Only four populations in the past and six present ones show  $N_{eLD}$  estimates above 500. In the past (1970s), 11 populations have  $N_{eLD} < 50$  while in the present (2010s) five populations show  $N_{eLD} < 50$ .  $N_{eLD}$  of separate populations appear to have increased over the 40 years between sampling ( $p = 0.048$ ; Wilcoxon matched pairs test), although this trend was not observed for metapopulations. None of the metapopulations had an  $N_{eLD}$  above 500 at any point in time (Table S7).

### 3.5 Indicators

Indicator classifications are shown in Figure 7. In summary, the  $\Delta H$  indicator detects reduction of genetic diversity within several populations (Figure 7a), but metapopulation systems buffer against some of these trends so that when metapopulations are considered – increase and decrease within separate subpopulations

levels out (Figure 7b). This is not the case in all systems however, and the  $N_e$ -indicator provides warning signals of low effective population sizes in 6 out of 10 systems studied (Figure 7b). The  $\Delta F_{ST}$  indicator shows that gene flow is significantly decreasing in the same metapopulation (Skåarnja 2) where we also observe  $\Delta H$  and  $N_e$  warning signals. However, the change of  $F_{ST}$  reflects an expected reduction genetic exchange that is less than the proposed threshold value, i.e., the number of migrants between subpopulation is estimated to have been reduced by less than 25% over the monitoring period (Figure 7b).

### 3.5.1 $\Delta H$ ινδισατορ

Applying the  $\Delta H$  indicator to the 29 separate populations, we observe that five populations in four different systems exhibit statistically significant decrease of diversity measures at a rate higher than the 0.05 percent decrease per year limit for the genetic status to be regarded as “Good” (Figure 7a). These populations exhibit reductions between 0.06 and 0.83 percent per year in several diversity measures, respectively (Table S3a). Population 3 in Skåarnja 1 is classified as “Alarm” with respect to  $H_E$  and  $P_L$ , and as “Warning” with respect to  $N_A$ . Population 4 in Skåarnja 1 is classified as “Warning” with respect to  $H_E$ . Population 1 in Skåarnja 2 has lost allelic diversity ( $A_R$  and  $N_A$ ) as well as expected heterozygosity ( $H_E$ ) at a rate reflecting a retention of 70-94% of genetic variation after 100 years classifying it as “Warning”. In addition, this population has experienced a decrease in proportion of polymorphic loci ( $P_L$ ) at a yearly rate of 0.74% resulting in the classification “Alarm”. Population 3 in Hotagen 3 is classified as “Warning” based on the rate of diversity loss of  $A_R$ , and  $N_A$ , and as “Alarm” based on loss rate of  $H_E$  and  $H_O$ . Finally, the population in Lake Saxvattnet had experienced a significant decrease in  $H_E$ , with an estimated diversity retention of 89% after 100 years, classifying this population as “Warning”. The remaining 24 populations were classified as “Good” for all five diversity measures, and in those, statistically significant increase of genetic diversity is observed in eight populations representing four systems (Figure 7a).

When applying the indicators to metapopulations, all but two were estimated to have retained more than 95% of genetic diversity after 100 years in all five measures and were classified as “Good”. Metapopulations Skåarnja 2 lost a substantial amount of allelic diversity and was classified as “Warning” with respect to  $A_R$ ,  $N_A$ , and as “Alarm” based on  $P_L$  (Figure 7). It should be noted that for Skåarnja 1, the green classification is due to the “new” population that occurred in the 2010 sample but was not found in the past sample (Figure 4; Figure S1).

### 3.5.2 $N_e$ indicator

We apply the  $N_e$  indicator (Figure 2, 3) using our  $N_{eV}$  estimates that are larger than the  $N_{eLD}$  estimates (Ryman et al. 2019; Table S7). One population (Population 2 in Hotagen 4) was classified as “Alarm” ( $N_{eV} < 50$ ) due to the low  $N_{eV}$  of 46 (Figure 7a). A total of 19 populations distributed over four metapopulations were classified as “Warning” ( $N_{eV} = 56-470$ ; Table S7, Figure 7a), and nine populations had an effective population size ( $N_{eV}$ ) of  $>500$  and were classified as “Good”. For ten full systems (eight metapopulations and the two independent lakes) six are below 500 and thus show “Warning” ( $N_{eV} = 65-428$ ), while four systems show  $N_{eV} \geq 500$  (605-?) and thus status “Good” (Figure 7b).

### 3.5.3 $\Delta \Phi_{ST}$ ινδισατορ

A statistically significant increase of  $F_{ST}$  is observed in one metapopulation (Skåarnja 2), and significant decrease of  $F_{ST}$  in two systems (Jougdadalen and Hotagen 4). However, when translating these changes to reflect changes in gene flow among subpopulations none of these three cases exceed the proposed limiting values for the  $\Delta F_{ST}$  indicator and they are thus classified as “Good” (Figure 7b). Skåarnja 2 exhibit an initial  $F_{ST}$  of 0.159 (in the 1970-80s sample), a 25% decrease in gene flow correspond to a limiting upper value for  $F_{ST}$  of 0.202 while the observed present (2010s)  $F_{ST}$  was  $F_{ST} = 0.180$  (corresponding to a 14% decrease in gene flow) and thus below the limit. In Jougdadalen and Hotagen 4  $F_{ST}$  has decreased significantly, indicating that gene flow has increased in these systems. In Jougdadalen,  $F_{ST}$  decreased from 0.054 (past) to 0.042 (present), corresponding to a 31% increase in genetic exchange. Similarly, gene flow increased by 30% in Hotagen 4 (past  $F_{ST} = 0.103$  and present  $F_{ST} = 0.080$ ). Since the increase in gene flow did not exceed 50% in neither of these metapopulations, they are both classified as “Good” (Figure 7b).

### 3.5.4 The CBD indicators

The indicators proposed for the CBD framework (Laikre et al., 2020; Hoban et al., 2020) showed that for indicator 1, four metapopulations had an  $N_e$  above 500, and six below 500 (Figure 7c). For indicator 2 we note that all populations were maintained over time so there is a 100 percent retention of populations. With respect to indicator 3, we use genotypic data in all ten systems monitored, so all populations in this study meet the criterion of this indicator.

## 4. DISCUSSION

We assessed and monitored genetic diversity within and between populations of brown trout over 40 years in a total of 27 small alpine lakes in ten geographically separate systems in central Sweden. We used genetic data to identify genetically separate populations and detected 29 such populations that were stable over time. Individual populations occurred in 1-4 alpine lakes within the same water system and at least 12 of the lakes harbored more than one population.

We quantified the genetic diversity within and between populations and applied recently proposed indicators to track genetic diversity over time, and we propose that these indicators can aid in biodiversity monitoring. All populations were located in protected areas where maintaining biodiversity is a common goal. While we found no general trend with respect to decrease or increase of genetic diversity among the 29 populations of the ten water systems we did observe considerably lower levels of genetic diversity in the two above-tree-line systems. In one of these systems we also found a decrease of genetic diversity at a magnitude exceeding the proposed limiting values for within population diversity change ( $\Delta H$  indicator).

In contrast, increased genetic diversity was observed in several separate populations as well as in three population systems (Figure 7). The effective population size ( $N_e$ ) was generally low; 20 out of 29 local populations and five out of eight metapopulations revealed  $N_e < 500$  which is below the threshold value proposed for this indicator. The between population indicator  $\Delta F_{ST}$  showed significant increase of divergence, potentially suggesting reduced gene flow in the system where the  $\Delta H$  and  $N_e$  indicators gave warning signals (Skåarnja 2; Figure 7b), although the change was within the proposed limiting values. In two systems  $\Delta F_{ST}$  showed a significant reduction, potentially indicating increased gene flow, but here too within the proposed limiting values (cf. Figure 3). Applying the CBD indicators to the present data provides conclusions consistent with the national indicators but with less detail (Figure 7c).

### 4.1 Detection of sympatric populations and divergence among them

Conspecific populations that coexist in the same habitat without obvious ecological divergence (cryptic sympatry) is a type of “hidden” biodiversity that we still have limited knowledge of (Struck et al., 2017). The present results suggest that the occurrence of cryptic sympatry is a common phenomenon in these mountain lake systems. We detected such structures in over 40 percent of the lakes, and they were stable over time in occurrence as well as in amount of divergence. Our observed  $F_{ST}$  among sympatric populations are of a similar order of magnitude as previously reported for cryptic, sympatric, salmonid populations (Andersson et al., 2017a; Aykanat et al., 2015; Marin et al., 2016; Wilson et al., 2004).

### 4.2 Identifying populations for monitoring

Recognizing genetic diversity occurring both within and between populations is essential for conservation management of structured populations (Caballero et al., 2010). We used the structure software to identify genetically distinct populations within systems of interconnected lakes, and then we monitored these structures over time. Our findings indicate that this approach was needed to identify and monitor genetically distinct populations. If we had focused on lakes only, the population diversity within them would have gone unnoticed.

To identify metapopulations we primarily used geographic location and knowledge of waterways for potential migration. For instance, metapopulations Skåarnja 1 and 2 were separated into two systems because of

waterfalls that most likely prevents migration between the two areas. Our inferred metapopulation structure was supported by the phylogenetic tree (Figure S2).

Life-history diversity of brown trout is complex, and the fish typically spawn in streams and creeks and feed in lakes, but lake spawning is also possible as well as river residency (e.g. Östergren et al., 2012; Ferguson et al., 2019). Ideally, sampling would occur at spawning grounds and during the reproductive period. In practice this is difficult in these systems because they are located in remote areas that are difficult to access, particularly in late fall/early winter when spawning occurs and when weather conditions are typically harsh. We were not able to sample all water bodies in any of the systems studied, and thus cannot rule out that additional subpopulations exist that can exchange migrants with the populations identified and monitored.

### 4.3 Difficulties in identifying subpopulations within metapopulations

Two populations were only found in the 2010s samples and were not observed in samples from the 1970-80s. These “new” populations were found in Lakes Daimatjärn 1 Ö and Munsvattnet (Skåarnja 1 and Hotagen 1, respectively) and indicate that our data do not represent the entire metapopulations present in these systems. We also found that the relative frequencies at which populations were observed in the samples fluctuated over time (Table S2), most likely as an effect of sampling.

The finding of the “new” populations complicate inference about the genetic diversity status of the two metapopulations involved. In metapopulation Skåarnja 1, for example, we observed a decrease or no change in diversity measures in all the three populations that occurred at both points in time. Yet, when regarding the whole metapopulation we see an increase in diversity over time (Figure 7a-b). This is due to the appearance of the new genetic cluster in the present sample, and this new population displays the highest levels of diversity in all measures when compared to the other populations in the present sample. Excluding the new population from the analysis results in a decrease in heterozygosity and no change of allelic diversity ( $A_R, N_A, P_L$ ) in this metapopulation. Like diversity, the estimate of metapopulation effective size  $N_{eV}$  is affected by the appearance of the new population; including it results in an  $N_{eV}$  of 65, while excluding this cluster doubles the effective size of the metapopulation ( $N_{eV}=135$ ). The reduction is due to the larger allele frequency change resulting from the new cluster. Similar observations can be made in the metapopulation Hotagen 1 (where a new population appears in the 2010s sample), but the effects of including it in the estimates for the whole system are less striking. Thus, recognizing the population genetic structure and identifying existing genetically distinct populations is important for the interpretation of indicator values.

### 4.4 The relationship between $N_e$ and genetic diversity

According to the neutral model (Kimura, 1983), larger populations are expected to exhibit greater levels of genetic diversity than smaller ones. However, empirical support for this theory has been conflicting. Some studies report a correlation between population size and diversity (Aho et al., 2006; Hague & Routman, 2016; Knaepkens et al., 2004) while others fail to do so (Bazin et al., 2006; Lonsinger et al., 2018). The present results are generally in line with the neutral model (Figure 6a). The variation is large, however, and in some metapopulations we find no correlation between  $N_e$  and the level of heterozygosity (Figure 6a).

There is no apparent correlation between expected retention of heterozygosity over 100 years and  $N_e$  (Figure 6b). Most of this lack of correlation is explained by the fact that heterozygosity is increasing in many of the populations monitored. The increase is likely due to genetic connectivity among populations with systems, and can also be due to connectivity with populations that we have not been able to detect in this screening. When considering only populations where heterozygosity is observed to decrease over the 40-year monitoring period we find a pattern which appears relatively consistent with expectations, i.e., that populations with high  $N_e$  tend to exhibit a higher degree of retention of  $H_E$  (Figure 6b).

### 4.5 Genetic diversity trends in protected areas

Several studies have reported similar levels of genetic diversity in populations located within and outside protected areas (PAs - Caló et al., 2016; Hedenäs, 2018; Novello et al., 2018; Wennerström et al., 2017; Zechini et al., 2018), but none of those studies consider the temporal aspect. Araguas et al. (2017) examined genetic

diversity and introgression in brown trout populations within PAs in north-western Spain, with samples taken at more than two points in time. They observed diversity fluctuations within localities over time (albeit without statistical testing), but no general trend among localities. We have also observed significant fluctuations in genetic diversity within some of the subpopulations and/or metapopulations over time. In seven of the eight separate water systems where more than one population was identified we find decrease in genetic diversity in some subpopulations, and increase in others. Clearly, we would need to continue monitoring these systems in coming decades to see if such fluctuations of genetic diversity within and between populations are random within systems, and if they vary among populations over time.

We observe a general trend of small local  $N_e$ , but with higher metapopulation  $N_e$ , and migration rates between subpopulations are crudely estimated as  $\sim 0.5$ - $2.5$  individuals per generation (assuming an island model of migration). These findings suggest that the metapopulation structure buffers the systems against loss of genetic diversity and that protected areas need to be large enough to support a large meta- $N_e$  (cf. Jorde & Ryman, 1996; Gompert et al., 2021).

#### 4.6 Indicators

When applying the three indicators proposed for the Swedish Agency of Marine and Water Management (SwAM; Johannesson & Laikre, 2020) we find that the proposed limiting threshold values are exceeded in some of the 29 monitored populations (Figure 7a). However, when considering the metapopulations that these populations belong to, positive trends in other populations of the same metapopulations compensate for the negative trends in several cases. Only two of ten full systems (Figure 7b) show warning signals for the  $\Delta H$  indicator. Metapopulation  $N_e$  is however, often below the 500-threshold resulting in warning signals from the  $N_e$  indicator. We underline that the true meta- $N_e$  can be larger in these systems because we have likely not sampled the full metapopulations in any of the present cases. Sampling over substantially larger areas are needed to resolve this issue. On the other hand, the generally low  $N_e$  estimates observed underlines the vulnerability of these systems – if they become fragmented and isolated, local  $N_e$  is low and reduced connectivity will rapidly result in elevated rates of diversity loss. Protecting large, interconnected systems are thus important for the conservation and viability of fish in small mountain lake systems. We also apply the indicators for CBD (Figure 7c), and in the present case the results are largely consistent with the results from the national (SwAM) indicators.

#### 4.7 Further developments of indicators

For the indicator  $\Delta H$ , recommendations for threshold values are based on the general guideline of retention of 95% heterozygosity over 100 years (Allendorf & Ryman, 2002). It should be noted, however, that this guiding principle refers to short term genetic conservation and may need to be revised for long term genetic resilience. Further, detecting changes of the magnitude proposed – i.e. a reduction of 5% over 100 years – can be statistically challenging. Sampling over extended periods (several generations) is warranted, but even then, it may be difficult to obtain a reasonable statistical power for detecting statistically significant changes.

Further work is needed to refine the indicators. The statistical power for detecting various levels of heterozygosity change from typically used sets of markers are needed. Also,  $N_e$  estimation in non-isolated populations is complex. For instance, no method for assessing inbreeding effective population size ( $N_{eI}$ ) from genotypic data is currently available for structured populations. In such situations, different types of  $N_e$  differ -  $N_{eV}$  and  $N_{eLD}$  reflect inbreeding rates of local population in isolation, but fail to provide estimates of inbreeding rates for populations in the face of migration (Ryman et al., 2019). If we are able to sample a full metapopulation with all subpopulations at proportions reflecting their contribution to the total system, we can use  $N_{eV}$  to get a good estimate of inbreeding  $N_{eI}$ . However, if we fail in identifying the full metapopulation, estimates of  $N_{eV}$  will underestimate the  $N_{eI}$  of the metapopulation (Ryman et al., 2019). As discussed above, we do not think we have been able to completely identify all subpopulations in the present case.

Further, to implement the  $\Delta F_{ST}$  indicator we used the simplifying assumption of an island model in

migration-drift equilibrium to translate temporal changes of  $F_{ST}$  among subpopulations into migration rates. Clearly, important future work includes investigating the effects of violating this assumption. The limiting values used here are also highly subjective, and it is unclear if they are sufficient to detect biologically important changes of connectivity. A temporal aspect of the change is also missing. The national indicators are now being applied to more species in Sweden including herring, cod, salmon, and eelgrass (Maria Jansson, SwAM, pers. comm.) and this work will provide more empirical information that can aid in modifying and improving the indicators and limiting values applied for them.

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## Author contributions

A.A., N.R., and L.L. designed the study and led the collection of present-day samples. N.R. initiated and organized the collection of samples from the 1970s and 1980s, and those samples have been stored in a frozen tissue bank maintained by N.R. and L.L. at the Department of Zoology, Stockholm University. S.K. led the laboratory analyses. A.A. performed population genetics analyses, provided the first draft of the manuscript, and led the writing with all authors contributing to the final manuscript.

## Data accessibility

Data supporting the findings of this paper are provided in the Supplemental Information (Tables S2, S3, and S6). Additional data are available at Dryad XXX (to be completed after manuscript is accepted for publication).

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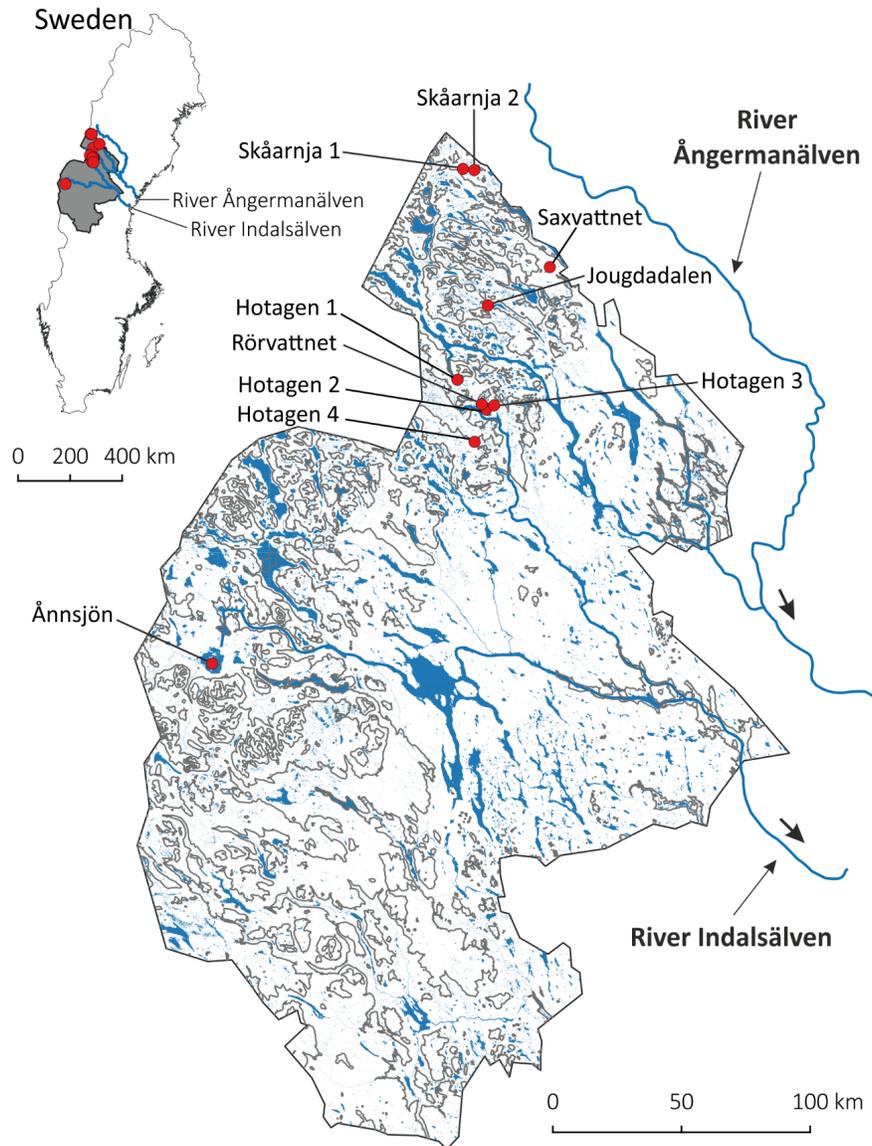
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**Table 1.** Average  $F_{ST}$  (over 96 SNPs) among populations (clusters) within metapopulations at the two time points (past=1970-80s; present=2010s) as well as divergence change over time ( $\Delta F_{ST}$ ) with associated significance levels. Significance levels for differences among populations were obtained from genepop, while p-values for  $\Delta F_{ST}$  are from the Wilcoxon matched pairs test. Lakes Saxvattnet and Rörvattnet are not included here because each of these lakes only harbors one population.

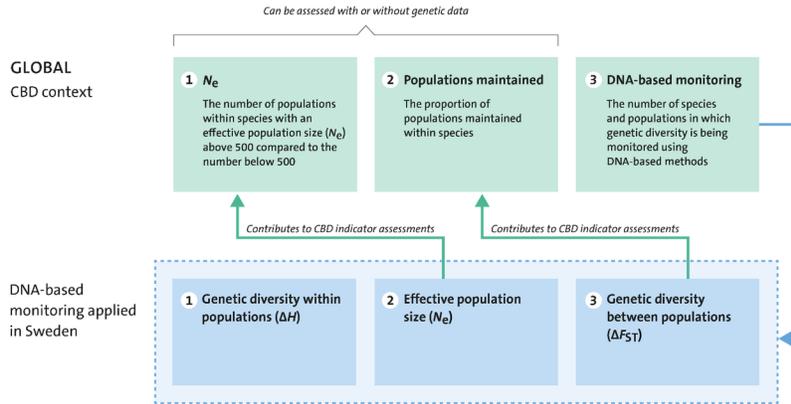
Metapopulation/Lake	$F_{ST}$ among populations (past)	p ( $F_{ST}$ past)	$F_{ST}$ among populations (present)
Skåarnja 1	0.063	<0.001	0.081
Skåarnja 2	0.159	<0.001	0.180
Jougdadalen	0.054	<0.001	0.042

Metapopulation/Lake	$F_{ST}$ among populations (past)	$p(F_{ST} \text{ past})$	$F_{ST}$ among populations (present)
Hotagen 1	0.071	<0.001	0.077
Hotagen 2	0.071	<0.001	0.080
Hotagen 3	0.092	<0.001	0.094
Hotagen 4	0.103	<0.001	0.080
Ånnsjön	0.068	<0.001	0.069



**Figure 1** . Map of the study area. Red dots indicate the locations of the seven sampled metapopulations and three single lakes (cf. Table 1, Supplemental Information Figure S1). Arrows next to the rivers indicate the direction of water flow.

### Indicators for genetic diversity

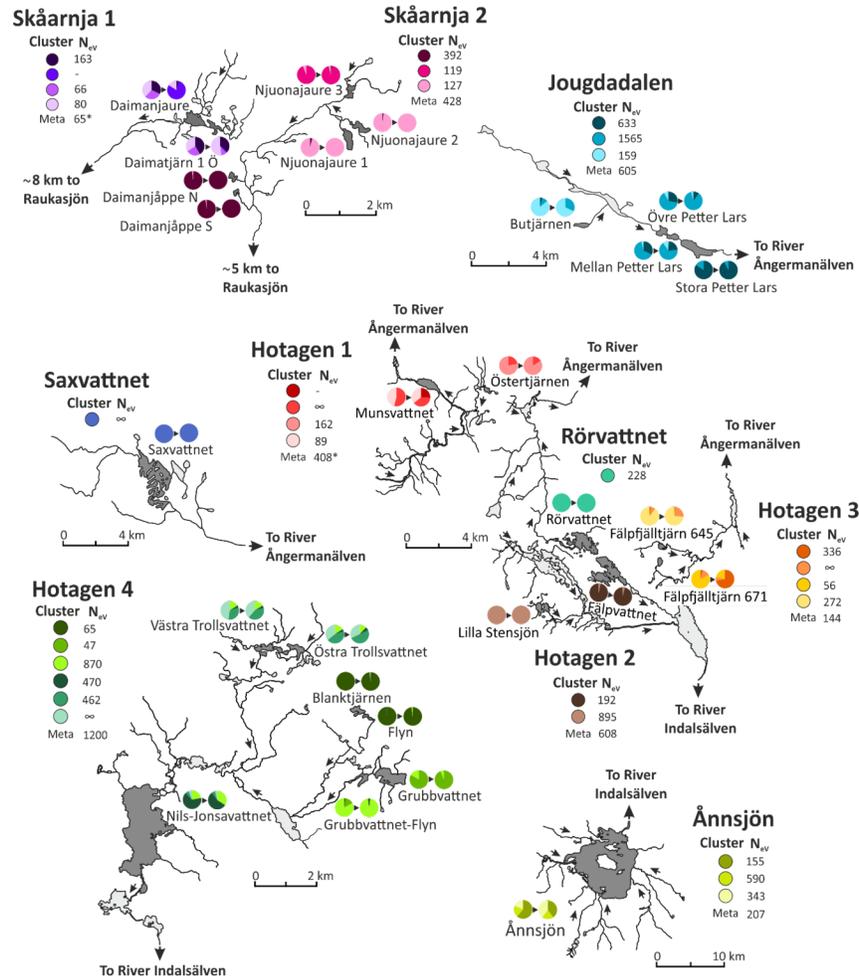


**Figure 2.** Indicators for genetic diversity proposed for the CBD “post 2020 framework” (green boxes) and for national monitoring and management of genetic resources in aquatic environments in Sweden (blue boxes).

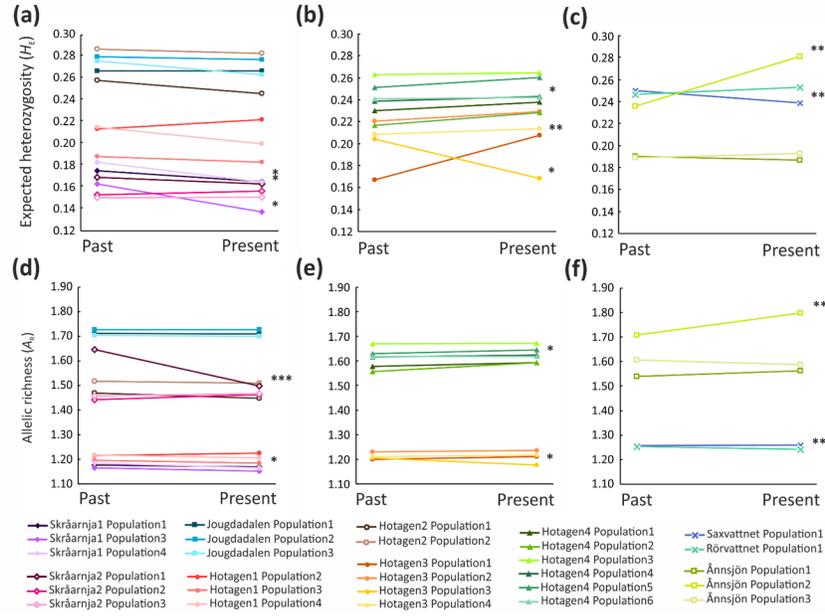
Indicator	Threshold	Interpretation	Comments
<b>Genetic diversity within populations (ΔH=H2-H1)</b> H1: Heterozygosity at first point in time H2: Heterozygosity at second point in time	≤ 0.05% reduction per year*	Good	Focus on $H_E$ but also considers $H_O$ , $A_R$ , $N_A$ , $P_L$ , as well as $\pi$ , $F_{ROH}$ , $HP$ when available.
	0.06-0.3% reduction per year**	Warning	
	> 0.3% reduction per year***	Alarm	
<b>Effective population size (<math>N_e</math>)</b>	$N_e \geq 500$	Good	Applies to single isolated populations, metapopulations, or sub-areas of the total range. For metapopulations, subpopulation $N_e$ is also considered.
	$50 < N_e < 500$	Warning	
	$N_e \leq 50$	Alarm	
<b>Genetic diversity between populations (ΔF<sub>ST</sub>=F<sub>ST2</sub>-F<sub>ST1</sub>)</b> F <sub>ST1</sub> : Genetic differences between two or more subpopulations at first point in time F <sub>ST2</sub> : Genetic differences between two or more subpopulations at second point in time	ΔF <sub>ST</sub> no/minor change	Good	Change of the number of genetically distinct populations is also considered in this indicator.
	ΔF <sub>ST</sub> reflects c. 25% reduction of genetic exchange between subpopulations, or c. 50% increase of genetic exchange among subpopulations	Warning	
	ΔF <sub>ST</sub> reflects >50% reduction of genetic exchange among subpopulations, or >100% increase of genetic exchange among subpopulations	Alarm	

\*≥95% retention of H over 100 years; \*\*75-94% retention of H over 100 years; \*\*\*<75% retention of H over 100 years

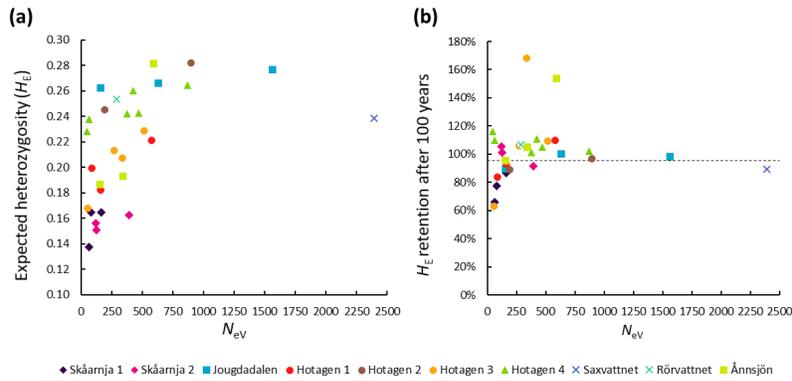
**Figure 3.** Three indicators proposed and currently applied for national use in Sweden and their proposed threshold criteria.  $H_E$ =expected heterozygosity,  $H_O$ =observed heterozygosity,  $A_R$ =allelic richness,  $N_A$ =average number of alleles per locus,  $P_L$ =proportion of polymorphic loci,  $\pi$ =nucleotide diversity,  $F_{ROH}$ =fraction of genome covered by ‘runs of homozygosity’; the two latter ones applicable only for sequence data.



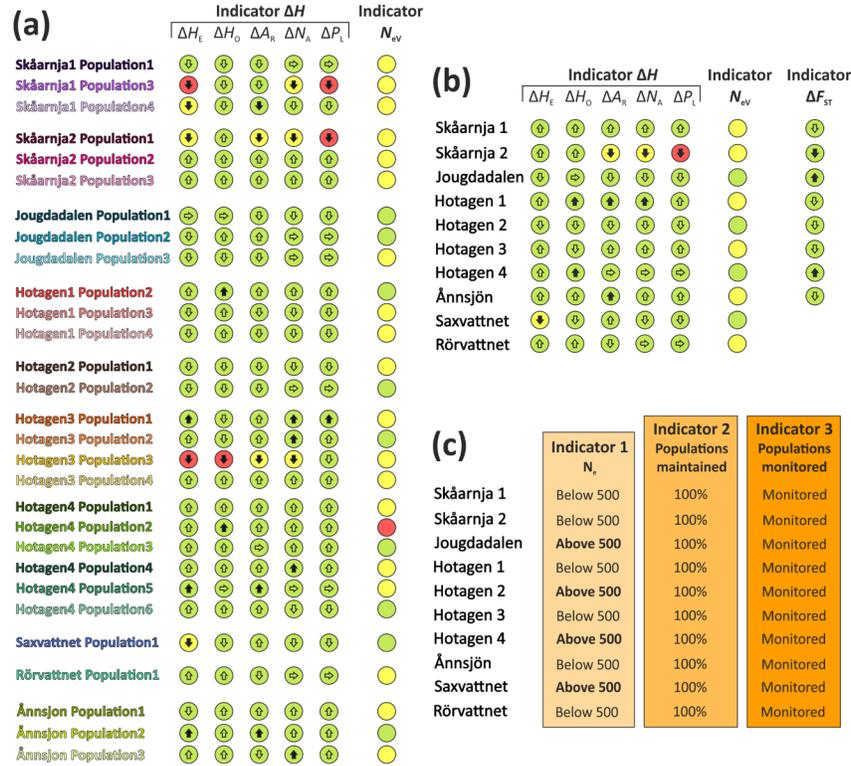
**Figure 4.** Occurrence and distribution of the 31 genetic clusters in the seven water systems and three individual lakes (Saxvattnet, Rörvattnet, Ånnsjön). Pie diagrams show the representation of populations in the present (leftmost diagram in each pair) and the past samples (rightmost diagram; detailed illustration of the genetic clusters in each system are provided in Figure S1). Estimates of effective population size measured as  $N_{eV}$  are indicated for each population occurring at both points in time. Black arrows indicate direction of water flow. Note the presence of “new” populations in the present samples from Skåarnja 1 (Lake Daimanjaure) and Hotagen 1 (Lake Munsvattnet).



**Figure 5.** Trends over time in expected heterozygosity ( $H_E$ ; a-c) and allelic richness ( $A_R$ ; d-f) for populations within metapopulations. (a,d) populations in Skårarnja 1, Skårarnja 2, Jougdadalen, Hotagen 1 and Hotagen 1; (b,e) populations in Hotagen 3 and Hotagen 4; (c,f) populations in Ännsjön, as well as Saxvattnet and Rörvattnet.



**Figure 6.** Correlation between estimated effective population size ( $N_{eV}$ ) and (a) genetic diversity measured as expected heterozygosity ( $H_E$ ) in the present, as well as (b) predicted retention of  $H_E$  over 100 years. Each point in the graph represents one of the 29 populations occurring at both points in time, classified into the seven metapopulations and the three single lakes monitored. Dashed line marks 95% retention after 100 years.

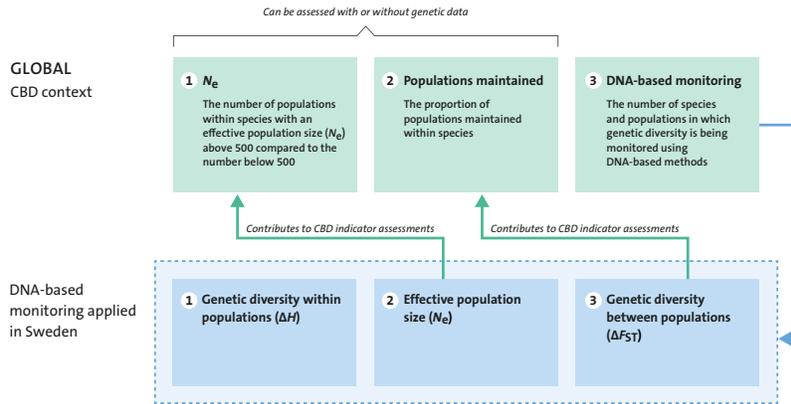


**Figure 7.** Genetic status of the 29 populations (clusters) occurring at both points in time according to the indicators proposed for use in aquatic systems in Sweden (a) as well as the eight metapopulations and two single lakes according to the Swedish (b) and the (c) CBD indicators. For the Swedish indicators, the colored circles indicate genetic status; green= “Good”, yellow= “Warning”, and red= “Alarm” (cf. Figure 3). Arrows inside circles show the direction of change, with horizontal arrows meaning stability (no change); black arrows indicate that the change is statistically significant ( $p < 0.05$ ); for  $\Delta F_{ST}$ , arrows indicate increase or decrease in connectivity. The  $\Delta F_{ST}$  indicator was not applicable for any of the 29 populations or two of the single lakes Saxvattnet and Rörvattnet because each of these lakes are isolated from other lakes in the study and each of these lakes only harbor one population.

### Hosted file

Figure1\_Sweden & J\selectlanguage{ngerman}ämtland map\_w flow arrows.pdf available at <https://authorea.com/users/388620/articles/548644-mapping-and-monitoring-genetic-diversity-of-an-alpine-freshwater-top-predator-by-applying-newly-proposed-indicators>

## Indicators for genetic diversity



Indicator	Threshold	Interpretation	Comments	
<b>Genetic diversity within populations (<math>\Delta H = H_2 - H_1</math>)</b>	$\leq 0.05\%$ reduction per year*	Good	Focus on $H_e$ but also considers $H_o$ , $A_R$ , $N_A$ , $P_L$ , as well as $\pi$ , $F_{ROH}$ , HP when available.	
	H1: Heterozygosity at first point in time H2: Heterozygosity at second point in time	0.06-0.3% reduction per year**		Warning
	> 0.3% reduction per year***	Alarm		
<b>Effective population size (<math>N_e</math>)</b>	$N_e \geq 500$	Good	Applies to single isolated populations, metapopulations, or sub-areas of the total range. For metapopulations, subpopulation $N_e$ is also considered.	
	$50 < N_e < 500$	Warning		
	$N_e \leq 50$	Alarm		
<b>Genetic diversity between populations (<math>\Delta F_{ST} = F_{ST2} - F_{ST1}</math>)</b>	$\Delta F_{ST}$ no/minor change	Good	Change of the number of genetically distinct populations is also considered in this indicator.	
	$F_{ST1}$ : Genetic differences between two or more subpopulations at first point in time $F_{ST2}$ : Genetic differences between two or more subpopulations at second point in time	$\Delta F_{ST}$ reflects c. 25% reduction of genetic exchange between subpopulations, or c. 50% increase of genetic exchange among subpopulations		Warning
	$\Delta F_{ST}$ reflects >50% reduction of genetic exchange among subpopulations, or >100% increase of genetic exchange among subpopulations	Alarm		

\* $\geq 95\%$  retention of H over 100 years; \*\*75-94% retention of H over 100 years; \*\*\*<75% retention of H over 100 years

