Received: 29 November 2021 | Revised: 8 September 2022 | Accepted: 15 September 2022

DOI: 10.1111/mec.16710

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Monitoring genetic diversity with new indicators applied to an alpine freshwater top predator

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Funding information
Svenska Forskningsrådet Formas, Grant/Award Number: 2020-01290; Vetenskapsrådet, Grant/Award Number: 2019-05503; Havs-och Vattenmyndigheten; Carl Tryggers Stiftelse för Vetenskaplig Forskning; Erik Philip-Sörensens stiftelse

Handling Editor: Andrew P. Kinziger

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. We monitored genetic diversity over 40 years (1970–2010) in metapopulations of brown trout (*Salmo trutta*) inhabiting 27 small mountain lakes representing 10 lake systems in central Sweden using >1200 fish per time point. We tested six newly proposed indicators; three were designed for broad, international use in the UN Convention on Biological Diversity (CBD) and are currently applied in several countries. The other three 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 (indicators ΔH and ΔF_{ST}, respectively) and assess the effective population size (N_e-indicator). We identified 29 genetically distinct populations, all retained over time. Twelve of the 27 lakes harboured 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 ($N_e \leq 500$) in 20 populations with five showing $N_e < 100$. Statistically significant genetic diversity reductions occurred in several populations. Metapopulation structure appears to buffer against diversity loss; applying the indicators to metapopulations suggest mostly acceptable rates of change in all but one system. The CBD indicators agreed with the Swedish ones but provided less detail. All these indicators are appropriate for managers to initiate monitoring of genetic biodiversity.

KEYWORDS
cryptic sympatry, hidden biodiversity, intraspecific biodiversity, protected area, sympatric populations

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 (Allendorf & Ryman, 2002; Hellmair & Kinziger, 2014; Spielman et al., 2004).

International policy, including the UN Convention on Biological Diversity (CBD; www.cbd.int), identifies intraspecific diversity (=genetic diversity within species) as one of the three pillars of biodiversity, also including species and ecosystem diversity, 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 (Bruford et al., 2017; Hoban et al., 2013; Laikre et al., 2010).

The CBD Strategic Plan for 2011–2020 had a goal to safeguard genetic diversity (www.cbd.int/sp; Goal C). The target associated with this goal focusses on cultivated species, their wild relatives and socioeconomically important species. The main indicator to monitor progress towards this target follows the 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; Hoban et al., 2020, 2022; Hoban, Paz-Vinas, et al., 2021; Laikre et al., 2020). The three pragmatic indicators for genetic diversity proposed for global use include (1) the proportion of populations within species with an effective population size $Ne \geq 500$, (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 (Hoban et al., 2020; Laikre et al., 2020). Several countries are starting to apply these indicators (Thurfjell et al., 2022; Drs. Jessica da Silva, Alicia Mastretta-Yanes personal communication).

Also, several countries have moved forward with respect to monitoring genetic diversity using DNA-based techniques (i.e., applying Indicator 3 of Hoban et al., 2020; Laikre et al., 2020). Countries in the forefront include Switzerland where five key species were recently identified for an ambitious pilot project involving a stratified random sampling over species ranges and using whole genome resequencing (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., 2021a, 2021b) 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). These indicators measure change in genetic diversity within populations (indicator called ΔH), between populations (indicator ΔF_{ST}), and the effective population size (indicator N_e). Here, we present and apply these indicators for the first time.

Specifically, we map and monitor genetic diversity within and between genetically distinct populations over time using brown trout (*Salmo trutta*) in alpine lake systems in protected areas in central Sweden as a model. This species carries a key ecological role in these waters where it is a top predator and often the only fish species; its cultural and socio- value is also high (Frank et al., 2011; Marco-Rius et al., 2013). 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 (Andersson, Jansson, et al., 2017; Ryman et al., 1979; Saha et al., 2022) 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).

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

The study area is located in the mountainous range of Jämtland 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 five 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 Ångermanälven or River Indalsälven, 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 Jämtland County Administrative Board at two points in time, the 1970–80s and 2010s reflecting 5–6 generations in the brown trout in these areas (generation time c. 6.8 years; Charlier et al., 2012; Palmé et al., 2013; Table S2a), 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, 1981, 1983; Ryman et al., 1979; Ryman & Ståhl, 1980), and we included $n = 1263$ 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 Sámi communities. One of the study areas (lake system of Hotagen 4; Figure 1) is part of a long-term genetic monitoring research effort that we (N.R.,

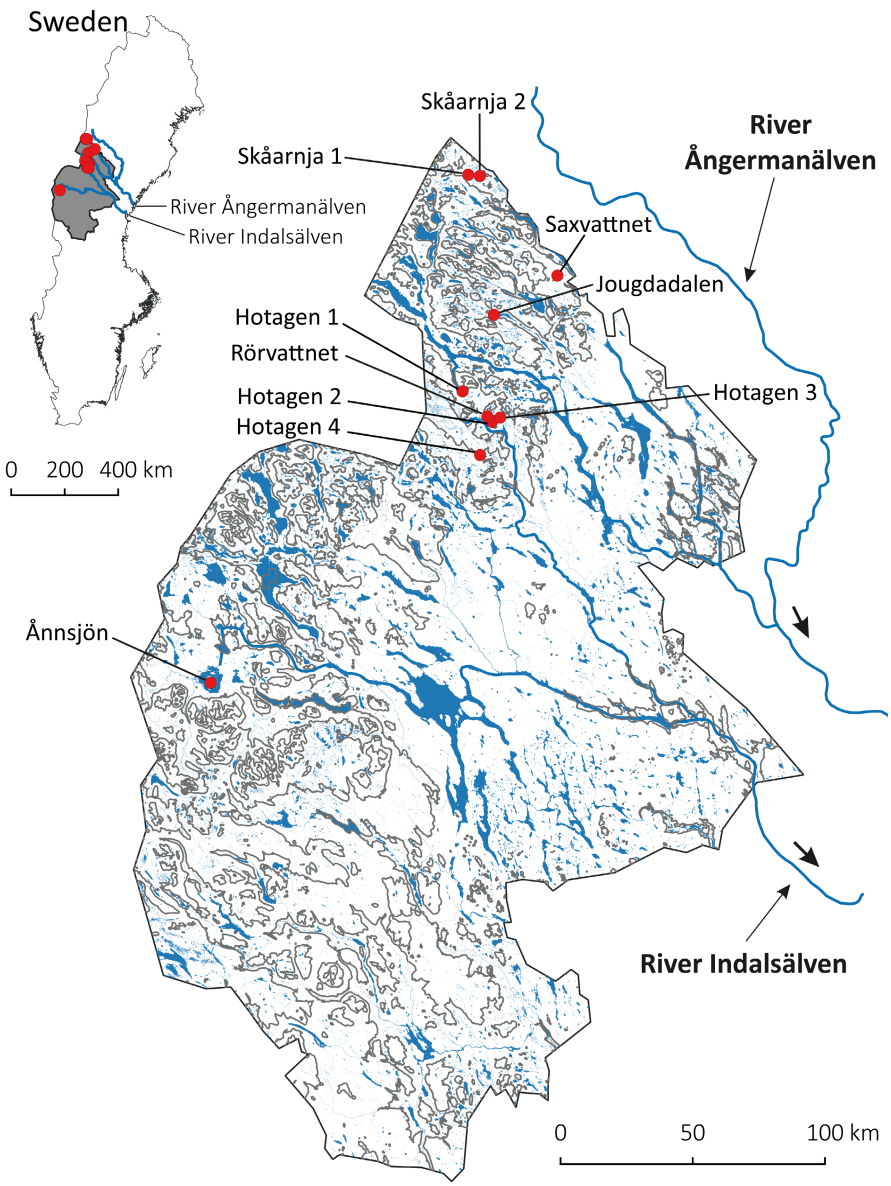

FIGURE 1 Map of the study area. Red dots indicate the locations of the seven sampled metapopulations and three single lakes (compare with Table S1). Arrows next to the rivers indicate the direction of water flow.

L.L.) have run since the 1970s (Andersson, Johansson, et al., 2017; Charlier et al., 2012; Jorde & Ryman, 1996; Laikre et al., 1998; Palmé et al., 2013). Present day samples (2010s) included $n = 1319$ fish collected in 2012, 2014, 2017, and 2018. Our study design was similar to that of Kinziger et al. (2015).

2.2 | Genotyping

In total, $n = 2582$ 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 µl 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 normalized to 30–50 ng/µl.

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 3782 SNPs variable in Danish brown trout (Bekkevold et al., 2020). The 96 SNPs are distributed across the genome, with 1–3 SNPs on each of the 40 chromosomes with a minimum of >190, 000 bases between them (Saha et al., 2022, their Figure S1). We have compared patterns of diversity and divergence using these 96 SNPs versus using the 3000 SNPs that they were selected from (Andersson, 2021; Andersson, Jansson, et al., 2017) as well as using whole genome sequencing (Kurland et al., 2019; Saha et al., 2022) and find they provide consistent information.

2.3 | Population genetic analysis

Individual fish with genotype call rates below 0.7 ($n = 10$) were excluded, resulting in a total of 2572 individuals used in further analyses. After this exclusion locus call rates ranged between 0.89 and

1.0, and all 96 loci were retained. We quantified genetic diversity in several ways described below, and these measures were used for the indicators (section 2.4). We assessed the most likely number of populations (*K*) using STRUCTURE (version 2.3.4; Falush et al., 2003; Pritchard et al., 2000). 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. We analysed lakes that are interconnected to each other (metapopulations) jointly. If migration is highly unlikely or impossible due to geographic distance and/or migratory obstacles, such lakes or lake systems were analysed separately. This resulted in seven metapopulations and three separate lakes being analysed (Table S1) 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 á priori information was used. The burnin 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 (version 1.0; Wang, 2019) and STRUCTURE HARVESTER (version 0.6.94; Earl & vonHoldt, 2012). Mean individual *Q* over the 20 runs was derived from the CLUMPP software (version 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 "populations" as genetic clusters of individuals identified by STRUCTURE, and further analyses are based on these populations. We use "population" and "cluster" synonymously from here on. The same population/cluster can occur within the same lake only or in multiple lakes within the same metapopulation (but not in different metapopulations), and we also use term "subpopulation" for such populations/clusters.

To verify genetic structuring with an additional approach we used DAPC (Jombart et al., 2010) implemented in the ADEGENET package (version 2.1.5; Jombart, 2008; Jombart & Ahmed, 2011) in R (version 4.1.2; R Core Team, 2021) although a previous study has suggested that STRUCTURE is more powerful than DAPC to detect multiple populations in a sample (Jorde et al., 2018). We used the find.clusters function and inferred the most likely *K* from the Bayesian information criterion (BIC) given by this approach (a detailed description is provided in Appendix S1).

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 version 6.5 (Peakall & Smouse, 2006, 2012), allelic richness (A_R) using FSTAT (version 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 (version 7.1; StatSoft, Inc., 2005). To test for changes of the genetic diversity measures over time we performed nonparametric Wilcoxon matched pairs test as well as Student's *t* test for paired samples (heterozygosity, average number of alleles per locus, allelic richness), and χ^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 (clusters) with the temporal method (N_{eV}) using TEMPOFS (sampling plan II; Jorde & Ryman, 1996, 2007), and with the linkage disequilibrium method (N_{eLD}; Hill, 1981; Waples, 2006; Waples & Do, 2010) as implemented in NEESTIMATOR version 2.1 (Do et al., 2014). Confidence intervals for N_e were obtained from the respective software.

Variance (N_{eV}) and linkage disequilibrium (N_{eLD}) effective size are expected to differ in substructured populations (Ryman et al., 2019), and we used N_{eV} rather than N_{eLD} as an indicator (below) for two reasons. First, the results showed that N_{eV} gives the largest estimate of effective size for the majority of the populations (Table S3) and thus represents the most conservative measure for an N_e indicator. Second, N_{eV} relates more directly to subpopulation effective size than N_{eLD} because it is only marginally affected by migration, whereas N_{eLD} is strongly dependent on the migration rate (Ryman et al., 2019). N_{eV} for metapopulations were estimated by pooling genotypic data from all samples within an interconnected lake system (Figure 1; Table S1) from each of the two points in time and then applying the temporal method.

F_{ST} (Weir & Cockerham, 1984) quantifying temporal genetic heterogeneity within populations and genetic heterogeneity among subpopulations within metapopulations was obtained using GENEPOP (version 4.3; Raymond & Rousset, 1995; Rousset, 2008). CHIFISH (version 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 neighbour-joining phylogenetic tree based on sample size corrected F_{ST}, constructed in POPTREE2 (Takezaki et al., 2010).

2.4 | Indicators

In general, by mapping genetic diversity within and between populations at one point in time we can identify populations with particularly low levels of genetic diversity in relation to other conspecific ones, and/or populations that appear isolated from other populations. Mapping data can thus tell us something about the genetic status at a particular time point 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; Figure 2). We also applied the three indicators proposed for the context of the UN Convention on Biological Diversity (CBD) context by Laikre et al. (2020) and elaborated by Hoban

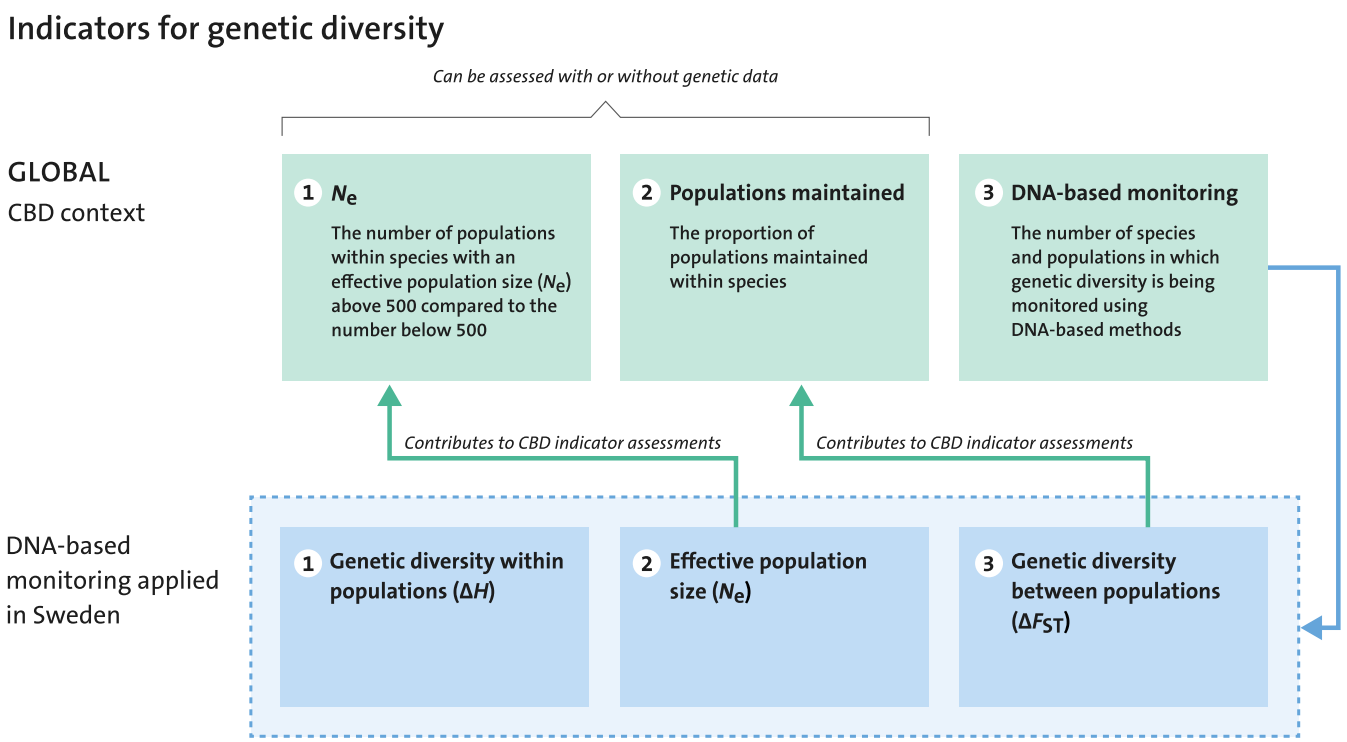

FIGURE 2 Indicators for genetic diversity proposed for the CBD "post 2020 framework" (green boxes; Laikre et al., 2020, 2021; Hoban et al., 2020; Hoban, Paz-Vinas, et al., 2021) and for national monitoring and management of genetic resources in aquatic environments in Sweden (blue boxes; Johannesson & Laikre, 2020; this study; compare with Figure 3).

et al. (2020); Hoban, Bruford, et al. (2021) 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; Hoban, Bruford, et al., 2021; Laikre et al., 2021).

The three indicators suggested by Johannesson and Laikre (2020) all represent application of proposed CBD indicator 3 (Figure 2) – that is, 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 ΔH, 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 (compare with Hoban, Paz-Vinas, et al., 2021). Indicator 3 is used to monitor between population genetic diversity. We call this indicator ΔF_{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. ΔF_{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 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, personal communication). Proposed threshold values for the ΔH 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 the indicator ΔH are: retention of c. 95% genetic variation after 100 years (reflecting less than 0.05% reduction per year, assuming a constant rate of change); this rate of reduction is suggested to reflect classification colour green for "Acceptable" (Figure 3). An expected retention of c. 75–94% variation over 100 years (reflecting a reduction between 0.06%–0.3% per year) is proposed to reflect "Warning"/yellow where further investigation of the reason for reduction is warranted. Finally, <75% of

genetic variation expected to be retained after 100 years (reduction of more than 0.3% per year) reflects "Alarm"/red alert where prompt measures are required for understanding the reason for decline, and thereafter taking steps to halt the reduction and restore genetically safe conditions.

Here, we apply indicator ΔH 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 nonparametric 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., "Acceptable", "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 ΔH indicator as green/"Acceptable". We apply the same threshold values for annual genetic reduction (i.e., ≤0.05%; 0.06%–0.3%; >0.3%; Figure 3) for all the measures of genetic diversity (H_E, 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 $N_e \geq 50$ and $N_e \geq 500$ is necessary for a population's short- and long-term survival, respectively (Franklin, 1980; Jamieson & Allendorf, 2012). The proposed thresholds are: $N_e \geq 500$ ("Acceptable"), $50 < N_e < 500$ ("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 a 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 $N_e \geq 500$ for long term viability is 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 indicator classifications on the estimate of these two that generally is the largest, in line with observation for nonisolated populations (Ryman et al., 2019). We apply this indicator to metapopulations as well as to separate subpopulations.

For the ΔF_{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) regarding threshold values, but stress that further evaluation is needed. We propose that without detectable (statistically significant) change of F_{ST} among populations over time, this indicator is classified as "Acceptable" (Figure 3). When statistically significant changes occur we propose to evaluate these as follows: 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 an increase can be expected following, for example, management activities to connect previously fragmented populations. However, decrease of F_{ST} can also be an effect of homogenization following for example, release activities. Such activities are not expected to have occurred in the present case since all monitored lakes are located in protected areas. Similarly, large scale release activities resulting in genetic homogenization have been documented in for example, 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 may have occurred. With a ΔF_{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 S2) for converting a statistically significant ΔF_{ST} into an indicator of change in genetic exchange (migration) between subpopulations. 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 observed for the studied 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 back 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). Again, we note that this is a first suggestion that will need considerable testing, evaluation, and probably, modification in the future.

3 | RESULTS

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 S4; Figure 4). A total of 29 of these 31 clusters occurred at both points in time and we refer to

Indicator	Threshold	Interpretation	Comments
Genetic diversity within populations ($\Delta H = H2 - H1$) H1: Genetic diversity at first point in time H2: Genetic diversity at second point in time	≤ 0.05% reduction per year (corresponding to ≥95% retention of H over 100 years)	Acceptable	This indicator incorporates several measures of within population diversity, such as H_E, H_O, A_R, N_A, and P_L, as well as π and F_{ROH}, when available. The same thresholds apply to all of them. Warrants a "warning" if there is a statistically significant increase in genetic diversity coupled to extra information of anthropogenic activity (with expected negative biological consequences) that is likely the cause of this change.
	0.06-0.3% reduction per year (corresponding to 75-94% retention of H over 100 years)	Warning	
	> 0.3% reduction per year (corresponding to <75% retention of H over 100 years)	Alarm	
Effective population size (N_e)	$N_e \geq 500$	Acceptable	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	
Genetic diversity between populations ($\Delta F_{ST} = F_{ST}2 - F_{ST}1$) $F_{ST}1$: Genetic differences between two or more subpopulations at first point in time $F_{ST}2$: Genetic differences between two or more subpopulations at second point in time	ΔF_{ST} no/minor change	Acceptable	This indicator particularly needs further application, evaluation, and modification.
	ΔF_{ST} reflects 25-50% reduction of genetic exchange between subpopulations, **or** 50-100% increase of genetic exchange among subpopulations	Warning	
	ΔF_{ST} reflects >50% reduction of genetic exchange among subpopulations, **or** >100% increase of genetic exchange among subpopulations **or** loss of population(s)	Alarm	

FIGURE 3 Three indicators proposed and currently applied for national use in Sweden (Johannesson & Laikre, 2020) 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; π, nucleotide diversity; F_{ROH}, fraction of genome covered by "runs of homozygosity"; the two latter ones applicable only for sequence data. See also Figure 2.

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; $\chi^2 = 6.16–47.60$; $p = .000–.046$). Results from DAPC are largely consistent with those from STRUCTURE (Appendix S1).

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 in the range $0 \leq F_{ST} < 0.03$ (Table S2a). In 15 of the 29 populations, genetic divergence between time points was significantly different from zero ($p = 0–.02$; Table S2a).

Sympatric populations (i.e., genetically divergent clusters coexisting within the same lake) were observed at both time points in 12 lakes (Figure 4; Table S4; Figure S1). These lakes were distributed across five metapopulations and the "independent" lake Ånnsjön. 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; *Pearson* $r = 0.29$.; $p = .14$), lake area (*Pearson* $r = 0.27$; $p = .18$), or average lake depth (*Pearson* $r = -0.31$; $p = .20$).

The genetic relationships among the identified populations reflect the geographic location of the lakes and water systems. Overall, the major branching of the phylogenetic tree (Figure S2) corresponds to the main river drainages (Figures 1 and 4). Lake Ånnsjön was found to harbour three subpopulations and is thus viewed as a metapopulation. The other two separate lakes (Saxvattnet and Rörvattnet) only harboured one population per lake. Thus, we had 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 above the tree-line (Skåarnja 1 and 2) as compared to those below the tree-line (Table S5, Figures 5 and S3–S7). In the above tree-line metapopulations, heterozygosities (expected as well as observed) were always below 20%, while the corresponding estimates for below tree-line areas were at or above 20% (Table S5). 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 metapopulations. 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 < .001 and .012; Table S5).

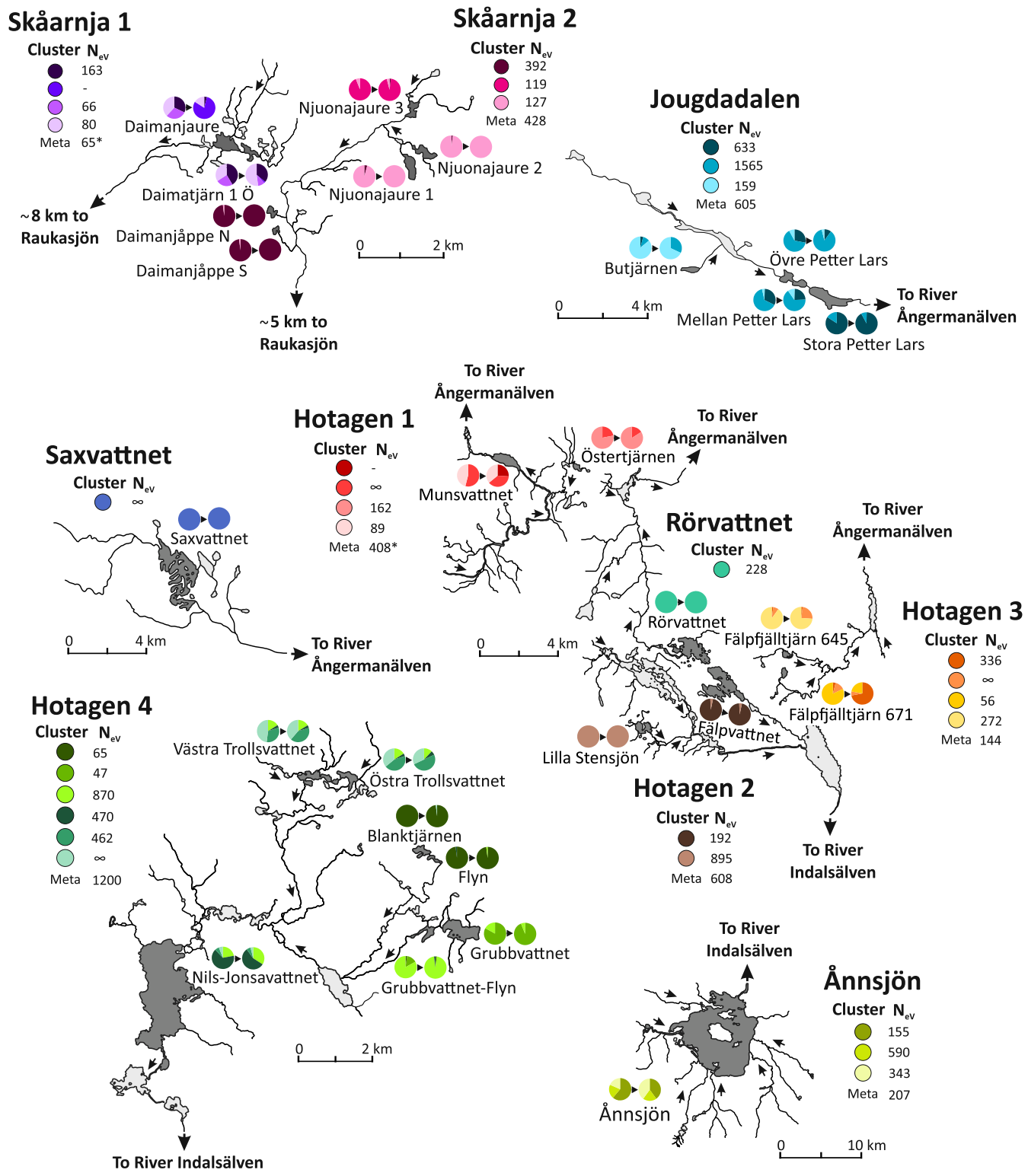

FIGURE 4 Occurrence and distribution of the 31 genetic clusters in the seven water systems and three individual lakes (Saxvattnet, Rörvattnet, Ånnsjön). Pie diagrams show the representation of populations in the past (leftmost diagram in each pair) and the present samples (rightmost diagram; detailed 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).

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 $p > .05$; Sign test; Figures 5 and S3-S5; Table S6). Most striking is the pronounced decrease of genetic diversity in

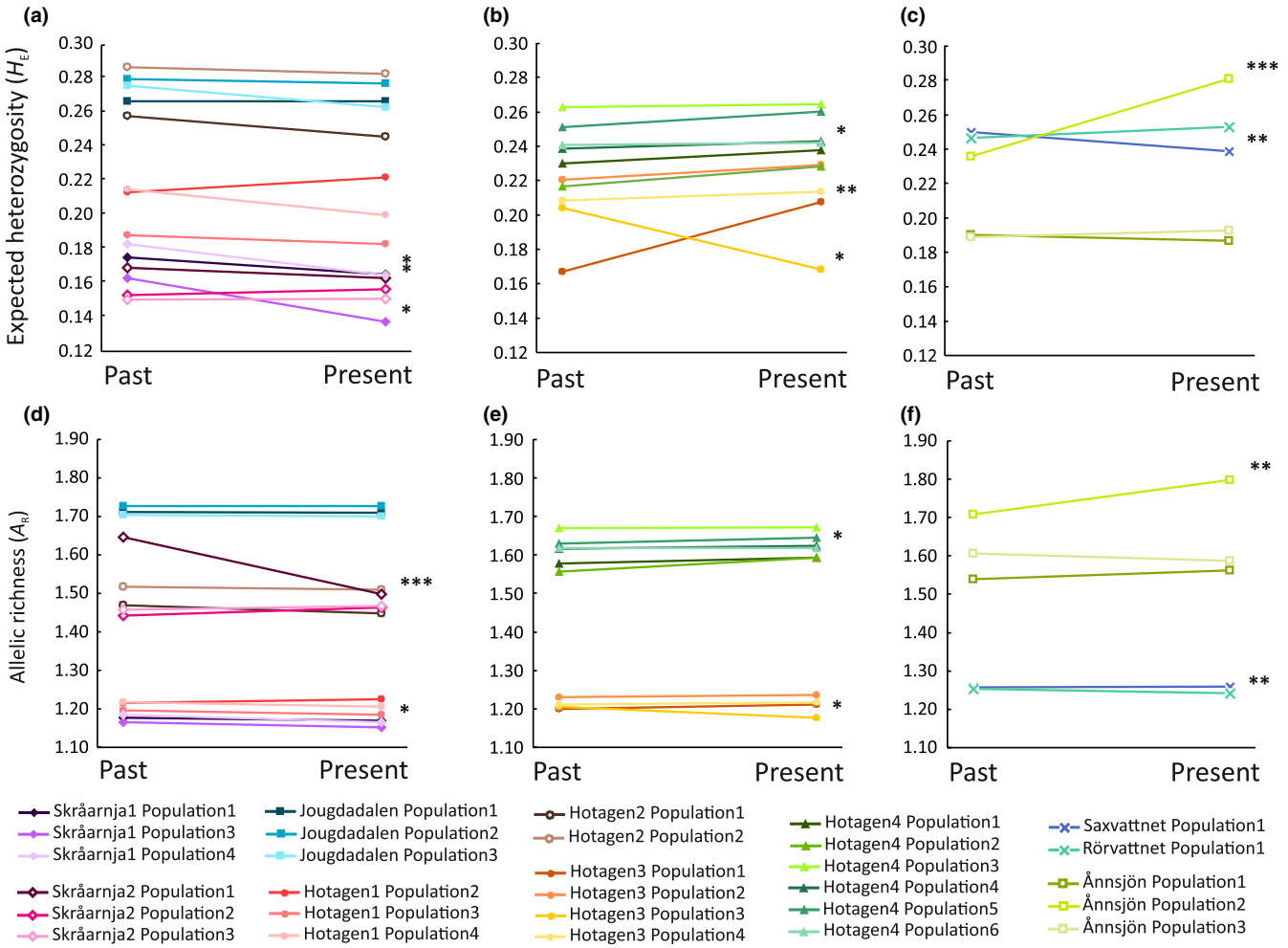

FIGURE 5 Trends over time in expected heterozygosity (H_E; a–c) and allelic richness (A_R; d–f) for populations within metapopulations. (a, d) populations in Skåarnja 1, Skåarnja 2, Jougdadalen, Hotagen 1 and Hotagen 1; (b, e) populations in Hotagen 3 and Hotagen 4; (c, f) populations in Ånnsjön, Saxvattnet, and Rörvattnet.

three out of six populations in the above tree-line metapopulations Skåarnja 1 and 2 (Figures 5, 6 and S3–S5; Table S6). The opposite trend is observed in for example metapopulation Hotagen 4 where genetic diversity is increasing in all subpopulations and significantly so in three out of seven (Figures 5 and 6).

3.3 | Genetic divergence between populations

Genetic divergence among populations measured as F_{ST} are summarized in Table 1 and Table S7. 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 (2010s) samples, and were significantly larger than zero in all lake systems at both points in time (all $p < .001$; Table 1). Among nonsympatric 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 lake systems where we have more than one population per system (Table 1). One case shows significant increase of F_{ST} (Skåarnja 2; $p = .007$; 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; $p = .013$ and .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 S3). 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 suggest (i.e., local N_{el} is expected to be larger than local N_{eV}, compare with 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

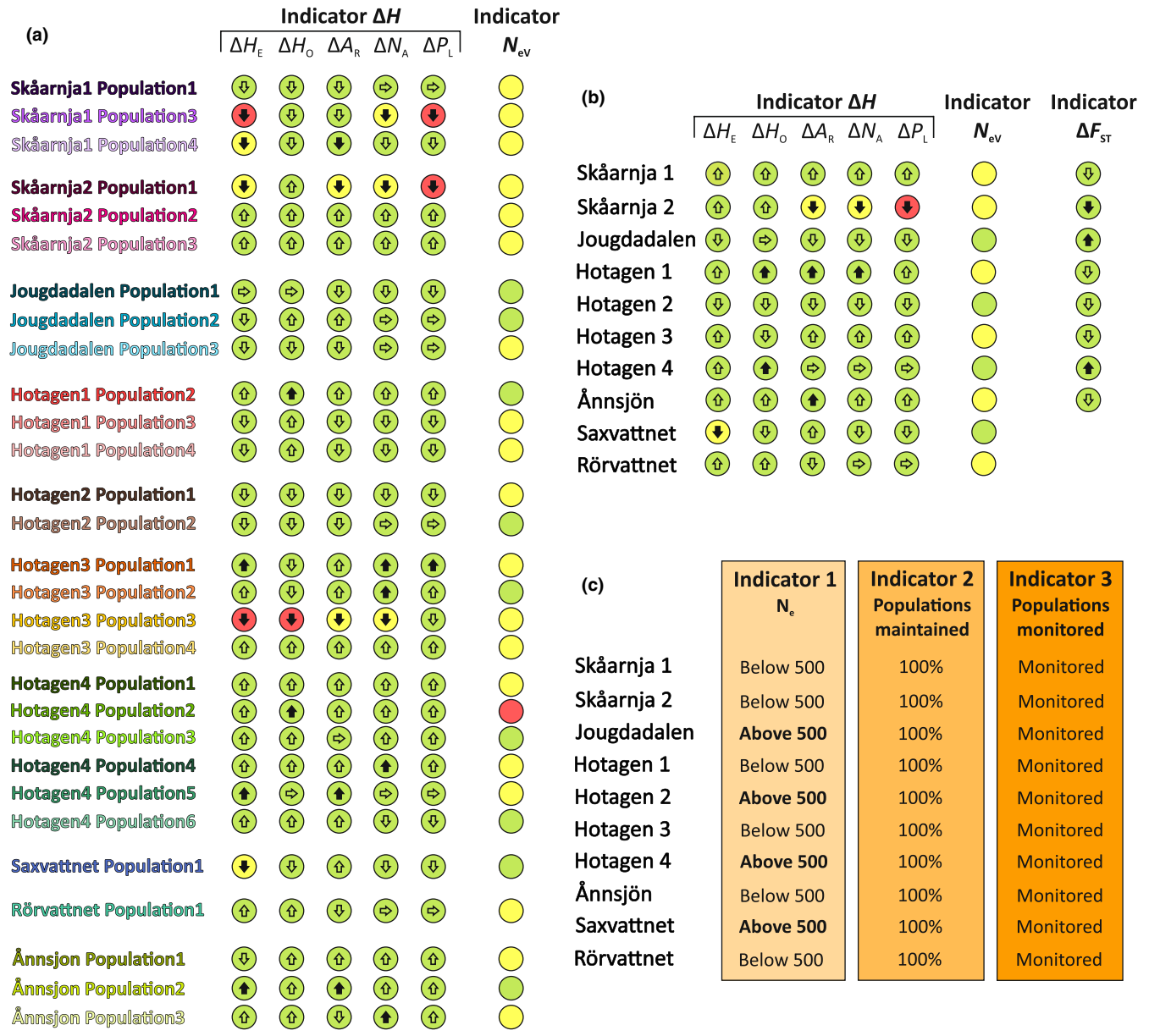

FIGURE 6 Genetic indicator classifications. (a) Swedish indicators applied to 29 genetically distinct brown trout populations/clusters, (b) 10 systems: Eight metapopulations and two single lakes, and (c) CBD indicators applied to the same 10 systems. For the Swedish indicators, the coloured circles indicate classification; green = "acceptable", yellow = "warning", and red = "alarm" (compare with Figure 3). Arrows inside circles show the direction of change, with horizontal arrows meaning apparent stability (no change); filled arrows indicate that the change is statistically significant ($p < .05$). For ΔF_{ST} arrows indicate increase or decrease in connectivity. The ΔF_{ST} indicator was not applicable to 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 harbour one population.

three metapopulations and in one of the single lakes N_{eV} was above 500 (Figure 4; Table S3).

There was a significant positive correlation between N_{eV} and expected heterozygosity (H_E) ($r = 0.48$ and $p = .008$ for present estimates; Figure 7a). 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 ($r = 0.39$; $p = .16$; Figure 7b).

N_e estimates based on the linkage disequilibrium method (N_{eLD}) where consistently lower than estimates based on the temporal method (N_{eV}) (Table S3). 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 ($p = .048$; 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 S3).

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 (ΔF_{ST}) with associated significance levels. Significance levels for differences among populations were obtained from GENEPOP, while *p*-values for ΔF_{ST} are from the Wilcoxon matched pairs test; Significant *p*-values are in bold. Lakes Saxvattnet and Rörvattnet are not included here because each of these lakes only harbours one population

Metapopulation/lake	F_{ST} among populations (past)	*p* (F_{ST} past)	F_{ST} among populations (present)	*p* (F_{ST} present)	ΔF_{ST}	*p* (ΔF_{ST})
Skåarnja 1	0.063	**<0.001**	0.081	**<0.001**	0.018	0.213
Skåarnja 2	0.159	**<0.001**	0.180	**<0.001**	0.021	**0.007**
Jougdadalen	0.054	**<0.001**	0.042	**<0.001**	−0.011	**0.013**
Hotagen 1	0.071	**<0.001**	0.077	**<0.001**	0.006	0.413
Hotagen 2	0.071	**<0.001**	0.080	**<0.001**	0.010	0.241
Hotagen 3	0.092	**<0.001**	0.094	**<0.001**	0.002	0.705
Hotagen 4	0.103	**<0.001**	0.080	**<0.001**	−0.023	**0.006**
Ånnsjön	0.068	**<0.001**	0.069	**<0.001**	0.000	0.347

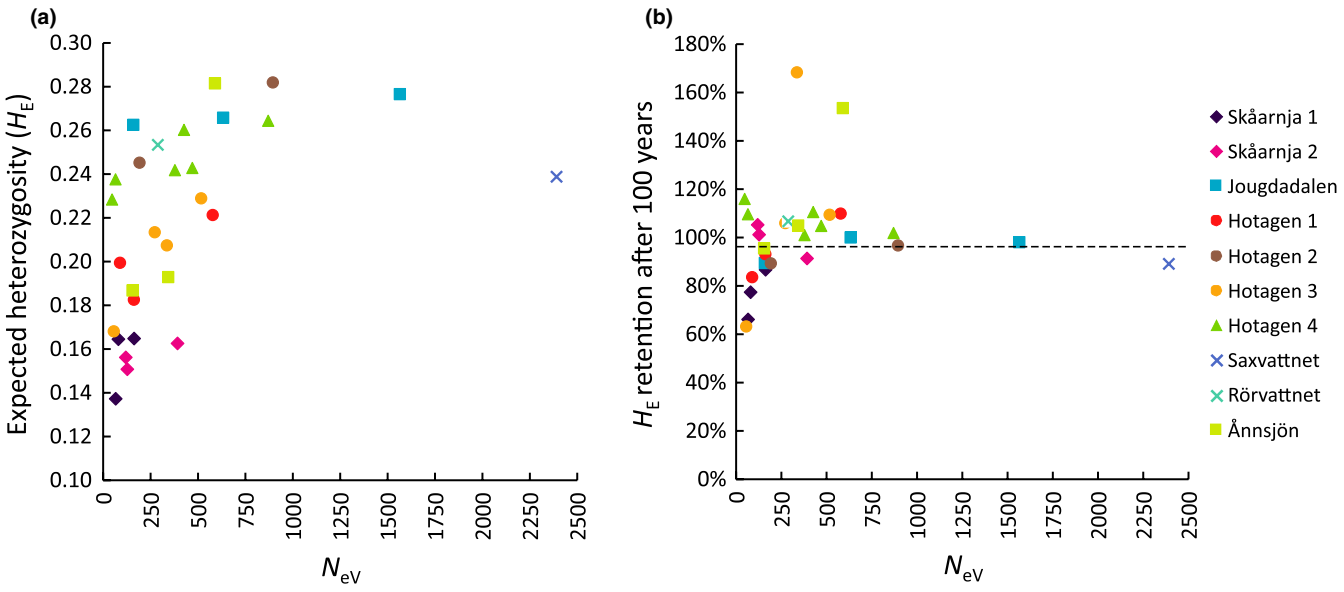

FIGURE 7 Correlation between estimated effective population size (N_{eV}) and (a) genetic diversity measured as expected heterozygosity (H_E) in the present, as well as (b) predicted retention of H_E over 100 years. Each point in the graph represents one of the 29 populations occurring at both points in time, classified into the seven metapopulations and the three single lakes monitored. Dashed line marks 95% retention after 100 years.

3.5 | Indicators

Indicator classifications are shown in Figure 6. In summary, the ΔH indicator detects reduction of genetic diversity within several populations (Figure 6a), but metapopulation systems buffer against some of these trends so that when metapopulations are considered – increase and decrease within separate subpopulations levels out (Figure 6b). This is not the case in all lake systems however, and the N_e-indicator provides warning signals of low effective population sizes in 6 out of 10 systems studied (Figure 6b). The ΔF_{ST} indicator shows that gene flow is significantly decreasing in the same metapopulation (Skåarnja 2) where we also observe ΔH 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, that is, the number of migrants between subpopulation is estimated to have been reduced by less than 25% over the monitoring period (Figure 6b).

3.5.1 | ΔH indicator

Applying the ΔH indicator to the 29 separate populations, we observe that five populations in four different lake systems exhibit statistically significant decrease of diversity measures at a rate higher than the 0.05% decrease per year limit for this indicator (Figure 6a). These populations show reductions between 0.06 and 0.83% per year (corresponding to loss of 6%–56% in 100 years) in several diversity measures, respectively (Table S2a). 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 75%–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 "Acceptable" for all five diversity measures, and in those, statistically significant increase of genetic diversity is observed in eight populations representing four lake systems (Figure 6a).

When applying the indicators to metapopulations, all but two were predicted to retain more than 95% of genetic diversity after 100 years in all five measures and were classified as "Acceptable". 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 6). 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

One population (Population 2 in Hotagen 4) was classified as "Alarm" ($N_{eV} < 50$) due to the low N_{eV} of 46 (Figure 6a). A total of 19 populations distributed over four metapopulations were classified as "Warning" ($N_{eV} = 56–470$; Table S3, Figure 6a), and nine populations had an effective population size (N_{eV}) of > 500 and were classified as "Acceptable". For the 10 lake systems (eight metapopulations and the two independent lakes) six were below 500 and thus showed "Warning" ($N_{eV} = 65–428$), while four displayed $N_{eV} \geq 500$ (605–∞) and thus classification "Acceptable" (Figure 6b).

3.5.3 | ΔF_{ST} indicator

A statistically significant increase of F_{ST} was observed in one metapopulation (Skåarnja 2), and significant decrease of F_{ST} in two metapopulations (Jougdadalen and Hotagen 4; Table 1; Table S2b). However, when translating these changes to reflect changes in gene flow among subpopulations none of these three cases exceeded the proposed limiting values for the ΔF_{ST} indicator and they are thus classified as "Acceptable" (Figure 6b). Skåarnja 2 exhibited an initial F_{ST} of 0.159 (in the 1970–80s sample); a 25% decrease in gene flow corresponds to a limiting upper value for F_{ST} in the present sample of 0.202. However, the observed present (2010s) F_{ST} was $F_{ST} = 0.180$ (corresponding to a 14% decrease in gene flow) and thus below this threshold value. In Jougdadalen and Hotagen 4 F_{ST} has decreased significantly, indicating that gene flow has increased in these lake systems. In Jogdadalen, 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 "Acceptable" (Figure 6b).

3.5.4 | The CBD indicators

The indicators proposed for the CBD framework (Hoban et al., 2020; Laikre et al., 2020) showed that for indicator 1, four metapopulations had an N_e above 500, and six below 500 (Figure 6c). For indicator 2 we note that all populations were maintained over time so there is a 100% retention of populations. With respect to indicator 3, we use genotypic data in all ten lake 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 10 geographically separate water systems in central Sweden. We used genetic data to identify genetically distinct populations and detected 29 such populations that were stable over time. At least 12 of the lakes harboured more than one population, and some populations/clusters occurred in more than one lake.

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. Thus, this study represents a case of monitoring genetic diversity in a natural system protected from severe anthropogenic changes, such as habitat destruction and fish stocking. While we found no general trend with respect to decrease or increase of genetic diversity among the 29 populations of the 10 water systems, we did observe considerably lower levels of genetic diversity in the two above-tree-line lake 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 (ΔH indicator).

In contrast, increased genetic diversity was observed in several separate populations as well as in three metapopulations (Figure 6). 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 confidence intervals for N_e estimation is sometimes large (Table S3). For more exact estimates sample sizes need to increase (number of loci and/or individuals). The between population indicator ΔF_{ST} showed significant increase of divergence, potentially suggesting reduced gene flow in the metapopulation where the ΔH and

N_e indicators gave warning signals (Skåarnja 2; Figure 6b), although the change was within the proposed limiting values. In two lake systems ΔF_{ST} showed a significant reduction, potentially indicating increased gene flow, but here too within the proposed limiting values (compare with Figure 3). Applying the CBD indicators to the present data provides conclusions consistent with the national indicators but with less detail (Figure 6c).

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., 2018). The present results suggest that the occurrence of cryptic sympatry is a common phenomenon in these alpine lake systems. We detected such structures in over 40% 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, Jansson, et al., 2017; 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 treated as two separate lake 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., Ferguson et al., 2019; Östergren & Nilsson, 2012). Ideally, sampling should also occur at spawning grounds and during the reproductive period, in order to understand the underlying reproductive barriers in time and space. In practice this is difficult in these water 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 water 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 found in the 2010s samples but absent 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 lake systems. We also found that the relative frequencies at which populations were observed in the samples fluctuated over time (Table S2a), most likely as an effect of our restricted sample size n = 50 per lake and point in time.

The finding of the "new" populations in the present sample (2010s) that were not seen in the past (1970–80) complicate inference on the genetic diversity change of the two metapopulations involved. In metapopulation Skåarnja 1, for example, we observed a decrease or no change in diversity measures within 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 6a–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 that 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). Including the new cluster results in a larger allele frequency change within the metapopulation over time, which causes the N_{eV} estimate to decrease. 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 metapopulation estimates 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

There is no apparent correlation between expected retention of heterozygosity over 100 years and N_e (Figure 7b). 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 lake 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, that is, that populations with high N_e tend to exhibit a higher degree of retention of H_E (Figure 7b).

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 - Calò 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. We do not know if these fluctuations represent a "natural state" or if they are a result of anthropogenic activities such as poaching and/or environmental change. Clearly, we would need to continue monitoring these water systems in coming decades to see if such fluctuations of genetic diversity within and between subpopulations are random within metapopulations, and if they vary among populations over time. The large effort in this study for monitoring genetic diversity in protected areas will make an important reference point when expanding the monitoring of nonprotected areas to understand the effect of large anthropogenic changes on genetic diversity.

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 ~0.5–2.5 individuals per generation (assuming an island model of migration). These findings suggest that the metapopulation structure buffers the lake systems against loss of genetic diversity and that protected areas need to be large enough to support a large meta-N_e (compare with Gompert et al., 2021; Jorde & Ryman, 1996).

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 6a). 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 systems show warning signals for the ΔH indicator (Figure 6b). 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 water systems because we have likely not sampled the full metapopulations in any of the present cases. Sampling over substantially larger areas is needed to resolve this issue. On the other hand, the generally low N_e estimates observed underlines the vulnerability of these lake systems – if they become fragmented and isolated, local N_e will be low, and reduced connectivity will rapidly result in elevated rates of diversity loss. Protecting large, interconnected water systems are thus important for the conservation and viability of fish in small mountain lake systems.

We also apply the indicators for CBD (Figure 6c), and in the present case the results are largely consistent with the results from the national (SwAM) indicators. When applying the CBD indicator 2 (proportion of populations remaining) we use the genetic populations/clusters that we have identified. All of them remain. In absence of genetic data this indicator would identify population on the basis of, for example, geographic location. In this case, occurrence of brown trout in each of the sampled lakes. If we had used the indicator that way, we would also find that all populations remain since the species has not disappeared from any of the lakes in this study.

We propose that all these indicators are appropriate and ready to be applied, typically in cooperation between managers and scientists, to implement conservation goals for genetic biodiversity although continuous testing, evaluation, revision and improvement is warranted. An important aspect is data storage and availability. If tissue and genotypic data is stored and made available, future improvement of indicators can be applied to already available data and/or material. SwAM is planning for such availability (Maria Jansson, SwAm, personal communication).

4.7 | Further developments of indicators

For the indicator ΔH, 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 – for example, a reduction of 5% over 100 years – can be statistically challenging because of the minor change expected over one or a few years even for species with a relatively short generation interval. 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 change of genetic diversity from typically used sets of genetic 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 of wild populations 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 water system, we can use N_{eV} to get a good estimate of inbreeding N_{el} (Hössjer & Tyvand, 2020). However, if we fail in identifying the full metapopulation, estimates of N_{eV} will underestimate the N_{el} 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 ΔF_{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. We are aware of the limitations of this approach, as pointed out by for example, Whitlock and McCauley (1999), but nevertheless suggest it as a first step in developing an indicator relating to among-population genetic variation. Clearly, important future work includes investigating the effects of violating the assumption used in this indicator. The threshold values used here are also highly subjective, and it is unclear if they are sufficient to detect biologically important changes of connectivity. Thus, extended work including empirical application to species with different population structuring, ecology, etc. is needed. The national indicators are now being applied to more species in Sweden including herring, cod, salmon, and eelgrass (Maria Jansson, SwAM, personal communication) and this study will provide more empirical information that can aid in modifying and improving the indicators and threshold values applied for them.

AUTHOR CONTRIBUTIONS

Anastasia Andersson, Nils Ryman, and Linda Laikre designed the study and led the collection of present day samples. Nils Ryman 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 Nils Ryman and Linda Laikre at the Department of Zoology, Stockholm University. Sten Karlsson led the laboratory analyses. Anastasia Andersson performed population genetics analyses, provided the first draft of the manuscript, and led the writing with all authors contributing to the final manuscript.

ACKNOWLEDGEMENTS

We acknowledge the comments and suggestions provided by the Subject Editor Dr Andrew Kinziger and four anonymous reviewers. The indicators for national use in Sweden were elaborated in a science-management joint effort supported by the Swedish Agency for Marine and Water Management (SwAM). We are grateful to Kerstin Johannesson at Gothenburg University and to Maria Jansson and Anna Hasslow who initiated (AH, MJ) and led (MJ) the efforts at SwAM. We also thank Elisabeth Sahlsten, Gry Sagebakken, Fredrik Ljunghager, Kristina Samuelsson, Norbert Häubner, Ulrika Stensdotter Blomberg and several other managers at SwAM for valuable discussions and suggestions. We thank Maria de la Paz Celorio Mancera and Diana Posledovich for laboratory assistance. We thank Ida Pernille Øystese Andersskog, Hege Brandsegg, Line Birkeland Eriksen, and Merethe Hagen Spets at the genetic laboratory at NINA for SNP-genotyping. Diana Posledovich is also acknowledged for help with illustrations as is Jerker Lokrantz and Elsa Wikander at Azote (Figure 2), and Rolf Gustavsson for analytical discussions. We are grateful for extensive help with sample collection and thank the following for field work assistance: Kurt Morin, Fredrik Andersson, Kristoffer Andersson, Olle Eriksson, Thomas Giegold, Rolf Gustafsson, Sara Kurland, Diana Posledovich, Gunnar Ståhl, Karin Tahvanainen, Jan Oscarsson and Sportfiskeklubben D30, Randi Olofsson-Lund and Håkan Lund (Almdalen Fjällgård), Tina Hedlund (Aquanord AB), the Sámi communities Jijnjevaerie, Voernese, Ohredahke, and Raedtievaerie, Sven Ringvall, Nils Hallberg, and Jan-Olof Andersson at Jämtland Läns Sportfiskeklubb, Jens Andersson, Ingemar Näslund, Mattias Lindell, and the County Administrative Board of Jämtland. We acknowledge support from the Swedish Research Council Formas (L.L.), the Swedish Research Council (L.L., N.R.) the Swedish Agency for Marine and Water Management (L.L.), the Carl Trygger Foundation (L.L.), and the Erik Philip-Sörensen Foundation (L.L.).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are provided in Supporting Information, including all measurements used for the indicators (Tables S2, S3a,b, and S6). Additional data are available in Dryad at https://doi.org/10.5061/dryad.fbg79cnx5. There is a two-year embargo on the individual genotype data.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Andersson, A., Karlsson, S., Ryman, N., & Laikre, L. (2022). Monitoring genetic diversity with new indicators applied to an alpine freshwater top predator. *Molecular Ecology*, *31*, 6422–6439. https://doi.org/10.1111/mec.16710