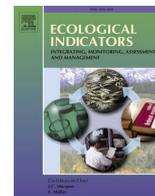


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Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Practical application of indicators for genetic diversity in CBD post-2020 global biodiversity framework implementation

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ARTICLE INFO

Keywords:

Monitoring
Headline indicator
Conservation genetics
Conservation policy
Maintaining genetic diversity
Biodiversity

ABSTRACT

Genetic diversity is a key aspect of biological variation for the adaptability and survival of populations of species and must be monitored to assure maintenance. We used data from the Swedish Red List 2020 and from published reviews to apply three indicators for genetic diversity proposed for the post-2020 Global Biodiversity Framework of the Convention on Biological Diversity (CBD). We studied a wide range of taxonomic groups, and made more detailed indicator assessments for mammals and herptiles.

For indicator 1, the proportion of populations with effective population size $N_e > 500$, 33% of 22,557 investigated species had a population size estimate that could be used as a proxy for N_e . For herptiles and mammals, 70% and 49% of populations of species, respectively, likely had $N_e > 500$.

Data for evaluation of indicator 2, the proportion of remaining populations or historical range, was available for 20% of all species evaluated for the Red List. Meanwhile, 32% of the herptile and 84% of the mammal populations are maintaining their populations and range.

For indicator 3, the number of species or populations in which genetic diversity is monitored using DNA-based methods, there are genetic studies on 3% of all species, and 0.3% are being monitored genetically. In contrast, 68% of mammals and 29% of herptiles are studied using DNA, and 8% of mammals and 24% of herptiles are genetically monitored.

We conclude that the Red List provides data that are suitable for evaluating the genetic indicators, but the data quality can be improved. We also show that the genetic indicators capture conservation issues of genetic erosion that the Red List misses. There is a synergy in estimating the genetic indicators in parallel with the Red Listing process. We propose that indicator values could be included in national Red Listing as a new category - "genetically threatened", based on the genetic indicators.

1. Introduction

The Convention on Biological Diversity (CBD; <https://www.cbd.int>) has, from its ratification in 1993, identified genetic diversity - biological variation within species - as one of three pillars of biodiversity. However, implementing the CBD with respect to genetic diversity has long lagged behind the other biodiversity pillars of species and ecosystems, particularly for wild species (Laikre et al., 2010). The initial draft from the CBD for a post-2020 biodiversity framework also largely neglected genetic diversity (CBD/WG2020/2/3 January 2020). In a letter to Science, Laikre et al. (2020) argues for all species to be genetically

monitored in order to maintain their genetic diversity and evolutionary potential. Hoban et al. (2020) develops this framework, and defines three genetic indicators, suitable for CBD reporting: 1) the number of populations with effective population size (N_e) above versus below 500, 2) The proportion of populations (or geographic range) maintained within species, 3) the number of species and populations in which genetic diversity is monitored using DNA-based methods (Hoban et al. 2020). Further elaboration of these proposed indicators have been provided in subsequent work, including the suggestion to use the census population size, $N_c > 5000$ as a proxy for $N_e > 500$ when the N_e/N_c ratio is not known for the focal species. In other words, to assume an

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<https://doi.org/10.1016/j.ecolind.2022.109167>

Received 5 April 2022; Received in revised form 9 July 2022; Accepted 11 July 2022

Available online 16 July 2022

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Ne/Nc ratio of 0.1 (Hoban et al., 2021a; Hoban et al., 2021b; Hoban et al., 2021c; Laikre et al., 2021). This may for many species be a conservative approach (Waples et al., 2013). Currently, the Ne-indicator is proposed as a Headline Indicator (an indicator all countries must report in their National Reports) in the post-2020 CBD Global Biodiversity Framework. The proportion of populations maintained-indicator is proposed as a Component Indicator (an optional but recommended indicator) and the DNA-based indicator is also suggested to be included (Convention on Biological Diversity, 2021). Conservation genetics researchers such as the newly formed Coalition for Conservation Genetics (<https://www.coalitionforconservationgenetics.org/>; Kershaw et al., 2022) are arguing for making the proportions of remaining populations indicator a Headline Indicator as well, and the DNA-based indicator a Component Indicator (Kershaw et al., 2022).

Several attempts are now being made to apply these indicators; in countries such as South Africa, Mexico, Japan, and Belgium indicators 1 and 2 are being tested in pilot work (Jessica da Silva, Alicia Mastretta-Yanes, Fumiko Ishihama, Joachim Mergeay, pers.comm.). In Switzerland and Sweden, indicator 3 is being elaborated (Fischer and Litsos, 2022; Johannesson and Laikre, 2020; Andersson et al., 2021). Through a review, the EU COST Action G-BiKE (g-bikegenetics.eu) is also developing a standardized summary for indicator 3 (Michael W. Bruford, pers. comm.). The main point of these studies is to assess genetic diversity in a more standardized way, monitor its change over time, and use the outcomes in prioritization and decision making for sustainable management and conservation. In a world where human induced climate change and ecological footprint are substantial (IPCC, 2014; Lin et al., 2018), strong pressures are induced on populations of species to rapidly adapt, genetic diversity within and among populations (reflected in indicator 1 and 2, respectively) is vital to support such adaptation, resilience and survival. Without relevant monitoring, it is impossible to assess the status of genetic diversity. The point of using Sweden as an example is to test the method for feasibility at a country level, for a country with well-developed biodiversity monitoring facilities. The idea was to find strengths and weaknesses using a dataset that is easily available, so the task could be achieved in a relatively short time frame and feed into the ongoing CBD related work to develop a post-2020 biodiversity monitoring framework.

Here, we report on our attempt to assess if national Red List data in Sweden include sufficient information for applying the indicators 1 and 2 (the Ne-indicator and the proportion of remaining populations-indicator), using already collected data on 22,571 species, subspecies and populations (henceforth referred to as species unless otherwise specified) gathered for the Swedish Red List classification (Ahrné et al., 2020). Species classified to the categories, Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR) or Regionally Extinct (RE)] were used to find out if, and for how many of these species sufficient data is available for assessing proposed CBD indicators for genetic diversity (Hoban et al., 2020). Species that were classified as Data Deficient (DD), Not Evaluated (NE) or Not Applicable (NA), were excluded. Note that DD species are typically rare, and often it is not even certain they are reproducing consistently within Sweden.

The data from the Red Listing was chosen as it is the most complete dataset we had access to, where a large amount of species has been evaluated thoroughly and consistently. The Red List is a globally recognized, standard, and rigorous inventory of conservation status of plant and animal species, using quantitative criteria to evaluate the extinction risk of species. The Red Listing is following strict guidelines (IUCN, 2012), and the results are reproducible given the same data-sources. For species that are less well known, there is more uncertainty in the results, as important data is often lacking. This uncertainty is visible through the variation in classification (a species may be classified as EN, at worst CR and at least VU to show variation). Red List assessments often contain an estimate of the global (or in this case national) census size of a species, and sometimes also contain census size of

individual populations or subspecies within species, and either number of, or trend in, populations or geographic range. The aim of this study was to investigate if national Red List data can be used for assessing and reporting on genetic indicators, and to quantify the number of species that can be assessed in this way for different taxonomic groups.

Both indicator 1 and 2 can be calculated and reported in the absence of genetic data (Hoban et al., 2020; Hoban et al., 2021a; Laikre et al., 2021). Hoban et al (2020) and Laikre et al (2020) suggested that numerous data sources could have information for reporting on these indicators including species' recovery plans or the Red List. The Red Listing in Sweden is conducted by committees of experts on specific organism groups, each headed by a chair employed at the Swedish Species Information Centre (<https://www.artdatabanken.se/en/>). The process follows an overarching standard but varies depending on the organism group, and how much information is available. Sweden has long data series on landscape and forest composition, enabling a data-driven process for habitat variables for many species (see Artfakta, 2022 and references therein). For more well-known groups such as vertebrates, most fields that can be estimated are filled, while for many invertebrate groups, plants and fungi, a short-list of likely candidates for Red Listing is made, and the focus is to estimate relevant values for those. The species not making the short-list are either common, or there is limited knowledge on habitat preferences. If there is no knowledge at all, the species can be classified as Data Deficient, DD. The Swedish Red List has been updated every fifth year since 2000.

We specifically addressed the proportion of the 22,557 species where data on population(s)' size and geographic range within species is available, whether monitoring of genetic diversity is or has been carried out, and how the data availability differs among species groups. We assessed indicator 3 based on data from already published reviews of studies on mapping and monitoring of genetic diversity in Sweden (Laikre et al., 2008; Posledovich et al., 2021).

We selected three species groups from the Swedish Red List for which more detailed information is available for an in-depth analysis, and calculated indicators 1, 2 and 3 for species in these groups. The species reproduction is sexual and they were (mostly) terrestrial. These groups were mammals, amphibians and reptiles; amphibians and reptiles were combined into "herptiles" in the analysis (Ahlen et al., 2020).

2. Materials and methods

2.1. Available data from the Red List

In the Red List, only species that were present within the country in the year 1800 are assessed. Species that have naturally immigrated to Sweden are included if they can be regarded as established, which is classified to have occurred after 10 consecutive years of reproduction. Further, in a few cases known distinct, isolated subpopulations within species are Red Listed separately (IUCN, 2012). In this Swedish example, such listing applies to the porpoise (*Phocoena phocoena*, L.), harbour seal (*Phoca vitulina*, L.), red deer (*Cervus elaphus*, L.) and European grass snake (*Natrix natrix*, L.), that have distinct subpopulations which are genetically separated from the other populations (Ahrné et al., 2020) and these populations are assessed separately.

The following information that could be used for calculating the suggested genetic indicators is gathered per assessed species in the Red Listing process (IUCN, 2012): distribution trends and size of the total population, area of occupancy (AOO, number of 2*2km grid cells occupied by the species), extent of occurrence (EOO, the minimum convex polygon or α -hull of species occurrences), degree of population fragmentation, size of the largest sub-population, and if there is a negative trend in the number of localities or sub-populations. The sources vary with species and the specific source is referenced in Artfakta (Artfakta, 2022). The most important sources of distribution data are the Species Observation System (<https://www.artportalen.se>), which contains both citizen science data and data from most

environmental inventories in Sweden. Data on trends is sparser, but for mammals and herptiles, many of the rare species are monitored in different programs as referenced in Artfakta (Artfakta, 2022). The whole assessment is documented in each edition of the Red List (Ahrné et al., 2020). An example with high quality data is the lynx (*Lynx lynx*, L.). A mix of citizen science data and formal inventories are used to determine the range of the lynx. Tracking and camera-trap data is used to estimate the population size based on the number of family groups, consecutive estimates of population size can be used to determine the trend (Ahrné et al., 2020). The polecat (*Mustela putorius*, L.) is an example of a less known mammal. The range is certain from citizen science data and hunting bag reports, while the population trend is more uncertain. The harvest data might be biased, and suggests there is a decrease, the historical range estimates suggest a recent range expansion, with the conclusion that the trend over the last decades is most likely relatively stable, but with large uncertainty (Thurffjell and Tomasson, 2017). The quality of the estimates varies greatly from best guesses of experts to very accurate monitoring data, but is generally best among well-studied groups such as vertebrates and plants.

2.2. Indicator 1: The proportion of populations within species with $N_e > 500$.

To calculate indicator 1, and its data availability, we used the proposed proxy of $N_e/N_c = 0.1$ and thus assuming that with an $N_c > 5000$ the target of $N_e > 500$ is reached. We classified all species with a value for total national population size estimate, size of largest subpopulation, or a classification for severely fragmented or Red Listed as RE, as having data. If any of these population estimates (national species census estimates or the size for the largest sub-population) were < 5000 , or if the species was classified as severely fragmented, or if it was Red Listed as RE, we classified it as having an $N_c < 5000$ individuals and thus an $N_e < 500$.

In the more detailed analysis of mammals and herptiles, we analyzed data at the species level (e.g. we merged subspecies or subpopulations of the same species). The best population estimate (in contrast to the minimum or maximum estimates) was used to assess whether or not the species' total population size in Sweden was > 5000 individuals. We assigned all species with a total population < 5000 or Red Listed as RE and extinct in the last 100 years as 0% reaching the target $N_e > 500$, as no subpopulation can be larger than the total population. One species that went extinct in Sweden > 100 years ago was excluded from analysis (the wild reindeer). For species with known distinct (sub) population structure, the exact proportion of subpopulations with $N_c > 5000$ was obtained. For species where we do not know the population structure, but we know the species are common and highly mobile (such as bats, predators, deer), we assume that $\approx 100\%$ of the populations of that species reach $N_e > 500$ through gene flow. We then assessed the rest of the mammals and herptiles as follows. LC (common) species that surely have some subpopulations on islands etc, and are less mobile, such as rodents, shrews, mole, hedgehog, amphibians and reptiles were given a value of "high", which corresponds to 90% in the later summary calculations. For species classified as severely fragmented, we set the value either as "medium" (50%) if fragmentation is described as a threat or "low" (10%) if fragmentation is described as the worst threat (Artfakta, 2022). This was done although the species may not be considered suffering from fragmentation as defined in the Red List criteria, this is because fragmentation can result in genetic threats (isolated populations can be small with $N_c < 5000$) that are not considered in the Red Listing.

An example here may be the sand lizard (*Lacerta agilis*, L.). There is one large ($N_c > 5000$), more or less continuous, population in southern Sweden, at no risk of extinction, and then a larger area in south-central Sweden with heavily fragmented relict populations. The fragmented relict populations are small and isolated and do not reach $N_c < 5000$ and thus not $N_e > 500$.

A couple of mammal species have been kept in game enclosures and

have escaped, been released, or translocated for hunting purposes. Those populations are commonly < 5000 individuals, but they hold little or no conservation value from a genetic standpoint, therefore they were assigned "anthropogenic" and excluded from the summary. Many of the Swedish red-deer populations are of introduced origin, but the nominate subspecies in the southernmost county of Sweden is indigenous and its population is estimated to exceed 5000 individuals (Ahrné et al., 2020). We summarized the average proportions per species group using the values calculated as described above.

2.3. Indicator 2. The proportion of populations or range maintained within species

To calculate indicator 2, all species that had data recorded in the Red List database (as yes or no) for any of the following criteria: decreasing extent of occurrence, decreasing area of occurrence, decrease in area or quality of habitat, or decrease in number of local areas or populations, were considered to have data. The time-frame considered in the Red List is three generations with a minimum of 10 years. If they were filled out as "yes" or Red Listed as RE they were classified as losing populations.

For mammals and herptiles, we analyzed data on the species level, by merging separately assessed taxa of the same species. All criteria for trends in the Red List are based on only a three-generation time-span, therefore we also looked at a longer historical context of 100 years, and if the species had a larger distribution in the past, we classified it as not maintaining populations. Species Red Listed as RE that went extinct > 100 years ago were excluded from the analysis. Current population range compared to historical population range was estimated based on information of historical range in the Swedish species information database Artfakta (Artfakta, 2022), which in turn is based on older literature. Given available data in the Red Listing database, it was hard to estimate indicator 2 "the proportion of populations maintained within species" for most species, partly because data has only been recorded in a standardized manner since 2000, and here we have chosen a longer time horizon, so the response was mostly binary yes or no, for the summary calculated as 0% (no) or 100% (yes). Many species were not assessed in the Red List as losing or not losing populations. Thus, if these species could not be assigned an exact value, they were estimated to $\approx 100\%$ maintenance of populations if they are good dispersers and likely not losing any populations, and "High" if they are common, but potentially some populations could have been lost. High corresponds to 90% in the summary.

2.4. Indicator 3: The number of species and populations in which genetic diversity is being monitored using DNA-based methods

To calculate indicator 3, we used already published reviews on genetic studies of species in Sweden. These reviews covered published work up until 2006 (Laikre et al., 2008) followed by published work over the period 2006–2019 (Posledovich et al., 2021). We combined the data from both of these studies to get as complete a picture as possible of genetic knowledge on species in Sweden. We compiled data at the species level to assess all species with any study on population genetics. All studies with samples from the same population from at least two time points (which could be considered monitoring), and all species with currently ongoing monitoring of genetic diversity were noted. Species where only a part of the total Swedish population were studied was included, while species where studies are planned but not started were excluded.

3. Results

3.1. Indicator 1

A total of 7336 species (33%) of the 22,557 assessed in the national Red List in Sweden had data on population size or structure. Of those

7336 species 60% potentially have populations with $N_c > 5000$ for indicator 1 while 40% have $N_c < 5000$ and thus $N_e < 500$ (Fig. 1).

The more detailed analysis of mammals and herptiles shows that 49% of herptile and 70% of mammal populations in Sweden are estimated to have $N_c > 5000$ individuals. All of them have a population estimate, or the population could easily be estimated to be ≥ 5000 individuals by us. For 19 (30%) mammal species and 6 (35%) herptile species, the exact proportions of populations with $N_c > 5000$ could be calculated. In 13 mammal and 1 herptile species, the exact number of populations was known, the rest can be calculated due to a low total population. For another 19 (30%) mammal species, very accurate proportions of populations with $N_c > 5000$ could be estimated given the total population > 5000 individuals and high mobility of species likely leading to a continuous national population. For 23 (36%) mammals and 8 (47%) herptiles, the proportion of populations with $N_c > 5000$ was estimated based on our knowledge of the groups.

3.2. Indicator 2

4470 (20%) of all 22,557 assessed species had data on if they were maintaining their populations, AOO or EOO or not, and 4142 (9%) had a potentially stable number of total populations or range for indicator 2 (Fig. 2).

The more detailed analysis shows that 32% of the herptile species and 84% of the mammals in Sweden have maintained their geographic subpopulations or distribution during the last 100 years.

3.3. Indicator 3

There are population genetic studies on 576 species (2.6%) of all 22,557 species in the Swedish Red List, 82 species (0.4%) have data from at least two time periods. There is ongoing genetic monitoring on 55 species (0.2%; Table 2; Fig. 3).

There is at least one population genetic study on 68% of mammals and 81% of herptiles, 13% of mammals and 6% of herptiles have studies with at least 2 time points included and there are ongoing genetic monitoring programs for 5 (8%) mammal species and 5 (29%) herptiles

(Table 1).

4. Discussion

Here we demonstrate that data already collected for the Red List is useful in assessing the suggested indicators for genetic diversity. The inclusion of genetic diversity indicators in the CBD post 2020 framework is a major advance in a long-standing problem- the neglect of this vital aspect of biodiversity in policy (Hoban et al., 2020; Laikre et al., 2020). Still, genetic indicators required demonstration of their applicability, as they have not been used as much as species and ecosystem indicators, such as the Red List Index. We also show that the genetic indicators capture conservation issues of genetic erosion that the Red List misses.

From the results (Fig. 1,2,3) we might conclude that we have data on a limited proportion of all species (certainly most species outside of mammals, birds, reptiles, fish and higher plants lack data), and the effort to apply the $N_e > 500$ across most or all species in a country would be extremely challenging. The other way to view the results is that good data exists when efforts have been made, but no effort is currently invested into a population estimation for many species, including common species that are nowhere near to be Red Listed. The high proportion of species with < 5000 individuals in groups, with a low proportion of species with population estimates, suggests that a neglect of effort for recording data has been applied to the lesser known groups (Fig. 1,2,3). As we see in Figs. 1, 2 and 3, there is a huge variation among taxonomic groups, both in the availability of data, as well as in the proportion of species fulfilling $N_c > 5000$. The reasons behind these differences vary greatly, as each group has its own individual challenges. Such variation in data quality and quantity for Red Lists has been noted in other studies (Bland et al., 2012; Butchart and Bird, 2010).

In the two more closely studied groups (mammals and herptiles), it is possible to apply indicator 1 (proportion of species where $N_c > 5000$) in better detail as most species in these groups have population estimates, or are known to be well over the census size threshold, and we often have well-informed ideas on population structure. No species with a population > 5000 is listed as heavily fragmented or have a very limited area of occurrence or localities for these groups (Table S1). For other

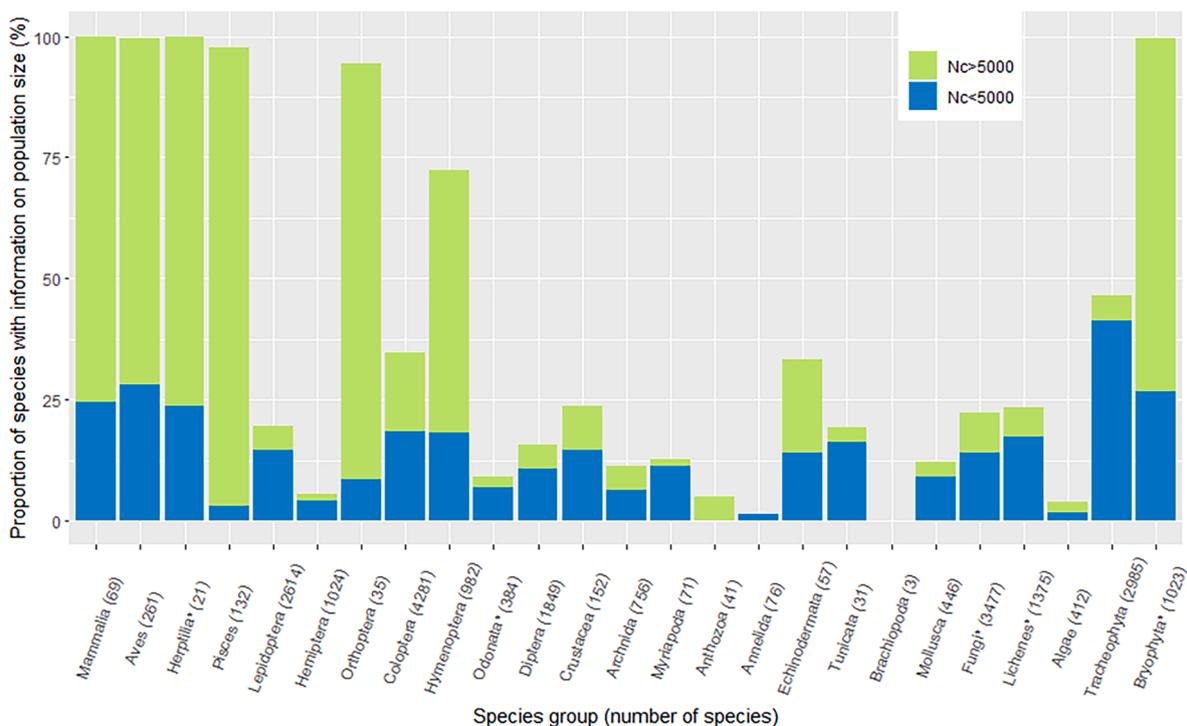


Fig. 1. Data availability for indicator 1 for different Red List taxonomic groups (cf. Table 2) from the Red List database. * Defined in Table 2.

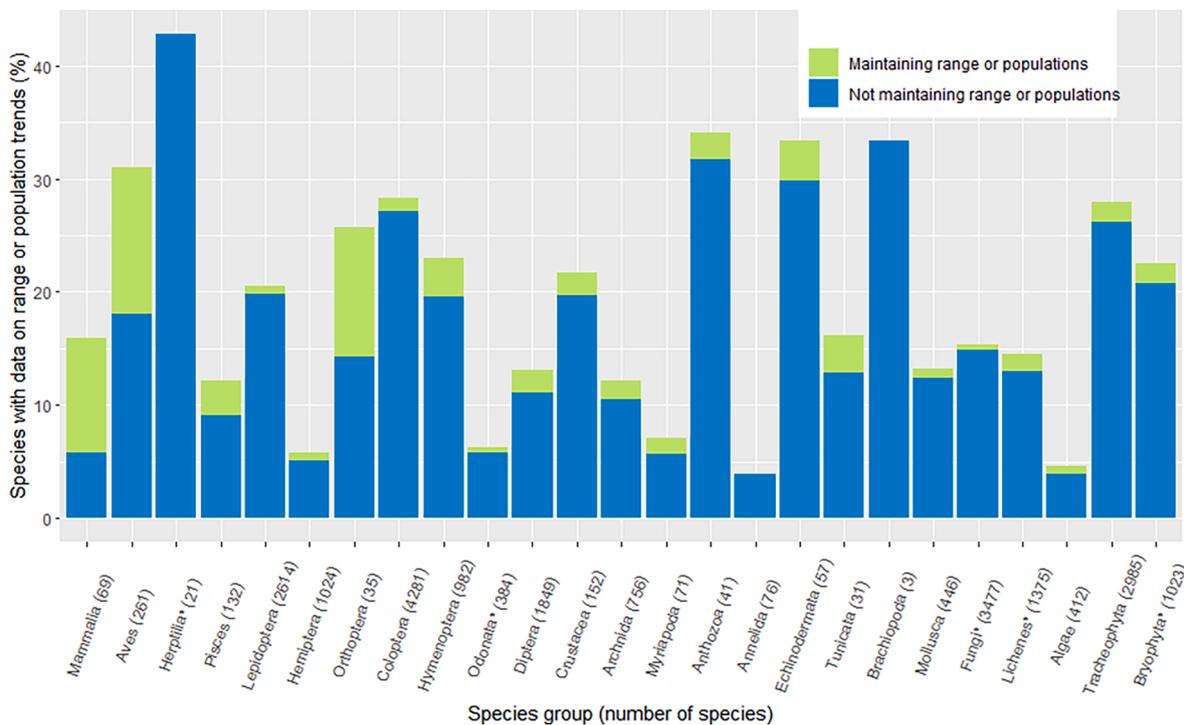


Fig. 2. Data availability for indicator 2 for different Red List taxonomic groups (cf. Table 2) from the Red List database. * Defined in Table 2

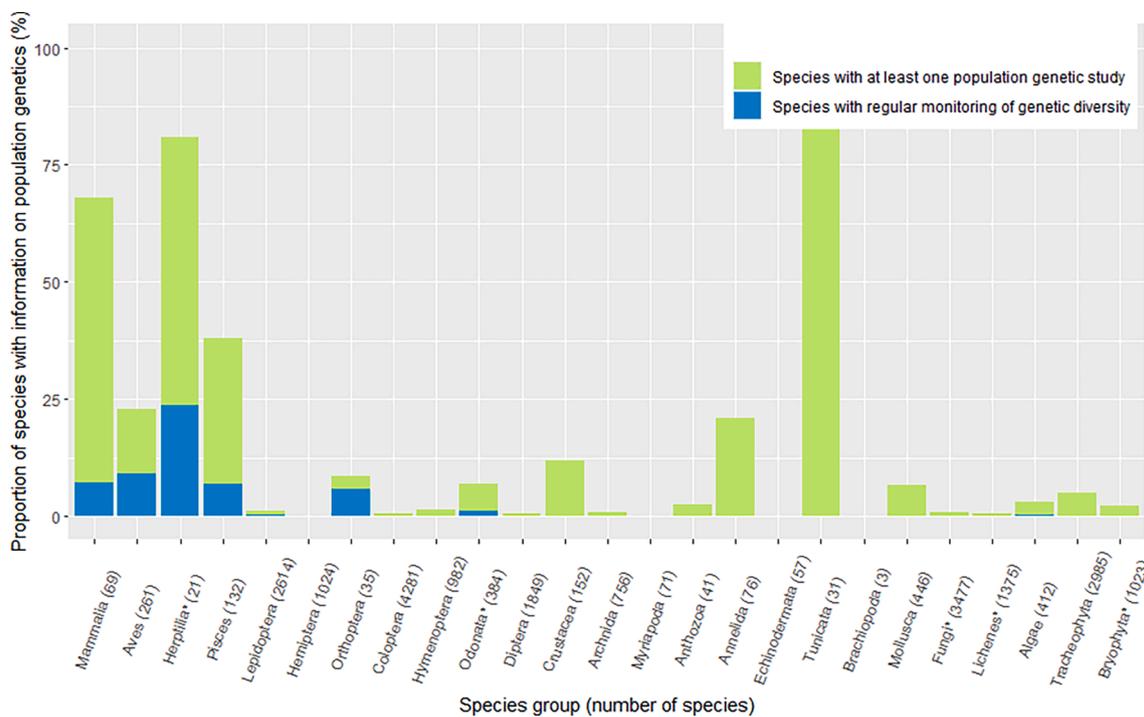


Fig. 3. Data availability for indicator 3 for different Red List taxonomic groups (cf. Table 2) from the Red List database. * Defined in Table 2.

groups, we may have to adjust how these criteria are used, depending on population dynamics within the group. For instance, fragmentation does not mean the same thing for a clonal plant species, or species with a seed bank, as it does for an amphibian.

Data availability for indicator 2 depends a lot on how each expert group approached the Red Listing process, and could depend on the baseline year (we used 100 years ago). The more detailed study on mammals and herptiles shows it is definitely possible to apply the

indicator, but a more in-depth evaluation of each species could render a better estimate instead of the current yes–no dichotomy (range declining or not) that can be derived from the Red Listing data. The proxy of current distribution < historical distribution that we used for the more closely studied groups requires an individual assessment that is not included in the Red List, and hence is time consuming for large groups. However, there are probably great synergy effects of making calculations for this indicator in parallel with the Red Listing process, as

Table 1

Detailed assessment of genetic indicators 1, 2 and 3 for mammals and herptiles. Percentage values come from studies or deduction. ≈ was given to very good estimates. A written “Yes” or “No” comes from the Red List (for indicator 1 or 2), while “High”, “Medium” or “Low” were estimated. Summaries for indicator 1 and 2 were calculated as averages, where High was set to 90%, Medium 50% and Low 10%. For indicator 2, “Yes” (from the Red List) was set as 100% and “No” 0%. For indicator 3 information on whether genetic diversity is monitored using DNA based techniques was obtained from Posledovich et al. (2021, data listed in their Appendix 3.1). ¹ Known population structure (through research and/or deduction). ² Most red deer populations are of a mixed anthropogenic origin, but there are two populations of the native subspecies (*C. elaphus elaphus*), the values for those are in here. The anthropogenic populations are excluded from analysis.

Scientific name	Group	Indicator 1 % of pop > 5000	Indicator 2 Maintains range or populations	Indicator 3 Monitoring genetic diversity with DNA-based techniques
<i>Sorex isodon</i>	Mammals	0%	No	No
<i>Sorex araneus</i>	Mammals	High	High	No
<i>Sorex minutus</i>	Mammals	High	High	No
<i>Sorex minutissimus</i>	Mammals	High	High	No
<i>Sorex caecutiens</i>	Mammals	High	High	No
<i>Neomys fodiens</i>	Mammals	High	High	No
<i>Canis lupus</i>	Mammals	0% ¹	100%	Yes
<i>Erinaceus europaeus</i>	Mammals	High	High	No
<i>Talpa europaea</i>	Mammals	High	High	No
<i>Lynx lynx</i>	Mammals	0% ¹	100%	No
<i>Ursus arctos</i>	Mammals	0% ¹	100%	Yes
<i>Vulpes lagopus</i>	Mammals	0% ¹	0%	Yes
<i>Vulpes vulpes</i>	Mammals	≈100%	≈100%	No
<i>Phoca vitulina</i>	Mammals	50% ¹	50%	No
<i>Halichoerus grypus</i>	Mammals	50% ¹	50%	No
<i>Pusa hispida</i>	Mammals	0% ¹	0%	No
<i>Lutra lutra</i>	Mammals	0% ¹	100%	No
<i>Meles meles</i>	Mammals	≈100%	≈100%	No
<i>Martes martes</i>	Mammals	≈100%	≈100%	No
<i>Mustela erminea</i>	Mammals	≈100%	No	No
<i>Mustela nivalis</i>	Mammals	≈100%	Yes	No
<i>Mustela putorius</i>	Mammals	≈100%	Yes	No
<i>Gulo gulo</i>	Mammals	0% ¹	100%	Yes
<i>Pipistrellus nathusii</i>	Mammals	≈100%	≈100%	No
<i>Pipistrellus pipistrellus</i>	Mammals	0%	High	No
<i>Pipistrellus pygmaeus</i>	Mammals	≈100%	≈100%	No
<i>Nyctalus noctula</i>	Mammals	≈100%	≈100%	No
<i>Nyctalus leisleri</i>	Mammals	0%	High	No
<i>Eptesicus serotinus</i>	Mammals	≈100%	≈100%	No
<i>Eptesicus nilssonii</i>	Mammals	≈100%	≈100%	No
<i>Myotis nattereri</i>	Mammals	≈100%	≈100%	No
<i>Myotis dasycneme</i>	Mammals	0%	High	No
<i>Myotis bechsteinii</i>	Mammals	0%	High	No
<i>Myotis mystacinus</i>	Mammals	≈100%	≈100%	No
<i>Myotis daubentonii</i>	Mammals	≈100%	≈100%	No
<i>Myotis myotis</i>	Mammals	0%	High	No
<i>Myotis brandtii</i>	Mammals	≈100%	≈100%	No
<i>Myotis alcathoe</i>	Mammals	0%	High	No
<i>Plecotus auritus</i>	Mammals	≈100%	≈100%	No
<i>Plecotus austriacus</i>	Mammals	0%	High	No

Table 1 (continued)

Scientific name	Group	Indicator 1 % of pop > 5000	Indicator 2 Maintains range or populations	Indicator 3 Monitoring genetic diversity with DNA-based techniques
<i>Vespertilio murinus</i>	Mammals	≈100%	≈100%	No
<i>Barbastella barbastellus</i>	Mammals	≈100%	≈100%	No
<i>Muscardinus avellanarius</i>	Mammals	High	High	No
<i>Castor fiber</i>	Mammals	100% ¹	100%	No
<i>Lepus timidus</i>	Mammals	High	No	No
<i>Alces alces</i>	Mammals	100% ¹	100%	Yes
<i>Capreolus capreolus</i>	Mammals	≈100%	≈100%	No
<i>Cervus elaphus</i>	Mammals	50% ²	100% ²	No
<i>Phocoena phocoena</i>	Mammals	67% ¹	67%	No
<i>Sciurus vulgaris</i>	Mammals	High	High	No
<i>Sicista betulina</i>	Mammals	High	High	No
<i>Mus musculus</i>	Mammals	High	High	No
<i>Apodemus flavicollis</i>	Mammals	High	High	No
<i>Apodemus sylvaticus</i>	Mammals	High	High	No
<i>Rattus norvegicus</i>	Mammals	High	High	No
<i>Lemmus lemmus</i>	Mammals	High	High	No
<i>Myopus schisticolor</i>	Mammals	High	High	No
<i>Myodes rutilus</i>	Mammals	High	High	No
<i>Myodes glareolus</i>	Mammals	High	High	No
<i>Craseomys rufocanus</i>	Mammals	High	No	No
<i>Microtus agrestis</i>	Mammals	High	High	No
<i>Arvicola amphibius</i>	Mammals	High	High	No
<i>Alexandromys oeconomus</i>	Mammals	High	High	No
Summary Mammals		70%	84%	8%
<i>Triturus cristatus</i>	Amphibians	Medium	No	No
<i>Lissotriton vulgaris</i>	Amphibians	High	High	No
<i>Bufo bufo</i>	Amphibians	High	High	Yes
<i>Bufo viridis</i>	Amphibians	0% ¹	No	Yes
<i>Epidalea calamita</i>	Amphibians	0%	No	Yes
<i>Rana dalmatina</i>	Amphibians	High	No	No
<i>Rana temporaria</i>	Amphibians	High	High	Yes
<i>Rana arvalis</i>	Amphibians	High	High	Yes
<i>Pelophylax lessonae</i>	Amphibians	0% ¹	No	No
<i>Pelophylax esculentus</i>	Amphibians	High	High	No
<i>Pelobates fuscus</i>	Amphibians	0%	No	No
<i>Hyla arborea</i>	Amphibians	0%	No	No
<i>Bombina orientalis</i>	Amphibians	0%	No	No
<i>Lacerta agilis</i>	Reptiles	Low	No	No
<i>Coronella austriaca</i>	Reptiles	Medium	No	No
<i>Natrix natrix</i>	Reptiles	High	No	No
<i>Vipera berus</i>	Reptiles	High	High	No
Summary Herptiles		49%	32%	29%
Total summary		65%	73%	12%

Table 2

Overview of organism groups and data availability for indicator 3 (the number of species and populations in which genetic diversity is being monitored using DNA-based methods).

Group	No. of taxa assessed in the Red List	At least one population genetic study	At least one population genetic study with temporally separate samples (not regular monitoring)	Regular monitoring of genetic diversity
Mammalia	69	47 (68%)	9 (13%)	5 (7%)
Aves	261	60 (23%)	25 (10%)	24 (9%)
Herpetilia (reptilia and amphibia)	21	17 (81%)	6 (6%)	5 (24%)
Pisces	132	50 (38%)	17 (13%)	9 (7%)
Lepidoptera	2614	27 (1%)	9 (0.3%)	5 (0.2%)
Hemiptera	1024	1(0.1%)	0 (0%)	0 (0%)
Orthoptera	35	3 (9%)	0 (0%)	2 (6%)
Coleoptera	4281	22 (1%)	1 (0.02%)	0 (0%)
Hymenoptera	982	14 (1%)	4 (0.4%)	0 (0%)
Odonata (and Plecoptera, Ephemeroptera, Trichoptera, Psochoptera)	384	27 (7%)	4 (1%)	4 (1%)
Diptera	1849	11 (1%)	2 (0.1%)	0 (0%)
Crustacea	152	18 (12%)	1 (1%)	0 (0%)
Arachnida	756	6 (1%)	0 (0%)	0 (0%)
Myriapoda	71	0 (0%)	0 (0%)	0 (0%)
Anthozoa	41	1 (2%)	0 (0%)	0 (0%)
Annelida (and Tricladida)	76	16 (21%)	0 (0%)	0 (0%)
Echinodermata	57	0 (0%)	0 (0%)	0 (0%)
Tunicata	31	0 (0%)	0 (0%)	0 (0%)
Brachiopoda	3	0 (0%)	0 (0%)	0 (0%)
Mollusca	446	30 (7%)	0 (0%)	0 (0%)
Fungi (Only Macrofungi)	3477	34 (1%)	0 (0%)	0 (0%)
Lichenes (Composite organism)	1375	8 (1%)	0 (0%)	0 (0%)
Algae	412	13 (3%)	0 (0%)	0 (0%)
Tracheophyta	2985	149 (5%)	2 (0.07%)	1 (0%)
Bryophyta (and, Antho-cerotophyta, Marchantiophyta)	1023	22 (2%)	0 (0%)	0 (0%)
Summary	22,557	576 (2.6%)	82 (0.4%)	55 (0.2%)

changes in habitat distribution and quality are often assessed for the species. For example, it contributed one species (*Hyla arborea*, L.) to the second indicator that would otherwise have been left out. It is worth noting that from the Red Listing process, there is more abundant data for the second indicator for less well-known groups (Fig. 2) and it is likely more robust than indicator 1 as population censuses are scarce. The second indicator scales well, as the loss of individual populations can be recorded on most scales. It is also less dependent on the definition of individual or census size, which can be tricky in many organism groups (especially some plants and insects). We also note there may be data sources available other than the Red List which could be applied across large numbers of species, such as large species occurrence data, which has been used in some metrics of population loss, including a proposed indicator for plants (Khoury et al., 2019; Powers and Jetz, 2019).

For indicator 3, we used previous literature reviews (Laike et al., 2008; Posledovich et al., 2021), and we note that the more well studied groups are also more commonly subjects to genetic studies (Fig. 3).

4.1. Comparisons with the Red List classifications

The main difference between the genetic indicators and the Red List is a focus on different levels of biodiversity. While the Red List mainly focuses on the species level and avoiding species' extinction, the genetic indicators are intended to monitor the population level - genetic variation within and among populations and thus avoiding genetic erosion (Exposito-Alonso et al., 2021). The two perspectives are derived from population ecology and evolutionary ecology, respectively, and are thus driven by different mechanisms, where species or populations may erode genetically before population effects can be seen (Kardos and Luikart, 2021; Spielman et al., 2004). Even in a country with substantial environmental and species monitoring programs, it is difficult to apply the genetic indicators to all organism groups, given current knowledge. On the other hand, the genetic indicators could be applied successfully to thousands of species. From this test using Swedish Red List data, the data availability is reasonably sufficient for mammals and herptiles, fish,

birds and some plants (Table 2, Fig. 1,2,3), and a few species in other groups.

Compared to the Red List, the genetic indicators 1 and 2 suggest that more species appear threatened under genetic criteria than under the Red Lists demographic criteria (S1). This is due to the higher threshold of 5000 instead of 2000 individuals for indicator 1, and the longer time span 100 years instead of three generations for indicator 2. The higher threshold may be of less importance. Looking at the population sizes (S1) it seems that changing the ratio $N_e/N_c = 0.1$ by 50% makes little difference regarding the estimated proportions of species having $N_e > 500$, so the results are fairly robust. On the other hand, the longer time scale will catch the depleted species that have plateaued at a low level with only a fraction of their former population. Depleted species are Red Listed as LC if their populations have been stable for the last three generations and exceeds 2000 individuals, even though their population may only have 1% of the original size left. If those populations are investigated using a genetic indicator, the longer time span enables us to identify species that may be at risk, as genetic erosion may have happened (Exposito-Alonso et al., 2021) and inbreeding may be a threat. This has potential to be of value for conservation, as it has been emphasized that population losses are ongoing in many species (Ceballos and Ehrlich, 2002; Ceballos et al., 2017), and we should not neglect these population losses for common species, even if they do not meet Red List criteria. Such population losses may represent loss of vital genetic diversity that reduces the adaptive capacity and long-term survival of remaining populations of the species.

There are a few more criteria in the Red List and combinations of criteria that may lead to species being classified as NT or threatened even if population size is > 2000 . In other words, genetic threats and Red List assessments are not aligned. The Red Listed species that did not meet genetic indicators were highly mobile species such as bats that have a small fraction of a larger European population in Sweden, but also decreasing widespread and common species. The species Red Listed as LC that met genetic indicators 1 and 2 were species with relatively low mobility such as herptiles, or species with a clear isolated population,

such as marine mammals that are split into Baltic and Atlantic populations (Table S1).

4.2. Further considerations

The proposed genetic indicators do not consider hybridization issues, where a native subspecies such as red deer (*Cervus elaphus elaphus*, L.), is being outbred with introduced animals of taxonomically mixed origin. For relatively well-known species groups, these factors could be included in some way in an alternative genetic indicator, such as the genetic scorecard developed recently in Scotland (Hollingsworth et al., 2020). Also, with more extensive use of genomic methods (indicator 3) potential genetic erosion due to introgression will be possible to monitor.

A genetic indicator may be less well suited to assess at a country level if populations are transboundary; the best practice may be to do joint assessments on the appropriate scale in such cases. For a population spanning several countries where a joint assessment is appropriate, there is also a joint responsibility for the conservation of the population. The second indicator seems less sensitive to spatial scales, as a loss of range or populations is always a loss of range or populations. However, a loss of range for a transboundary population may only be seen in some of the countries hosting the population.

An expanded database of Ne/Nc ratios could help apply a more species-tailored ratio than the conservative 0.1 ratio uniformly employed here. One approach could be to use published numbers where available, and to estimate Ne/Nc ratio using life-history based models (Waples et al., 2013) for the rest.

One issue encountered when working with the genetic indicators proposed by Laikre et al (2020) and Hoban et al (2020) is the lack of publicly available, detailed guidelines for applying them in practice. This will need to be developed as assumptions may currently be needed for their application. For example, in this study, the level of fragmentation was used to determine the proportion of populations might be below Ne 500, which we admit is a strong assumption. In addition, it was not possible to evaluate population structure for most species, hence the simplified dichotomy of ‘maintaining populations’ or ‘not maintaining populations’. Clearly, further guidance on this aspect is needed. Without detailed guidance, there will likely be differences in results depending on who did the assessment, something that can reduce the credibility and repeatability of indicators over time. The Red List, definitions are well documented, and for the genetic indicators to take a more prominent role in conservation, definitions and guidelines thus need to be developed.

4.3. The next steps

When assessing species for the Red List, there are synergies if genetic assessment is done in parallel, as information is often gathered species by species and literature and experts for each species are consulted. Therefore, the easiest way to apply the genetic indicators to a larger proportion of species may be to do it simultaneously with the Red List reassessment. The first step would be to instruct Red Listing committees to fill out all checkboxes that can be estimated, i.e. if “has a declining AOO” is not filled out as “yes”, it should be filled out as “no” if that can be estimated. The number of populations should always be recorded if possible. Another simple change is that for species where there is some knowledge on population size, there should be instructions to enter the population size as >20,000 (20,000 is the population size that can potentially affect the Red Listing status in combination with other estimates) if that can be estimated. An additional step would be to add info that is specific for the genetic indicators, such as the sizes of different populations, historical range estimates (maybe AOO and EOO for comparability), quantified Ne/Nc ratio, and information on genetic monitoring and knowledge from assessments of population genetic structure.

A suggestion may be that genetic indicators such as these could be implemented in the Red List, adding a new criterion, “Genetically threatened”. A similar suggestion to include a ‘genetic threat’ in the Red List process has been made several times (Garner et al., 2020; Willoughby et al., 2015). We truly think that it is now feasible and necessary for the Red List to do so, as the genetic threats to species are growing in impact, and as genetic technologies and knowledge of genetic processes are ever more accessible (Hoban et al., 2021b; Parli et al., 2021; Taft et al., 2020).

Overall, we conclude that genetic indicators could be evaluated for a substantial fraction, but not a majority of species in a country, and that the genetic indicators have the potential to identify threatened species and populations that are missed by the Red List. We encourage other countries to perform similar applications of these indicators so comparisons become possible.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was funded by the Swedish Environmental Protection Agency (SEPA), and they had a role in defining the questions, but not in the outcome. The work of LL was funded by the Swedish Research Council Formas (grant 2020-01290) and the Swedish Research Council (grant 2019-05503).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2022.109167>.

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