

The rarity of Invertebrates prevents reliable application of IUCN Red-List criteria.

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Abstract

Among the most widely used information underpinning international conservation efforts is the IUCN Red List of endangered species. The Red List designates species extinction risk based on geographic range, population size, or declines in either. However, the Red-List has poor

representation of invertebrates which comprise the majority of animal diversity, and it has frequently been questioned whether Red List criteria are appropriate for these organisms. Due to their small size, difficulty in identification, and general rarity, many invertebrates are hard to study, making Red List criteria difficult to apply. Here we discuss these criticisms in the context of empirical evidence from one of the largest terrestrial arthropod surveys to date, documenting the abundance and distribution of over 13,000 species in Sweden. Using simple empirical examples from these data, we argue that even the most ambitious monitoring efforts are unlikely to produce enough observations to reliably estimate population sizes and ranges for more than a fraction of species. Thus, there is likely to be substantial uncertainty in classifying most species according to current criteria. In response, we discuss the introduction of potential new IUCN criteria to more accurately capture the conservation needs of invertebrates, and to increase the representation of invertebrates on the IUCN Red List.

Red-Listing and terrestrial arthropods.

Rapid rates of environmental degradation threaten biodiversity worldwide (Garcia *et al.*, 2014, and concerted conservation efforts are required to mitigate the impacts of human driven global

change (Synes *et al.*, 2020; Williams *et al.*, 2021). The International Union for Conservation of Nature (IUCN) Red List underpins many international conservation policies that aim to preserve threatened species and ecosystems (Vie *et al.*, 2009). Assessments provided by the Red List often influence the allocation of funding to large numbers of conservation projects and have demonstrable success in protecting threatened species (Rodrigues *et al.*, 2006; Bland *et al.*, 2019; Betts *et al.*, 2020).

The current Red-Listing process involves categorisation of species using a set of criteria developed over the course of several decades (Mace *et al.*, 1992, Mace *et al.*, 2008). These criteria build on ecological theory to use quantitative data to provide a framework to assess which species are at risk of extinction. Assessments categorise organisms based on criteria such as minimum viable population size (criteria C & D) geographic range (criterion B), analyses of extinction risk (criteria E), and those that examine risk based on trends in either range or population size (criteria A) (IUCN, 2022; Appendix A).

The Red List faces several challenges (Rondinini *et al.*, 2014; Bachman *et al.*, 2019; Cazalis *et al.*, 2022), but a major criticism is the overwhelming taxonomic bias of assessments, which are skewed towards large and (relatively) easy to observe vertebrates. Almost all mammals (91%), amphibians (88%), reptiles (87%) and fishes (69%), have received an assessment, and all 11,188 bird species have each received multiple assessments (IUCN, 2021). However, the overwhelming majority of species on earth are invertebrates (Stork, 2018; Srivathsan *et al.*, 2022), yet of the one million described species of insects, only 1.2% (~12,000) have received an assessment (IUCN, 2021) (Figure 1A). Of assessed invertebrates a considerably higher proportion of species are listed as data deficient (Figure 1B), meaning the Red List has reduced capacity to inform conservation action. What's more the invertebrates that do have

representation on the Red List still display bias in the taxa assessed, with most entries composed of well-studied taxa. For example, within Hymenoptera, which is one of the best studied groups, the majority of assessments come from Apidae (5988 species, 186 assessments) and Formicidae (10,213 species, 149 assessments). On the other hand two of the most speciose groups across all insects, Ichneumonidae (23,765 species) and Braconidae (18,579 species), have only 2 and 6 assessments respectively (Bánki *et al.*, 2023).

The poor representation of invertebrates on the Red List is alarming as recent research has demonstrated severe declines in global invertebrate populations (van Klink *et al.*, 2020; Wagner *et al.*, 2021), and high sensitivities to global change (Millard *et al.*, 2021; Outhwaite, McCann and Newbold, 2022). It is therefore essential that we increase efforts to identify threatened organisms and provide risk assessments to guide international conservation efforts. Yet, despite the strong evidence to suggest that major components of global biodiversity are threatened by global change, the bias in Red-List assessments means our current perspective of which organisms are threatened relies substantially on selective information (Cardoso *et al.*, 2011; Eisenhauer, Bonn and A. Guerra, 2019) from taxonomically and regionally restricted monitoring efforts.

The rarity of invertebrates and applying red list criteria.

The relatively poor representation of invertebrates on the Red List is likely symptomatic of several interacting issues. The hyper-diverse nature of invertebrate fauna mean that they contain vast numbers of species, however, the majority of these organisms rarely provide more than a

handful of observations for analysis of population or range sizes. A seminal paper published in 1943 (Fisher, Corbet & Williams) used data from a 5-year Lepidoptera survey in Rothamsted, to demonstrate that the majority of species were rare. In this study approximately 14% of species were observed only once (Figure 2A). After 80 years of empirical work the same pattern has remained, for terrestrial arthropods globally, most are only encountered in low numbers or at single sites (Morse, Stork and Lawton, 1988; Basset and Kitching, 1991; Novotný and Basset, 2000; Coddington *et al.*, 2009, Hudson *et al.*, 2017, Dornelas *et al.*, 2018, Srivathsan *et al.*, 2022). In 2019 we conducted a comprehensive and systematic survey of all terrestrial arthropods in Sweden (Box 1), this survey represents one of the largest and most sophisticated arthropod surveys to date. In total we collected over 4700 samples of arthropod communities from 198 sites, representing over a million hours of survey effort. Despite the enormous sampling effort, and molecular identification (Iwaszkiewicz-Eggebrecht *et al.*, 2023) (Appendix Figure S1), most of the organisms we surveyed were still rarely observed with 13% of species found at only a single site (1% of total sites). Over 40% of organisms occupied five or fewer sites (2.5% of total sites), and less than 1% occupied more than half of the sites (Figure 2B). Our findings compound the evidence for a long-standing pattern in ecology - rarity is an inherent feature of invertebrate communities.

Previous debates have focused on whether criteria (Table S1) are potentially inapplicable to many invertebrate taxa, since the standard thresholds might provide inappropriate measures of relative risk for small organisms with high reproductive rates (Tschardtke *et al.*, 2007; Cardoso, Borges, *et al.*, 2011; Collen and Böhm, 2012; Cardoso *et al.*, 2012; Eisenhauer, Bonn and A. Guerra, 2019; Fox *et al.*, 2019; Akçakaya *et al.*, 2021). However, the rarity of invertebrate species means that even if appropriate, criteria (Table 1) are difficult to apply using quantitative

data.

For example, the majority of invertebrates that have received Red List assessments are classified under criterion B. Yet, in our survey a high proportion of species contain too few observations to reliably estimate species distributions (van Proosdij *et al.*, 2016; Jeliaskov *et al.*, 2022; Yoccoz, 2022; Erickson and Smith, 2023), and 24% of the species we detect produce fewer than three observations, preventing application of Criteria B1 (using extent of occurrence as the metric of range size). Similarly, estimating population sizes for insects is often extremely difficult, which is perhaps why only 0.0016% of total insect assessments are completed under Criteria C, and 96% of D criteria assessments are completed under D2 (restricted range and limited number of locations).

Without adequate quantitative evidence on abundance and distribution the process is dependent on the taxonomic expertise. However, the supply of such experts is limited (Hochkirch *et al.*, 2022) and for the majority of species (i.e. those that have yet to be described taxonomically and ecologically), this expertise has yet to be established. This leaves practitioners with a difficult decision during the assessment process for rarely observed organisms – classify species in the absence of adequate quantitative data and high uncertainty, relying on potentially subjective viewpoints from taxon experts. Or place organisms into either the “Data Deficient” or “Not Evaluated” categories and accept that limited conservation action can result from the IUCN assessment.

An empirical example using trend-based criteria.

Due to the inherent statistical relationship between sample size and uncertainty, low abundances or occurrences are intrinsically linked to low statistical power. Therefore, trends may be difficult to estimate without considerable degrees of uncertainty (Jeliazkov *et al.*, 2022; Yoccoz, 2022; Erickson and Smith, 2023). To illustrate how classification using trend-based criteria may produce uncertain estimates and classification we use the empirical incidence and abundance distributions revealed by the data described in Box 1 to establish whether trends can actually be detected. We simulate the minimum trends based on observed occurrence and abundance frequencies for each “Threatened” Red List category from Criteria A (Box 2), and attempt to retrieve the true trend by fitting statistical models to these simulated data. These simulations demonstrate high rates of misclassification in terms of trend-based IUCN categories. As most species produce very low numbers of observations, assigning Red List categories is associated with high levels of uncertainty and high risk of misclassification. (Figure 3).

Estimated trends in occurrence and abundance for rare species were associated with a high percentage error (Figure 3), as deviations away from zero were larger for organisms with lower occurrence frequencies. This resulted in high levels of misclassification for both Vulnerable (Figure 3A & D), and Endangered (Figure 4B & E) organisms: 46% & 49% (for occurrence and abundance trends respectively) of Vulnerable organisms were misclassified as “Lower risk” (i.e., any category lower than Vulnerable) whilst 18% & 20% were misclassified as Endangered, and 7% & 11% as Critically Endangered. Only 30% & 18% received the correct classification. Of Endangered organisms, 18% & 33% were misclassified as “Lower risk”, 25% & 18% as Vulnerable, and 12% & 15% as Endangered, whilst 45% & 33% were correctly classified. For both of these categories, the majority of organisms were misclassified into less severe Red List

categories than the true category they belonged to. On the other hand, Critically Endangered organisms received mostly correct classifications, with 67% & 51% being placed within the correct category, but 33% & 49% still received incorrect classifications into lower risk categories, with 28% & 33% placed in Endangered, 2% & 4% in Vulnerable and 3% & 12% in Lower risk.

These simulations are a simple demonstration of how statistical uncertainty can impact category designation in the context of small sample sizes. Even with the best available data for terrestrial invertebrates, it becomes extremely difficult to quantify changes to most invertebrate populations reliably. For rare species, there is a high degree of uncertainty associated with applying Red List criteria, and estimates of trends in occurrence or population size and results in high degrees of misclassification. These misclassification rates are even worse for lower sampling intensities (50 & 100 sampling sites - Figures S2 to S5) which are more reflective of long-term monitoring efforts (Hallmann *et al.*, 2017; Crossley *et al.*, 2020). Smaller changes in abundance are harder to estimate accurately, especially with low sample sizes. As rare species are particularly at risk of being threatened (Purvis *et al.*, 2000a; Purvis *et al.*, 2000b, Jetz & Freckleton 2015), high uncertainty in range or population size trends will constrain our decision making for those organisms most urgently needing an assessment. More severe trends are easier to detect, but the most severely declining species will also be the hardest to protect. The accurate designation of less severe categories is therefore critical to planning effective conservation action, as this is the stage when it may be easier and more cost-effective to reverse the changes.

Avenues for increasing invertebrate representation on the Red List.

By examining the data from a highly-resolved and national-scale survey of terrestrial invertebrates we can make several conclusions. First, despite high intensity sampling and modern molecular techniques, we show that most species are demonstrably hard to detect. This observation is in line with over 80 years of empirical work and collated data across local, regional and global scales (Morse, Stork and Lawton, 1988; Basset and Kitching, 1991; Novotný and Basset, 2000; Coddington *et al.*, 2009, Hudson *et al.*, 2017; Dornelas *et al.*, 2018, Srivathsan, *et al.* 2022). Due to the fact that most invertebrate species have yet to be described (Stork 2018), it is highly likely that dominance of rare species will remain into the foreseeable future. To provide protection for the planets most diverse and potentially vulnerable organisms we must rapidly extend our assessment of nature from a taxonomically biased subset of species to a broader and more representative sample of biodiversity (*Fraixedas et al.*, 2022). For invertebrates, we must move away from a reliance on information that is currently unobtainable even with the most advanced methods. We argue that applying the current Red-Listing guidelines (IUCN, 2022) to even the best possible data for invertebrate species will result in one of two outcomes: a) potentially inaccurate designation of Red List categories for many organisms, or b) listing a large portion of global biodiversity as data deficient (DD – IUCN Red List guidelines pg. 77). Whether rarity is a fundamental feature of invertebrate fauna or a function of imperfect sampling, there are consequences for conservation as many rare taxa will not benefit from the Red-Listing process.

Importantly, the extensive data generated by our study (Box 1) are an exception, as most monitoring efforts contain fewer sites, and are generally restricted to protected areas (Forister *et al.* 2023). Since overall data on arthropod abundances and distributions are scarce, the application of current criteria will call for a heavy reliance on expert opinion for most taxa.

However, as neither ranges nor abundances can be reliably estimated using empirical data and statistical models, the process requires experts to make inferences about patterns *without* quantitative evidence. As a result, Red-Listing will be further influenced by limited observations of particular populations within the home range of the experts. That, we argue, renders the Red-Listing process difficult to replicate, will limit the representation of invertebrates on the Red List, and potentially deflate the credibility of conservation science. Clearly, any modification of the Red List criteria to facilitate the proper assessment of conservation priorities for the vast majority of the planet's species will require broad community consensus. As a contribution to this process, we suggest three possible routes towards better Red-Listing practice for invertebrates.

First, standardization of new methodology should be explored. Despite the lack of data for individual species, community-level datasets often contain large numbers of observations. There is ample opportunity for hierarchical models to be used to improve inference by harnessing the structure of community data. Inferring ecological similarities among species based on phylogeny, spatiotemporal distributions, or shared traits, could allow more robust estimates to be made for organisms with sparse records (*Ovaskainen et al., 2017; Jeliaskov et al., 2022*). Current Red List practice could be updated to include advice on how ranges and population trends can be estimated using hierarchical modelling, but any changes made to advice should attempt to retain the flexibility of the assessment process. Importantly, the distribution of observations across phylogenies or ecological niches will influence how much extra information we can glean from organisms with good occurrence records (*Erickson and Smith, 2023*), so as a key priority for advancing the state of conservation science, the practical implementation of our first recommendation should be investigated.

Second, for those species with low occurrences or abundances, a shift away from the single-species focus of current Red List criteria might be prudent until we can gather the data required for reliable species-level inference. This would complement the already established criteria that already work well for many taxonomic groups. One potential avenue would be to create Red List criteria that allow the identification of *groups or guilds* of invertebrates that are particularly threatened. These groups could constitute the entirety of higher taxonomic levels (e.g. sub-genus), groups of spatially aggregated species within a taxon, or organisms with shared traits (e.g. feeding guild). If there are too few observations for single species Red-Listing, then assessments containing groups of organisms could provide a suitable proxy. For example, using current criteria a group of organisms could be classified as threatened if members of a group are regionally restricted and exhibiting decreases in range size. Identification of appropriate taxonomic levels at which to assess organisms will require taxon-specific knowledge of the size of the group, as well as the development of criteria to appropriately convey the risk of extinction of one or more species. However, a multi-taxon approach would increase the rate of assessments and representation of invertebrates on the Red List.

Third, an alternative could be to identify spatially distinct *communities*, without pre-defined taxonomic relationships, that might be sensitive to environmental change. Conservation of *habitats rather than species* has almost always proven a cost-effective and implementable process for species conservation (*Fahrig, 1997; Lawton, 1999; Mantyka-pringle, Martin and Rhodes, 2012; Segan, Murray and Watson, 2016*). Identifying communities with a high number of endemic species with relatively small ranges, a high degree of fluctuation in community composition, or negative trends in an index of diversity, might identify communities containing

at risk species. The IUCN Red List of threatened ecosystems (*Rodríguez et al., 2011; Bland et al., 2019*) provides a useful framework to begin to develop a community-level Red List for invertebrates, and has success in channeling resources into habitat conservation. However, the major roadblock is deciding on sensible criteria to define what constitutes an at-risk community. Meta-community theory might provide useful metrics of what indicates a threatened community, based on temporal and spatial shifts in community composition, functional diversity, and local extinctions (*Iknayan and Beissinger, 2018; Saravia and Momo, 2018; Roberts et al., 2019*). However, the lack of taxonomically comprehensive long-term monitoring programs globally will make it difficult to distinguish dangerous declines from what might constitute the expected dynamics of highly diverse communities in fluctuating environments (*Zheng et al., 2015; Blanchet et al., 2018*).

All of our suggestions, as well as continued effective use of current criteria, are contingent on the establishment of suitable monitoring programs. Urgent investment in comprehensive and well-designed monitoring schemes is required if we wish to accurately detect the ranges, abundances and temporal and spatial trends in invertebrates as major components of global biodiversity. We first need to identify groups and ecosystems that contain large numbers of endemic or threatened invertebrates, and then monitor these communities to assess the effectiveness conservation efforts. This, we feel, will lay the ground work for providing better protection of threatened organisms for which we struggle to obtain sufficient data.

Table S1. Summary of IUCN Red-list. AOO – area of occupancy. EOO – extent of occurrence. More detailed explanations of the cases for criteria A can be found in table S1 of the supplementary information.

Criteria	Vulnerable	Endangered	Critically Endangered
<p>A. Declines in population size / geographic range</p> <p>A1 - Causes of the reduction are clearly reversible AND understood AND have ceased.</p> <p>A2 - Causes of reduction may not have ceased OR may not be understood OR may not be reversible.</p> <p>A3 - Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years)</p> <p>A4 - The time period must include both the past and the future (up to a max. of 100 years in future), and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.</p>	<p>≥ 30% OR ≥ 50% for case A1</p> <p>Based on:</p> <ul style="list-style-type: none"> a) Direct observation b) An index of abundance c) Decline in range d) Levels of exploitation e) Effects of introduced taxa 	<p>50% OR ≥ 70% for case A1</p> <p>Based on:</p> <ul style="list-style-type: none"> a) Direct observation b) An index of abundance c) Decline in range d) Levels of exploitation e) Effects of introduced taxa 	<p>80% OR ≥ 90% for case A1</p> <p>Based on:</p> <ul style="list-style-type: none"> a) Direct observation b) An index of abundance c) Decline in range d) Levels of exploitation e) Effects of introduced taxa
<p>B . Geographic range</p>	<p>B1 AOO < 2000 km² OR</p> <p>B2 EOO < 20000 km²</p> <p>AND two of:</p> <ul style="list-style-type: none"> a) No. locations ≤ 10 b) Continuing decline c) Extreme fluctuations 	<p>B1 AOO < 500 km² OR</p> <p>B2 EOO < 5000 km²</p> <p>AND two of:</p> <ul style="list-style-type: none"> a) No. locations ≤ 5 b) Continuing decline c) Extreme fluctuations 	<p>B1 AOO < 10 km² OR</p> <p>B2 EOO < 100 km²</p> <p>AND two of:</p> <ul style="list-style-type: none"> a) No. locations = 1 b) Continuing decline c) Extreme fluctuations

C. Small population size AND decline	No. of mature individuals < 10,000 AND ONE OF: C1) decline of 10% C2) a) ≤ 1000 mature individuals b) 100% of individuals are mature c) Extreme fluctuations	No. of mature individuals < 2500 AND ONE OF: C1) decline of 20% C2) a) ≤ 250 mature individuals b) 95-100% of individuals are mature c) Extreme fluctuations	No. of mature individuals < 250 AND ONE OF: C1) decline of 25% C2) a) ≤ 50 mature individuals b) 90-100% of individuals are mature c) Extreme fluctuations
D. Very small or restricted population	D1) No. of mature individuals < 1000 OR D2) AOO < 20 km ² or number of locations ≤ 5	No. of mature individuals < 250	No. of mature individuals < 50
E. Quantitative analysis of extinction risk.	$\geq 10\%$ in 100 years	$\geq 20\%$ in 20 years or 5 generations.	$\geq 50\%$ in 10 years or 3 generations.

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Box 1. A spatially and taxonomically extensive national survey of Swedish arthropods.

To pinpoint the challenges associated with the application of trend-based Red List criteria to invertebrates, we examined the data produced by a systematic effort to characterise the current distribution and diversity of the Swedish arthropod fauna. This survey consisted of 198 malaise traps across Sweden, which were sampled weekly to produce 4748 community-level samples, comprising 26 kg of invertebrate biomass and an estimated 3.3 million individuals. Using a high-throughput molecular pipeline (Iwaszkiewicz-Eggebrecht *et al.*, 2023) we matched over 13,000 with a species-level reference and identified over 30,000 unique OTUs. This dataset (with details given in the supplementary material) is one of the most comprehensive systematic surveys of arthropod diversity, in terms of spatiotemporal scale and taxonomic coverage. These data produced represent the gold-standard in terms of national-scale arthropod biodiversity monitoring and are derived from one of the best known faunas in the world (Ronquist *et al.*, 2020).

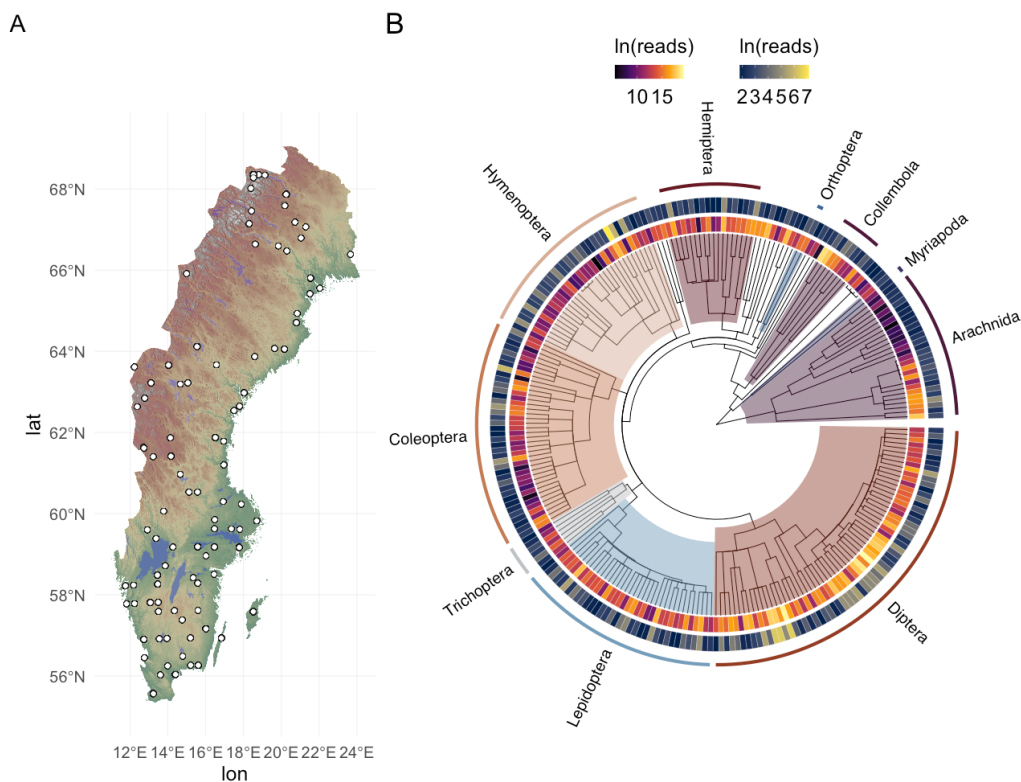


Figure B1. (A) The spatial layout of trap locations within Sweden, with the map illustrating elevation and major bodies of water. Traps were emptied weekly between April and October, and monthly in the remainder of the year. (B) An illustration of the diversity of organisms detected in the survey. The survey detected over 13,144 species across 556 arthropod families. The tree is an arthropod taxonomy where the terminal nodes represent the 253 families containing over 5 species-level operational taxonomic units (OTUs). Major arthropod clades are highlighted by the external bars and shaded regions across sections of the taxonomy. The outer ring of the heatmap illustrates the number of species level OTUs found in each family, and the inner ring illustrates the number of reads in each family.

Box 2. Simulating declines in abundance and occurrence of Swedish arthropods.

To highlight the problems when classifying infrequently observed species based on trends in range or population size, we simulated the minimum trends from criteria A (Table 1). More specifically, we focus on criteria A2b and A2c, which refer to reductions in an index of population size, and trends in geographic range (IUCN, 2022). For these criteria, Vulnerable (VU), Endangered (EN), and Critically Endangered (CR) categories are defined by observing at least 30%, 50%, or 80% declines in abundance or geographic range over a 10-year period. We simulated the corresponding declines, estimated the trends from the simulated data, and calculated the error between simulated and estimated trends. In more detail, for each Red List category trend, we simulated the occurrences or abundances from either a generalised linear model, or a zero-inflated mixture model respectively. We then fitted the same model to the simulated data, retrieved the estimated parameters, and calculated the error in the simulated and estimated trend (Figure 3), and the rate of misclassification into IUCN categories. Details of the simulation exercises can be found in the appendix.

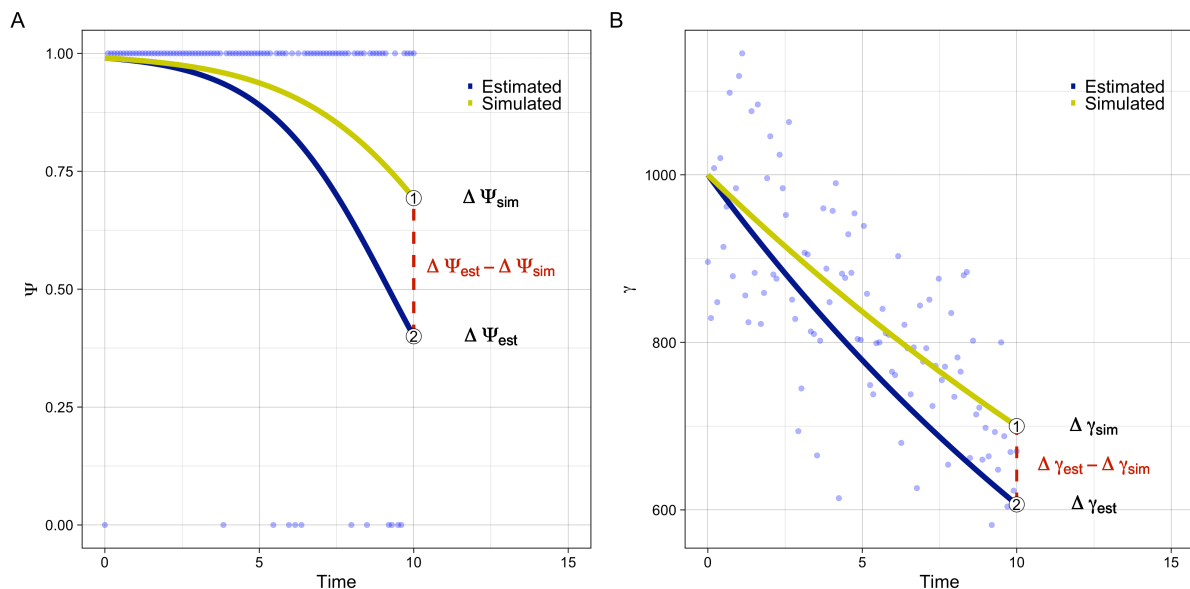


Figure B2. Illustration of calculating estimation error for simulated trends in occurrence probability (A), and read count (B). For each species detected in the survey we simulated IUCN specified trends (yellow lines) in occurrence probability (ψ) or read count as a proxy for relative abundance (γ). From these simulated trends we draw observations (light blue points) from a binomial distribution for occurrence and a zero-inflated Poisson distribution for abundance. The changes at year 10, i.e. the simulated trends in occurrence or read count ($\Delta \Psi_{sim}$, $\Delta \gamma_{sim}$ respectively), are indicated by the points labelled 1. The appropriate model was then fitted and the trend estimated (blue line) from the sets of simulated data. The estimated trends at year 10 ($\Delta \Psi_{est}$, $\Delta \gamma_{est}$) in each model are indicated by points labelled 2. The error (red dashed lines) is calculated by subtracting estimated trends from simulated trends.

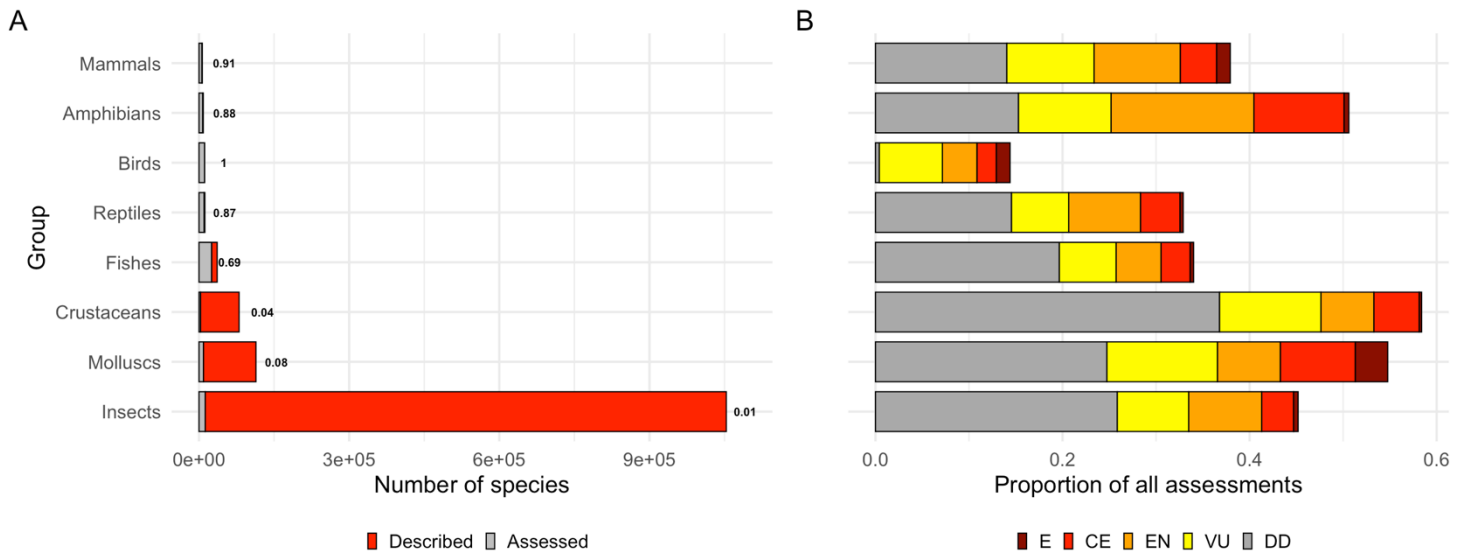


Figure 1. (A) Shows the number of described and assessed species in the groups with major representation (over 1000 assessments), on the IUCN red list. Red bars represent the total number of described species, and grey bars the number of species assessed by the IUCN. The numbers next to each bar represent the proportion of each group assessed. The proportion of assessments in each group that fall under the “Extinct” or “Extinct in the wild” category (“E” – dark red bars), “Critically Endangered” (“CE” – red bars), “Endangered” (“EN” – orange bars), “Vulnerable” (“VU” – yellow bars), and “Data deficient” (“DD” – grey bars). The remainder of assessments in each group consist of organisms classified as “Near threatened”, “Lower risk”, or “Least concern”. The important feature of (B), is that invertebrate species have considerably larger numbers of Data Deficient species than other animal groups.

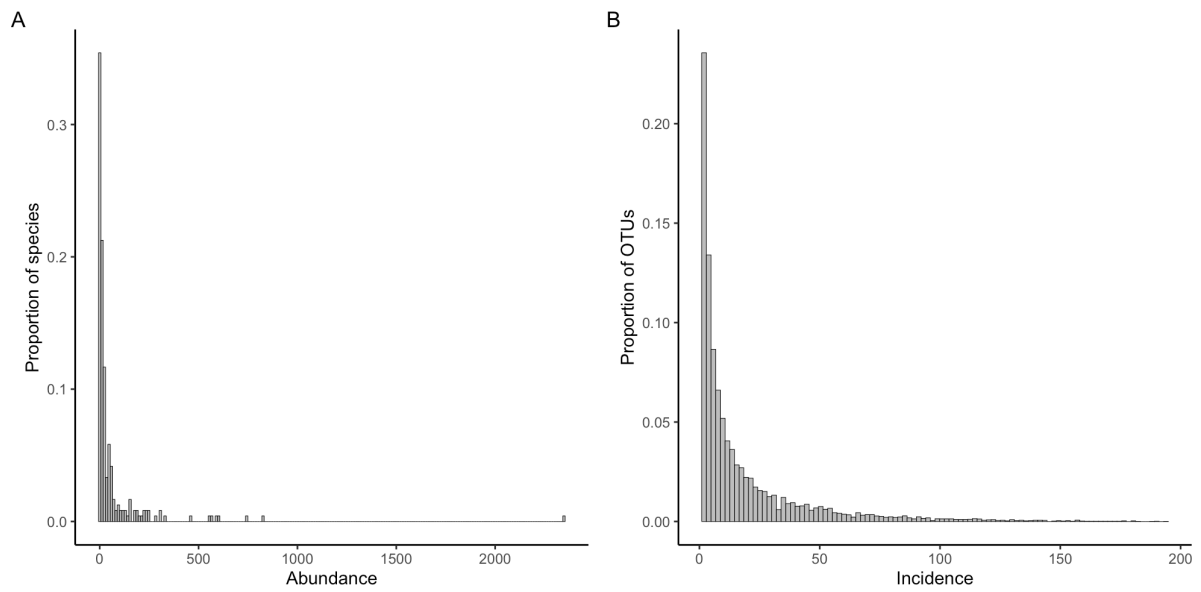


Figure 2. Displaying the similarities in the incidence and abundance frequencies of species caught from a single location in rothamsted UK between 1933-36 (A) and from the single year 198 site sampling effort in Sweden (B).

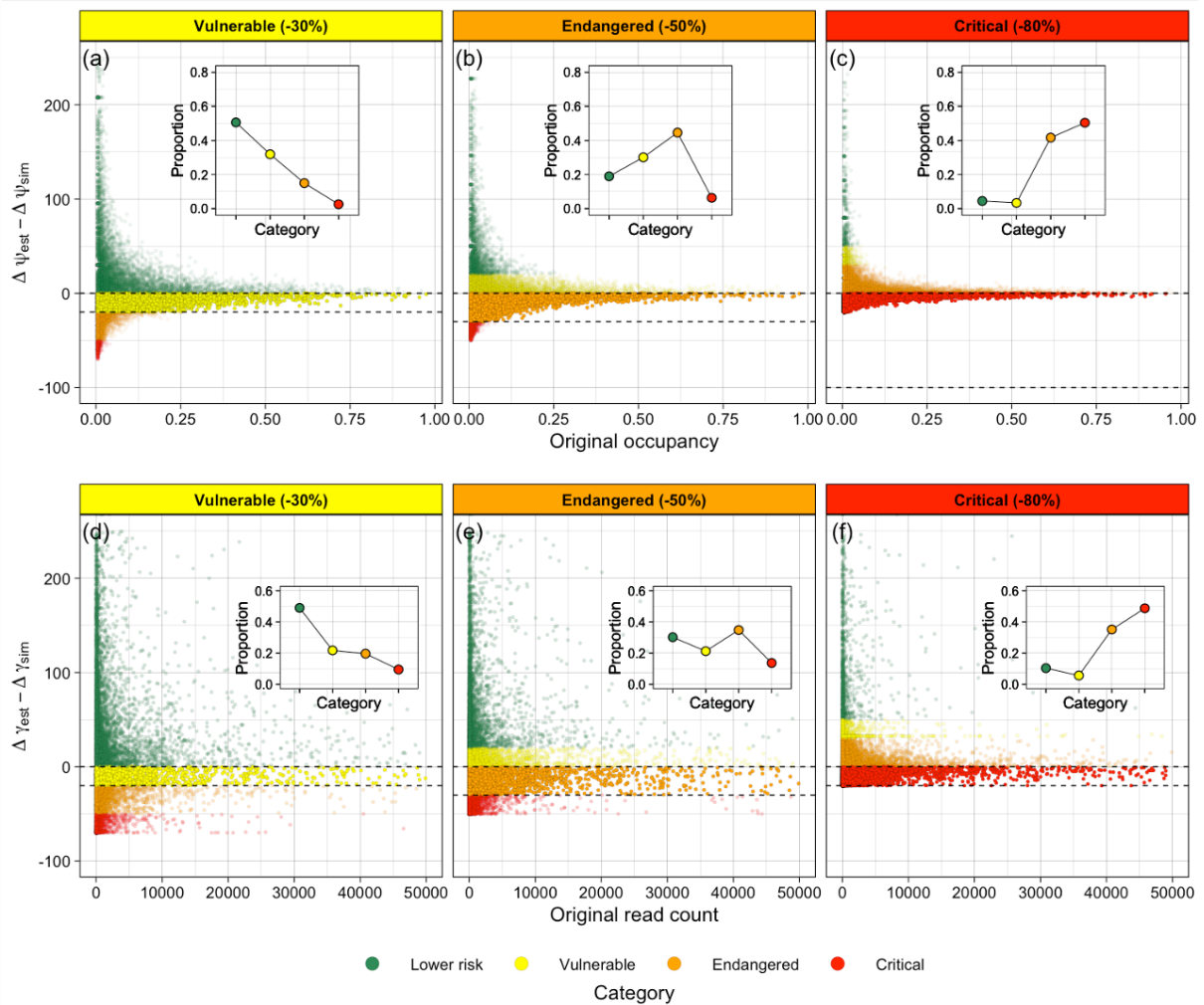


Figure 3. The percentage error in occurrence (a:c) and abundance (d:f) trends. $\hat{\psi} - \psi$ represents the estimated occurrence – simulated occurrence and $\hat{\gamma} - \gamma$ represents estimated abundance – simulated abundance. Occurrence trends are displayed versus the original occurrence frequency for organisms, and abundance trends versus original read count as a proxy for abundance. Each organism was simulated to experience the minimum trends classifying them as “Vulnerable (-30%)”, “Endangered (-50%)”, or “Critically Endangered (-80%)” according to IUCN red-list Criteria A. Each individual point represents the lower confidence interval around the point estimate of the error in the simulated and estimated trend for a single species detected in our data. The colour of each point highlights the category to which the species would be classified based on the estimated trend in population size and horizontal dashed lines border outcomes with a correct classification. Near Threatened (NT) and Least Concern (LC) categories have been merged to a single category (“Lower risk”). Both axes have been truncated to allow easier visualization of the distribution. Insets illustrate the proportion of all species that were classified as each of these categories.

Methods:

Arthropod surveys:

Arthropod samples were collected using malaise trap sampling across Sweden over a 12-month period in 2019. Traps collected arthropods directly into 0.5L bottles containing approximately 400ml of 95% ethanol. Samples were collected by a network of volunteers who also maintained traps throughout the year. Traps were emptied weekly between April to October, and monthly in the remainder of the year. Traps in northern latitudes were not sampled in the portion of the year in which there was too much snow to operate a malaise trap. The spatial layout of the traps was designed to sample arthropod communities present in all of the major Swedish eco-regions and climates. In total we collected 4707 insect community samples.

Molecular pipeline:

After completing all steps of the DNA extraction and purification, we amplified 418 bp of the cytochrome b mitochondrial gene following the FAVIS protocol (add ref). Samples were then sequenced on an Illumina NovaSeq 6000 SPrime flow cell and sequencing data was processed bioinformatically following pipelines that can be accessed via the following links: <https://github.com/biodiversitydata-se/amplicon-multi-cutadapt> (read trimming and filtering); <https://nf-co.re/ampliseq> (ASV reconstruction and taxonomic annotation). In short, we use cutadapt v.3.2 (Martin, 2011) for primer trimming and R package DADA2 v.4.2.1 for denoising (Callahan *et al.*, 2016). Then we use SINTAX (Edgar, 2016) in order to get the taxonomic assignment for all ASVs using a custom-made reference COI database (<https://doi.org/10.17044/scilifelab.20514192.v4>). Then we used a *uchime* algorithm implemented in *vsearch* (Rognes *et al.*, 2016) to filter out chimeric sequences and perform clustering with SWARM (Mahé *et al.*, 2014) with $d=13$. Additional cleaning up steps to filter out unassigned or ambiguous ASVs, remove ASVs present in more than 5% of negative controls and eliminate ASVs with a very small number of reads (<3 total reads) were done with a custom-made script (https://github.com/johnne/clean_asv_data). The bioinformatic processing and filtering resulted in 442,409 cleaned ASV sequences grouped into 33,888 clusters.

Statistical analysis:

Simulations:

To demonstrate the effect of small sample sizes on classification into IUCN Red List categories, we simulated observations using models often used in ecology to estimate species occurrence probabilities and trends in population size. For each Red List category trend, we simulated the occurrences or abundances from either a generalised linear model, or a zero-inflated mixture model respectively. We then fitted the same model to the simulated data, retrieved the estimated parameters, and calculated the error in the simulated and estimated trend (Figure 3), and the rate of misclassification into IUCN categories. Details of the simulation exercises can be found in the appendix.

To establish the structure of species incidence across a hyper-diverse community, we use the occurrence data generated by the “Insect Biome Atlas” (IBA) project, one of the largest inventories of invertebrate biodiversity worldwide (Figure 1). This survey represents one of the largest design-based monitoring programs of terrestrial arthropod diversity. The project involved weekly sample collection from a network of 198 malaise traps in Sweden, consisting of a total of 4748 samples and 26 kg of invertebrates, collected over the course of a single year. Samples were collected between February and December 2018. Samples were processed and species were identified using a high throughput molecular pipeline (described in detail in Iwaszkiewicz-Eggbrecht et al 2023b). From this pipeline, we used data for 13144 arthropod OTUs that were assigned species-level taxonomies from a reference database. We used the average incidence frequency of each species (i.e. the average observed occurrence of a species across all sites in Sweden – Figure S1A) to represent occurrence probability as an indicator of range size. We used read counts as a proxy for within species abundance (Figure S1B). This is rapidly becoming a standard measure of approximate abundances in surveys of taxonomically challenging and/ or hyperdiverse taxa, including insects (Aizpurua et al., 2018; Bista et al., 2018; Deagle et al., 2019, Piper et al., 2019; Vasar et al., 2022). While relative (i.e. between species) abundances in environmental data may be affected by a number of biases (Iwaszkiewicz-Eggbrecht 2023a), we use these to approximate the emergent distribution of species abundances in our data, noting that the same distributions are supported by independent, non-molecular data.

Changes in range size:

We used generalised linear models to simulate changes in range size as they are often used in ecology to estimate geographic ranges (Norberg *et al.*, 2019). For range size simulations we used occurrence probability as an estimate of geographic extent and we simulated changes in range sizes using logistic regression:

$$\begin{aligned} \text{logit}(\Psi_{its}) &= \beta_s + x_{it}\beta_{1s} \\ Y_{its} &\sim \text{bernoulli}(\Psi_{its}), \end{aligned}$$

β_{0s} is the logit of the occurrence of species s calculated from the IBA incidence data (i.e. the number of sites that species occurred in/ the total number of sites), and β_{1s} is the species-specific slope coefficient that corresponds to the minimum trend in occurrence of a given Red List category (i.e. -30% , -50%, -80%). Ψ_{its} is therefore the probability of occurrence of species s at time t at sampling site i . For each Red List category trend, we simulated the occurrence by drawing 200 observations from a bernoulli distribution $Y_{its} \sim \text{bernoulli}(N, \Psi_{its})$. We then fitted the same model to the simulated data and calculated estimated change in occurrence probability (i.e. the trend) for each species at year 10, which was used to classify them into IUCN categories.

Changes in an index of population size:

Population dynamics of sparsely occurring organisms are often driven by two different ecological processes, one that dictates whether organisms occupy a site, and one that drives the local abundance at that site. For population size analysis we therefore used a zero inflated mixture model (Wenger and Freeman, 2008) to model the probability of occurrence, and changes in abundance at each individual site that a species occurs:

$$\begin{aligned} \text{logit}(\Psi_s) &= \alpha_s \\ \log(\gamma_{its}) &= \beta_{0s} + x_{it}\beta_{1s} \end{aligned}$$

$$\pi_{its} \sim \text{bernoulli}(\Psi_{its})$$

$$Y_{its} = \begin{cases} 0 & , \text{ if } \pi_{its} = 0 \\ \text{negbinom}(\gamma_{its}, \theta) & , \text{ if } \pi_{its} = 1 \end{cases}$$

Here we assume that only the index of abundance (γ_{its}), is sensitive to change over time, and probability of occurrence (Ψ_s), remains constant. The parameter α_s is again the logit of the occurrence of species s calculated from the IBA incidence data. β_{0s} is the initial log abundance of species s and β_{1s} is the species-specific slope coefficient that corresponds to the trend in abundance of a given Red List category. x is the value of the covariate representing the year, taking the values 0-10. π is a Bernoulli distributed indicator of whether a site was occupied or not, and controls whether the abundance is a true 0 due to the site being unoccupied, or produced as a result of sampling stochasticity by drawing observations from a negative binomial distribution $Y_{its} = \text{negbinom}(\lambda_{its}, \theta)$. The parameter θ that controls overdispersion in the negative binomial distribution is set to the average value estimated across all species ($\theta = 0.23$).

For both models we simulated each IUCN trend over 10 years of data (i.e $\mathbf{X} = x_1 \dots x_{10}$) and compared the simulated trends in occurrence probability ($\Delta \Psi_{sim}$) and our abundance proxy ($\Delta \gamma_{sim}$) to estimates produced by fitting the same models to the simulated observations. For each species we calculated the percentage error in the predicted and simulated occurrence probability ($\Delta \Psi_{est} - \Delta \Psi_{sim}$) and population trends ($\Delta \gamma_{est} - \Delta \gamma_{sim}$) at the end of the 10-year time period. We compared the number which, according to the IUCN categories, were classified correctly, or were assigned a less severe or more severe Red List status. We classify any category lower than “Threatened” as “Lower risk” which encompasses the “Least Concern” (LC) and “Near Threatened” (NT), categories. All models were fitted using maximum likelihood estimation. Models were inspected for convergence errors and models that failed to converge were excluded from further analysis.

All analyses were run in the R programming language version 4.2.2 (2022-10-31) (R Core Team 2022). We used the ‘pscl’ package to run our zero-inflated mixture models of abundance (Zeileis, Kleiber and Jackman, 2008), and the ‘glm’ function included with

base R to run models of occurrence probability. For both models we take the lower end of the confidence interval around the point estimate according to IUCN red-listing guidelines. For zero-inflated models, these intervals were calculated using bootstrapping, implemented in the 'boot' package (Cantey & Ripley 2022).

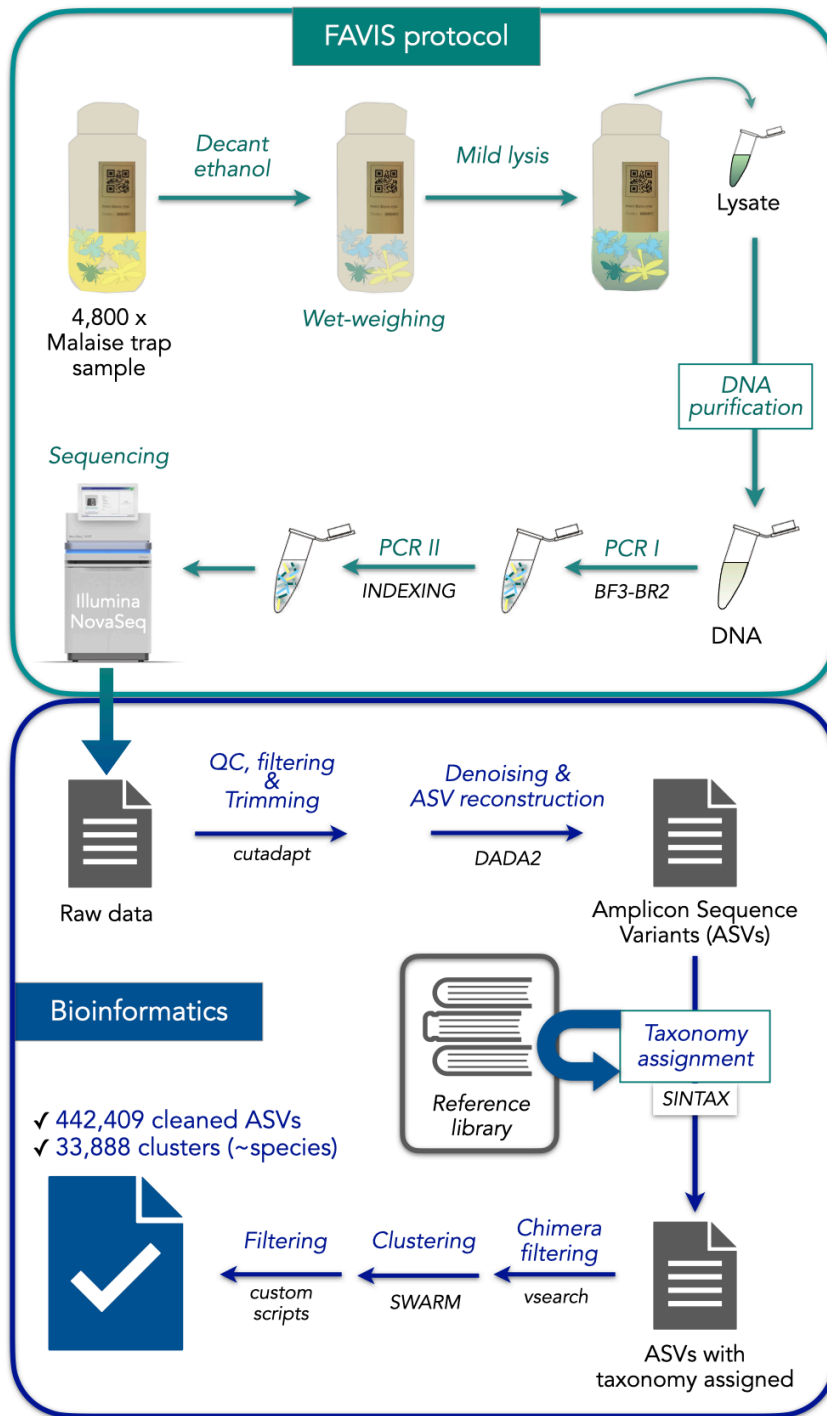


Figure S1. An overview of the FAVIS protocol for DNA extraction, purification, library preparation, and sequencing (Top panel) and the bioinformatics pipeline used to prepare sequences for analysis.

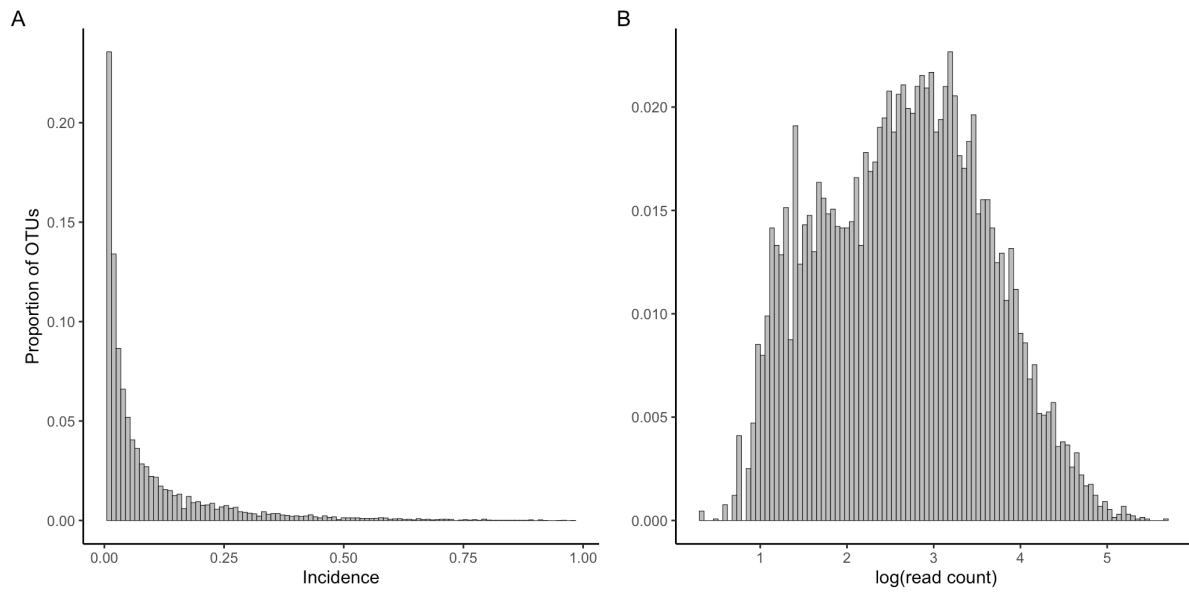


Figure S2. Data used in the simulation of abundance or occurrence trends. (A) The incidence (occurrence) frequencies for each operational taxonomic unit (OTU) assigned a species-level identification in the Swedish data, i.e. proportion of total sites occupied by each species. (B) The log of read counts for each OTU in the Swedish data, corresponding to a proxy of within-species abundance.

Criteria	Vulnerable	Endangered	Critically Endangered
A. Declines in population size / geographic range	<p>≥ 30% OR ≥ 50% for case A1</p> <p>Based on:</p> <ul style="list-style-type: none"> a) Direct observation b) An index of abundance c) Decline in range d) Levels of exploitation e) Effects of introduced taxa 	<p>50% OR ≥ 70% for case A1</p> <p>Based on:</p> <ul style="list-style-type: none"> a) Direct observation b) An index of abundance c) Decline in range d) Levels of exploitation e) Effects of introduced taxa 	<p>80% OR ≥ 90% for case A1</p> <p>Based on:</p> <ul style="list-style-type: none"> a) Direct observation b) An index of abundance c) Decline in range d) Levels of exploitation e) Effects of introduced taxa
B . Geographic range	<p>B1 AOO < 2000 km² OR</p> <p>B2 EOO < 20000 km²</p> <p>AND two of:</p> <ul style="list-style-type: none"> a) No. locations ≤ 10 b) Continuing decline c) Extreme fluctuations 	<p>B1 AOO < 500 km² OR</p> <p>B2 EOO < 5000 km²</p> <p>AND two of:</p> <ul style="list-style-type: none"> a) No. locations ≤ 5 b) Continuing decline c) Extreme fluctuations 	<p>B1 AOO < 10 km² OR</p> <p>B2 EOO < 100 km²</p> <p>AND two of:</p> <ul style="list-style-type: none"> a) No. locations = 1 b) Continuing decline c) Extreme fluctuations

C. Small population size AND decline	No. of mature individuals < 10,000 AND ONE OF: C1) decline of 10% C2) a) ≤ 1000 mature individuals b) 100% of individuals are mature c) Extreme fluctuations	No. of mature individuals < 2500 AND ONE OF: C1) decline of 20% C2) a) ≤ 250 mature individuals b) 95-100% of individuals are mature c) Extreme fluctuations	No. of mature individuals < 250 AND ONE OF: C1) decline of 25% C2) a) ≤ 50 mature individuals b) 90-100% of individuals are mature c) Extreme fluctuations
D. Very small or restricted population	No. of mature individuals < 1000	No. of mature individuals < 250	No. of mature individuals < 50
E. Quantitative analysis of extinction risk.	≥ 10% in 100 years	≥ 20% in 20 years or 5 generations.	≥ 50% in 10 years or 3 generations.

Table 1. Summary of IUCN Red-list criteria not used in our analyses. AOO – area of occupancy. EOO – extent of occurrence. More detailed explanations of the cases for criteria A can be found in table S1 of the supplementary information.

Results:

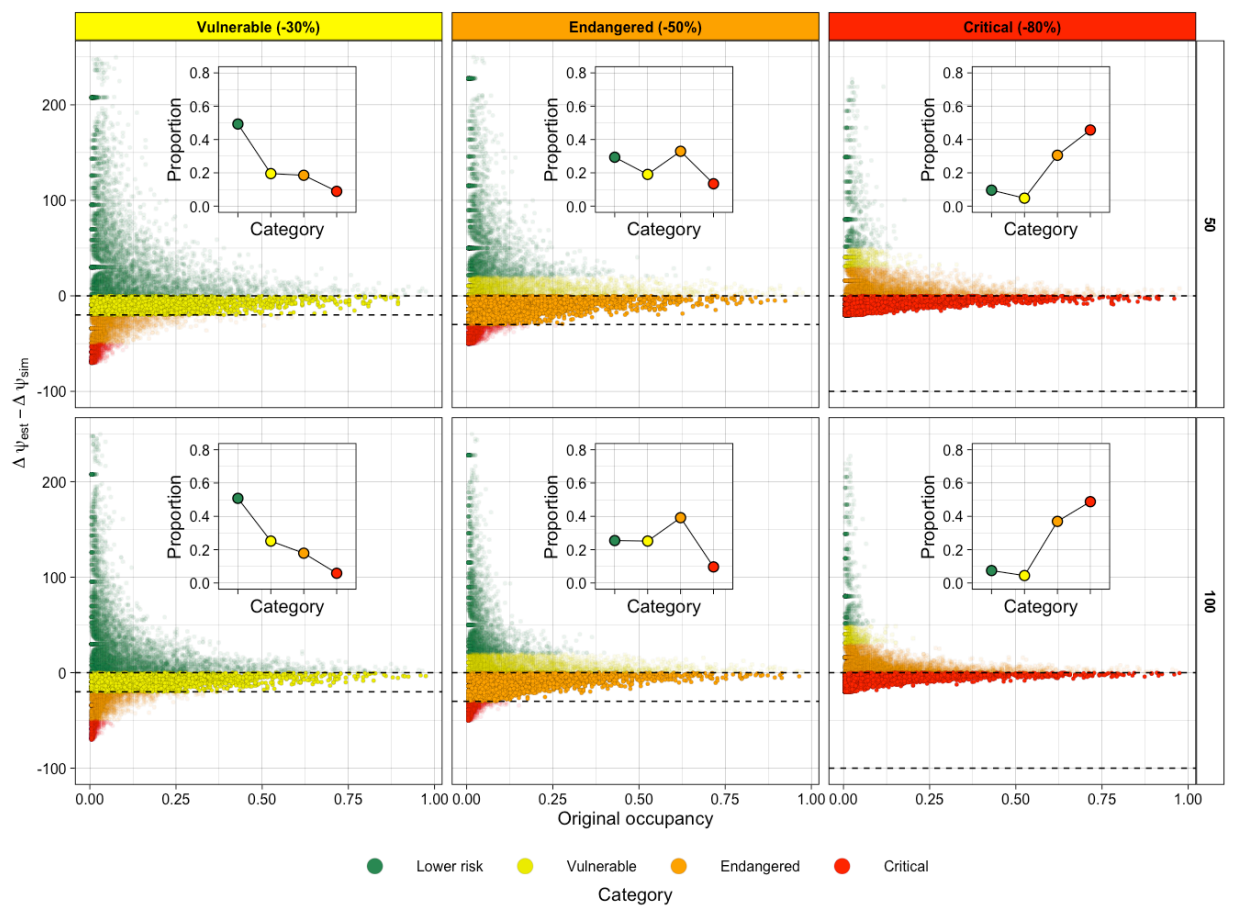


Figure S3. The percentage error in occurrence trends ($\hat{\Psi} - \Psi$: estimated occurrence – simulated occurrence) versus original incidence frequency for organisms simulated to display trends classifying them as “Vulnerable” (a) , “Endangered” (b), or “Critically Endangered” (c) according to IUCN red-list criteria. Each row represents these error distributions for different sample sizes. Each individual point represents the error in the simulated and estimated trend for a single species detected in the IBA data. The colour of each point highlights the category to which the species would be classified based on the estimated trend in population size and horizontal dashed lines border outcomes with a correct classification. Near Threatened (NT) and Least Concern (LC) categories have been merged to a single category (“Lower risk”). Both axes have been truncated to allow easier visualization of the distribution. Insets illustrate the proportion of all species that were classified as each of these categories.

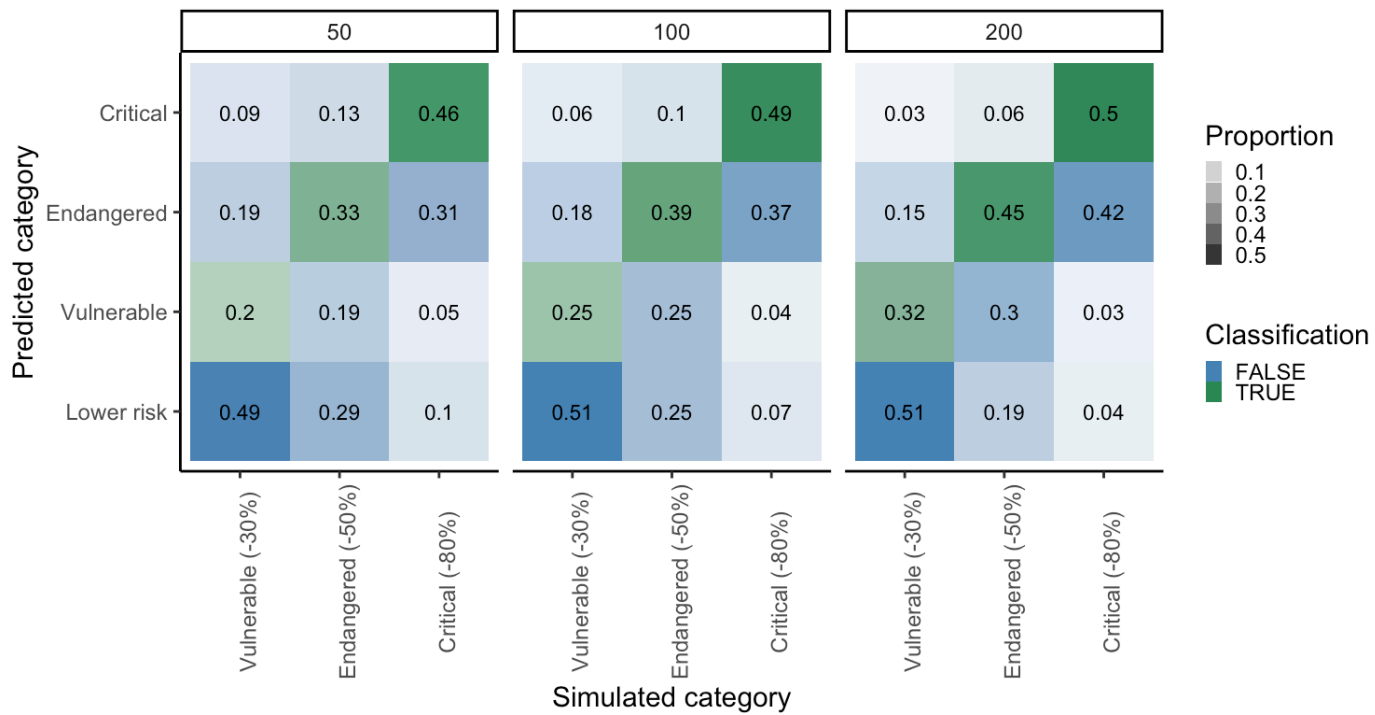


Figure S4. Confusion matrices showing the proportion of all species estimated to lie within each IUCN category against their simulated range size trend categories. The colour of each grid square indicates whether classifications were correct (green), or incorrect (blue). The transparency indicates the total proportion of species within each simulated & estimated category combination. Each panel illustrates the prediction errors for different sampling efforts (50,100,200 samples respectively).

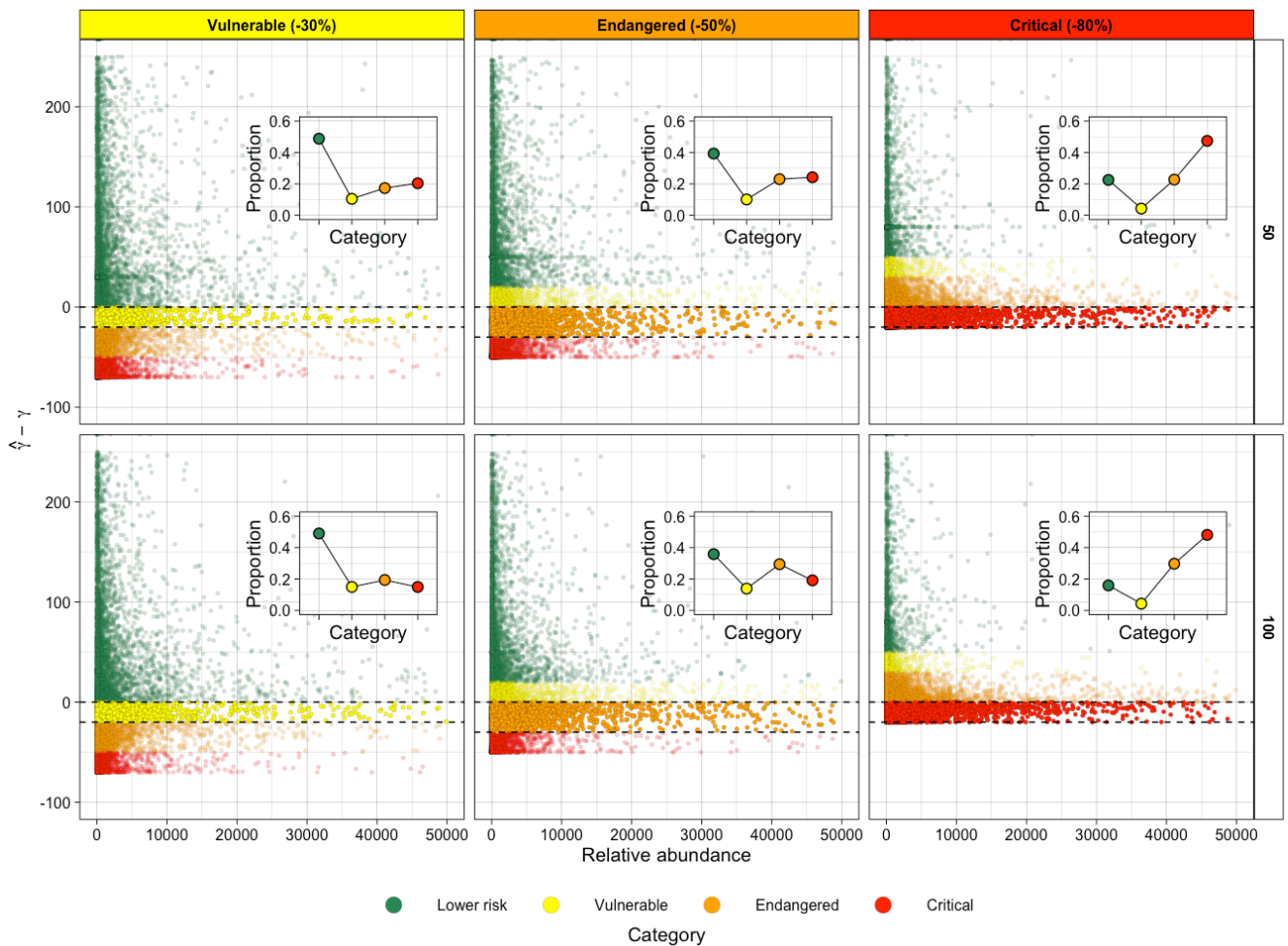


Figure S5. The percentage error in abundance trends ($\hat{\gamma} - \gamma$: estimated abundance – simulated abundance) versus original species abundance for organisms simulated to display trends classifying them as “Vulnerable” (a), “Endangered” (b), or “Critically Endangered” (c) according to IUCN red-list criteria. Each row represents these error distributions for different sample sizes. Each individual point represents the error in the simulated and estimated trend for a single species detected in the IBA data. The colour of each point highlights the category to which the species would be classified based on the estimated trend in population size and horizontal dashed lines border outcomes with a correct classification. Near Threatened (NT) and Least Concern (LC) categories have been merged to a single category (“Lower risk”). Both axes have been truncated to allow easier visualization of the distribution. Insets illustrate the proportion of all species that were classified as each of these categories.

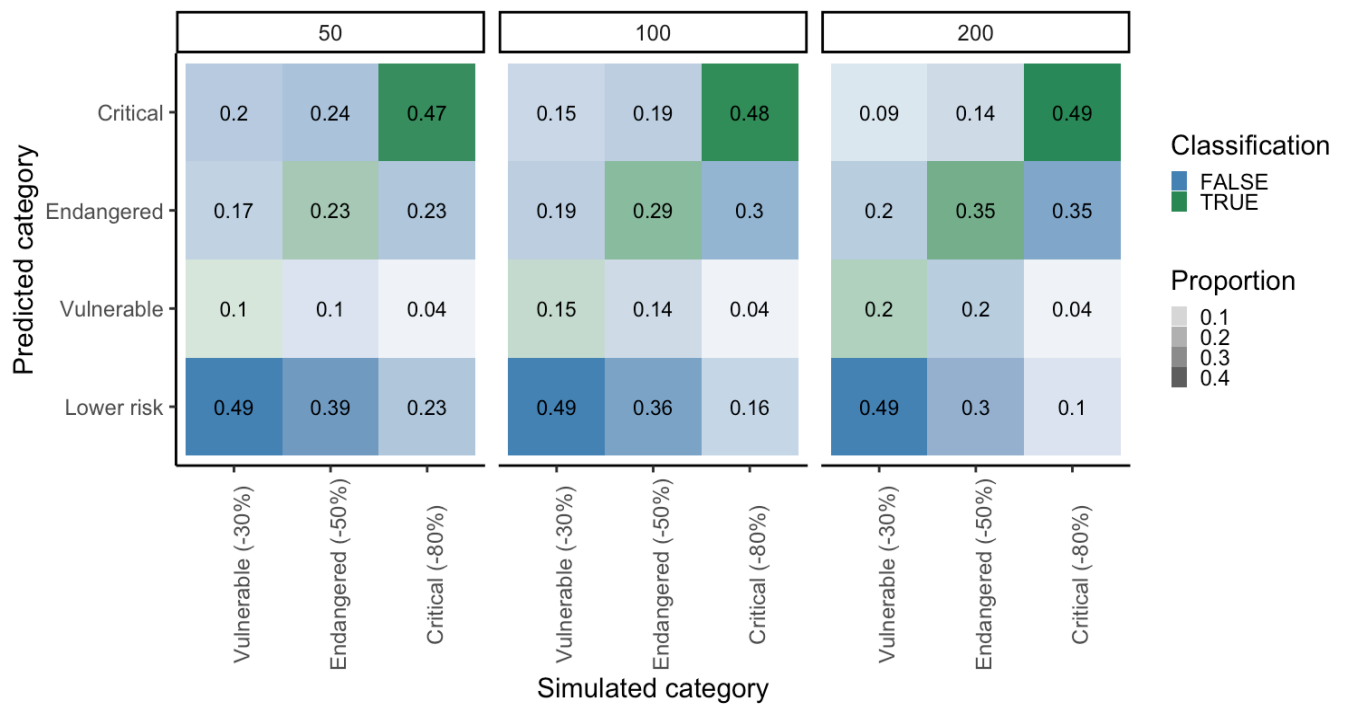


Figure S6. Confusion matrices showing the proportion of all species estimated to lie within each IUCN category against their simulated abundance trend categories. The colour of each grid square indicates whether classifications were correct (green), or incorrect (blue). The transparency indicates the total proportion of species within each simulated & estimated category combination. Each panel illustrates the prediction errors for different sampling efforts (50,100,200 samples respectively).

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