Moving towards better risk assessment for invertebrate conservation.

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Abstract

Global change threatens a vast number of species with severe population declines or even extinction. The threat status of an organism is often designated based on geographic range, population size, or declines in either. However, invertebrates, which comprise the bulk of animal diversity, are conspicuously absent from global frameworks that assess extinction risk. Many invertebrates are hard to study, and it has been questioned whether current risk assessments are appropriate for the majority of these organisms. As the majority of invertebrates are rare, we contend that the lack of data for these organisms makes current criteria hard to apply. Using empirical evidence from one of the largest terrestrial arthropod surveys to date, consisting of over 33,000 species collected from over a million hours of survey effort, we demonstrate that estimates of trends based on low sample sizes are associated with major uncertainty and a risk of misclassification under criteria defined by the IUCN. 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, and there is likely to be substantial uncertainty in assessing risk for the majority of global biodiversity using species-level trends. In response, we discuss the need to focus on metrics we can currently measure when conducting risk assessments for these organisms. We highlight modern statistical methods that allow quantification of metrics that could incorporate observations of rare invertebrates into global conservation frameworks, and suggest how current criteria might be adapted to meet the needs of the majority of global biodiversity.

The underrepresentation of invertebrates.

Rapid rates of environmental degradation threaten biodiversity worldwide (Garcia *et al.*, 2014) and concerted conservation efforts are required to mitigate the impacts of global change (Synes *et al.*, 2020; Williams *et al.*, 2021). Invertebrates are at the forefront of this crisis, comprising the majority of species, as well as some of the organisms most vulnerable to environmental pressure. Recent research has provided evidence for 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). However, representation of these organisms in monitoring programs and global conservation efforts is notoriously poor.

A glaring example of the neglect of invertebrates in assessments of nature is their poor representation on the IUCN Red List, which is a central pillar of global biodiversity conservation. Assessments provided by the Red List often underpin 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) (Rodrigues *et al.*, 2006; Vie *et al.*, 2009; Bland *et al.*, 2019; Betts *et al.*, 2020). Under this framework the majority of vertebrates have received an assessment, and notably all 11,188 bird species have received multiple assessments each (IUCN, 2021). Yet, of the one million described species of insects, only 1.2% (~12,000) have received an assessment (IUCN, 2021) (Figure 1A), and a considerably higher proportion of invertebrate (compared to vertebrate) species are listed as data deficient (Figure 1B).

The current status quo is alarming. There is strong evidence to suggest that major components of global biodiversity are threatened by global change, whilst our current perspective of which organisms are threatened relies on selective information (Cardoso, Borges, *et al.*, 2011; Cardoso *et al.*, 2012; Eisenhauer, Bonn and A. Guerra, 2019), invertebrate populations are suffering widespread and rapid changes world-wide, and our monitoring efforts are often limited in their ability to detect the full scope of these changes (Forister *et al.*, 2023). Meanwhile, our conservation frameworks and policy instruments fail to sufficiently represent the majority of global biodiversity and the risk that they face.

Impediments to invertebrate monitoring and conservation.

Invertebrates are notoriously difficult to identify and study, and these issues produce several fundamental impediments to invertebrate conservation (Cardoso et al 2011). These include their relative unpopularity with the public, policy makers, and scientists, their overwhelming *under*-description compared to their diversity, and dwindling taxonomic expertise (Hochkirch *et al.*, 2022). It is generally accepted that these impediments not only limit our understanding of invertebrate communities, but also prevent the widespread assessment of invertebrate extinction risk under global conservation frameworks. Previous debates have also focused on whether risk assessment criteria themselves (such as those used by the IUCN Red List - Table S1) are applicable to many invertebrate taxa, since data might be hard to acquire or the standard thresholds might provide inappropriate measures of relative risk for small organisms with high reproductive rates (Tscharntke *et al.*, 2007; Cardoso, Erwin, *et al.*, 2011; Cardoso *et al.*, 2012; Collen and Böhm, 2012; Eisenhauer, Bonn and A. Guerra, 2019; Fox *et al.*, 2019; Akçakaya *et al.*, 2021). For example, estimating *total* population sizes for insects is often extremely difficult, which might explain why only 0.0016% of total insect assessments are completed under the IUCN criteria that designates risk due to absolute population size (Criteria C). The well-established impediments to invertebrate conservation, and the potentially poor fit of some assessment criteria for a hyper-diverse group of organisms, result in a reduced set of tools by which we can provide an assessment, and limit the rate of invertebrate threat assessments.

The rarity of invertebrates

Despite the applicability of some criteria being questioned, assessments based on abundance and range size *trends* are fundamentally useful measures of the threat faced by an organism, and the majority of invertebrate assessments are performed using these metrics. However, a consistent feature of invertebrate communities is that the majority of organisms are extremely rare. This pattern was documented in a seminal paper in 1943 (Figure 2 A), using data from a 5-year Lepidoptera survey in Rothamsted UK. In this study approximately 14% of species were observed only once. After 80 years of empirical work, the same pattern remains. In studies of invertebrate fauna; 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). Figure 2C:D illustrates the persistence of this pattern - across high profile datasets and biodiversity data bases, invertebrate communities are still dominated by rare organisms.

Modern sampling techniques, using high throughput DNA sequencing and molecular taxonomy provide a route to rapid identification of invertebrates, and a key tool in improving our understanding of their diversity and ecology. In 2019 we conducted an intensive and systematic molecular survey of terrestrial arthropods in Sweden (Box 1) (Miraldo *et al.*, 2024), this survey represents one of the largest and most sophisticated arthropod surveys to date. In total we collected over 4700 weekly samples of arthropod communities from 198 sites, representing over 1.5 million hours of survey effort. Despite the enormous sampling effort, and state of the art 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 – an abundance of rarity is an inherent feature of invertebrate communities.

Assessing trends and distributions

Unfortunately, the reality of this consistent pattern in invertebrate community data is that, for the majority of species, we are unable to reliably estimate the changes in their population sizes or ranges due to a lack of sufficient data. Due to the inherent statistical relationship between sample size and uncertainty, low abundances or occurrences are intrinsically linked to low statistical power, and any estimands are therefore difficult to quantify without considerable degrees of uncertainty (van Proosdij *et al.*, 2016; Jeliazkov *et al.*, 2022; Yoccoz, 2022; Erickson and Smith, 2023). To illustrate how classification using quantitative criteria may produce uncertain estimates we use the empirical incidence and abundance distributions revealed by the data described in Box 1 to demonstrate the uncertainty in classification of risks.

We simulate decreases in occurrence and an index of population size (Box 2) using the empirically derived measures from our data (Box 1). We focus on trends, as being able to reliably detect changes in range or population size is a pre-requisite for evaluating whether species are in decline, but also whether any interventions are effective conservation measures. We use criteria defined by the IUCN Red List, as it is the most well-known conservation framework to assess extinction risk. Although we only directly apply the thresholds for Criteria A, it should be noted that reliable detection of trends are also requirements for Criteria C, and Criteria B. Estimated trends in occurrence and abundance for rare species is associated with a high percentage error (Figure 3), when applying IUCN thresholds to determine a Red List category this resulted in high levels of misclassification for both Vulnerable (Figure 3A & D), and Endangered (Figure 3B & 3E) organisms.

In the light of the consistent pattern of rarity in invertebrates, it is extremely difficult to reliably quantify changes to most populations– even with the best available data. For rare species, there is a high degree of uncertainty when estimating trends in occurrence or abundance, and using established criteria to evaluate risks results in high degrees of misclassification. This uncertainty and the inherent dangers will be even worse for lower sampling intensities (Figures S2 to S5) which are more reflective of long-term monitoring efforts (Hallmann *et al.*, 2017; Crossley *et al.*, 2020). We show that smaller changes in abundance are harder to estimate accurately, especially with low sample sizes. Yet, as rare species are particularly at risk of being threatened (Purvis and Hector, 2000; Purvis *et al.*, 2000; Jetz and Freckleton, 2015), high uncertainty in range or population size trends will constrain our decision making for those organisms most urgently needing an assessment. Similarly, 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 risk 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.

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. In the absence of adequate data to estimate population and range size trends, the process is reliant on taxonomic expertise. However, the supply of such experts is limited (Hochkirch *et al.*, 2022) and, critically, 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, resign to the fact that an organism cannot be assessed and must be categorized as “Data deficient”. This, we argue, renders the process difficult to replicate, and will limit the representation of invertebrates in our global conservation efforts.

Avenues for increasing invertebrate representation in conservation efforts

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 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 using species-level trends under current frameworks (IUCN, 2022) in conjunction with the best possible data, will result in one of two outcomes: a failure to provide risk assessment for the majority of earths organisms, or potentially inaccurate classification of threat categories for many organisms.

Importantly, the extensive data generated by our study (Box 1) are an exception, as most monitoring efforts contain fewer sites, and are often restricted to protected areas (Forister *et al.*, 2023). For conservation efforts to succeed they must be based on quantitative evidence, as such we suggest three possible routes towards better risk assessment for invertebrates (Figure 4), all of which rely on information that is currently measurable from standard and molecular surveys. We highlight statistical approaches that can reliably quantify metrics of changes to invertebrate communities that incorporate information for rarely observed organisms. We also outline existing frameworks that can be used to guide the development of new criteria to classify risk to these organisms.

A) Improving single species inference

Although for many species data are limited, practitioners should initially try to understand risks to individual organisms, as a classification into a risk or Red List category is a useful tool for conservation, public engagement, and policy making (Rodrigues *et al.*, 2006; Bennun *et al.*, 2018; Bachman *et al.*, 2019; Bland *et al.*, 2019; Betts *et al.*, 2020). The first approach is, therefore, to improve the species-level inference and assessment by employing statistical techniques that leverage the structure of community data (Figure 4A). There is a growing appreciation of the importance and dominance of rare species in the ecological monitoring literature (Jeliazkov *et al.*, 2022; Yoccoz, 2022), and numerous techniques have been suggested to improve species-level inference for rare organisms. Despite the lack of data for individual organisms, community-level datasets often contain large numbers of observations across many thousands of species with shared evolutionary histories, spatiotemporal distributions, and ecological traits. This structure within community data can be used to allow data poor species to borrow strength from closely related or ecologically similar organisms. Inferring similarities among species based on these features is now common practice among quantitative ecologists, allowing more robust estimates to be made for organisms with sparse records (Ovaskainen *et al.*, 2017; Norberg *et al.*, 2019; Jeliazkov *et al.*, 2022). As the appreciation for the importance and dominance of rare species has grown, more sophisticated methods have emerged. For example, Ovaskainen et al (2024) demonstrate a transfer learning approach to improve species level inference for hundreds of thousands of species, most of which only provide a handful of observations. The benefit of a combining the single-species approach with information obtainable from the community level is that it offers tangible assessments linked to individual species for practitioners, the public, and policymakers. These statistical techniques can be directly incorporated into current risk assessment practice, such as the IUCN Red Listing process, since they can be used to derive the single-species metrics on which current assessments are focused. A relatively simple improvement would be to update IUCN guidelines to include advice on how ranges and population trends can be estimated using hierarchical modelling. Collaboration with quantitative ecologists on the best practices when these techniques, and how to use them with current data will be necessary to help improve single species inference. However, it is important that any changes made to advice should attempt to retain the flexibility of the original assessment process.

B) Improving group-level inference

Despite growing appreciation of the issue of rare species and advances in statistical methodology, alternatives to the single-species focus of current frameworks might be prudent to allow including species with too few observations even for more sophisticated statistical techniques. A group-level approach could provide a tractable way of using currently available data to inform conservation decisions for multiple species simultaneously. As many rare organisms often come from similar taxonomic groups, share traits, or display similar ecological responses or distributions, the second option is to assess trends across *groups* of organisms. Similar to our first approach (*Improving single species inference*), integrated models can be used to improve inference for community level metrics using the structure within community data or integrating data from different sources (Miller *et al.*, 2019; Simmonds *et al.*, 2020; Zulian, Miller and Ferraz, 2021; Doser *et al.*, 2022; Lauret *et al.*, 2023; Zipkin *et al.*, 2023). This approach can then be used to quantify changes in community-level diversity metrics that might indicate risks. Approaches that pool observations across taxonomic groups can also be used to improve species level inference, whilst simultaneously modelling group-level responses (Adjei *et al.*, 2024). We envision a potential approach where data-poor species are pooled into higher taxonomic levels (e.g. a genus), and abundance or distributional trends evaluated as a part of this cluster. Many of the statistical techniques that can be used to improve single-species approaches may also guide group-level assessment of trends. Estimates are often derived hierarchically for both the group and its members (see *Improving single species inference*), and where estimates at the level of species prove too uncertain, estimates at the group level may provide a less specific, but useful measure of risk. For example, tropical endemic groups with limited distributions but too few observations for single species assessments would make good candidates for group level metric estimation. Another useful approach is to group species based on sensitivities to change, something that would naturally reflect a measure of extinction risk with regards to a changing environment. Species archetype models (Dunstan, Foster and Darnell, 2011; Hui *et al.*, 2013; Rognstad *et al.*, 2021; Yu *et al.*, 2022) can achieve this by clustering organisms by their environmental responses and assigning an 'archetype' defining how different groups respond to different environmental variables. The distributions and trends of groups (as well as individual species) can then be estimated over time, and used to assess risks of co-localised groups of organisms. Estimating and clustering organisms based on their sensitivities to environmental change synergises well with the capacity of the current Red List criteria that allow for risk designation based on *projected* trends. For example, if group-level sensitivities to habitat cover covariates predict distributional or abundance declines, then current IUCN criteria can be adapted to classify these risks.

Importantly, group-level metric estimation should only be considered for organisms for which individual assessments are unobtainable, and the conservation requirements of organisms should be considered *before* group-level assessment. An essential criterion for group-level assessment is spatial association - *sensu* sharing the same habitat and resource use, and showing largely overlapping distributions. Firstly, this acts as a safeguard against potentially inappropriate groupings, organisms with wildly different distributions (e.g. localised in completely different habitats or regions), should clearly not be included in a group trend estimate. Second, it allows targeted conservation efforts to particular regions, with the same goal, e.g. habitat preservation or restoration.

A major benefit of these methods is that ecological recording schemes often already collect information at the group-level (e.g. O'Connor *et al.*, 2019; Breeze *et al.*, 2021), and these methods can take advantage of pre-existing data from standardised and citizen science recording programs. However, the metrics produced by these methods do not immediately complement existing risk assessment criteria, and new criteria must be developed to categorise threat levels from these metrics. Identification of appropriate taxonomic levels at which to assess organisms will require specific knowledge of the group or community of organisms, and the criteria must appropriately convey the threat posed to one or more species. However, a multi-taxon approach could increase the uptake of assessments and representation of invertebrates in global conservation assessments.

C) Improving bio-regional inference

An alternative approach is to incorporate observations of rare species into models that allow estimation of ecological or biological regions. Assessment would involve the monitoring of changes in the distribution and community composition of distinct 'bio-regions' quantified by a statistical model. Typically, statistical methods of this type define regions of geographical or environmental space that display similar community compositions (Figure 4C). Numerous quantitative methods have been developed to cluster regions in this manner (Hill *et al.*, 2020; Woolley *et al.*, 2020). Methods are generally divided up into those that cluster regions first then estimate distributions, and those that estimate species distributions first and cluster after. The most rigorous and robust however are those that conduct this analysis simultaneously (Foster *et al.*, 2013, Vanhatalo, Foster and Hosack, 2021) and we therefore recommend these methods wherever possible. Some of the most well defined of these methods are those that designate 'regions of common profile' (RCP's) (Foster *et al.*, 2013). These approaches define regions via a community 'profile', i.e. a common occurrence pattern of organisms displayed across its extent, which is governed by environmental variables. Changes in ranges of RCP's can then be quantified reliably with respect to changes in their community profiles, or due to changing environmental variables. A key strength of this approach is that statistical techniques to estimate bioregions are diverse and therefore flexible - methods are capable of using often a variety of input data, ranging from individual measures of occurrence to measures of community turnover (Leaper *et al.*, 2011; Stephenson *et al.*, 2018).

Another major benefit of this approach is that from a management perspective, regional level management is much more tractable than managing thousands of species individually. If there is one truism in conservation it is that management 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) (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, or negative trends in the extent of the modelled bio-region would provide quantifiable metrics on which to assess risk. Although no current frameworks exist specifically for bio-regional assessment, the IUCN Red List of threatened ecosystems (RLE) *(*Rodríguez *et al.*, 2011; Bland *et al.*, 2019*)* provides a useful framework to guide development of regional level risk assessment criteria. From a practical standpoint, the RLE has directly adopted many of the risk thresholds (e.g 30%, 50% and 80% range size decreases for RLE criteria A) from the Red-list of threatened species. Conceptually these same thresholds could then be applied to bioregional distribution changes, with the added benefit that species occurrences would be directly tied to the quantification of changes.

A basis in adequate monitoring

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 of invertebrates as major components of global biodiversity (Jeliazkov *et al.*, 2022). Fortunately, the techniques for doing so at scale are becoming more available, making this a more achievable goal in the near-term (Van Klink *et al.*, 2024). Applying these methods to identify groups and ecosystems that contain large numbers of endemic or threatened invertebrates, and then monitor these communities is essential to assess the effectiveness of conservation efforts. This, we feel, will lay the groundwork for providing better protection of threatened organisms for which we struggle to obtain sufficient data.

Table 1.

Summary of Criteria A of the IUCN Red List for Vulnerable (VU), Endangered (EN) and Critically Endangered organisms (CR).

Criteria		Based on:	VU	EN	CR
A1	Causes are reversible AND have ceased	a. Direct observation (except A3). b. An index of abundance. c. A decline in geographic range (Area of occupancy/ Extent of occurrence). d. Actual or potential levels of exploitation.	≥ 50%	≥ 70%	≥ 90%
A2	Causes are irreversible OR have not ceased		≥ 30%	≥ 50%	≥ 80%
A3	Reduction projected/ inferred/ suspected in the future (up to 100 years)		≥ 30%	≥ 50%	≥ 80%
A4	Reduction projected/ inferred/ suspected, causes have not ceased OR understood OR irreversible		≥ 30%	≥ 50%	≥ 80%

		e. Effects of introduced taxa, hybridization, pathogens, pollutants, competitors, or parasites.			

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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 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 33,000 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).

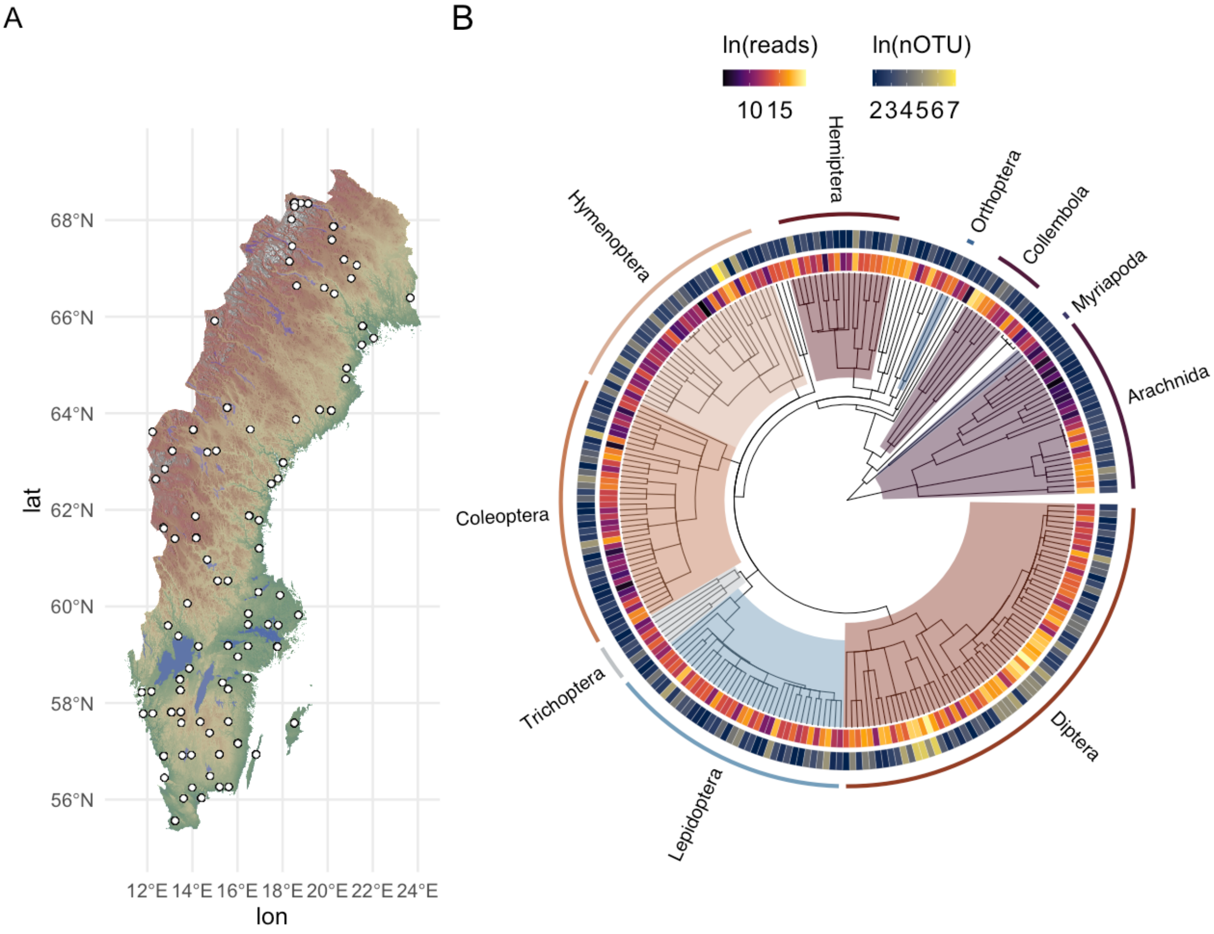

Figure B1. (A) The spatial layout of trap locations within Sweden, with the map illustrating elevation and major bodies of water. Traps were emptied weekly between April and October, and monthly in the remainder of the year. (B) An illustration of the diversity of organisms detected in the survey. The survey detected over 13,144 annotated 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 of the IUCN Red List (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 using observered incidences frequencies from the data in Box 1, and then estimated the trends from the simulated data by fitting statistical models. We then calculated the error between simulated (i.e. real) and estimated trends to illustrate the issues in retrieving real trends from rarely occurring organisms. For each trend category, 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.

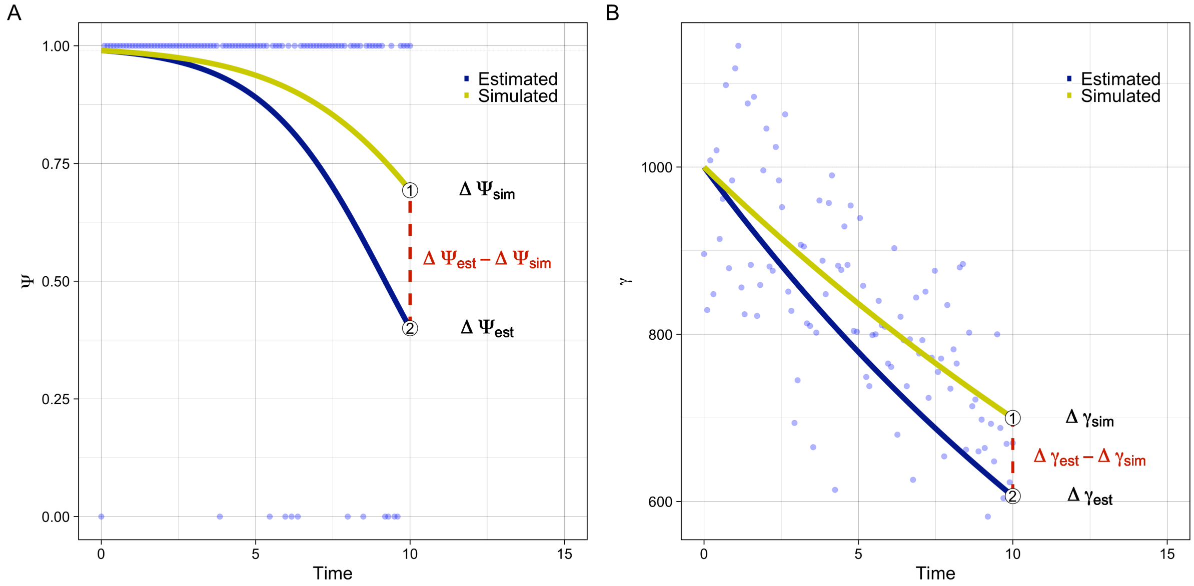

Figure B2. Illustration of calculating estimation error for simulated trends in occurrence probability (A), and read count (B). For each species detected in the survey we simulated IUCN specified trends (yellow lines) in occurrence probability (ψ) or read count as a proxy for relative abundance (γ). 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.

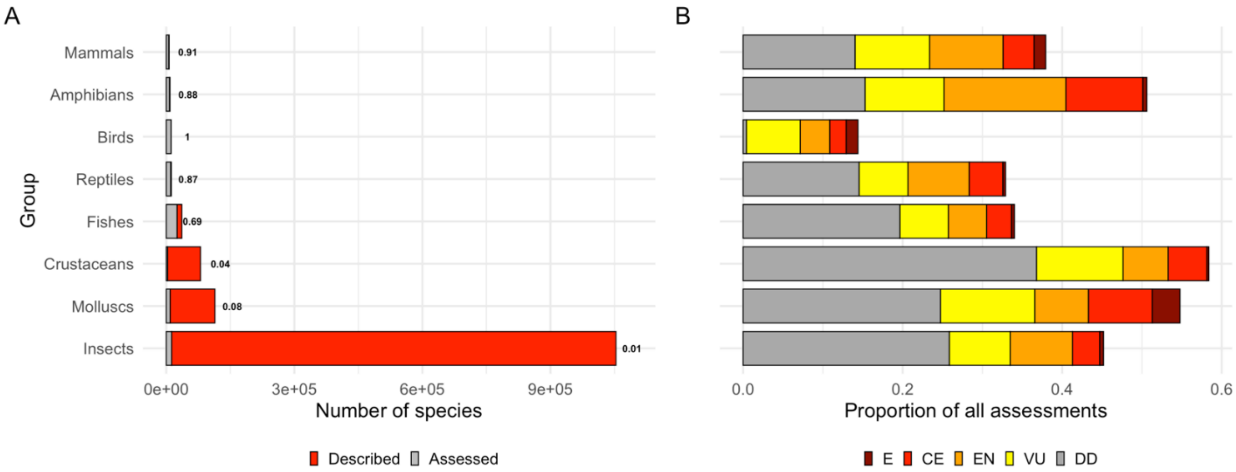

Figure 1. Taxonomic bias in IUCN red list coverage. Panel (A) Shows the number of described and assessed species in the groups with major representation (over 1000 assessments), on the IUCN red list. Red bars represent the total number of described species, and grey bars the number of species assessed 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”. Panel (B) shows the imbalance in assessment categories, with invertebrate species having considerably larger numbers of Data Deficient species than other animal groups.

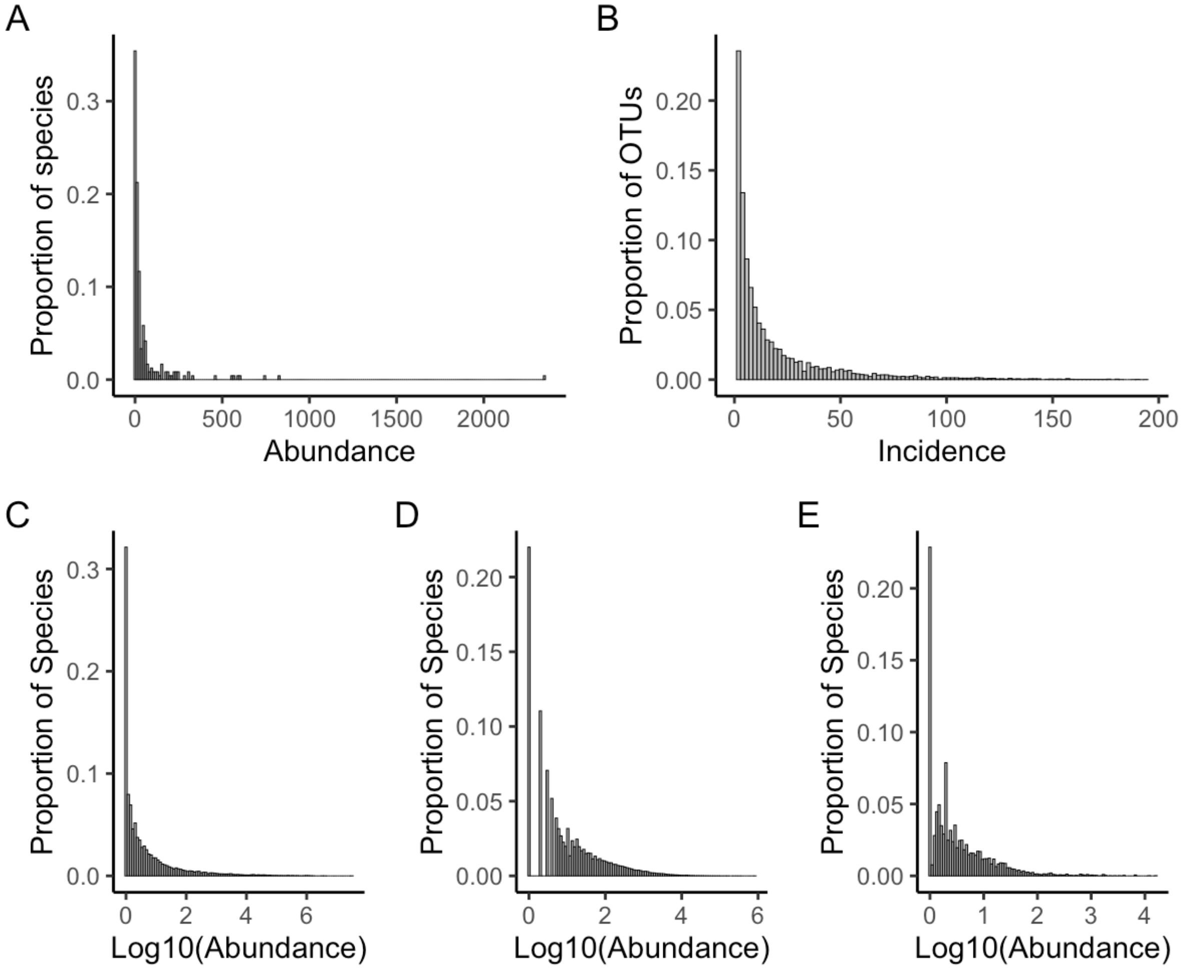

Figure 2. Abundance / Incidence distributions illustrating the pervasiveness of rare invertebrates. Panel A displays abundance frequencies of species caught from a single location in Rothamsted (UK) between 1933-36 (from Fisher, Corbett & Williams, 1943), whereas panel B represents data from the high-intensity molecular survey effort in Sweden in 2019 (Box 1). Panels C-D display the log10 abundance distributions from all organisms in three high profile data sets used in scientific research; C) Biotime (Dornelas *et al* 2018), D) GBIF (2000-2024; GBIF.org 2024), and E) the Predicts database (Hudson *et al* 2017).

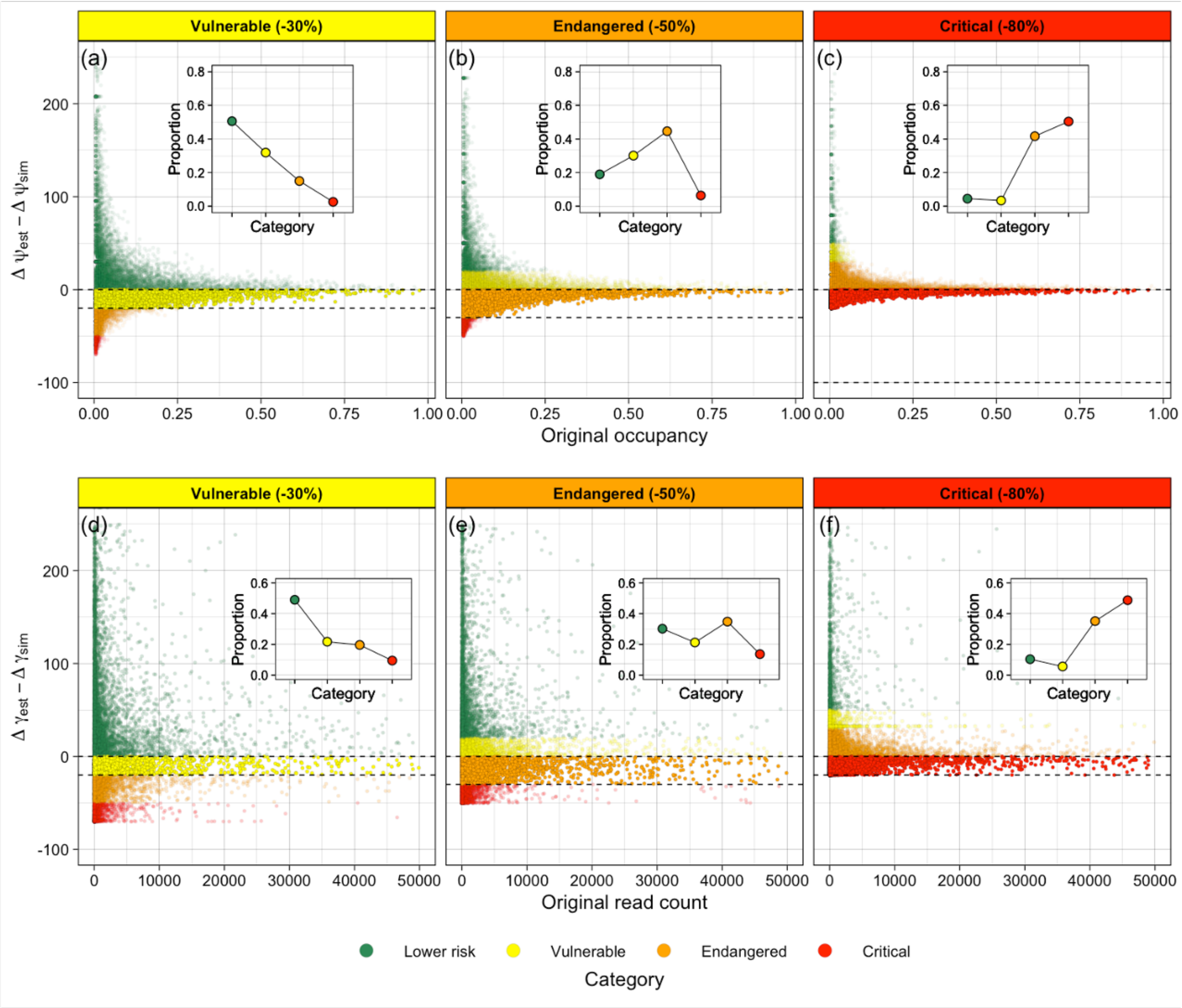

Figure 3. The percentage error in occurrence (a:c) and abundance (d:f) trends recovered by methods outlined in Box 2. $\widehat{\psi} - \psi$ represents the estimated occurrence – simulated occurrence, and $\hat{\gamma} - \gamma$ represents estimated abundance – simulated abundance. Occurrence trends 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 difference between the simulated trend and the lower confidence interval around the point estimate of the 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.

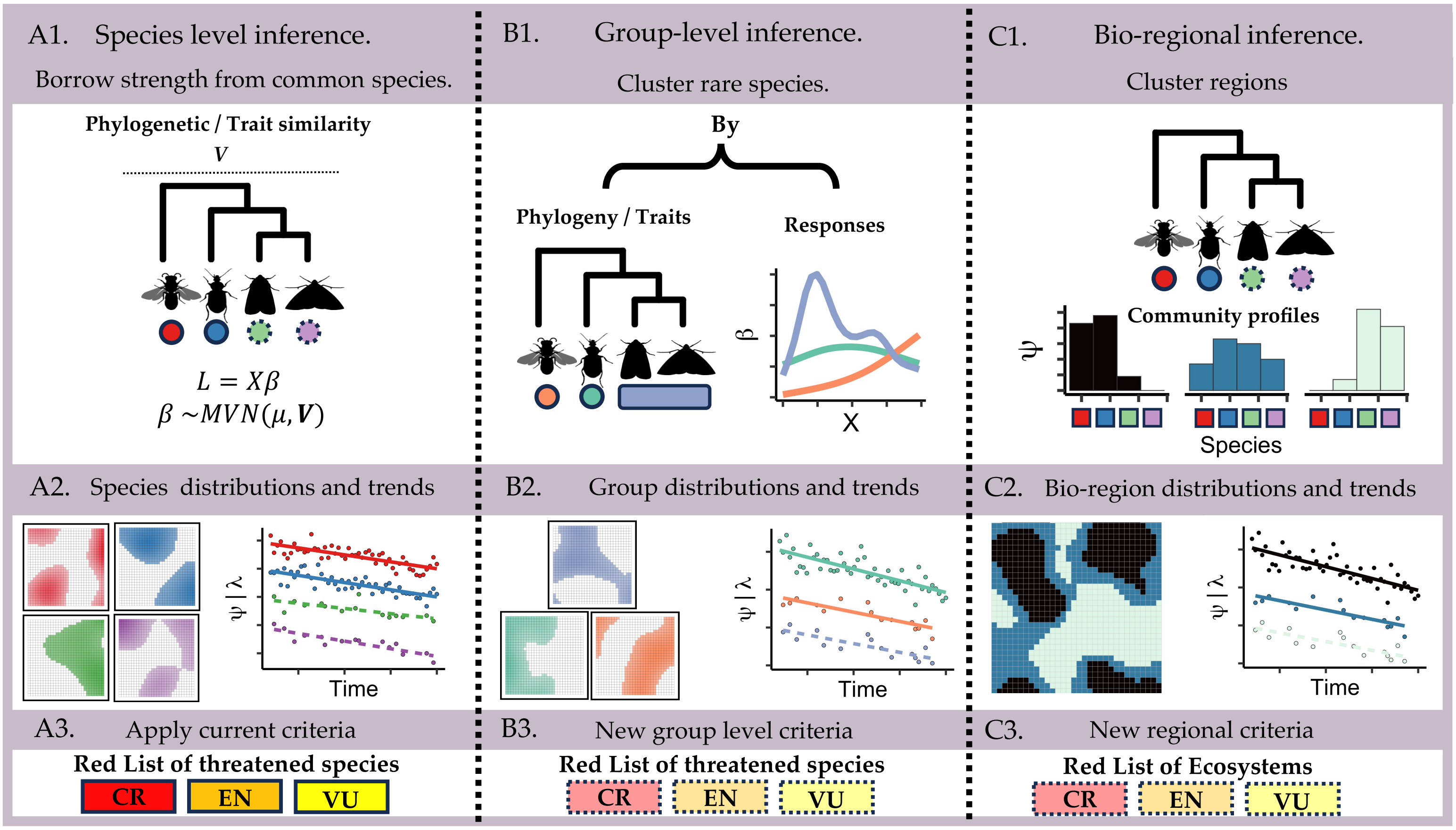

Figure 4. A conceptual overview of three proposed approaches to improved threat assessment for rare invertebrates, focusing on improved inference at the levels of A) single species; B) species groups and C) bio regions. For *improved species level inference*, we propose using hierarchical models (A1), which can improve the estimates of environmental responses (β), through 'borrowing of strength' for data poor species through, for example phylogenetic relationships ($\boldsymbol{V}$), with more common species. This can improve inference for species level distributions and trends (ψ/λ) (A2) – which are directly compatible with the IUCN Red List of threatened species (A3). For *improved group-level inference*, we envisage the clustering of species by phylogeny, shared traits, or environmental responses (B1), which can then be used to quantify group and species level distributions and trends (B2). Quantification of these metrics are not directly compatible with any existing framework, but the Red List of threatened species may provide a useful guide to develop new group-level assessment criteria (B3). For *improved inference at the level of bio-regions*, we highlight statistical methods that quantify the occurrence probabilities of different communities across regions, to designate 'regions of common profile' (RCP's) or statistical bioregions (C1). Monitoring the distributions of bio-regions or RCP's can then be used to assess the vulnerability of spatially associated species through assessing distributional extents or trends (C2). Bio-regional criteria do not complement any existing framework but the Red List of Threatened Ecosystems may guide development of new bio-regional criteria (C3).

Supplementary material

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 (Iwaszkiewicz-Eggebrecht, et al 2023). 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-Eggrebrecht 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-Eggrebrecht 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:

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

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 $Y_{its} \sim bernoulli(N, \Psi_{its})$. 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:

$$logit(\Psi_s) = \alpha_s$$

$$log(\gamma_{its}) = \beta_{0s} + x_{it}\beta_{1s}$$

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

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

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 $Y_{its} = negbinom(\lambda_{its}, \theta)$. 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 $\mathbf{X} = x_1 \ldots x_{10}$) 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).

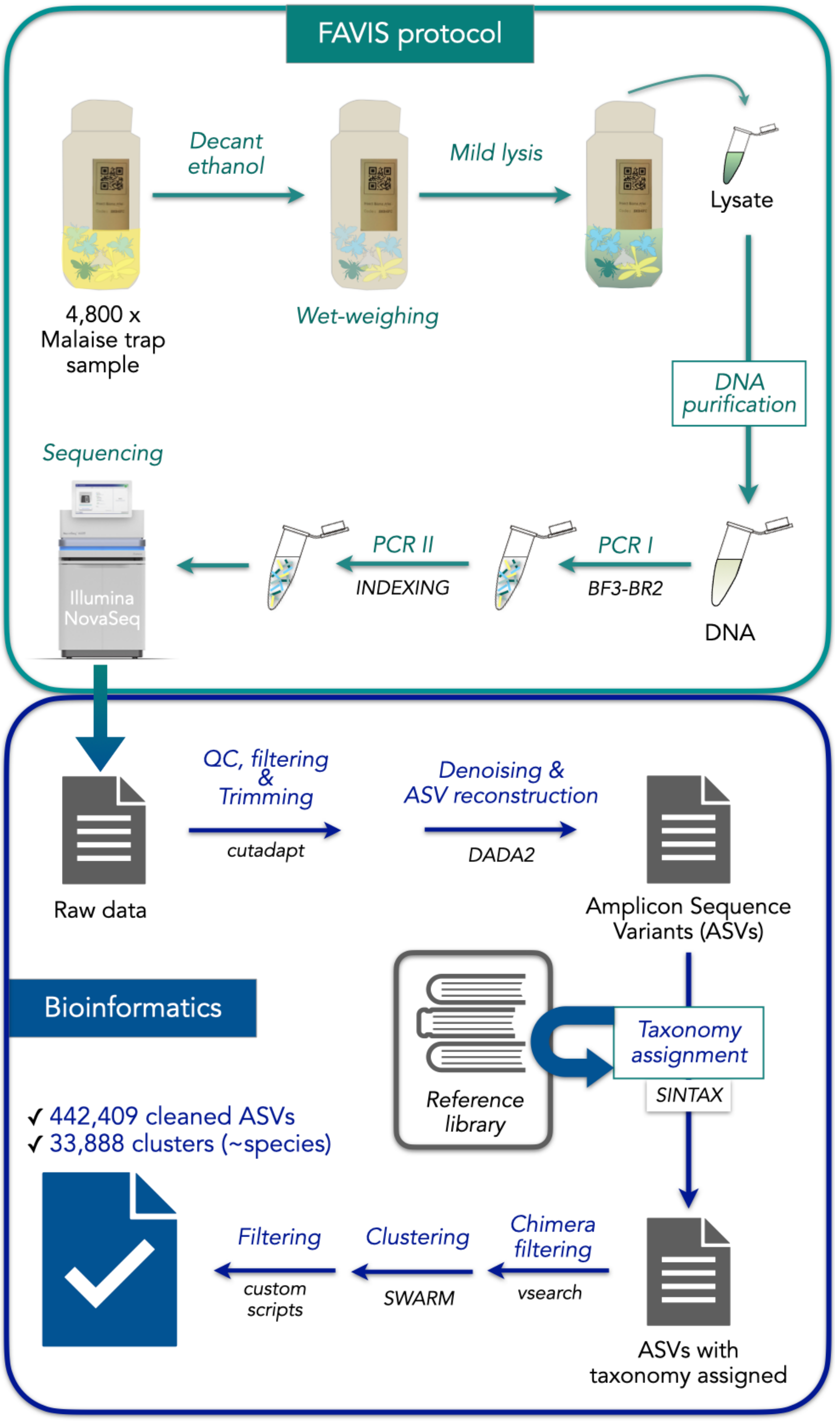

Figure S1. An overview of the FAVIS protocol for DNA extraction, purification, library preparation, and sequencing (Top panel) and the bioinformatics pipeline used to prepare sequences for analysis.

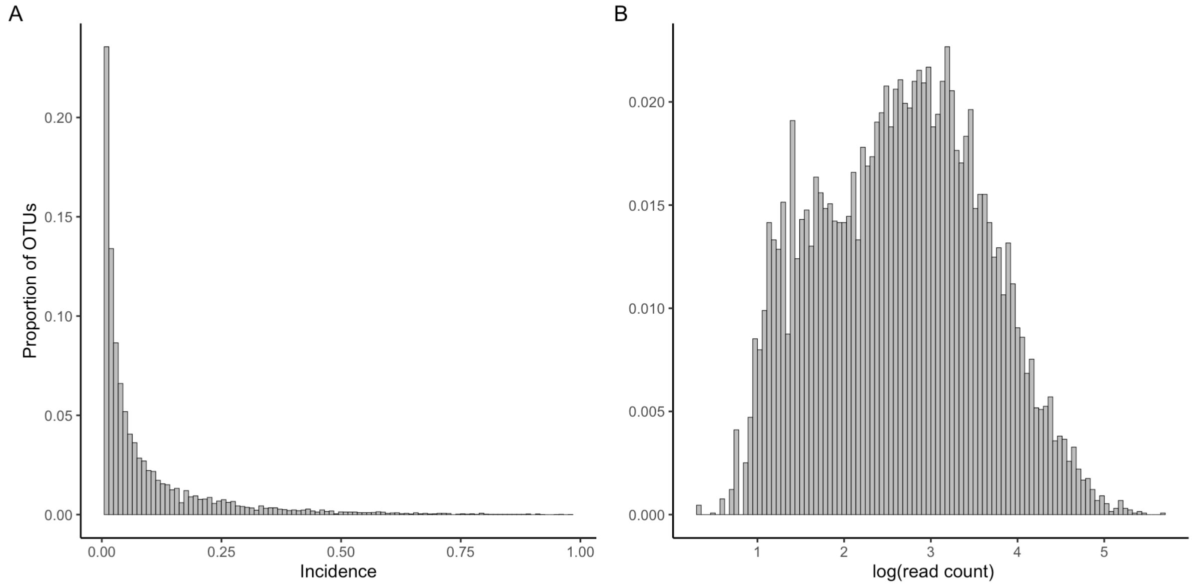

Figure S2. Data used in the simulation of abundance or occurrence trends. (A) The incidence (occurrence) frequencies for each operational taxonomic unit (OTU) assigned a species-level identification in the Swedish data, i.e. proportion of total sites occupied by each species. (B) The log of read counts 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	≥ 30% OR ≥ 50% for case A1 Based on: a) Direct observation b) An index of abundance c) Decline in range d) Levels of explotation e) Effects of introduced taxa	50% OR ≥ 70% for case A1 Based on: a) Direct observation b) An index of abundance c) Decline in range d) Levels of explotation e) Effects of introduced taxa	80% OR ≥ 90% for case A1 Based on: a) Direct observation b) An index of abundance c) Decline in range d) Levels of explotation e) Effects of introduced taxa
B . Geographic range	B1 AOO < 2000 km^2 OR B2 EOO < 20000 km^2 AND two of: a) No. locations ≤ 10 b) Continuing decline c) Extreme fluctuations	B1 AOO < 500 km^2 OR B2 EOO < 5000 km^2 AND two of: a) No. locations ≤ 5 b) Continuing decline c) Extreme fluctuations	B1 AOO < 10 km^2 OR B2 EOO < 100 km^2 AND two of: 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:

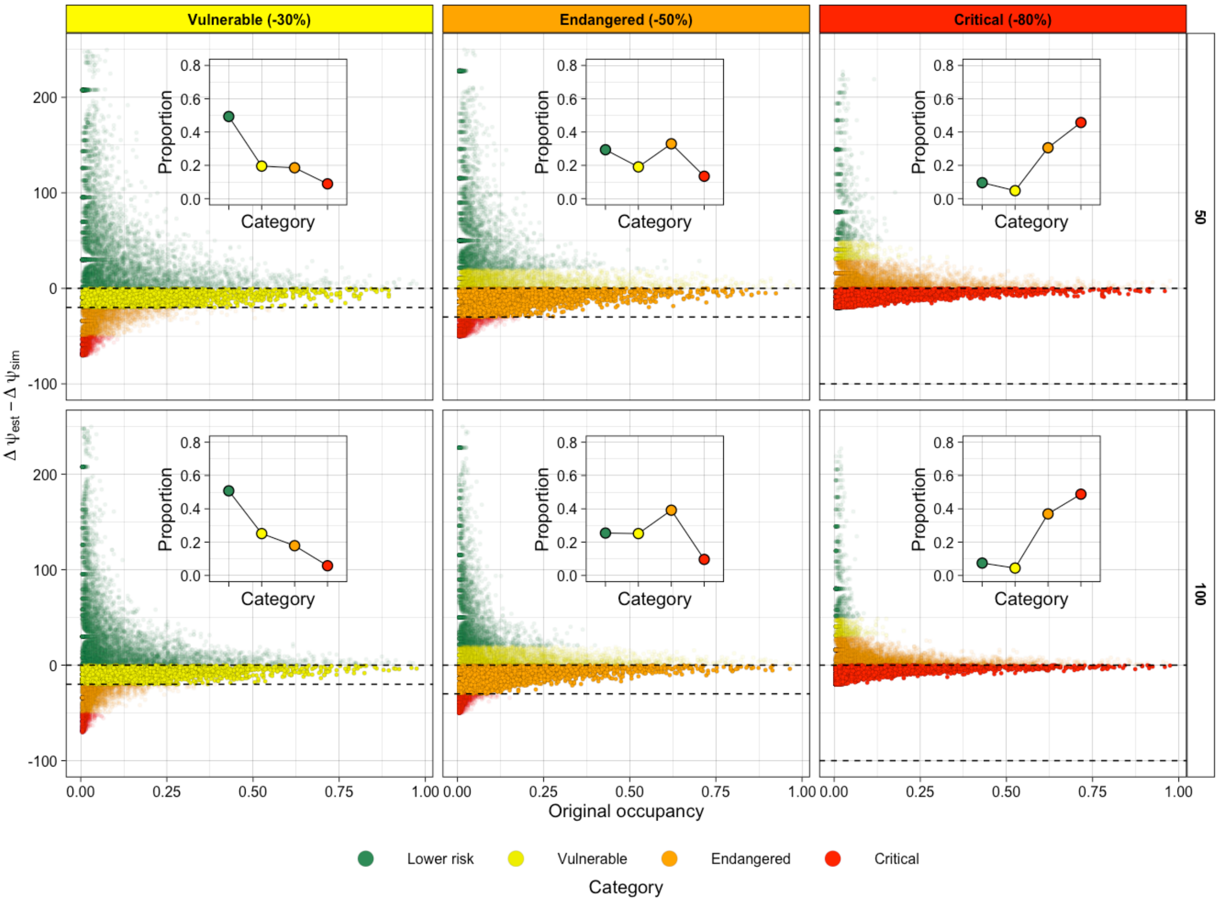

Figure S3. The percentage error in occurrence trends ($\widehat{\psi} - \psi$: estimated occurrence – simulated occurrence) versus original incidence frequency for organisms simulated to display trends classifying them as "Vulnerable" (a) , "Endangered" (b), or "Critically Endangered" (c) according to 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.

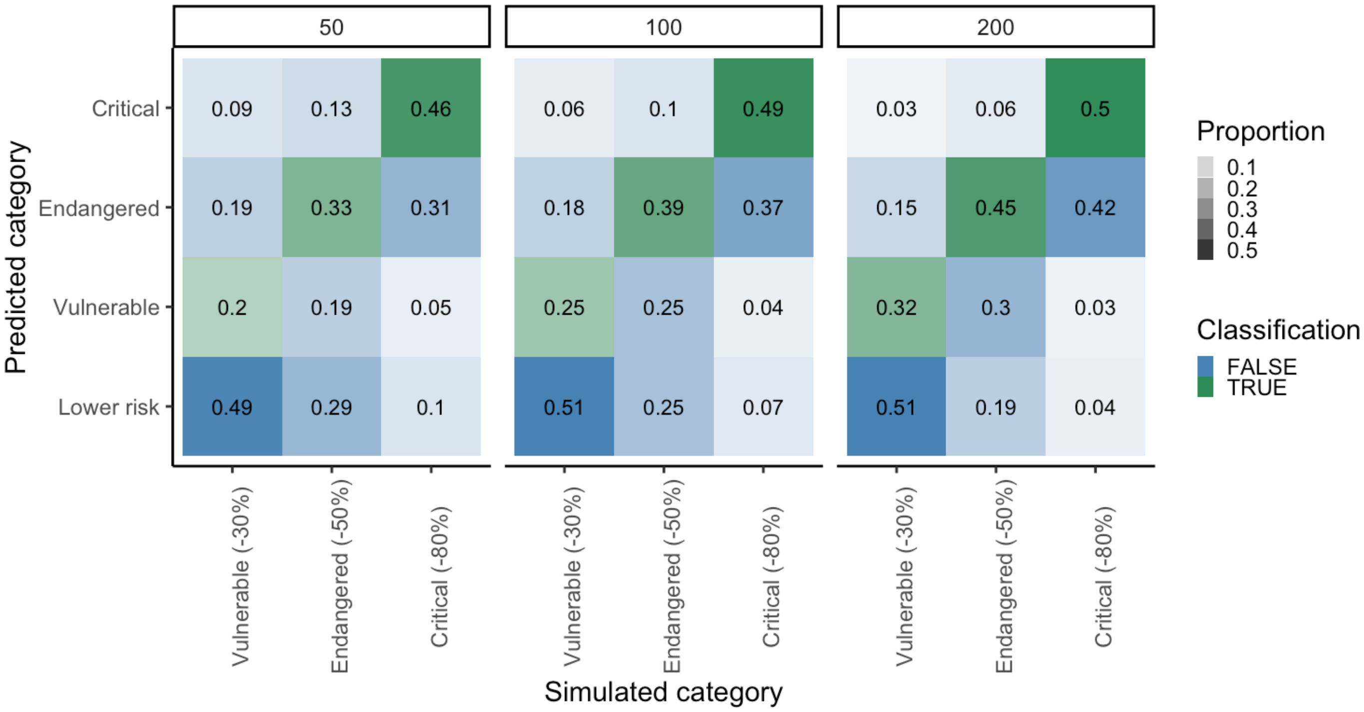

Figure S4. Confusion matrices showing the proportion of all species estimated to lie within each IUCN category against their simulated range size trend categories. The colour of each grid square indicates whether classifications were correct (green), or incorrect (blue). The transparency indicates the 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).

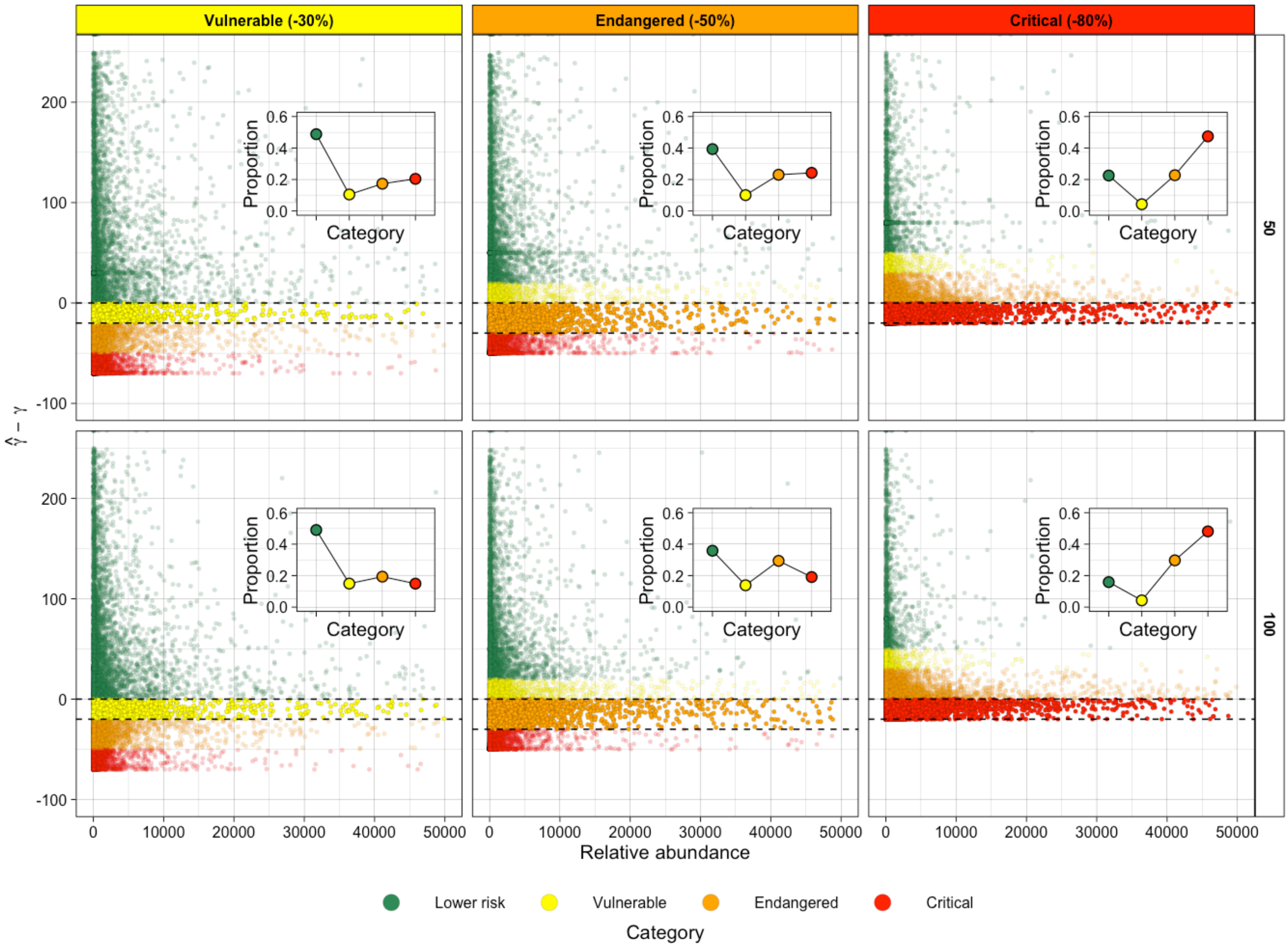

Figure S5. The percentage error in abundance trends ($\hat{\gamma} - \gamma$: estimated abundance – simulated abundance) versus original species abundance for organisms simulated to display trends classifying them as "Vulnerable" (a) , "Endangered" (b), or "Critically Endangered" (c) according to IUCN 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.

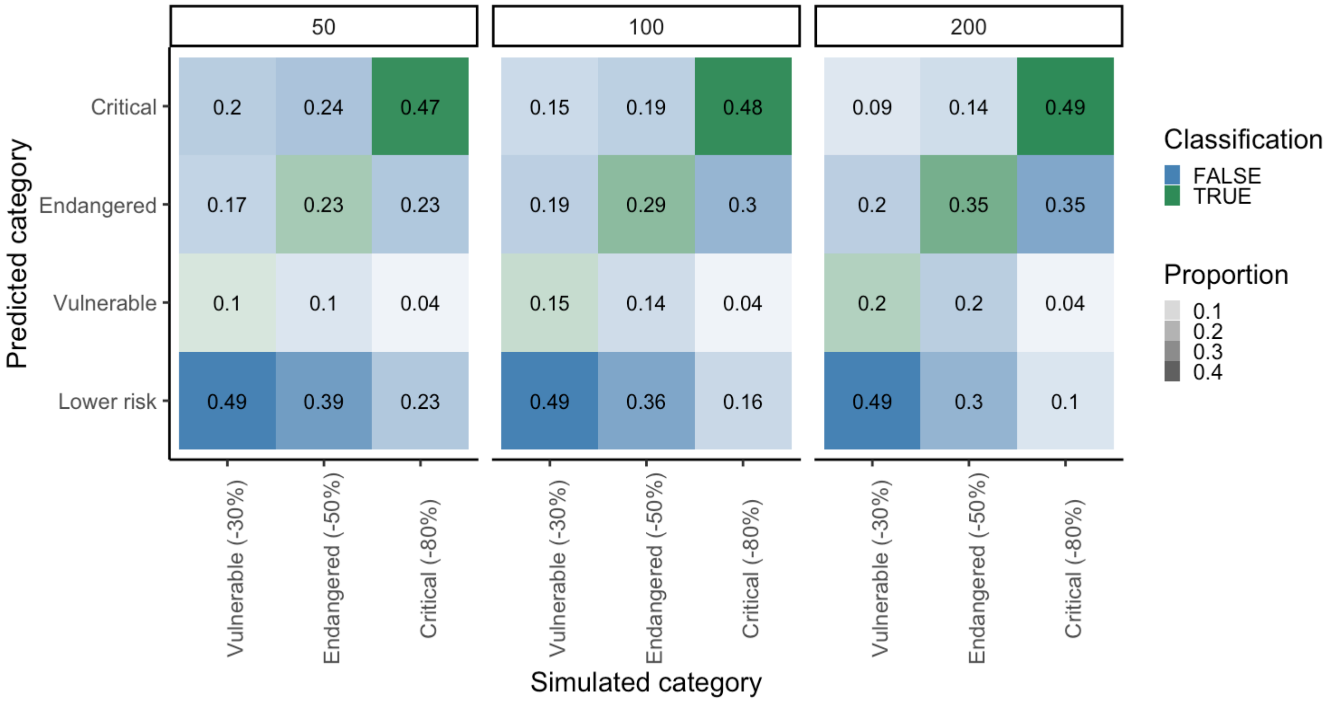

Figure S6. Confusion matrices showing the proportion of all species estimated to lie within each IUCN category against their simulated abundance trend categories. The colour of each grid square indicates whether classifications were correct (green), or incorrect (blue). The transparency indicates the 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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