



A red listing gap analysis of molluscs and crustaceans in Northern Europe: What has happened in the last 10 years?

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ABSTRACT

At the current rates of species extinction on a global level, Red List assessments need to speed up to inform conservation management in a timely manner. This study analyzed the progress made over the last 10 years in red listing aquatic invertebrates in Northern Europe. A survey of 43 freshwater molluscs and 1492 marine crustaceans was carried out for their Red List status in twelve countries during a twenty year interval (2003–2022). Our survey demonstrated that many countries have no national Red List or outdated Red Lists for the freshwater molluscs and only four countries have assessed their existing crustacean species. Alarmingly, we find 13 % fewer occurrence records for the crustaceans and 48 % fewer records for the freshwater molluscs in GBIF in the last 10 years (2013–2022) than in the 10 years previously (2003–2012). A barcode gap analysis reveals more barcodes for the 16S gene (77 %) than for the COI gene (63 %) for the freshwater molluscs and even fewer barcodes for the marine crustaceans (17 % for 16S and 40 % for the COI gene). With the current methods, regular comprehensive red listing of aquatic invertebrates is unrealistic. Here we present a set of scripts that allow automated occurrence and barcode gap analyses on unrepresented species groups. Finally, we discuss ways to increase the number of occurrence records and speed up red listing under existing European frameworks through whole community screening of ecosystems using molecular and other emerging tools.

1. Introduction

Globally, only 7 % of the >2.1 million species described by scientists are evaluated on the IUCN (International Union for Conservation of Nature) Red List of threatened species (IUCN Red List version 2022–1). Assessments mostly cover a few groups (e.g., birds, mammals, amphibians, fish and vascular plants), whereas for other groups, such as the invertebrates (e.g., insects, molluscs, crustaceans and worms) as well as algae, mosses, fungi and lichens, data and assessments are severely lacking. The IUCN Red List 2022 officially evaluated 26,581 invertebrate species, of which 385 have the status *extinct* (EX), inferring that ‘there is no reasonable doubt that the last individual has died’ and 16 species are *extinct in the wild* (EW), but may still live in zoos and animal parks (IUCN Standards and Petitions Committee, 2022). As of 2022, >6000 threatened invertebrate species are in the categories *critically endangered* (CR: 1420 species), *endangered* (EN: 1919 species) and *vulnerable* (VU: 2727 species) (IUCN Red List, 2022–1). Additionally,

1791 species are *near threatened* (NT) and 8759 are in the category *least concern* (LC), but this almost certainly represents a vast underestimate as many invertebrate species have not been evaluated or even described yet (Chapman, 2009). *Data deficiency* (DD), which currently includes 6119 invertebrates (IUCN, 2022), represents one of the major hurdles for determining the conservation status of most invertebrate groups. Reasons why a species might be classified as ‘*data deficient*’ and what type of information might be missing are manifold, they range from uncertain taxonomy to too few or old occurrence records and uncertain population status. This has been reviewed in detail by Bland et al. (2017) for 6 terrestrial and freshwater animal groups, who recommend the use of justification tags and research actions necessary to support the reassessment of DD species in a more strategic way, but this does not solve the problem of DD species in practice. Cardoso et al. (2011a, 2011b, 2012) already pointed out that traditional red listing criteria of the vertebrates are not readily applicable to invertebrate groups that have a small body size, restricted distribution and are often highly diverse. The

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IUCN Red List Criterion (a reduction in population size over 10 years) has led to biases in the assessment of insect taxa that might be valid for other invertebrate groups, for example groups which have short life-cycles and are very responsive to local weather changes (Fox et al., 2019). The authors suggested instead the evaluation of long-term data (>10 years) and linear trends that might abate the effects of annual variation better and would therefore provide a more robust assessment. Other studies stated that the main problems for invertebrates are the missing long-term and standardized data on distribution (e.g., Akçakaya et al., 2021).

In general, red listing helps to understand the extinction risk of species and is therefore an essential tool for the establishment of conservation plans for species that are most threatened with extinction. It also provides the basic data needed to achieve target 12 of the Aichi Biodiversity Targets of the Convention on Biological Diversity on the conservation of species (<https://www.cbd.int/sp/targets/>). Target 12 specifies that 'by 2020 the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained', however, this target has completely failed to be met (Hochkirch et al., 2020). Novel, faster methods are urgently needed to achieve target 12 on a local and global scale. Today, the Red List has no legal status, but is an independent expert source of knowledge about the species listed in each country and should serve as a guideline for conservation planning and decision making (Rodrigues et al., 2006).

In technical terms, red listing measures risk of extinction for a species and/or a population and is applied to wild populations in their natural distribution area. The IUCN Regional Guidelines are applied to understand and determine the extinction risk of the species in a certain region (IUCN RedList Guidelines, 2022). For this, five main evaluation criteria are used in the assessment process, the (A) population decline, (B) restricted distribution and decline, fluctuation and/or fragmentation, (C) small population size and decline, (D) very small population size or area of occupancy (AOO), and (E) quantitative analysis, so called population viability analysis (PVA). Such PVAs are often missing for most species analyzed, and their use in nature conservation is controversial (e.g., Ellner et al., 2002; Green and Bailey, 2015). In practice, there exist different commercial and free software for Red List evaluation (e.g., RAMAS© Red List Pro, GeoCAT (Bachman et al., 2011)), and different R packages like *red* (Cardoso, 2017), *ConR* (Dauby et al., 2017), *rCAT* (Moat and Bachman, 2017) and *redlistr* (Lee and Murray, 2017), providing numerous tools and guidelines, but the challenge lies in the regular monitoring of species and obtaining the required up to date data on the species' population status.

The evaluation of extinction risk for a species/population is helpful for stakeholders in a conservation priority setting process, but besides the Red List status, other factors should be considered as well (e.g., the global taxon level, cultural values, ecological traits) (IUCN RedList Guidelines, 2022). For example, the first IUCN conservation plan that was established for a terrestrial invertebrate was for the Crau plain grasshopper (*Prionotropis rhodcamica*). It was categorized as *Critically Endangered* on the IUCN Red List and as a consequence of this a conservation plan was developed in France (Hochkirch et al., 2014; Hochkirch, 2016).

Traditional methods such as direct counting methods, transect inventories, feces and acoustic analysis, are costly and time consuming, and are mostly applicable to higher taxa (mammals, birds, reptiles, amphibians, fish and vascular plants). Invertebrates (e.g., insects, molluscs, crustaceans and worms) or algae, gymnosperms, fungi and lichens, which represent the highest biodiversity on earth (Chapman, 2009), require on the other hand a new generation of monitoring methods and new sources of data to bridge the gap in species knowledge and push conservation tasks forward (e.g., citizen science, remote sensing or genetic sampling, Maes et al., 2015; Collen et al., 2016; Hochkirch et al., 2020).

Molecular tools are often suggested to detect rare species (e.g.,

metabarcoding, qPCR and ddPCR, Bohman et al., 2014; Sepulveda et al., 2019), but community-based methods such as metabarcoding are mostly useful when reference databases specific to the monitored habitats are available. In the last decades, the number of records in DNA barcode reference libraries has increased rapidly (Kress et al., 2015; Fontes et al., 2021), but extensive gaps still exist for aquatic organisms in Europe (Weigand et al., 2019). Several Red Lists targeting terrestrial invertebrate groups were published by the European Union around ten years ago (e.g., European Red List of Grasshoppers, Crickets and Bush-crickets: Hochkirch, 2016, European Red List of bees: Nieto, 2014), but no such European-wide lists exist for aquatic invertebrates, aside from a European Red List of non-marine molluscs published twelve years ago (Cuttelod et al., 2011).

To bridge this gap, we aim to analyze the progress made in the last 10 years in the red listing of two aquatic invertebrate groups, the marine crustacea and the freshwater molluscs, in 12 Northern European countries from 2003 to 2022. In addition, we provide a gap analysis of occurrence records and genetic barcodes (16S and CO1) for our target species. Finally, we aim to provide recommendations about how the Red List assessment for aquatic invertebrates could be improved in practice in future, based on novel sources of occurrence records.

2. Material and methods

2.1. Target region and organism groups

The target taxa assessed in this study were 1492 species of marine crustaceans and 43 species of freshwater molluscs found in 12 countries across Northern Europe (Belgium, Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Netherlands, Norway, Poland, Sweden and the United Kingdom). The selection of countries was determined by having coasts bordering the same North European seas, namely the North Sea and Baltic Sea, which assumes that those countries also harbor similar North European species.

The marine crustaceans (MC) selected consist of six different orders including Decapoda, Amphipoda, Thecostraca, Cumacea, Isopoda and Mysida. The freshwater molluscs (FM) belong to the families Margaritiferidae, Unionidae, Dreissenidae and Cyrenidae (see Supplementary Tables A.1. and A.2. for a full list of the species). To validate the species names, the nomenclature of WoRMS (WoRMS, 2023) was followed for the marine crustaceans (<http://www.marinespecies.org>, database accessed on 15/03/2023) and MolluscaBase (MolluscaBase, 2023) was used for the freshwater molluscs (<https://www.molluscabase.org>, database accessed on 15/03/2023). When searching for relevant data on the species, we used the most recent accepted name as well as the synonyms (Tables A.1. and A.2.).

2.2. Information on Red List per country

Firstly, we created a target species list for the MC and FM for each country (Belgium, Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Netherlands, Norway – without Svalbard, Poland, Sweden and the United Kingdom). For most countries, there exists a digital database listing species that occur in the country (see Table 1 for references). For some countries, there was no digital information available. In those cases, we used scientific articles and books showing the distributions of our species of interest (e.g., for the FM: Kuiper et al., 1989 in Denmark or Pierchocki and Wawrzyniak-Wydrowska 2016 in Poland; for MC: Jazdzewski et al., 2005) (Table 1) (Tables A.1. and A.2.). Secondly, we searched for a national Red List for each country to check if our target species were assessed on national level according to IUCN criteria or not, which means if there exists a Red List for the country, how old the latest Red List was and at which intervals the species were evaluated during the last 10 years. Some countries (Denmark, Finland, Norway and Sweden) have digitized databases where both, the species lists and Red Lists, could be found (often combined with additional

information on species description, occurrence maps, photographs etc.). In other countries, where this was not the case, we used scientific and/or Red List reports and books (De Bruyne et al., 2003; Jungbluth and von Knorre, 2011; Rachor et al., 2013; Seddon et al., 2014; Piechocki and Wawrzyniak-Wydrowska, 2016; Rudzite et al., 2018; Hyvärinen et al., 2019; Maes et al., 2019; SLU Artdatabanken, 2020) (Table 1) to find as much information as possible on the Red List category of each species. Additionally, we communicated directly with the relevant red listing organization and/or species expert(s) in each country to obtain species lists (see acknowledgements for details of contacted persons). We also searched on the European level for information and took into account the existing European Red List for Freshwater Molluscs (Cuttelod et al., 2011) and the HELCOM Red List of Baltic Sea Species (HELCOM, 2012, 2020). These data, compiled in Tables A.1. (FM) and A.2. (MC), formed the basis for our analysis. To obtain an overview of the different IUCN Red List categories for our target species in each country, we calculated in Tables 2 and 3 a) the number of species with an IUCN Red List category, b) the percentage of species that can be found in the country out of the total number of species analyzed in Northern Europe ($n = 43$ for FM and $n = 1492$ for MC, respectively), c) the percentage of species with status *data deficient* (DD) in the country and d) the percentage of threatened species (nr. of species in VU, EN, CR categories together) per country, for both the FM and MC.

2.3. Retrieving occurrence records and barcode data

Using a custom Python script, we queried the Global Biodiversity Information Facility database (GBIF) for existing occurrence records and BOLD and GenBank for barcode data for all 1492 crustacean and 43 molluscan species (<https://www.gbif.org>, <https://www.boldsystems.org>, <https://www.ncbi.nlm.nih.gov/genbank/>, accessed on 16.03.2023). The custom Python script is available on GitHub: https://gitlab.leibniz-lib.de/smartin/collect_data_footprint, and generates a single CSV file as output (Tables B.1. and B.2.). The script retrieves information via the application programming interface (API) and uses both the valid scientific names and the synonyms as an initial search. GBIF was queried for existing occurrence records between 2003-2012 and 2013-2022 in Belgium, Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Netherlands, Norway, Poland, Sweden and the United Kingdom. Records were categorized according to 11 different record types, and classified into three different categories 1) relevant for red listing: human observation, living specimen, material sample, observation, occurrence, 2) might be relevant for red listing: material citation, machine observation, 3) irrelevant for red listing: fossil specimen, preserved specimen and unknown. We summarized the total number of species occurrence records relevant for red listing (category 1 only) found per organism group (marine crustaceans and freshwater molluscs) for each country between 2003 and 2022 (Tables C.1. and C.2.). The Python script can also pipe the data to an ad hoc R script for the on-the-fly creation of choropleth maps. These maps show the total number of occurrence records for the investigated countries as follows: a) between 2003 and 2012 and b) between 2013 and 2022, to compare the records available in the database for those time intervals. The different values for each country are represented by color differences, the color scheme for this was selected based on recommendations by Brewer et al. (2013). Outliers can skew the color scale so much that differences in bulk data cannot be distinguished anymore. Therefore, all outliers were assigned their own, single color. Outliers are defined here as those data points, which have values exceeding the upper quartile range (Q3) plus 1.5 times the interquartile range (IQR) (Hedderich and Sachs, 2020). Outliers occurred only in the upper, not the lower range of the data. The detection of outliers was implemented in R in such a way that the map plots adjust automatically. All data processing for the maps was performed using R version 4.2.3 (R Core Team, 2023). In addition, the following R packages were used: 'dplyr' for processing the raw data (Wickham et al., 2023), the Natural Earth dataset implemented in

'rnaturalearth' for the background map (South, 2017), 'ggplot2' (Wickham, 2016) and 'sf' (Pebesma, 2018) for calculating and plotting the maps, and 'ggspatial' (Dunnington, 2021) and 'scales' (Wickham and Seidel, 2022) for additional map-related features.

For the barcode gap analysis, BOLD and GenBank were queried for COI and 16S records (<https://www.boldsystems.org> (Ratnasingham and Hebert, 2007), <https://www.ncbi.nlm.nih.gov/genbank/>) for all species and their associated synonyms, using the custom python script (https://gitlab.leibniz-lib.de/smartin/collect_data_footprint) (same dates as above). The bar plots with the proportion of barcodes available for each organism group were created in R version 4.2.3 (R Core Team, 2023).

3. Results

3.1. Information on Red List and red listed species per country

The 12 Northern European countries considered in our analysis display very varied levels of information, both in terms of species analyzed and number of red listed FM and MC records, which hinders a unified approach to controlling biodiversity losses in aquatic invertebrates. Five of twelve countries (Belgium, Denmark, Latvia, Netherlands and Poland) still have no official national Red List available for FM (Tables 2 and Table A.1.), but some information on threatened species and an IUCN category could be found in other publications (see Table 1 for details). Germany has an outdated FM Red List which is older than 10 years and follows a different classification system than the IUCN assessment criteria. The European Red List for FM has not been updated since 2011 (Table 1). Normally, the European Red List is a useful complement to the regular reporting system under the EU's Habitat Directive (Council Directive 92/43/EEC, 1992), because it addresses all species of a specific taxonomic group, and not only the protected ones by the EU legislation (Cuttelod et al., 2011).

The situation is even more disappointing for the MC (Tables 1, 3 and Table A.2.). None of the countries have assessed all 1492 crustacean species compiled here, and Finland, Germany, Norway and Sweden are the only countries that have assessed most of the crustaceans (Tables 1 and 3). Other countries including Estonia, Latvia, Lithuania and Poland are included in the European Red List of the Baltic Sea species that lists at least some crustacean species (HELCOM checklist 2.0, 2020). Remarkably, countries on the Scandinavian peninsula (Norway, Sweden and Finland) have complete digital species databases that summarize distribution pattern, species description and conservation status, and carry out regular red listing at 5 years intervals for Sweden and Finland and every 6 years in Norway (Tables A.1. and A.2.). Denmark and Germany recently started to use digital databases (2019 and 2021, respectively), but these are not as complete as those from the Scandinavian countries.

For the FM, less than half of the countries analyzed harbor >30 different FM species (e.g., Germany ($n = 38$), Poland ($n = 35$), Netherlands ($n = 33$), Sweden ($n = 33$) and the UK ($n = 32$)) (Table 2). Estonia has the highest number of *not evaluated* species ($n = 11$). For the countries without a national Red List for FM (Belgium, Denmark, Netherlands and Poland) other references were found that mention some IUCN categories for some single species. All other species were sorted for this study into the category (Table 2), but did not get this category officially. Finland showed the highest percentage of species in the *data deficiency* category (21 %, $n = 6/29$), followed by Estonia (15 %, $n = 4/27$), Norway (13 %, $n = 3/24$), and Sweden (12 %, $n = 4/33$) (Table 2). Germany and the Netherlands are the countries with the highest percentage of threatened FM species (50 %, $n = 19/38$ and 27 %, $n = 9/33$, respectively), followed by Latvia with 25 % ($n = 4/16$) of threatened species according to the existing national Red Lists (Table 2). The Netherlands has the highest number of *recently extinct* species (5 in total), followed by Belgium, Germany and Poland with one *recently extinct* species per country (Table 2).

For the MC, most countries have a species list and only four countries

Table 1

An overview of the latest Red List in each country and the source and/or database link for the freshwater molluscs (FM) and marine crustaceans (MC).

Country	Latest Red List of FM	Source of database	Latest Red List of MC	Source of database
Belgium	NO RED LIST (2019)	Maes et al., 2019 (Flanders only); https://nl.wikipedia.org/wiki/Lijst_van_zoetwatermollusken_in_Nederland,_Belgi%C3%AB_en_Luxemburg	NO RED LIST	VLIZ Belgian Marine Species Consortium (2010 onwards). Belgian Register of Marine Species at http://www.marinespecies.org/berms (species list)
Denmark	NO RED LIST (2019)	https://bios.au.dk/forskningraadgivning/temasider/redlistframe/soeg-en-art/ ; https://allearter-databasen.dk/ ; Kuiper et al., 1989	NO RED LIST (2019)	https://bios.au.dk/forskningraadgivning/temasider/redlistframe/soeg-en-art/ (species list)
Estonia	2017	https://elurikkus.ee/generic-hub/occurrences/search?#records	NO RED LIST	(HELCOM checklist 2.0 only)
Finland	2019	Hyvärinen et al., 2019; https://punainenkirja.laji.fi/en/results?type=species&year=2019&redListGroup=MVL.761	2019	Hyvärinen et al., 2019; https://punainenkirja.laji.fi/en/results?type=status&year=2019&redListGroup=MVL.766 https://www.nic.funet.fi/index/Tree_of_life/warp/crustacea-fi-Finnish-list.html (species list)
Germany	2011	Jungbluth and von Knorre, 2011; https://www.rote-liste-zentrum.de	2013	Rachor et al., 2013
Latvia	2018	Rudzite et al., 2018	NO RED LIST	(HELCOM checklist 2.0 only)
Lithuania	NO RED LIST	–	NO RED LIST	(HELCOM checklist 2.0 only)
Netherlands	NO RED LIST (2003)	De Bruyne et al., 2003; https://nl.wikipedia.org/wiki/Lijst_van_zoetwatermollusken_in_Nederland,_Belgi%C3%AB_en_Luxemburg	NO RED LIST	https://www.nederlandsesoorten.nl/linnaeus_nlg/app/views/species/tree.php (species list)
Norway	2021	Artsdatabanken, 2021. Norsk rødliste for arter 2021. Artsdatabanken, Trondheim. https://www.artsdatabanken.no/lister/rodlisterforarter/2021	2021	Artsdatabanken, 2021. Norsk rødliste for arter 2021. Artsdatabanken, Trondheim. https://www.artsdatabanken.no/lister/rodlisterforarter/2021
Poland	NO RED LIST (2004–2009; 2016)	Piechocki and Wawrzyniak-Wydrowska, 2016; http://www.iop.krakow.pl/pckz/defaultadf8.html?nazwa=default&je=en	NO RED LIST	(HELCOM checklist 2.0 only); Jążdżewski et al., 2005 (species list)
Sweden	2020	SLU Artdatabanken (2020); https://www.dyntaxa.se ; http://artfakta.se/artbestamning	2020	SLU Artdatabanken, 2020; https://www.dyntaxa.se ; http://artfakta.se/artbestamning
United Kingdom	2013	Seddon et al., 2014	NO RED LIST	Marine Species of the British Isles and Adjacent Seas (MSBIAS) http://www.marinespecies.org/msbias (species list)
European Red List	2011	Cuttelod et al., 2011	NO RED LIST	–
HELCOM Red List of Baltic Sea species	–	–	2012 (+2020)	https://helcom.fi/baltic-sea-trends/biodiversity/red-list-of-baltic-species/red-list-of-benthic-invertebrates/

have a Red List (Finland, Germany, Norway and Sweden) (Tables 1 and 3). Those Red Lists are far from complete for all the analyzed crustacean species (Table A.2.). Even without an official national Red List, information was found that Denmark has assessed three species and Poland ten (Table 3). Germany has its own assessment system, which does not follow the IUCN criteria and is ten years old now. Additionally, there exists a Red List for the Baltic Sea/HELCOM area, that has assessed 24 species found in the region (Tables 3 and Table A.2.). The United Kingdom harbors most of the known species in Northern Europe (74 %, $n = 1106$) followed by Norway (65 %, $n = 964$) and Sweden (44 %, $n = 663$). Finland only harbors few marine crustaceans ($n = 36$) because the country is surrounded by mainly brackish-freshwater of the Baltic Sea. Norway, in contrast, has a long marine coastline, and evaluated nearly all MC species. Most of the species ($n = 821$) have the category *least concern* (Table 3). Sweden has a Red List, but most MC species are *not evaluated* ($n = 453$) or assessed with *data deficiency* ($n = 21$) (Table 3). The highest percentage of species with *data deficiency* is found in Germany, which also has the highest numbers of threatened species (22 %, $n = 70/318$) (Table 3).

3.2. Occurrence records

The occurrence records for both organism groups differ between the different analyzed categories. While the category for human

observation, material sample and occurrence showed the highest record numbers, the category with material citation and machine observation is very low, but increased between the different time intervals (e.g., an increase in machine observation in the time interval 2013–2022 compared with 2002–2013) (see Tables C.1. and C.2. for more details). For each organism group (FM and MC), occurrence records were available in all twelve countries and both time intervals (Figs. 1–2; Table 4). The registered records differed between the organisms, countries and years and will be closer discussed per organism group in the following part.

The species records available in GBIF for the FM differed extremely between the countries and the time intervals (Figs. 1A–B). Three different categories could be found: 1) countries with <35/300 records, 2) <2000 records and 3) <10,000/4000 records in the first and second time period, respectively (Table 4). The Netherlands were an exception from those categories, due to extremely high numbers of occurrence records in the first (>25,000) and second time period (>5000 records). Seven countries (Estonia, Finland, Latvia, Lithuania, Norway, Poland and Sweden) out of 12 have more occurrence records for FM in the last 10 years (2013–2022) compared to the 10 years prior (Table 4).

For the MC, similar disparate patterns were found. While countries such as Denmark, Finland, Latvia, Lithuania and Poland have fewer than 500 occurrence records in the first time period, other countries (Belgium, Estonia, Netherlands, Sweden) have between 10,000 and

Table 2

Red listing summary table: number of freshwater mollusc species per country, assessed species per category, percentage of species harbored in the country, percentage of species with data deficient and percentage of threatened species. Background marked grey: categories defined as 'threatened species'. [NA: Not Applicable, NE: Not Evaluated, DD: Data Deficient, LC: Least Concern, NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered, RE: Regionally extinct; (NE): means that they were not officially put in this category, but sorted during this study as not evaluated].

Country	Latest Red List of FM	IUCN Red List Categories										Σ species per country	% country's species over Σ species in Northern Europe	% DD over Σ species in each country	% threatened over Σ species in each country	
		NE	NA	DD	LC	NT	VU	EN	CR	RE						
Belgium	<i>no Red List</i> (2019)	(28)										1	29	67.44		
Denmark	<i>no Red List</i> (2019)	(27)											27	62.79		
Estonia	2017	11	1	4	8	2						1	27	62.79	14.81	3.70
Finland	2019	1	6	19	1	1	1	1					29	67.44	20.69	6.90
Germany	2011	5	2	1	9	1	6	6	7	1			38	88.37	2.63	50.00
Latvia	2018				11	1	2	2					16	37.21		25.00
Lithuania	<i>no Red List</i>	-	-	-	-	-	-	-	-	-			-	-	-	-
Netherlands	<i>no Red List</i> (2003)	(18)	1				7	2				5	33	76.74		27.27
Norway	2021			3	18	1	2						24	55.81	12.50	8.33
Poland	<i>no Red List</i> (2004)	(28)				1	1	4				1	35	81.40		14.29
Sweden	2020		2	4	23	2		2					33	76.74	12.12	6.06
United Kingdom	2013		3	1	26	4	1		1				32	74.42	3.13	6.25

Table 3

Red listing summary table: number of marine crustacean species per country, assessed species per category, percentage of species harbored in the country, percentage of species with data deficient and percentage of threatened species. Background marked grey: categories defined as 'threatened species'. [NA: Not Applicable, NE: Not Evaluated, DD: Data Deficient, LC: Least Concern, NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered, RE: Regionally extinct].

Country	Latest Red List of MC	IUCN Red List Categories										no of species per country	% country's species over Σ species in Northern Europe	% DD over Σ species in each country	% threatened over Σ species in each country	
		NE	NA	DD	LC	NT	VU	EN	CR	RE						
Belgium	<i>no Red List</i>	-	-	-	-	-	-	-	-	-	-	-	247	16.55		
Denmark	<i>no Red List</i> (2019)	(302)									2		305	20.44		0.66
Estonia	<i>no Red List</i>	-	-	-	-	-	-	-	-	-	-	-	45	3.02		
Finland	2019		7		29								36	2.41		
Germany	2013		11	106	109	15	45	4	21	7			318	21.31	33.33	22.01
Latvia	<i>no Red List</i>	-	-	-	-	-	-	-	-	-			31	2.08		
Lithuania	<i>no Red List</i>	-	-	-	-	-	-	-	-	-			27	1.81		
Netherlands	<i>no Red List</i>	-	-	-	-	-	-	-	-	-			371	24.87		
Norway	2021	5	72	49	821	11	5	1					964	64.61	5.08	0.62
Poland	(<i>no Red List</i>)	(38)		3	3	3	1						48	3.22	6.25	2.08
Sweden	2020	453	64	21	86	14	19	2	2	2			663	44.44	3.17	3.47
United Kingdom	<i>no Red List</i>	-	-	-	-	-	-	-	-	-			1106	74.13		
Baltic Sea/ HELCOM	2012/2020			13	3	3	4	1					344	23.06	3.78	1.45

40,000 occurrence records (Figs. 2A-B, Table 4). Norway and the United Kingdom have the highest numbers, but only Norway has increased the number of occurrence records in GBIF for MC in the last 10 years (Figs. 2A-B, Table 4). Half of the countries (6 of 12) have fewer registered occurrence records in the last 10 years, those are Belgium, Estonia, Netherlands, Poland, Sweden and the UK.

3.3. Barcode data

The gap analysis for the analyzed FM species ($n = 43$) shows that more barcodes exist for the 16S gene (77 %, $n = 33$) than for the COI gene (63 %, $n = 27$) for our species list (Table A.1.) (Fig. 3). For the MC ($n = 1492$) the gaps are bigger with only 16 % ($n = 246$) of the species having a 16S barcode and 40 % ($n = 604$) having a COI barcode available for our species list (Table A.2.) (Fig. 3).

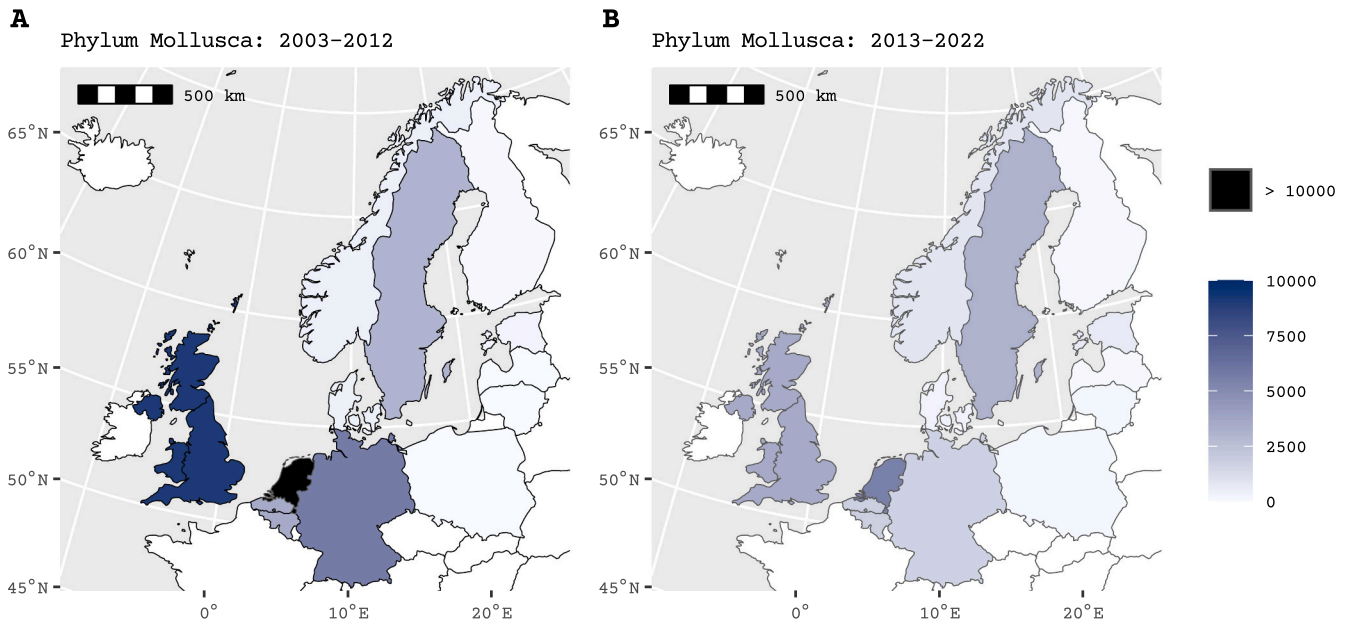


Fig. 1. A-B: Number of occurrence records found in GBIF for each country for the freshwater molluscs (43 species) A) for 2003–2012 and B) for 2013–2022.

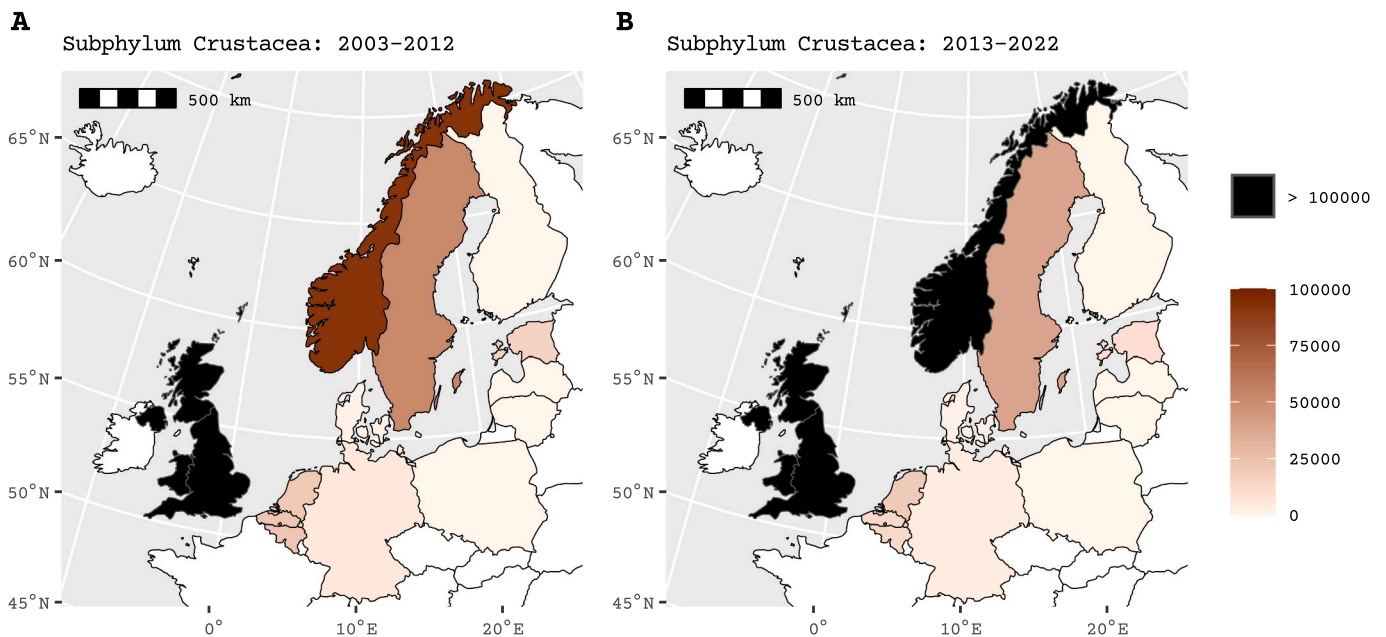


Fig. 2. A-B: Number of occurrence records found in GBIF in each country for the marine crustaceans (1496 species) A) for 2003–2012 and B) for 2013–2022.

4. Discussion

In this study, we showed with our bioinformatic tools that there is a clear lack of red listing for the two target groups of freshwater molluscs and marine crustaceans in Northwestern Europe. Thus, what has really happened during the last ten years? Even in advanced European countries only a small proportion of the aquatic invertebrate fauna has been assessed and is being effectively monitored. When comparing the last two decades, the effort between the countries differs greatly. While some countries have made progress in red listing and assess more and more species with time (e.g., Finland, Norway, Sweden), there are still many northwestern countries lagging behind that have no Red List at all for MC and incomplete or outdated ones for FM. There have previously been discussions about whether red listing is even applicable to its full extent

for invertebrates (see discussions of [Cardoso et al., 2012](#); [Fox et al., 2019](#); [Akçakaya et al., 2021](#); [Cowie et al., 2022](#)). Our analysis casts doubt on whether the red listing processing can ever be implemented for all species and in all countries, and at a time interval that allows for effective conservation action for threatened species by stakeholders (e.g., through action plans). It seems to be a utopic goal, especially in the short time frames available for effective conservation action, given the current rapid biodiversity losses on a global scale ('Sixth Mass Extinction'; [Cowie et al., 2022](#); [Finn et al., 2023](#)). Reporting systems and regular monitoring are fundamental to monitor and preserve biodiversity, but how can this be accelerated?

Looking closer at the occurrence records submitted to GBIF during the last two decades, for the MC all countries analyzed together have on average 13 % fewer occurrence records available in the last 10 years

Table 4

Number of occurrence records per country, time interval and organism group. Red background color means a decrease in the number of records in GBIF for the second time interval (2013–2022). Green background indicates an increase and red background a decrease in occurrence records for the second time interval. The differences between the two time intervals are calculated in the third column.

Country/years	Freshwater Molluscs			Marine Crustaceans		
	2003_2012	2013_2022	Difference	2003_2012	2013_2022	Difference
Belgium	3441	1785	-1656	22101	12385	-9716
Denmark	455	300	-155	320	1649	1329
Estonia	273	659	386	15561	9472	-6089
Finland	34	101	67	15	226	211
Germany	5702	1651	-4051	5376	3008	-2368
Latvia	0	27	27	2	24	22
Lithuania	0	163	163	2	60	58
Netherlands	28791	5510	-23281	20755	19610	-1145
Norway	473	858	385	91479	95692	4213
Poland	8	119	111	141	58	-83
Sweden	3027	3133	106	52061	38451	-13610
United Kingdom	9065	3502	-5563	138736	84547	-54189

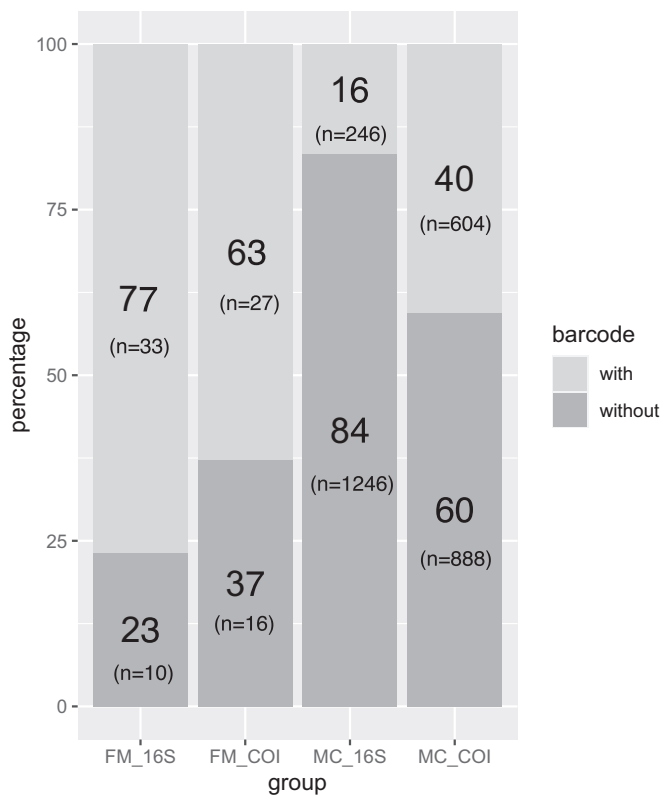


Fig. 3. Number of species from the species list that have a CO1 barcode (combined from BOLD/GenBank) and a 16S barcode (GenBank). In brackets, the absolute numbers are given. Light grey: percentage of species with a barcode. Dark grey: percentage of species without a barcode. FM: Freshwater molluscs; MC: Marine crustaceans.

(2013–2022) than in the 10 years previously (2002–2013) (Table 4). For FM, there were 48 % fewer records in the last 10 years (2013–2022) than recorded in the 10 years previously. Even if many smaller countries increased their numbers of occurrence records in the ten year period, an extreme decrease was observed from the Netherlands, Germany and the United Kingdom. The decrease in numbers of observed occurrence records for our target organisms can have two causes i) sampling effort was less in the last 10 years (2013–2022) than in the 10 years previously (2002–2013) due to less organized monitoring efforts in the country (e. g., through a decrease in the number of taxonomists that are able to

classify species in these groups, Hochkirch et al., 2022); or ii) the population of the species has declined (fewer individuals overall) resulting in fewer observations, but without standardized monitoring datasets it is difficult to support this claim. However, it is interesting to see this trend, because independently of the causes, the target goals of safeguarding biodiversity and reversing biodiversity loss are not fulfilled.

This study clearly shows that consistent monitoring and distribution data are severely lacking for invertebrates, impeding the red listing process. To improve the number of observations available in public databases such as GBIF, new types of occurrence data need to flow into the red listing process, such as citizen science observations from international platforms such as [Observation.org](https://www.observation.org), or national reporting systems such as [Artportalen](https://www.artportalen.se) in Sweden (Sjödin Skarp, 2019) or [Artsoebe rvasjoner](https://www.artsoebe.rvasjoner.no) in Norway. Mobile phone applications such as [Obsidentify](https://www.obsidentify.com) or [iNaturalist](https://www.inaturalist.org) are user friendly, do not require prior taxonomic knowledge, have high potential to facilitate observations for all known species around the world and most importantly have an open data policy (Scherner and Hogeweg, 2018). To ensure taxonomic accuracy, [Observation.org](https://www.observation.org) sightings are validated by experts or by server processes before being shared on GBIF, now comprising a dataset of >71 million observations recorded by >175,000 volunteers (<https://www.gbif.org/dataset/8a863029-f435-446a-821e-275f4f641165>). [iNaturalist](https://www.inaturalist.org) uses image recognition to identify organisms and since 2020, most of the species' occurrences in GBIF have come from [iNaturalist](https://www.inaturalist.org/blog/76606-thank-you-for-helping-generate-most-gbif-records-for-most-species-since-2020) (<https://www.inaturalist.org/blog/76606-thank-you-for-helping-generate-most-gbif-records-for-most-species-since-2020>). In addition, a concerted effort should be made to increase the number of occurrence records available for invertebrates by involving citizen scientists through eDNA monitoring initiatives, which are especially relevant in aquatic environments. Very successful examples of this are the CALEDNA programme (<https://uc.edna.com/>), and a national campaign to map coastal fish in Denmark, where 360 citizen scientists were engaged to collect filtered seawater samples from 100 sites across Denmark over two seasons (Agersnap et al., 2022).

The gap analysis shows that for the 43 FM species in Northern Europe there are more species with a barcode than without a barcode, but there are still many gaps to be filled through systematic barcoding initiatives. Here, more work is needed for COI (37 % without a barcode) than for the 16S primer (23 % without a barcode) (Fig. 3), which is known to work better for freshwater molluscs (Elbrecht et al., 2016). Barcodes are often missing for species that occur in one country only (Weigand et al., 2019) or are rare in contrast to species that are highly abundant and found in several countries. For the 1492 MC species analyzed here, the situation is different, because less than half of the species still have no COI barcode and >83 % still have no 16S barcode (Fig. 3). Already >10 years

ago, several studies pointed out the enormous delay in barcoding for one of the most ancient and structurally diverse groups of arthropods (Costa et al., 2007; da Silva et al., 2011). A recent study showed that many new species can still be detected, even in well-known study areas like the North Sea, by upscaling barcoding projects using novel methodology such as MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) (Rossel and Martínez Arbizu, 2019). Similarly, ‘megabarcoding’ initiatives using high-throughput sequencing technologies such as MinION or PacBio can significantly speed up the barcoding process compared to traditional Sanger sequencing (Hebert et al., 2018; Srivathsan et al., 2021). For both organisms' groups analyzed here, coverage is quite different between European countries and there are still many barcodes marked as private data on BOLD, underlying the need to make data open-access more quickly to benefit the scientific community (Weigand et al., 2019). To come one step further, detailed, automated gap analyses, such as the one presented here are needed, where group-specific gap analyses can be run per country and the relevant barcode gaps addressed through country-specific or European-wide barcoding initiatives, such as the Biodiversity Genomics Europe project.

Other data types that need to be taken into account for red-listing are observations derived from eDNA metabarcoding studies. There exist large numbers of published metabarcoding studies on eDNA, sediment and plankton from aquatic environments (Leray and Knowlton, 2015; Haenel et al., 2017), but biological observations derived from these eDNA metabarcoding data are not yet widely available in repositories such as GBIF. As these types of DNA-based monitoring studies rise exponentially, they represent a treasure trove of observational data that could be captured in the red listing process. Raw sequencing data from such studies are available on databases such as the GenBank sequence read archive (<https://www.ncbi.nlm.nih.gov/sra>), but new tools are urgently needed to enable the easier publishing of the biological observations derived from these eDNA metabarcoding studies after bioinformatic processing of the raw data into Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs) (Callahan et al., 2017). These tools should support the publication of ASV and OTU tables, including comprehensive metadata such as the European Nucleotide Archive checklists for mixed sample registration (<https://www.ebi.ac.uk/ena/browser/checklists>) that facilitate data re-use according to FAIR principles (<https://www.go-fair.org/fair-principles/>). Since 2021, the Swedish Biodiversity Data Infrastructure, has implemented an Amplicon Sequence Variants portal (<https://asv-portal.biodiversitydata.se/>) to fill this gap. Raw data are submitted to the European Nucleotide Archive and after analysis of the raw data using the nf-core/ampliseq workflow (<https://nf-co.re/ampliseq>) and ASVs are submitted to the portal. From this portal, resulting occurrence records go to the Swedish Bioatlas, whose data are integrated into GBIF. Through regular updates, ASVs are re-annotated. At the moment the Swedish ASV portal only works for the 16S gene of prokaryotes, 18S for eukaryotes and ITS for fungi, but should be extended to metazoa and COI.

Similarly, the PlutoF data management system (<https://plutof.ut.ee/>) (Kõljalg et al., 2019) already enables the registration of environmental sample metadata according to ENA checklists and the publication of occurrence records derived from ASVs and OTUs on GBIF, together with a host of other data curation and sharing tools. An environmental sample can be already registered, photographed and basic metadata collected in the field using the PlutoF GO app, thus greatly simplifying and streamlining the gathering of biodiversity data (observations, specimens, material samples). Another on-going project is BiCIKL that aims to link research infrastructures, researchers, citizen scientists and other stakeholders in the biodiversity and life sciences through access to data, tools and services based on open science practices.

In Australia, the ‘Threatened Species Initiative’ has started to fill the implementation gap, and demonstrates how genome resources of threatened species can help conservation practitioners to integrate

genomics into threatened species recovery (Hogg et al., 2022).

All the above-mentioned tools help produce large amounts of presence data with which red listing criteria can in theory be calculated, such as Red List criterion B (restricted distribution and decline, fluctuation and/or fragmentation) and criterion D (area of occupancy: AOO). Criterion A (population decline) as a consequence of restricted distribution/fragmented distribution areas or smaller AOO could also be calculated using presence/absence data, but the number of fertile individuals in a population and therefore criteria pertaining to population size cannot be measured. As discussed in Akçakaya et al. (2021), the collection of standardized datasets over a long time period would allow robust and objective Red List assessments for invertebrates according to IUCN Red List Criteria.

Obtaining population level data from eDNA metabarcoding studies for the purpose of red listing is in theory possible, as ASVs obtained by denoising are able to provide haplotype level information at the population level, if fast evolving DNA regions are chosen that allow the resolution of intraspecific genetic variation, and with the application of appropriate bioinformatic filtering steps (Andújar et al., 2021; Andújar et al., 2022; Tsuji et al., 2020).

5. Conclusion

Here, we demonstrate that national red listing efforts for our target organism groups are not comprehensive, not comparable between countries both in terms of effort and time frames, and unrealistic if all invertebrate groups are to be covered. Instead, we propose the introduction of standardized European-wide assessments at the ecosystem level at regular intervals. Previous such initiatives of the European Red List, e.g., Cuttelod et al. (2011) for non-marine molluscs, or from the Baltic Marine Environment Protection Commission HELCOM for the Baltic Sea (HELCOM, 2012, 2020), were useful initiatives, but need to be regularly repeated in a standardized form to be useful for long-term monitoring. To our knowledge, there currently exist no such European Red Lists younger than 10 years for the molluscs or for the crustaceans. To monitor population size, distribution and trends it might be more efficient to monitor whole habitats as a proxy, not only for threatened and *data deficient* species, as suggested by Cuttelod et al., 2011, but for all species present in the habitat. Whole community screening, with alpha, beta and gamma diversity metrics (Whittaker, 1960) could be used for rapid monitoring as an alternative to red listing. Community screening using eDNA methods or deep learning based on GBIF records to estimate alpha, beta and gamma diversity metrics (Andermann et al., 2022) are promising tools that might speed up the red listing and conservation process. In Europe, the possibility to combine the European Red List of Habitats (Gubbay et al., 2016; Janssen et al., 2016) with the European Species Red Lists would be more cost-efficient and offer possibilities for regular Europe-wide assessments at the ecosystem level through the novel methods discussed here. Currently, European stakeholders have the European Habitats Directive (Article 12–16, Council Directive 92/43/EEC), the Marine Strategy Framework Directive (MSFD; 2008/56/EC) and the Water Framework Directive (WFD; Directive 2000/60/EC) as existing frameworks to evaluate the biodiversity status at six-year intervals (Fig. 4). These directives cover all aquatic habitats and have the potential to protect their biodiversity now and in the future, but they could be synchronized through i) the same reporting time intervals and ii) whole community screening with novel methods as discussed here (Fig. 4). The tools developed here can easily be used for other regions and organism groups to detect underrepresented species in databases, allowing stakeholders to fill those gaps. The next ten years will be critical in finding out if newly developed tools have the power to speed up biodiversity monitoring and help decision makers reverse biodiversity loss through direct conservation actions.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2023.110247>.

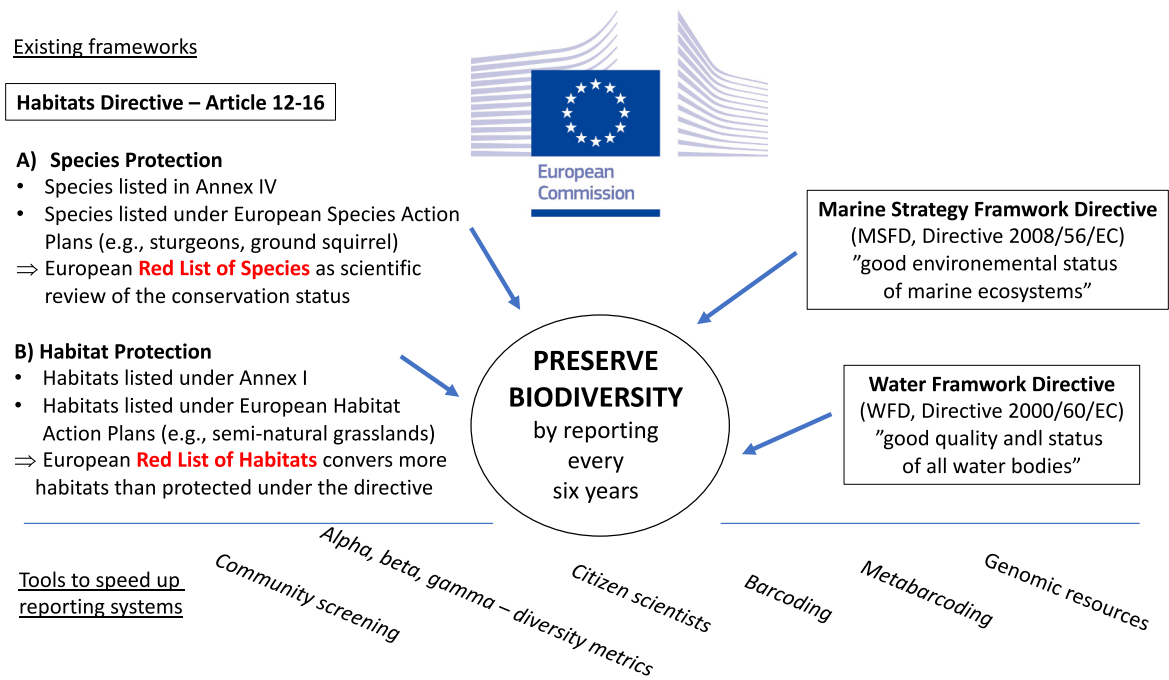


Fig. 4. Overview of existing frameworks to conserve biodiversity in the European Union and potential tools that speed up the reporting of species occurrences during the next ten years.

Declaration of competing interest

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

Data availability

All data are shared in the supplementary material. All scripts used to compile the dataset are available from [GitLab](#).

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