PLOS Water

REVIEW

Harmonization of aggregated freshwater biotic data to support continental and global assessment

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OPEN ACCESS

Citation: Lento J, Laske SM, Culp JM, Goedkoop W, Kahlert M, Lau DCP, et al. (2026) Harmonization of aggregated freshwater biotic data to support continental and global assessment. PLOS Water 5(1): e0000502. https://doi.org/10.1371/journal.pwat.0000502

Editor: Peng Wu, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, CHINA

Published: January 20, 2026

Funding: JL received a contribution agreement from the Government of the Northwest Territories through the Northwest Territories Cumulative Impact Monitoring Program (NWT CIMP) to review analysis opportunities using aggregated data from NWT CIMP-funded projects, which led to the initial drafting of section 2 of this review by JL as general

Abstract

Biodiversity loss and conservation are increasingly coming into focus in global policy fora, requiring information and assessments at wider spatial and temporal scales than previously considered. However, the monitoring framework required to support such data collection and assessment is lacking in many countries and is not harmonized across countries, hampering these efforts. Aggregation of existing freshwater data offers a solution to the problem of assessing status and trends of ecosystems and biodiversity at large spatial scales in the absence of nationally coordinated monitoring efforts. Analysis of aggregated data from different sources, collected using different protocols and with varying levels of metadata and supporting data, can be challenging and requires decisions regarding data comparability. In this paper, we identify the challenges inherent in harmonizing aggregated freshwater data for analysis, including general concerns related to research goals, spatial and temporal scale, sample selection, sampling effort, and site integrity. We also discuss the challenges related to measured parameters, sampled habitats, sample collection and processing methods, and data integrity for phytoplankton, benthic algae, macrophytes, zooplankton, benthic macroinvertebrates, fish, and supporting variables such as water and sediment chemistry. We provide a workflow to evaluate each of these challenges and make decisions about how best to work with the data. Finally, we review a case study from a large-scale analysis of freshwater data from the circumpolar Arctic region that exemplifies the encountered challenges and the chosen solutions. Through the

guidance on harmonization of aggregated data. Funders at NWT CIMP provided feedback on an earlier draft of section 2 and consented to its further development as part of this review. The author team was convened through a Belmont Forum and EU-Biodiversa grant for the Arctic-BIODIVER project to JC, IL, KSC, and WG. KSC's contributions to the CBMP Freshwater working group were supported by the National Environmental Agency. DCPL was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning for funding support (FORMAS; dnr 2021-01062). These funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

description of the case study, we provide practical solutions to support aggregation and analysis of existing freshwater data. As global conversations about biodiversity status and trends continue, the demand for large-scale analyses of data from different sources will only grow. In the absence of globally harmonized monitoring, we are faced with the need to ensure comparability of data, making expert judgements where needed to support sound conclusions.

1. Introduction

Loss of biodiversity continues globally despite conservation efforts [1], with the greatest losses occurring in freshwater ecosystems [2,3]. Furthermore, ongoing and emerging threats to biodiversity and ecosystem integrity in freshwaters [4–6] highlight the need for coordinated monitoring approaches to support large-scale assessment of biodiversity status and trends [7,8]. This idea is fundamental to the Convention on Biological Diversity's Kunming-Montreal Global Biodiversity Framework, which aims to halt and reverse biodiversity loss globally by 2050 [9]. Biodiversity monitoring frameworks can coordinate conservation or other policy efforts, and compiled datasets (built by gathering and combining multiple smaller and independent datasets together) can be rapidly referenced to detect and attribute causes of changes in biodiversity and can be used to build models that project future change [9–11]. However, monitoring of freshwater biodiversity or ecosystem integrity is still lacking in many countries, driven in part by low capacity, weak governance, and poor links to national policy [12,13].

In the absence of nationally coordinated monitoring programs, the use of aggregated databases (i.e., combinations of multiple independent datasets, usually from many different sources that may include government, academia, industry, and others into a single database) can establish baselines, trends, and ecological drivers of biodiversity and ecosystem integrity [14,15]. Large-scale databases have immense potential for assessing biodiversity change because of their taxonomic, geographic, and temporal scope [9,16]. Furthermore, the current push for open and accessible data [e.g., FAIR data principles, 17] and advancements in big data [18,19] are facilitating larger-scale analyses of existing datasets [15]. Increased access to species occurrence and abundance data from multiple sources (e.g., citizen science, community-based monitoring, remote sensing, open data portals and data aggregators, metabarcoding, and synthesis databases) provides avenues to investigate large-scale questions [15,16,19]. These large-scale assessments are important for evaluating change in species populations or distributions [16,19] and the effects of climate change and/or land use patterns that result in a biodiversity response [20,21].

Despite their many advantages, large, compiled datasets often contain spatial and/or temporal gaps [16,19], with data collected using disparate and non-standardized methods [14]. Such gaps and inconsistencies in datasets limit large-scale comparisons and inferences, and the ability to reduce bias and improve inference depends on understanding the factors responsible for the gaps [16].

Nevertheless, this should not be considered a deterrent to conducting large-scale analyses because the vast amount of existing data across continents and (eco)regions has high potential to be included in meaningful analysis [15,20]. To accomplish this, datasets must be harmonized, i.e., made similar enough for use together in comparative analysis without introducing variability from inherent differences in, for example, how data were collected. The key for undertaking such analyses of combined data sets is to understand how harmonization can optimize their comparability, how to handle incompatibilities due to different methodologies and/or insufficient metadata, and when differences in data characteristics require the exclusion of data.

Our objectives are to (1) provide an overview of the challenges inherent in combining freshwater data from different sources for large-scale assessments and (2) share insights and recommendations from our collective experiences building a circumpolar biodiversity baseline for Arctic freshwaters [22]. We identify pitfalls in analysis of aggregated freshwater data and offer guidance regarding the acceptable variability in source data and methods for such assessments (i.e., the amount of variability that is small enough to not have a large impact on the results and is therefore acceptable; variability that does not need to be controlled or removed). Through our case study in Arctic freshwaters, we describe approaches to achieve high-quality assessments with data from multiple sources. Our goal is to indicate how freshwater data from multiple, heterogeneous sources can be developed into high value data resources that can be combined for assessments rather than providing specific recommendations for monitoring protocols.

2. Challenges and considerations for analysis of aggregated data

Analysis of aggregated freshwater data requires considerable thought and evaluation to determine whether datasets are comparable and interoperable [19], which we define as samples that were collected and processed in a similar enough manner to allow them to be compared and analyzed together without introducing high levels of variability due to methodological differences. Evaluating comparability includes consideration of the original sampling goals, study design, spatial and temporal sample coverage, and sample collection or processing methods [23]. Each data attribute impacts the final dataset and affects whether a dataset can be used to meet the goals of analysis. Here, we provide an overview of these considerations and their relevance to assessing dataset comparability. The approaches to harmonize aggregated data for freshwaters are summarized in Fig 1.

Within this paper, we do not describe the approaches to be taken initially to acquire data and format data to match a schema (i.e., data template) with common fields (i.e., data column headers), because these steps are evolving rapidly as computational tools are developed [19]. However, the importance of this step should not be understated because it is necessary to facilitate the data harmonization process. The Global Biodiversity Information Facility (GBIF) is an example of a data repository that houses aggregated data, and GBIF offers guidance and schemas for formatting data that can be used to support these efforts [24], including a guide specific to formatting freshwater data [25] using the international data standard DarwinCore [26].

2.1. General challenges

2.1.1. Research goal. Categorization of studies by their research goal is an important first step to understanding whether data from these studies are comparable [23]. For example, if the analysis of aggregated data is focused on large-scale assessment of biodiversity of the fish assemblage, it will be necessary to retain only datasets that were collected with a goal to sample the full assemblage, rather than datasets that targeted individual species (e.g., only sampling Atlantic salmon *Salmo salar*) (Table 1). Even if datasets are focused on the same ecosystem components (e.g., aspects of the ecosystem that are monitored, including biotic assemblages or abiotic measurements), differences in research goals among datasets may introduce variability in spatial and temporal scales, site selection and integrity, measured parameters (i.e., the aspects of an ecosystem component that are actually measured/collected or portion of an assemblage sampled), sample collection effort and/or processing methods, and potentially data integrity.

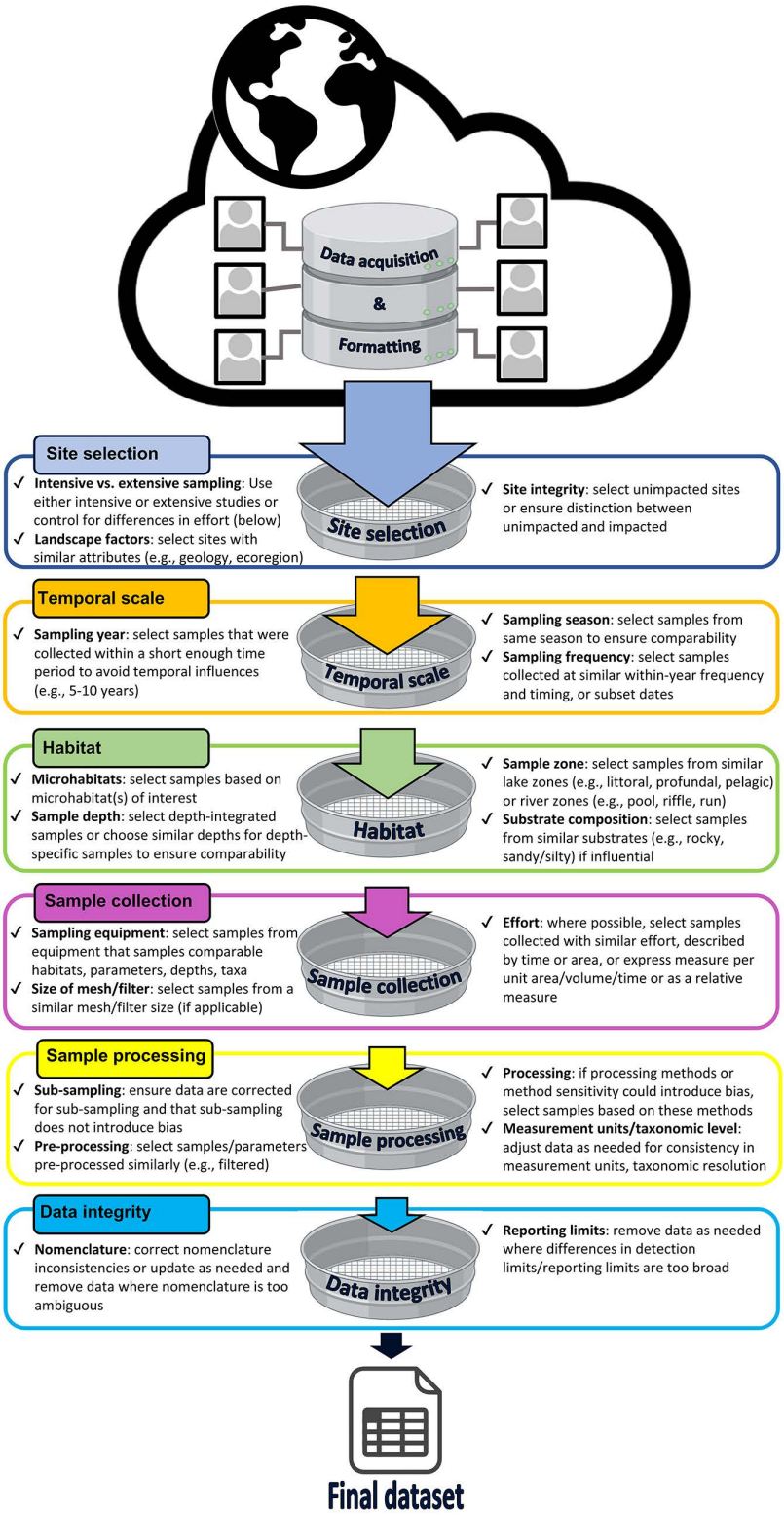

Fig 1. A flow chart of the steps for harmonizing aggregated freshwater data for analysis, highlighting the steps after initial data acquisition and formatting when there is a need to consider site selection, temporal scale, habitat, sample collection, sample processing, and data integrity before achieving the final dataset. For each step, details are provided about each aspect of the data that must be considered and how data may be filtered to reach the final, harmonized dataset.

https://doi.org/10.1371/journal.pwat.0000502.g001

Table 1. Summary of major challenges and considerations in analysis of aggregated freshwater data from different sources, specifically related to the research goal(s), spatial scale, temporal scale, site selection, site integrity, and sampling effort of data sources.

Data Aspect	Challenges and considerations
Research goal(s)	• Differences in research goals lead to the challenges listed here and in Table 2, and should be considered when selecting and harmonizing data • Data aspects (as listed here and in Table 2) may differ depending on whether data were collected for biomonitoring or bioassessment, and whether they were collected as part of routine monitoring or scientific research (e.g., including population/habitat range studies, experiments, mesocosm or water body manipulation)
Spatial scale	• Datasets can include extensive sampling of sites across a large area or intensive sampling within a localized area, which can introduce differences in data due to differences in sampling effort (described below) • Biotic samples collected at the microhabitat level may not be comparable with samples collected at a larger spatial scale (across multiple microhabitats), though this effect might be dependent on the organism group
Temporal scale	• Differences in the years of data collection (e.g., by a decade or more) may introduce temporal changes that confound spatial differences in datasets • Datasets collected during different seasons (e.g., winter and summer) are not comparable for many organism groups due to seasonal variability in environmental parameters and phenological changes in biota • Environmental parameters and biotic indicators differ in the temporal scale over which they vary (e.g., water chemistry can change over hours or days, whereas biotic change may be evident over weeks to years, depending on generation time, dispersal, and colonization rates), and this should be considered when considering reliability of data and potential for Type I and Type II errors
Sample selection	• Sample selection for analysis should ideally control for potential confounding factors, e.g., sites for analysis of water chemistry should be selected to have similar underlying geology • Metadata must include site characteristics such as habitat descriptions and measurement of relevant environmental parameters to allow for the selection of samples while controlling for variability in confounding factors
Site integrity	• Information about the degree of impact by environmental or anthropogenic stressors is necessary to ensure comparable samples are selected for analysis • Information about whether water bodies are natural or artificial is necessary because of potential differences in system morphology and abiotic and biotic ecosystem components
Sampling effort	• Analysis must control for the level of sampling effort across space and time, as differences in effort during sample collection (e.g., different spatial/temporal coverage and replication) will naturally yield different results (e.g., greater effort leads to greater chance of detecting environmental pulses and more accurate measurement of biodiversity).

https://doi.org/10.1371/journal.pwat.0000502.t001

2.1.2. Spatial scale. Differences in the spatial coverage of datasets often reflect contrasts in sampling effort [23] (Table 1). For example, spatially restricted investigations may have higher sample site density [27] or may allow resources to be allocated to repeated sampling over time (refer to sec. 2.1.3). These forms of spatially restricted studies with higher sample effort can increase the detectability of species or patterns that may be missed by large spatially extensive surveys [28] and may lead to higher and more accurate estimates of diversity or population size over the sampled area [27,29].

Spatially extensive sampling may not allow for the same concentrated sampling effort across space and time due to the resource constraints associated with sampling a larger geographic area. Consequently, spatially extensive sampling data may not be able to detect seasonal or inter-annual trends or episodic perturbations; though they may sample more regions, habitats, and environmental conditions, thereby increasing the number of species detected [28]. Spatially extensive sampling designs also have the potential to estimate species distributions, though care should be taken not to treat lack of presence at a site as a true species absence because of the high risk of a type II error [30].

Within-site variation in spatial scale can also be an issue because comparisons of data that were collected in different microhabitats may not be valid for all analytical purposes [31]. Abiotic and biotic conditions may vary between microhabitats, potentially confounding the patterns of interest [32,33]. Furthermore, community studies that focus on a larger spatial scale within a site (i.e., sampling across multiple microhabitats) are expected to have higher diversity than those that focus on a single microhabitat [29]. Stratification of samples by habitat type may be necessary to control for this variability (Fig 1).

2.1.3. Temporal scale. Temporal aspects of datasets that may affect comparability in analysis of aggregated data include the years and seasons of sampling and the time between sampling events (for repeated sampling; Table 1).

When comparing across regions, the temporal scope of sample collection should be considered. Data collected during the same time frame at multiple locations will prevent confusion over whether differences are due to space or time. This is particularly relevant due to ongoing climate change contributing to rapid shifts in freshwater ecosystems [34–36]. Samples collected at the same locations over decades may consequently differ solely due to natural inter-annual variability and/or the influence of changing climate. Caution must therefore be used when comparing data from different regions that were collected over very different time frames because spatial differences could be confounded by temporal changes.

The season in which sampling occurs plays an important role in determining the natural range of variability in habitat conditions (Table 1), including water chemistry, temperature, snow/ice cover, and hydrology [16,37,38]. For several biotic components, seasonal abundance and composition relate to phenological traits that are driven by habitat conditions such as temperature and flow, including the timing of aquatic insect emergence and spawning and migration of fish [39]. For example, the timing of sampling in relation to insect life cycles and emergence periods can result in different dominant taxa and decreased capture of early instars, and samples of benthic macroinvertebrates should be collected in the same season(s) to be comparable among sites or among years [31]. If the full annual cycle is not the focus of research questions, analysis of aggregated biotic or abiotic data should apply a seasonal classification to ensure natural seasonality and population cycles do not introduce variability into the analysis [16] (Fig 1).

Sampling frequency and how it relates to the expected rate of change in measured ecosystem components must also be considered [27,40]. Supporting variables, which are environmental measurements, such as water chemistry that are used to relate to or better understand variability in biotic measurements, can vary over short time scales, including diurnal cycles for some parameters [40,41]. Impacts of perturbation may be evident over different temporal scales depending on whether it is a lentic or lotic system and depending on water residence time [42]. While seasonal spot measurements may be effective for characterizing water chemistry, albeit with errors [40], spot measurements taken once per year may not be an accurate reflection of habitat conditions, particularly for parameters that are highly affected by flow and lake thermal stratification [42].

Algal communities, though less variable than water chemistry, can change over a matter of days or weeks and will thus integrate environmental changes over a relatively short period of time [43,44]. In contrast, benthic macroinvertebrates and fish will integrate environmental change over longer periods of time [45] and therefore require less frequent sampling, although sampling of fish must recognize differences in habitat use, life history, and catchability at different times [46]. The differences in rates of change for different organism groups and environmental variables influence the level of sampling effort (including temporal frequency) that is required to effectively characterize sites or answer research questions, and they also have implications for the temporal variability captured by the data. Therefore, single-sampling events for water chemistry data provide a less time-integrated measure of condition than single-sampling events for benthic macroinvertebrate or fish. Repeated sampling can detect seasonal and inter-annual trends and increase the chances of detection of episodic perturbation in organism groups or supporting variables with a fast turnover rate. Repeated sampling can also be used in hierarchical modelling frameworks that yield species detection probabilities for individuals in communities [e.g., multi-species occupancy models; 47–49].

2.1.4. Sample selection. Prior to any analysis of aggregated data, it is important to determine the questions and hypotheses to be addressed, as this will act as a guide for the types of data that are needed [19]. For example, will the analysis investigate genetic diversity [e.g., 50], intraspecific diversity [e.g., 51], richness [i.e., taxonomic diversity, e.g., 22], or ecosystem diversity [e.g., 52]? Datasets from multiple studies may apply or may be devoid of the information necessary to address a particular question. If the study aims to examine the population-level metrics of diversity, then it could be necessary for the collected datasets to include abundance, age, sex, or size information. Conversely, if the study focuses on species richness, then incidence or abundance information would likely suffice.

Considerations for sample selection in analyses of aggregated data require controlling variability that is unrelated to the questions or hypotheses (Table 1). The ability to detect patterns and differences in response variables is dependent on

both the magnitude of response and the amount of variability in the data [23]. If the combined data come from sites that differ naturally in terms of climate, ecoregion, underlying geology, system size, or other potentially confounding factors, then variability in the response to the stressor in question may be obscured or covary with multiple environmental gradients. For example, underlying geology should be taken into consideration when selecting diatom and benthic macroinvertebrate samples or analyzing data because geology plays a large role in determining driving factors like water and sediment chemistry (Fig 1) [53–55].

Due to the need to control for confounding factors and ensure similar sampling approaches, metadata (i.e., information about the data) and supporting data must offer sufficient detail to provide the context for the data and indicate comparability [56–58]. Metadata should include detailed site and habitat descriptions as well as specifics of sampling and sample processing methodology, and information about who collected the data [56,57]. Furthermore, data on relevant supporting variables that characterize sample habitats and seasons (e.g., flow, physical habitat measurements such as depth and width, substrate composition, and water chemistry, if not already included as part of the main sampling design) make it possible to control for confounding factors.

2.1.5. Site integrity. Site integrity indicates to what extent sites have been impacted by natural or anthropogenic stressors, thereby providing information about comparability of sites (Table 1). For example, a site in an area of resource development with active construction likely differs from unimpacted sites with respect to water chemistry, sediment load, physical habitat, and biotic community structure [59, 60]. Selection of samples for analysis therefore requires that sites can at minimum be classified as unimpacted (i.e., representing reference conditions) or impacted to ensure that human disturbance does not confound the assessment if it is not the focus of the research question. If possible, more detailed descriptions of site integrity may be provided by classifying sites in a hierarchy of reference conditions, such as natural condition, minimally impacted, least disturbed, or best attainable [61,62]. In addition to site integrity classification, information about potential stressors should ideally be present in the metadata, ensuring that analysis is focused on sites/samples with a similar level of integrity.

Although not directly a measure of site integrity, it is also necessary to consider whether sampled water bodies are natural or artificial (e.g., canals, reservoirs, and other human-made water bodies). This has implications for aspects of habitat such as channel structure (e.g., tortuosity), flow, water body shape, and habitat complexity, all of which may contribute to differences in biotic and abiotic ecosystem components [63,64].

2.1.6. Sampling effort. Sampling effort depends on factors such as the number of sample sites or reaches within a water body (often affected by ecosystem size), the number of replicate samples collected at a site during a sampling event, the length of time spent during a sampling event, the different sampling methods used at a site, and the temporal frequency of sampling events (Table 1). Analysis of aggregated data must control for these differences in sampling effort because they naturally yield different results [65]. Sampling effort can be affected by the spatial and temporal extent of sampling [23] and often differs among datasets.

Approaches to control for sampling effort in analysis of aggregated data depend on the extent of differences, the sampled parameters, and the measures of interest. For example, it may be necessary to stratify the data and omit data for sites that include sampling across multiple seasons while most other sites were sampled in only one or two seasons (Fig 1). If multiple sampling methods were used to collect taxa, it may be necessary to select a subset of data based on sampling methods that are common among studies in the aggregated data. Alternatively, use of integrated community models or other hierarchical frameworks (e.g., joint species distribution models) may allow for analyses that simultaneously model multiple data sources for multiple species [66,67]. In biotic studies, there are specific approaches to control for differences in sampling effort when estimating taxonomic richness. Sampling effort can be standardized for estimates of taxonomic richness through rarefaction [29,68] when the number of sample sites differs among studies or regions [e.g., refer to [21] and other papers in the same issue]. Also, abundance can be reported as catch per unit effort (CPUE) to control for differences in time or area sampled [69]. When these measures are not appropriate or possible, scaling the data

(to a mean of 0 and standard deviation of 1) or converting counts/abundance to incidence data (presence = 1, absence = 0) allows for the comparison of general trends across sites/studies while controlling for differences in magnitude. Options that are specific to particular freshwater ecosystem components or taxonomic groups, whether to control for differences in sampling or sample processing, are discussed in more detail in the next section.

2.2. Component-specific challenges

Combining freshwater biodiversity information from a variety of sources presents several challenges and considerations that are specific to the ecosystem component being studied. Table 2 identifies many of these challenges for water/sediment chemistry, fish, benthic macroinvertebrates, plankton, benthic algae, and macrophytes. While not an exhaustive list of ecosystem components or challenges, this information highlights some of the primary considerations that need to be made when tackling analysis of aggregated data for these freshwater components.

2.2.1. Measured parameters. Many challenges related to measured parameters for biotic sampling involve studies that focus on different subsets of an assemblage (Table 2). For example, analyses that aim to estimate taxonomic diversity should include datasets on the full assemblage of species within a particular organism group rather than data from studies that target specific or limited taxa [e.g., species-targeted sampling of fish; 70]. This is also a challenge for measures of abundance, biomass, and habitat use or occupancy by species. Similar issues exist when sampling equipment or study goals (e.g., a focus on certain size fractions) do not allow for the identification and enumeration of all species, which is a common concern for data on zooplankton [e.g., exclusion of rotifers; 71], phytoplankton [e.g., exclusion of picoplankton and nanoplankton; 72], and benthic algae [e.g., focus on only diatoms; 73]. To avoid introducing analytical bias in all biotic components, only samples that have measured a similar portion/group of the assemblage should be used for the analysis (Fig 1).

Water and sediment chemistry data can provide important supporting information to understand habitat conditions in freshwaters, but data cannot be compared if the same parameters have not been measured for all samples [74] (Table 2). Virro et al. [75] compared five different water quality databases that ranged from national to global scale and found variable degrees of overlap in parameters across databases. For nutrient data, such differences in comparability among studies depend upon whether elemental/molecular forms, organic or inorganic forms, and dissolved and particulate phases have been measured [74]. Similarly, the comparison of metal concentrations in water can include dissolved, total, or free metals, and data may not always be clear on the sampled fraction. Incomplete or incorrect metadata, including missing or unclear reporting of units, combined with ambiguous terminology can further contribute to a lack of clarity regarding the comparability of water chemistry data [57,74]. Such issues highlight the need for collection of a standard suite of water chemistry parameters to ensure comparability, and the need for detailed information about which parameters, fractions, and units are being reported. Some data repositories attempt to circumvent such issues through the use of a strict data schema that must be followed when providing data, including both required fields and controlled vocabulary [e.g., the Gordon Foundation DataStream in Canada; 76]. These efforts facilitate greater comparability between datasets.

2.2.2. Sampled habitat. Lake limnologic zone (littoral, pelagic, profundal), stream morphology (riffle, run, pool), tortuosity, depth, water body size, and connectivity are relevant characteristics of the habitat that was chosen for sampling because each can affect biotic composition and environmental characteristics [77] (Table 2). The distinction between lake limnologic zones is one of the most important considerations for analyses of aggregated lake biotic data. Habitat conditions in the shallow littoral zone are vastly different from those in the deeper profundal zone, resulting in different communities. For example, whereas littoral zones may often be oxygenated, with high primary productivity and a large variability of microhabitats (e.g., different size substrata, water plants, wave action), profundal zones of lakes generally have soft sediments, with low (or no) primary productivity and potentially low oxygen or anoxic conditions below the light-compensation level [77,78]. Benthic macroinvertebrate assemblages of soft sediments in the profundal zone therefore tend to be dominated by Diptera and worms that are adapted to low oxygen conditions, whereas those in the littoral zone

Table 2. Summary of major challenges and considerations in analysis of aggregated freshwater data from different sources, specifically related to measured parameters, sampled habitat, sample methods, and data integrity of data sources, with examples provided for a selection of ecosystem components (water chemistry, sediment chemistry, and contaminants; fish; benthic macroinvertebrates; plankton; benthic algae; and macrophytes).

Data aspect	Ecosystem component	Challenges and considerations
Measured parameters	**Water chemistry, sediment chemistry, and contaminants**	• Different studies may not measure the same suite of elements, compounds, and forms, or may not measure the full suite, precluding data comparisons • Measured elements, compounds, or forms may not be the most relevant for biotic components • Metals in water: studies may not include measurement of both total and dissolved metals, or may not specify which fraction is reported in the data
	Fish	• Data from studies that target one or more specific species and data from studies that sample the entire fish assemblage cannot be combined for estimates of diversity, abundance, biomass, or habitat use/occupancy • Studies may take different measurements of fish size, health, and fecundity or may only focus on abundance
	Benthic macroinvertebrates	• Density can be estimated from quantitative sampling methods, but semi-quantitative or qualitative methods provide estimates of abundance/relative abundance
	Plankton	• Studies that are focused on a particular group (e.g., cladocerans, copepods, rotifers for zooplankton; macroplankton, microplankton, nanoplankton, or picoplankton for phytoplankton) will not be fully comparable with data from studies that sample all groups
	Benthic algae	• Studies may focus on diatoms, soft algae (non-diatoms), or the full assemblage
	Macrophytes	• Studies may not include all classes (e.g., they may exclude mosses or macroalgae)
Sampled habitat	**Water chemistry, sediment chemistry, and contaminants**	• Depth of sampling affects water chemistry results (particularly in stratified lakes), and information about sample depth is an important component of metadata • Depth of sediment chemistry sample relates to time frame of described conditions, but also has the potential to affect chemical speciation
	Fish	• Sample depth, habitat, and thermal stratification may affect composition/size/age of collected fish • Water body size and connection to the hydrologic network may affect the composition/size/age of collected fish
	Benthic macroinvertebrates	• In rivers, samples may be focused on a particular microhabitat or fixed area (e.g., Surber or Hess sampler), or they may be multi-habitat samples (e.g., travelling kick sample), which can affect composition of samples • In lakes, the littoral zone has naturally higher diversity and a different invertebrate composition than the profundal zone • Benthic macroinvertebrate assemblage composition differs depending on substrate composition (e.g., sand/silt, cobble, or bedrock)
	Plankton	• Samples may be depth-specific (i.e., collected at a particular water depth) or composites (i.e., collected through the water column, as with a plankton tow) • Plankton assemblages differ in composition between the littoral and pelagic zones of lakes, and differ in relation to thermal stratification of the pelagic zone
	Benthic algae	• Benthic algal assemblages differ in composition between lake zones and river microhabitats • Benthic algal assemblages differ in composition among substrates (e.g., hard substrates such as cobble and rocks, soft substrates such as sand/silt/mud)
	Macrophytes	• Macrophyte growth forms and species differ depending on water depth and light penetration
Sample collection and processing methods	**Water chemistry, sediment chemistry, and contaminants**	• Different sampling equipment and sampling methods may collect water from different depths, and may collect depth-specific or integrated water column samples, affecting sample comparability • Filtration of samples (either in the field or in the laboratory) leads to estimates of a different fraction than unfiltered samples • Different laboratories have different sensitivity levels for their analyses, leading to differences in the minimum reporting limit
	Fish	• Depending on the mesh size of nets, samples may be biased against small- or large-bodied individuals • There may be differences in sampling effort among studies, with some studies using multiple different types and/or mesh sizes of sampling gear to get a more complete sample of the fish assemblage

(Continued)

Table 2. (Continued)

Data aspect	Ecosystem component	Challenges and considerations
	Benthic macroinvertebrates	• Different types of sampling equipment may be biased towards particular taxa and/or sizes, and samples may not be comparable among equipment types • Sampling effort (size, area, or time limitation of sampling) may differ among studies • Samples collected with different mesh sizes may have differences in taxonomic composition and size of individuals, and larger mesh sizes may be biased against smaller-bodied taxa • Studies may differ with respect to the amount of sample that is processed (i.e., full sample or sub-sampling), which can affect estimates of abundance as well as probability of detecting rare taxa • Studies may differ in their taxonomic resolution, and harmonization may be necessary
	Plankton	• Phytoplankton: Depending on the sampling equipment and the filter pore size used, some studies may not target the smallest phytoplankton size classes, excluding picoplankton and nanoplankton • Zooplankton: Depending on the mesh size of the sampling equipment, some studies may not target rotifers, and may represent only a portion of the zooplankton assemblage (excluding smaller-bodied individuals and taxa) • Phytoplankton: studies may report density (individuals/L) or may report biomass as wet weight (μg/L) or carbon (μgC/L)
	Benthic algae	• Studies may report density (individuals/cm^2), biomass (μg/cm^2), relative abundance, or counts (not standardized per unit area) • Differences in how cells and colonies are quantified (e.g., what is counted as a single unit) may lead to differences in estimates of abundance/biomass • The number of cells or colonies counted per sample may affect diversity estimates • Differences in sample cleaning (burning or oxidation) could affect quality of the sample prior to identification
	Macrophytes	• Studies may collect opportunistic observations (i.e., no specific effort level) or qualitative, semi-quantitative, or quantitative samples, with data ranging from taxon lists to abundance and diversity • Depending on how quantitative the sampling is, effort may differ between studies
Data integrity	**Water chemistry, sediment chemistry, and contaminants**	• Detection limits (or reporting limits) are necessary to ensure that samples are comparable, but they may not be provided with the data • Information about any data pre-treatment (e.g., if values below detection limit have been adjusted prior to sharing data) is necessary to ensure comparability • Samples collected from remote locations may exceed holding times, and data quality may be compromised
	Fish	• Common names (e.g., whitefish) may apply to multiple species, and data must be screened to ensure taxonomic ambiguities are resolved
	Benthic macroinvertebrates	• It may be necessary to check taxonomy of older samples for any required nomenclature updates • When sorting has been done through subsampling (rather than full enumeration), it is important to check whether the data are corrected for subsampling or not
	Plankton	• Algal nomenclature changes frequently, and it is necessary to check taxonomy of older samples for any required updates • Photos of algal taxa are valuable for quality assurance and quality control (QA/QC) of data, particularly as nomenclature updates are required
	Benthic algae	• Algal nomenclature changes frequently, and it is necessary to check taxonomy of older samples for any required updates and, where possible, to check the level of certainty in the identification of samples • Photographs and measurements of taxa are valuable for QA/QC of data, particularly as nomenclature updates are required
	Macrophytes	• It may be necessary to check taxonomy of older samples for any required nomenclature updates

https://doi.org/10.1371/journal.pwat.0000502.t002

hold a higher diversity [79] that is more similar to those of stream habitats [22]. Zooplankton assemblages are usually more diverse in littoral than in pelagic zones; however, most emphasis has been on sampling of pelagic habitats [80]. Fish are known to rely on energy resources in the littoral zone [81], but some species may shift to a reliance on pelagic food sources in the summer when zooplankton abundance is high [82]. As a result of differences in habitat use, some studies have focused on characterising fish assemblages in a single zone (e.g., only littoral or only pelagic) to circumvent

the difficulties associated with trying to efficiently sample across all lake habitats [46]. When conducting analysis of aggregated data for lake biotic communities, it may therefore be important to stratify the data based on the sampled habitat (Fig 1). Furthermore, for pelagic data, it may be important to have information about lake mixing regimes and thermal stratification in relation to sampling depth because this may affect composition of biotic samples [83].

Many water chemistry parameters vary strongly along the vertical profile of a lake, including temperature, oxygen, and nutrients [77]. Therefore, it is important to check the metadata to determine how/where the samples were collected, from which depth(s), and the sample position relative to thermally stratified layers, if applicable [57,58,84]. For example, water samples may be collected from just below the surface, as discrete samples collected from a specific depth(s), or as composite samples that integrate water over multiple depths in part of or the entire water column, which can influence the comparability of the data [85]. The location at which samples were collected (e.g., closer to the shore or in mid-lake) can also impact results [85] and should be taken into consideration. In rivers and streams, representative samples should be collected from the thalweg (deepest and fastest flowing location) or another area where water is flowing and mixing because results for water chemistry parameters might be affected by sampling in stagnant waters or pools that are not well mixed. Metadata for water chemistry samples should be used to subset based on the collection depth.

2.2.3. Sample collection and processing methods. Differences in gear function, mesh (pore) size, effort, and reported units of sampling lead to differences in either the capture of organisms (e.g., missing small size classes of zooplankton) or differences in reporting (e.g., individuals/unit or biomass) [23,72,86,87] (Table 2). It may be necessary to omit samples collected with very different methods or to conduct a qualitative assessment (e.g., presence/absence) rather than a quantitative assessment (e.g., abundance, density) if methods are not comparable [21]. For example, fish are often collected in lakes using multiple sampling methods because methods are size- or habitat-specific and will differ in effectiveness at capturing different species/sizes of fish [46], which complicates the harmonisation of data. In locations where replication of sampling with a single gear is sufficient to determine species detection probabilities, the addition of data from supplemental gear types can be used in hierarchical frameworks to improve understanding of community dynamics and processes that structure assemblages [67,88]. The conversion of fish data collected with different methods to presence/absence may be used to account for variability in abundance estimates, but even here, caution should be used when estimating diversity across studies, particularly for methods that are biased toward particular taxa or sizes [89]. For microscopic organisms, samples should be selected for analysis based on whether collection methods were quantitative or qualitative and based on comparable mesh/filter size. Some mesh sizes of zooplankton nets exclude small organisms (e.g., rotifers), and this may result in lower diversity and abundance estimates [71,87]. Larger mesh sizes may also exclude young zooplankton (e.g., nauplii and early copepodites) [87]. For phytoplankton, the exclusion of small size classes (e.g., picoplankton) due to filter pore size may lead to reduced estimates of diversity, biomass, and primary productivity [72]. It is important to ensure all samples combined for analysis are effectively sampling the same portion of the assemblage to avoid introducing sampling bias (Fig 1).

Different sample processing methods can introduce bias if they vary with respect to sub-sampling, enumeration, and species identification (Table 2). For example, benthic macroinvertebrate samples may be subsampled or fully enumerated, and identification may be at different taxonomic levels, including family, genus, or lowest practical taxonomic unit (species or coarser, as possible) [31,90]. Where taxonomic level differs, taxonomic harmonisation will be necessary, potentially by using a standardized list of taxa or applying a coarser taxonomic resolution and dropping some taxa that are redundant with finer resolution data [91]. Microscopic counting procedures for zooplankton can affect abundance and density estimates [71]. Similarly, information on how colonies are enumerated in benthic algae data is helpful to understand potential biases in abundance estimates. For both phytoplankton and benthic algae, it may be necessary to harmonise data on (relative) abundance/density/biomass if these data are reported in different ways in different studies. However, due to the level of expertise required to identify algal taxa, caution is necessary to ensure confidence in taxonomic identification because differences among taxonomists can have a large effect on sample comparability [92,93]. For all organism groups,

information on sample processing must be discerned from the metadata to ensure comparable data are retained for analysis.

Ambiguities can also be introduced into analysis of aggregated water chemistry data when information about sample filtration (whether it was done, and with what pore size) is lacking in the metadata [74], and this can result in increasing the unexplained variance in the analysis [75]. The issue of differences in processing methods, equipment, and sensitivity levels among laboratories is also difficult to control in analysis of aggregated chemistry samples [58]. In some cases, issues that arise due to among-laboratory differences can be handled and harmonized by simple conversion of units and values [75]. When lower detection limits or reporting limits differ among samples, decisions must be made for how to deal with these discrepancies [58]. Simple removal of non-detects (values below detection limit or reporting limit) biases the data towards higher values [94]. Common practice is to replace non-detects with other values (for example, some fraction of the detection limit), but this may have a particularly strong effect on the results and may introduce erroneous patterns into the data [94]. Statistical techniques such as maximum likelihood estimates offer a potential solution that may provide less biased options for replacing non-detects [94], but these approaches should still be used with caution when detection limits vary across studies [58].

2.2.4. Data integrity. Missing or ambiguous metadata is a common data concern that affects the comparability and integrity of data [56,58,74] (Table 2). For example, in their review of 25 million records of nutrient data from water chemistry samples, Sprague, Oelsner and Argue [74] found that over half had missing or ambiguous metadata that compromised the user's ability to analyse the data. The minimum detection limit or reporting limit for water chemistry and sediment chemistry analyses are required to determine whether low values are comparable among studies, but this information is not consistently reported [58]. Furthermore, chemistry data may be provided after some pre-treatment of the data (e.g., adjustment of data below detection limit/reporting limit), and it is critical that the information about the chosen pre-treatment be provided with the data [58]. In the absence of the required metadata, it is often necessary to omit the data to avoid making assumptions about the missing/ambiguous information (Fig 1).

Nomenclature issues are a common concern for data integrity of both abiotic and biotic data (Table 2). Sprague, Oelsner and Argue [74] found that the 10 most common nutrient parameters reported in water chemistry data from the USA were reported with 1046 different parameter names, of which 115 could not be clearly assigned to a single parameter. The fact that a single parameter can be labelled and reported with more than 100 different names (e.g., nitrite + nitrate was reported with 130 different names), leads to issues both in data harmonisation and in the potential for ambiguity and error. The same is true for biotic data [74,91]. Fish are often known by a wide variety of common or regional/local names [95] which complicates joining information across large spatial scales or among regions. This is particularly problematic if the same name is used across regions/communities to refer to different genotypes, or if a single fish species is known by multiple common names [96].

When combining data collected across a wide range of years, further effort must be made to ensure that nomenclature for biotic data is recently updated. Changes to taxonomic nomenclature occur as new species are discovered and existing species are re-classified (e.g., taxonomic groups are split or joined, or taxa are moved to different taxonomic groups) [91]. An early step in an analysis of aggregated biotic data should therefore be to examine the taxonomic list and ensure that all taxonomic names are current and updated [91]. Across broader regions (e.g., when comparing data between continents), it may be necessary to consider whether regional naming conventions exist and require additional harmonisation steps. For example, there are thousands of diatom species [97], and nomenclature is rapidly changing. Harmonization of nomenclature requires expert review of taxonomic lists and should make use of accepted taxonomic backbones, such as the Freshwater Animal Diversity Assessment (FADA) project [98]. New tools are emerging to simplify this process [91].

3. Case study: Data harmonization challenges and solutions

We provide a case study to demonstrate how aggregated freshwater data can successfully be harmonized to support large-scale analysis. This study from the circumpolar Arctic region was conducted by the Circumpolar Biodiversity

Monitoring Program's Freshwater Group [CBMP-Freshwater; 22] and exemplifies the multiple challenges that may be encountered in such harmonization efforts and the potential solutions (Table 3). The CBMP-Freshwater study was the culmination of an international collaboration among scientists from all Arctic countries that involved the collection, harmonization, and analysis of data from varied sources within each country [e.g., government monitoring, industry monitoring, academic research, primary and grey literature; 14].

Table 3 highlights the key challenges that were encountered during the data harmonization process for each freshwater ecosystem component and provides recommendations for how to deal with these challenges based on the decisions and approaches in the case study. These recommendations are based on a goal of large-scale biodiversity assessment, but they may also be applicable in the harmonization of large, aggregated datasets for other research purposes. In the following sections, details are provided regarding each of the challenges encountered in data harmonization and the rationale behind the choices (and therefore the recommendations in Table 3). Greater detail for each challenge and recommendation can be found in the cited papers.

Data included in the circumpolar analysis were generally from sites classified as least impacted or in reference condition, except for specific regional case studies [e.g., analysis of permafrost thaw impacts on water chemistry; 99]. Sites in different ecoregions and with different habitat characteristics were retained because the goal was to understand spatial variability across climatic and habitat gradients in the Arctic. Sites were grouped by ecoregion [Terrestrial Ecoregions of the World; 100] to compare across climatic regions. As the compiled data covered a large timespan, the focus for analyses of contemporary data was on samples collected in 2000 or later, except where this would exclude countries or in the case of paleo-sediment cores for diatoms.

The analysis for all organism groups had a goal of describing contemporary patterns in taxonomic diversity, but differences in sampling effort across ecoregions introduced bias in estimates of taxonomic richness, as richness increases with increasing sampling effort [29]. We therefore used interpolation and extraction of rarefaction curves [101] to provide standardized estimates of taxonomic richness that were unbiased by differences in effort. For a given taxonomic group, separate rarefaction curves were developed for each ecoregion and estimates of taxonomic richness at the same number of sites for all ecoregions were extracted through interpolation or extrapolation of the curve, as needed [21].

3.1. Phytoplankton

Lake phytoplankton samples were collected with a bottle, a volume-specific composite sampler or depth-specific sampler, or plankton net (10 or 20 µm mesh; Table A in S1 Text). Some samples were filtered, with mesh size varying by country and dataset (or unreported; Table A in S1 Text), which had the potential to affect the size fractions of phytoplankton present in samples (Table 3). Schartau et al. [102] retained all data for their analysis but acknowledged the need for greater harmonization of sample collection methods in future sampling. There was also a mix of depth-integrated and depth-specific samples among the datasets. All depth-specific samples for a site were combined prior to analysis to ensure all data represented a composite of the water column [102]. Samples from the littoral zone or from benthic habitats were excluded to ensure only pelagic species were assessed (sec 2.2.2; Table 3).

Harmonization of nomenclature was challenging due to evolving taxonomy of algae (Table 3). Schartau et al. [102] worked with developers of AlgaeBase [103], a global algal database of taxonomic, nomenclatural and distributional information, who allowed the taxonomic list to be matched against the AlgaeBase taxonomic database. While this required manual correction of typographic errors, it was an efficient way to check the extensive taxonomic list. Some taxa were grouped at a coarser level where necessary, if there was no match found in AlgaeBase (sec 2.2.4). A newly developed package (algaeClassify) in R v4.4.2 could be used to automate and speed up this taxonomic harmonization process for phytoplankton in the future [104,105].

The largest challenge in analysis of aggregated phytoplankton data was related to quantification differences. Across all datasets, phytoplankton were quantified as density (individuals/L), biomass (wet weight in µg/L or µg carbon/L), relative

Table 3. Key challenges encountered in the harmonization of aggregated freshwater data for the broad-scale analysis of Arctic freshwater biodiversity described in the case study and recommendations for how to deal with similar challenges, based on the approaches chosen in the case study. Described approaches were applied to the challenges encountered in the data and the general question of evaluating biodiversity status across a large spatial scale.

Ecosystem Component	Challenges Encountered	Recommended Approaches
Phytoplankton	• Most samples were collected in pelagic habitats, while some were littoral • Pore size for sample filtering differed across datasets • Datasets varyingly quantified phytoplankton as density, biomass, relative abundance, biovolume, or presence/absence • Taxonomy included, e.g., regional differences, outdated names, and typographic errors	• Constrain samples to those collected in the pelagic zone (or most commonly sampled zone) • Acknowledge the potential for size-based bias due to differing filter pore sizes • Convert data to incidence (presence/absence) because differences in quantification/measurement units introduce unavoidable bias • Harmonize nomenclature to adjust for changing taxonomy
Benthic Algae	• Samples collected from rivers, lakes, littoral, profundal, and hard and soft sediments • Most countries only identified diatoms and did not process soft algae portion • Differences in sample processing methods, with some approaches affecting visibility of characteristics for identification • Taxonomy included, e.g., regional differences, outdated names, and typographic errors	• Partition samples for analysis by habitat (lentic/lotic, littoral/profundal) and surface type (hard/soft sediments) • Focus on the diatom algal assemblage if soft algae data are not available • Constrain samples to those processed using chemical oxidation to ensure more accurate identification of diatoms • Harmonize nomenclature to adjust for changing taxonomy
Macrophytes	• Datasets varied between routine sampling with quantitative methods and opportunistic, qualitative observations • Datasets differed in terms of the portion of the macrophyte assemblage that was sampled (e.g., mosses, bryophytes)	• Separate data for qualitative and quantitative analyses when data and sampling effort vary greatly, with separate analysis of subsets of data where necessary • Constrain samples to those with common vascular macrophytes if aquatic mosses and bryophytes are inconsistently sampled
Zooplankton	• Samples collected at discrete depths or composite water column samples • Samples collected from profundal, littoral, benthic zones • Inconsistency in net/filter mesh size, covering a wide range across datasets • Large number of datasets did not identify or enumerate rotifers due to the need for smaller mesh size to collect efficiently • Taxonomy included, e.g., regional differences, outdated names, and typographic errors	• Combine depth-specific samples to provide comparable water column data to compare with composite samples • Constrain samples by lake zone, for example, use only pelagic samples if most common • Constrain analysis to the most prevalent range of mesh sizes (e.g., 20–156 μm) • Exclude rotifers but conduct follow-up analyses on a subset of sites that collected rotifers to ensure they are represented in diversity assessments • Harmonize nomenclature to adjust for changing taxonomy
Benthic Macroinvertebrates	• Timing of sampling (season) differed among datasets • Samples collected from rivers and lakes, littoral and profundal zones • Samples collected from soft or hard substrates and single or multi-habitats using different sample gear and methods • Differences in mesh size of sampling equipment among datasets • Differences in taxonomic resolution among datasets, ranging from genus/species to order level as the finest resolution	• Target season that was most commonly sampled (e.g., late summer and early fall), adjusting for latitude • Stratify analysis by rivers and lakes; constrain lake analysis to most common zone (e.g., littoral) • Constrain analyses to data collected with gear/methods that sample a similar portion of the assemblage from similar habitat(s) • Constrain to a specific mesh size range or convert data to presence/absence where mesh size varies strongly • Combine data at the family level as this creates larger datasets for assessing large-scale spatial patterns in diversity, but remove coarse order-level data

(Continued)

Table 3. (Continued)

Ecosystem Component	Challenges Encountered	Recommended Approaches
Fish	• Some datasets focused only on particular species or commercial fishing yields • Large differences in fish sampling methods and susceptibility to capture of different types of gear • Differences in the number of gear types used, sampling effort, and use of active and/or passive methods • Some datasets with single records and some with multi-year records, affecting detectability of species	• Constrain analysis to datasets that are focused on the portion of the fish assemblage that is of interest • Focus on datasets that use gear inclusive of all fish species or use in parallel the gear types highly effective for targeted species-specific sampling • Convert abundance data to incidence (presence/ absence) to account for bias introduced by differences in effort among commonly used gear types • Consolidate multi-year data records to maximize diversity estimates while remaining thoughtful of the importance/ relevance of scale
Supporting Variables	• Inconsistencies in the environmental data, measured parameters, and supporting metadata available for sites • Some datasets appeared to have data reported in the incorrect units • Water chemistry samples collected at surface, specific depths, or unknown depths • Differences in lower detection limit among datasets due to laboratory differences • Limited site-scale data collected consistently across large number of sites • Large number of sites and difficult topography complicate catchment delineation	• Exclude sites that do not have the full suite of critical environmental parameters/metadata needed for study objectives • Plot data to ensure no errors or discrepancies in reported measurement units • Exclude water chemistry samples collected at different or unknown depths, retaining only surface samples • Apply adjustment to water chemistry nondetects (e.g., half lowest detection limit) • Use geospatial data for consistent, large-scale supporting variables • Make use of Hydrobasins, globally-standardized flow-based basins available at a range of scales and with geospatially-derived supporting variables

https://doi.org/10.1371/journal.pwat.0000502.t003

abundance, biovolume (mm^3/m^3), or presence/absence. Due to this diversity, all data were converted to presence/absence for analysis to ensure comparability [102] (sec 2.2.3; Table 3). While this additionally controlled for differences in sample methodology that may have affected abundance/biomass estimates, it did not provide a solution for potential differences in size fractions introduced by these methodological differences. The choice was made to retain samples despite these differences because the spatial distribution of samples was patchy across the Arctic.

3.2. Benthic algae

Though the intention in the circumpolar analysis was to assess the full benthic algal community, including diatoms and soft algae, it became evident that this would not be possible for most countries [22]. In national monitoring programs, only Norway sampled soft algae. Additional soft algae data only originated from a small number of research programs. Because the bulk of the data across the circumpolar region was on benthic diatoms, the choice was made to exclude soft algae (sec 2.2.1; Table 3).

Benthic diatom sampling methods in the data compiled for analysis were generally focused on collecting a subsample from the top portion of soft sediments (lakes, some rivers) or scraping hard surfaces (lake littorals, most rivers; Table B in S1 Text; Table C in S1 Text). Methods were largely comparable, and benthic diatom samples were stratified by habitat (e.g., lentic/lotic, littoral/profundal) to control for natural differences in assemblages [106] (sec 2.2.2; Table 3). Furthermore, taxa were excluded from stream samples if they were known to be primarily planktonic because these taxa were only counted in some countries. Datasets were also retained based on sample processing and enumeration methods (Table 3). While not all samples were expected to be processed in the same manner, sample retention was dependent on the use of chemical oxidation in sample processing, as this results in clean diatom frustules and retains detailed ornamentation that is important for species identification [107,108]. Enumeration varied among datasets, with some including only

presence/absence data while others included relative abundance. Because relative abundance was important information for our study, presence/absence-based datasets were excluded (sec 2.2.3). Datasets were also excluded if taxa lists indicated that identification was not to the lowest possible taxonomic level [106].

The main challenge in compiling benthic diatom data for analysis is differences in taxa identification by the analysts [92]. There are widespread taxa complexes where identification is challenging due to incomplete knowledge of the taxonomy of these groups, sometimes imprecise descriptions of differential characteristics in the literature, and often overlapping morphological characters, making species identification difficult. Laboratories may also use different identification literature, leading to differences in identification of the same species due to the ever-changing taxonomy. The large number of diatom species also leads to a high risk of misspelling if no standard taxa list is used. In the EU intercalibration efforts for phytobenthos for the Water Framework Directive, metric development would not have been possible without taxonomic harmonization due to country-specific differences in nomenclature [109].

As a first step in the harmonization of nomenclature for the circumpolar analysis, diatomists met face-to-face to discuss challenging taxa complexes and set up a plan on how they should be treated in a merged dataset (sec 2.2.4). They discussed taxa groups and assessed different laboratory procedures for handling such complexes by reviewing images of diatom species and comparing identifications of the taxa. A full nomenclature list was corrected for errors and updated using AlgaeBase [103] to find synonyms and merge them to an accepted species name. To further reduce errors, they developed a taxonomic nomenclature for the data that combined taxa into groups and complexes for those diatoms that are easily misidentified, whose taxonomy was ambiguous, or that were frequently identified at different levels of taxonomic precision [106]. Although data harmonization can limit taxonomic resolution, it improves data comparability (i.e., the ability to compare data accurately) by reducing noise associated with errors resulting from taxonomic inconsistencies in large datasets (Table 3) [106,109,110].

3.3. Macrophytes

Analysis of aggregated macrophyte data was challenging due to the large variability in sampling effort among countries (Table D in S1 Text). Data coverage in lakes was good in countries with established, routine monitoring of macrophytes (e.g., Fennoscandia and Iceland), but data elsewhere were sparse, which complicated efforts to compare diversity estimates even with rarefaction [22]. To circumvent this issue, diversity estimates were created using only data from countries with established monitoring and separately using all countries with data, with the latter estimates being rarefied to a much smaller number of samples [22] (sec 2.1.6; Table 3).

Sampling effort within sites was also variable, ranging from systematic surveys in countries with established monitoring to opportunistic observations in some data sources from North America (Table D in S1 Text). Analysis therefore required consideration of both qualitative and quantitative data (sec 2.2.3). Sub-setting the data to run a detailed analysis with data from Fennoscandia and Iceland and a coarser analysis with all data once again provided a solution to account for these differences (Table 3).

It was also necessary to stratify data based on the portion of the macrophyte assemblage that was sampled. Monitoring of macrophytes does not always include enumeration of aquatic mosses, helophytes, and bryophytes, and it was necessary to determine whether these taxa were excluded as part of the methodology or whether they were truly absent in surveys (sec 2.1.6; Table 3).

3.4. Zooplankton

Sampling of zooplankton in lakes was primarily by volume-specific sampler at discrete depths or by composite water column samples using a plankton net (Table E in S1 Text), and depth-specific samples were combined for each site to provide comparable water column data [Table 3; 102]. Despite the general consistency in sampling approach, mesh sizes of plankton nets or filters were highly variable both within and among countries, ranging from 16 to 335 μm for all possible

samples (Table E in S1 Text), with potential implications for comparability of samples (sec 2.2.3). Exclusion of samples (due to sample timing, a focus on only one zooplankton group such as cladocerans, or a different collection method) narrowed the range to 20–156 μm [Table 3; 102]. Because mesh size for this dataset was not correlated with species richness, analysis continued with this range. However, it was noted that efforts should be made to improve harmonization of mesh sizes for zooplankton samplers.

One of the largest challenges for the comparability of zooplankton datasets was related to targeted sampling. Some datasets were only focused on cladocerans and did not include enumeration of other taxonomic groups. But a larger problem was inconsistency related to the inclusion of rotifers. Only a subset of datasets included rotifers due to the inefficiency with which this group is sampled by larger mesh sizes, and as a result, samples had to be stratified based on whether they included this group (Table 3). As a solution to ensure rotifers were not excluded completely, one analysis was run using all samples but excluding rotifers, while a second and separate analysis of a subset of samples included rotifers in diversity estimates [102].

Nomenclature was harmonized for zooplankton taxa across datasets, and it was necessary to combine some taxa due to hybridization [102]. Samples were excluded when taxonomic resolution was coarse (i.e., only at the level of Cladocera or Copepoda). The analysis focused on pelagic zooplankton, excluding benthic or littoral samples and species that typically live close to or in the sediments or associated with underwater vegetation (Table 3). Differences in the enumeration of these taxa contributed to variability in diversity estimates among datasets. Harmonization efforts to exclude taxa and/or samples were needed to provide comparable data (sec 2.2.4; Table 3).

3.5. Benthic macroinvertebrates

Macroinvertebrate data were available for both lakes and rivers, and data were harmonized separately for each water body type due to natural differences in assemblages [111]. Timing of sample collection varied across datasets due to latitudinal and longitudinal differences in timing of ice-off and accessibility of sites. Winter samples were excluded and samples from summer-early fall were retained for analysis because this range was deemed acceptable when accounting for climatic differences among ecoregions (sec 2.1.3; Table 3).

Differences in sampling methods and effort were the primary challenges in the analysis (sec 2.2.3). Samples were retained from the available data based on comparability of methods, initially selecting samples based on sampling equipment and the sampled habitat [Table 3; 111]. For lakes, there was a contrast between samples that were collected from the littoral zone with equipment that sampled hard surfaces (e.g., kick nets, stone scrub, scraper) and samples that were collected from the profundal zone or from soft sediments in the littoral zone using a grab sampler or corer (Table F in S1 Text). Because the soft sediment samples from the littoral and profundal zone would be expected to have lower diversity and represent primarily soft-bodied burrowing taxa, they were excluded from analysis [111]. This resulted in the exclusion of all lake data from Canada but removed substantial bias introduced by the difference in sampling method and habitat. The equipment used for the remaining stony littoral samples differed within and among countries (Table F in S1 Text), but data from kick nets, stone scrubs, surber samplers, and hydrobiological scrapers were considered to represent similar portions of the assemblage and were retained for analysis. Though there was some variety in sampling methods used for macroinvertebrates in rivers, six countries had sampling of hard substrates using a kick net (or similar D-frame net) while the remaining two countries sampled similar habitats using stone scrubs or scrapers (Table G in S1 Text). These methods were all deemed to sample similar portions of the macroinvertebrate assemblage and were thus considered comparable and retained for analysis [111].

Mesh size varied within and among countries, generally ranging between 250–500 μm for both lakes and rivers. For river samples, 400–500 μm was the most common mesh size for all countries except Iceland (250 μm; Table G in S1 Text). Lake data were more variable, and there was an approximately even split of countries with either 200–250 μm or 400–500 μm as the most common mesh size (Table G in S1 Text). Although differences in mesh size can affect the

abundance, size structure, and composition of taxa in macroinvertebrate samples, samples with 200–250 μm mesh were retained where necessary to ensure countries were not excluded (Table 3). Data were converted to presence/absence to control for abundance differences that were due to mesh size and differences in effort (time/area sampled) (sec 2.1.6).

Taxonomy was updated and regional differences in nomenclature were harmonized to ensure data comparability (sec 2.2.4; Table 3). Taxa that were not enumerated in all countries (e.g., nematodes, mites, and other taxa not efficiently captured with standard mesh sizes) were removed. There were regional differences in taxonomic resolution, with samples from European countries generally identified to genus and samples from North America often identified to family or order as is typical based on regional protocols [90]. Data were combined at the family level for analysis (order-level data omitted) because this retained spatial patterns in diversity and allowed for the retention of a larger number of samples.

3.6. Fish

The focus of the circumpolar analysis was to assess spatial patterns in diversity, and as such, an initial effort was made with fish data to select datasets that were focused on the full fish assemblage, rather than species-specific targeted datasets or those associated with commercial fishing [112,113].

Fish sampling methods varied widely among data sources (Table H in S1 Text; Table I in S1 Text), and differences in susceptibility to capture among gear types (i.e., gear-related sampling bias) had the potential to affect estimates of species richness or relative abundance. For example, gill nets provided an assessment of community composition or temporal change, but they were size-selective (sec. 2.2.3), and there was concern over missing size classes of fish, particularly small bodied species (e.g., Gasterosteidae) [112]. Similarly, the use of a single gear type like hook-and-line angling, while highly effective on a species-specific basis, must be used with complementary gears to assess the full fish assemblage.

Countries with national monitoring programs used standard gear types; for example, in Sweden, gill nets were used for sampling fish in lakes [114]. In the United States and Canada, studies were commonly conducted by individual programs at universities, agencies, or by private firms, and variability in methods was high (Table H in S1 Text; Table I in S1 Text). However, in the analysis of Arctic North America's freshwater fish biodiversity [113], 96% of the sites came from studies that used multiple gear types, which provided information on the full assemblage. Furthermore, gear types in different countries included active methods (e.g., angling or electrofishing) and/or passive methods (e.g., gill nets), which have different values of effort that may not be equivalent. In our assessments of fish biodiversity, we used incidence rather than catch-per-unit-effort (CPUE) data (sec. 2.1.6; Table 3). While this resulted in the loss of abundance information, it was the most reliable method for comparing across locations.

Aggregated fish data for circumpolar analysis included both lake and stream habitats, which would be expected to contain different assemblages of fish species. A choice was made for this global-scale analysis to keep all data because any divisions or selections based on habitat would have reduced spatial scope and sample size because in some locations data came from a mix of habitats (e.g., Iceland), while in others, fish data were limited to streams (e.g., Alaska's Seward Peninsula) or lakes (e.g., Canada's high Arctic) [112]. For smaller-scale analyses or those with habitat-related questions, other habitat considerations should be evaluated (sec 2.1.4, 2.2.2). In the North American analysis, fish data were constrained to rivers where a robust dataset allowed for comparisons with other taxonomic groups [113].

For sample stations with multi-year records, all presences were consolidated as a single detection. In the North American assessment [113], less than 4% of sites had multi-year records, while in the circumpolar assessment [112], 32% of sites were sampled more than once, due in part to the routine and repeated sampling in monitoring programs outside North America. Repeated sampling improves the likelihood of capturing rare taxa, which is important in biodiversity analyses. While this contributed to more accurate assessments of gamma diversity, consolidation of the data masks temporal changes (sec 2.1.1), which are known to occur [e.g., 115] and should be acknowledged.

3.7. Supporting variables

Site-scale abiotic ecosystem components such as water chemistry and physical habitat (e.g., substrate composition) were used for a circumpolar analysis of aggregated water chemistry parameters [99] and as supporting variables in the analysis of biodiversity data [14]. Both uses of the data came with challenges due to inconsistency in measured parameters and a lack of metadata (sec 2.2.4).

Analysis of aggregated water chemistry data generally focused on data that were collected as part of abiotic monitoring programs (i.e., not collected alongside biotic data) because these records were the most complete (i.e., they generally included pH, alkalinity, dissolved oxygen, carbon, nutrients, major ions, and metals). Continuous time series were not available in all regions, but the available data allowed for spatial and temporal analysis of bulk trends, comparing groups of sites across decades, as well as the comparison of bulk trends in unimpacted water bodies with a subset of water chemistry measurements associated with permafrost thaw slumps in Canada [99]. However, metadata detailing site integrity and sampled water depth were often missing, which necessitated the exclusion of samples [Table 3; 99]. Furthermore, not all parameters were measured at all sites, and they were only retained for analysis if they were measured in at least 60% of sites.

The largest challenges associated with analysis of aggregated water chemistry data were variability in measurement units and differences in the lower detection limit among datasets (sec 2.2.3, 2.2.4). An important component of harmonizing measurement units was plotting the data to check whether any datasets differed from the bulk of the samples by orders of magnitude because this generally indicated that measurement units were reported incorrectly in the metadata, and it allowed for removal or correction of data following a review of the source data (Table 3). Although lower detection limits differed among datasets due to differences in laboratory equipment sensitivity, spatial and temporal analysis of these values did not reveal any patterns, and the choice was made to use half the detection limit for any samples below these limits [99].

When water chemistry and physical habitat parameters were used as supporting variables, there were similar issues that arose for many of the biotic analyses. The main challenge resulted from inconsistent information on sampled habitat, including variability in the field data collected across datasets [22]. In many cases, this was a result of differing objectives associated with the collection of each dataset (sec 2.1.1). Datasets had varying levels of information on river flow, sampling depth, and riparian vegetation. Streambed composition was sampled across several datasets, but the particle size ranges and size classes used to quantify substrate composition varied [111,113]. There were large inconsistencies in measured parameters of water chemistry data across datasets (sec 2.2.1). For example, Lento et al. [111] found that conductivity and total phosphorus were the only water chemistry parameters consistently sampled across the eight Arctic countries as part of macroinvertebrate sampling programs. In some cases, whole suites of parameters were excluded (e.g., major ions). In other cases, there were regional differences in the elements, compounds, or forms that were measured (e.g., some regions sampled nitrate/nitrite while others sampled either Total Kjeldahl Nitrogen, TKN, or total nitrogen). The choice of which water chemistry or physical habitat parameters to include was driven solely by data availability across sites (Table 3).

Geospatial data provided a solution to deal with the lack of consistency in the collection of site-scale supporting variables and differences in measured parameters (Table 3). Although geospatial data represented a different, coarser scale for describing the habitat, the use of data layers with a continental or circumpolar extent ensured consistency across the circumpolar region. Furthermore, geology is known to correlate with water chemistry and can act as a proxy to characterize the chemical environment of samples at larger scales. One of the challenges of extracting geospatial data for large, aggregated datasets, however, is the need to have an estimate of catchment spatial area over which geospatial variables can be summarized. For example, the full database compiled by CBMP-Freshwater for the circumpolar biodiversity assessment included over 9000 sample locations across the circumpolar region, and delineations of catchments for all stations were not feasible, particularly considering the flat terrain in some areas. As a solution to this problem, global

hydrologic basin layers [116] were used to approximate catchments for all sites [Table 3; 22]. The HydroSHEDS database includes standardized global flow-based catchments (hydrobasins) delineated at different spatial scales from continental scale to small-scale sub-basins [116]. In the case study, each of the sample locations was aligned with the mid-scale hydrobasin in which it was located, and this basin was used to extract geospatial data in a standardized way across all sites [22]. Though this was not equivalent to the upstream catchment area, it was a close approximation that facilitated estimation of geospatial variables for many sample locations. To further improve representativeness of the hydrobasins, the intersection of the hydrobasin and the ecoregion in which each site was found was used for geospatial variable calculation to focus on the portion of the standardized catchment that was most representative of climate conditions at each site.

Climate, habitat, and landscape descriptors such as bedrock geology, permafrost coverage, and land cover were extracted for the hydrobasin/ecoregion intersection for each sample location. Where possible, global geospatial layers were chosen to ensure consistency across the full spatial extent of the biotic data. For example, the global layer WorldClim Version 2 [http://worldclim.org/version2; 117] was used to extract long-term average temperature and precipitation data, and the Circum-Arctic Map of Permafrost and Ground-Ice Conditions [Version 2; https://nsidc.org/data/ggd318; 118] provided permafrost and glacier data across the full region. Where consistent geospatial data were not available for the full spatial extent [e.g., the Geological Map of the Arctic only extended south to 60°N; 119], these layers were supplemented after comparing the level of consistency with other available layers. However, the expansion of HydroSHEDS to include many supporting variables derived for all hydrobasins [called HydroATLAS; 120] potentially offers the opportunity to expand the use of geospatial data in this manner.

4. Conclusions

For freshwater data, there are differences in methods that will affect data comparability and that may limit the extent to which data can be analyzed. The key to success is to recognize potential limitations and sources of bias in the data and determine best practices to proceed with assessment of the available data. While detailed assessments might be restricted to smaller-scale regional datasets, there is some level of comparison that is possible across continents, biomes, or ecoregions after accounting for the challenges described.

The extensive biodiversity assessment described in the case study provides an example of the benefits of harmonizing large, aggregated databases because it allowed for circumpolar patterns in biodiversity to be derived from a large number of independently collected datasets. Harmonization of the data enabled estimation of rarefied diversity in ecoregions and made it possible to compare these estimates at national and circumpolar scales, locate hot spots and cool spots for biodiversity across the Arctic, assess driver-response relationships and identify potential indicators of future change, and use paleolimnology and space-for-time approaches to develop projections of future biodiversity change in relation to climate change [21,22]. This information can be used to fill important gaps in our knowledge of remote Arctic ecosystems, supporting broad conclusions about the current state of biodiversity despite a lack of coordination in monitoring approaches [7,21]. The analyses would not have been possible without harmonization of sample methods, effort, and taxonomy. While coordination of future efforts is ultimately the goal to facilitate tracking of future change, this was an effective means to establish baseline conditions across a large spatial extent of the Arctic [21].

In many cases, the decisions made in the case study reflected the goal to include as many samples as possible to maximize spatial coverage of the assessment. Where possible, data were assessed to test the effect of different data choices or evaluate whether variability in sample methods might have impacted the results. For example, Schartau et al. [102] tested whether mesh size was correlated with zooplankton species richness and found that there was not an increase in richness with decreasing mesh size despite the potential to capture more species. Lento et al. [111] selected a subset of sites with benthic macroinvertebrate data at the genus level and analyzed the data at both the genus and family level to understand whether using the coarser taxonomic resolution impacted the ability to detect trends in the data, and they found that similar spatial patterns were evident at both taxonomic resolutions. In other cases, diversity patterns were

estimated at small spatial scales using the highest effort, most quantitative data available, and more coarse patterns were derived at larger scales using more qualitative data [22]. These approaches allowed for the limitations of the data to be tested, and for results from lower-quality data to be put into context by assessing higher-quality data at a smaller spatial scale. Similar approaches could be applied to assess simulated data as part of a detailed sensitivity analysis to understand the effect of different data decisions.

Some of the importance of these large-scale analyses of aggregated data stems from the fact that they can highlight where there are inconsistencies in sampling methods, effort, and reporting that preclude comparison of data across regions. This information can, in turn, be used to drive recommendations for the development of harmonized monitoring approaches [12,31] to support a more coordinated approach to monitoring. For example, Huser et al. [99] recommended developing an internationally recognized subset of simple-to-measure, critical water chemistry variables that are relevant to aquatic biodiversity, thus building a framework for long-term monitoring to support large-scale assessments of climate change impacts. Nomenclature harmonization and inconsistencies among taxonomists were recognized as important problems for analysis of aggregated data, particularly for phytoplankton and benthic diatoms, which provides support for the use of standardized, automated methods such as metabarcoding or automated image analysis for more objectively comparable data. Furthermore, the lack of supporting data (water chemistry and physical habitat data collected at biotic sampling sites) or inconsistencies in such data across datasets indicated the need for greater harmonization in such monitoring and reporting. While geospatial data can supplant such field data, and many options exist for continental or global data sources, that information may not be at a fine enough resolution to be associated with site-level variability. Detailed metadata information such as habitat descriptors and overall objectives of the research/monitoring program help control for variability across harmonized datasets and within analyses of aggregated data.

As global conversations about biodiversity status and trends continue, the demand for large-scale analyses of aggregated data from different sources will grow. In the absence of globally harmonized protocols, scientists will be faced with the need to ensure comparability of data and to make expert judgments on data comparability where needed to ensure sound conclusions.

Supporting information

S1 Text. Table A: Phytoplankton sampling methods and filter or mesh sizes from data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019), summarized as the number of sites in each country with data collected by a particular method and the range and most common filter/mesh sizes. Depth-integrated or depth-specific samplers were combined across sampler types (e.g., Limnos, Schindler, Van Dorn) to generalize about the portion of the water column that was sampled. **Table B:** Sampling methods used to collect algae from benthic habitats of lakes, summarized from data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019) as the number of sites in each country where a particular method was used. **Table C:** Sampling methods used to collect algae from benthic habitats of rivers, summarized from data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019) as the number of sites in each country where a particular method was used. **Table D:** Macrophyte sampling methods summarized from data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019) as the number of sites in each country with data collected by a particular method or combination of methods. **Table E:** Zooplankton sampling methods and filter or mesh sizes from data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019), summarized as the number of sites in each country with data collected by a particular method and the range and most common filter/mesh sizes. Some depth-specific samplers had nets attached while others did not (all were combined for the summary table). **Table F:** Benthic macroinvertebrate lake sampling methods and mesh sizes from data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019), summarized as the number of sites in each country with data collected by a particular method and the range and most common mesh sizes. **Table G:** Benthic macroinvertebrate river sampling methods and mesh sizes from data compiled for

the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019), summarized as the number of sites in each country with data collected by a particular method and the range and most common mesh sizes. **Table H:** Fish sampling methods used in lakes in data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019), summarized as the number of sites in each country with data collected by a particular method and the most common gill net mesh size for each country. **Table I:** Fish sampling methods used in rivers in data compiled for the CBMP-Freshwater circumpolar biodiversity assessment (Lento et al. 2019), summarized as the number of sites in each country with data collected by a particular method and the most common gill net mesh size for each country.
(DOCX)

Acknowledgments

The authors wish to thank all data providers and contributors to the Circumpolar Biodiversity Monitoring Program (CBMP) Freshwater Group's State of Arctic Freshwater Biodiversity Report and special issue. Without their generous contribution of data, time, and expertise, the first analysis of freshwater biodiversity for the circumpolar region would not have been possible and this guidance would not be as grounded in real-world data harmonization challenges and solutions. We thank V. Patil and R. Bailey for thoughtful feedback on an earlier version of this manuscript. JL further thanks the Northwest Territories Cumulative Impact Monitoring Program (NWT CIMP) of the Government of the Northwest Territories who provided both the funding and inspiration to develop this guidance on data harmonization to support analysis of aggregated data. KSC thanks the National Environmental Agency for continuous support to be part of the CBMP Freshwater working group. DCPL thanks the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning for funding support (FORMAS; dnr 2021–01062). This paper was further developed by the co-authors through the Arctic-BIODIVER program, which was funded by a grant from the Belmont Forum and EU-Biodiversa to JC, IL, KSC, and WG. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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