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Long-term archival of environmental samples empowers biodiversity monitoring and ecological research

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Abstract

Human-induced biodiversity loss and changes in community composition are major challenges of the present time, urgently calling for comprehensive biomonitoring approaches to understand system dynamics and to inform policy-making. In this regard, molecular methods are increasingly applied. They provide tools for fast and high-resolution biodiversity assessments and can also focus on population dynamics or functional diversity. If samples are stored under appropriate conditions, this will enable the analysis of DNA, but also RNA and proteins from tissue or from non-biological substrates such as soil, water, or sediments, so-called environmental DNA (eDNA) or eRNA. Until now, most biodiversity studies using molecular methods rely on recent sampling events, although the benefit of analyzing long-time series is obvious. In this context Environmental Specimen Banks (ESBs) can play a crucial role, supplying diverse and well-documented samples collected in periodically repeated sampling events, and following standardized protocols. Mainly assembled for integrative monitoring of chemical compounds, ESB collections are largely accessible to third parties and can in principle be used for molecular analysis. While ESBs hold great potential for the standardized long-time storage of environmental samples, the cooperation with Biodiversity Biobanks as scientific collections guarantees the long-time storage of nucleotide (DNA, RNA) extracts together with links to analytical results and metadata. The present contribution aims to raise the awareness of the biodiversity research community regarding the high-quality samples accessible through ESBs, encourages ESBs to collect and store samples in DNA-friendly ways, and points out the high potential of combining DNA-based approaches with monitoring chemicals and other environmental stressors.

Keywords: Environmental specimen banks, Biomonitoring, eDNA, Metabarcoding, Biodiversity biobanks

Introduction

Chemical pollution is one of the main drivers behind biodiversity decline [38]. Still, many questions remain about the specific modes of action of chemicals on organisms and populations and the mechanisms triggering biodiversity loss. Consequently, both environmental policy and research have identified an increasing need for investigations into the links between chemical pollution and the loss of biodiversity [25, 27, 48, 83, 84]. In this respect, Environmental Specimen Banks (ESBs) fulfil an important role in ecosystem monitoring by ensuring sample collection and long-term storage based on standardized protocols and extensive documentation [14]. Stored ESB samples are to date mainly used for analyses of chemical pollutants but are generally accessible for scientific purposes. Thus, they provide the opportunity to link chemical parameters with biodiversity pattern analysis, also allowing for a perspective backwards in time. In this context, DNA-based methods such as environmental DNA (eDNA) metabarcoding or metagenomics show a

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high potential for retrospective biodiversity assessments and correlation with prevailing contaminants or other environmental stressors, e.g., climate change, nutrients and anthropogenic land use [4, 10, 22, 49, 75, 93]. Concomitantly, the increased application of DNA-based approaches induces the need for storage of extracted DNA samples linked with associated metadata. In this context, Biodiversity Biobanks-in close collaboration with ESBs-can guarantee the appropriate deposition of DNA samples and associated data, such as extraction methods, DNA storage parameters, links to performed studies and to international nucleotide databases.

Environmental specimen banks (ESBs)

Currently more than 20 ESBs exist around the world, mainly distributed throughout Europe, Asia and North America. To foster global harmonization of ESB activities within the growing community, the International Environmental Specimen Bank Group (IESB) was initiated. The consortium promotes the development of techniques and strategies of ESBs as well as the cooperation and standardization among repositories [47, 79]. For a detailed overview of ESBs and stored sample types see Chaplow et al. [14]. ESBs are part of the precautionary principle in environmental policy as they continuously document the state of the environment and subsequently store the samples. Present and future generations can thus use the archived samples at any time to retrospectively analyse and better understand emerging environmental problems and trends. In addition, the samples allow for the investigation of stressors that could not be measured or were not known to be problematic at the time the samples were collected. Monitoring purposes are often associated with regional or national screening programs and are mostly related to persistent and toxic chemical contaminants (e.g., chlorinated, brominated and fluorinated organic contaminants, heavy metals) and their effects on terrestrial, freshwater and marine environments also including natural background and conurbation areas [5, 43]. Beside the main focus on chemical monitoring, samples are used for manifold other approaches such as, e.g., ecological status assessments or population structure analyses [19, 33, 63]. ESB collections include samples from around the globe, some of them being collected annually for more than 40 years, thereby allowing for a comprehensive analysis of pollution residues and changes through time, functioning as a basis for political decisions and appropriate restrictions in chemical compounds management. Next to real-time monitoring, specimen storage and documentation enables retrospective sample analysis for chemicals of emerging concern or with newly developed analytical tools [14]. ESBs periodically collect a variety of specified samples at selected sampling sites, ranging from human tissues to plant and animal samples from different ecosystem types, including top predators. In addition, abiotic samples as soil, sediment, suspended particulate matter, waste water, sewage sludge or atmospheric samples (airborne particulate matter) are collected and archived in ESBs [14].

To ensure sample integrity, collection is conducted according to standardized protocols. These cover sampling, transportation, processing and storage of material. Depending on ESB, sample storage is implemented in cold (−20 °C) or ultra-cold freezers (−80 °C) or in liquid nitrogen vapor tanks (around −190 °C) to ensure the integrity of the samples' biological and chemical composition over a long time period. Together with chemical and biological analyses, protocols and metadata are accessible through reports or peer-reviewed publications and the release in publicly available databases [8, 47, 69]. The research strategy of environmental specimen banks over the last 40 years reflects the progress in environmental chemistry. Common substances analysed in the twentieth century were metals, organochlorine pesticides, dioxins, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons [51, 72]. During this century, ESB samples were also analysed for elemental isotope signatures [19, 90], per- and polyfluorinated alkylated substances [26, 28], plasticizers [61, 92], pharmaceuticals [11], biocides [45], modern pesticides, flame retardants [31], and other chemicals of emerging concern. However, the scientific potential of ESB collections is not yet exhausted, especially when considering how newly developed techniques as high-resolution mass spectrometry or high-throughput sequencing open up novel analytical tools and possibilities in real-time and retrospective chemical analysis, including targeted and Non-Target Screening (NTS), effects based methods, and biodiversity assessments [36].

Environmental genomics

Developments in environmental genomics in particular High-Throughput Sequencing (HTS) techniques revolutionized species identification and biodiversity assessment throughout the tree of life. Using short DNA fragments up to whole genomic or transcriptomic information, possible applications of the method focus on assessing species richness and interactions, population genetic structure, functional trait expression and diversity of complex communities [17]. 'DNA barcoding' describes the DNA isolation and amplification of a short gene fragment from a single individual and the subsequent comparison to a reference database. It is used for simple and fast specimen identification in problematic life stages (e.g., larvae, seeds), incomplete specimens (tissue pieces),

or to separate 'lookalikes' [34, 82]. Based on the same principle and using the same reference databases, DNA metabarcoding uncovers the biodiversity of sample mixtures, comprising high numbers of individuals with different taxonomies [52, 91]. The method uses DNA mass extraction from bulk samples or their preservation fluids, followed by the application of HTS techniques and bioinformatic pipelines to assess biodiversity up to geno-species level [2, 77, 94]. DNA metabarcoding can also be applied on environmental, non-biological samples (e.g., soil, water, air) targeting intra- as much as extracellular DNA molecules (mitochondria, intact cells, free DNA) released from organisms into the environment. So-called environmental DNA (eDNA) metabarcoding is tempting through its non-invasiveness with a huge potential for large-scale biodiversity assessment [20, 78]. Due to the relative stability of DNA, the molecule persists in the environment even after cell death and can be detected by metabarcoding for a given period of time (depending on substrate). In contrast to DNA, RNA is very unstable and degrades in the environment minutes to hours after cell death. Environmental RNA-based markers, therefore, target metabolically active organisms and might be the more suitable tool to indicate living biotic assemblages or even gene expression patterns [18, 65]. In comparison to the above-mentioned approaches that are based on PCR amplification of a standardized short gene fragment, metagenomic techniques are PCR free, targeting the whole genomic material of an environmental sample [17]. Due to the absence of the PCR step, metagenomics approaches are hence assumed to provide more accurate abundance predictions but induces much greater costs and a higher complexity in laboratory and bioinformatic protocols [46]. Whole-genome information can also be extracted from single individuals providing a tremendous increase in molecular information compared to marker-based approaches with applications in phylogenetic or functional analysis [66]. Bypassing marker amplification through PCR, also transcribed RNA (mRNA, rRNA) can be used as a template for sample analysis [53, 57]. While transcriptomic approaches target transcribed RNA from single individuals, metatranscriptomics provide information of simultaneously expressed genes in mixed communities under given environmental conditions. However, with the instability of RNA molecules sampling and processing is accompanied with challenging collection and storage efforts.

Opening up environmental specimen banks for molecular analysis, and the role of biodiversity biobanks

First biodiversity studies already utilize ESB samples, as for example [21], where scientists retrospectively applied eDNA metabarcoding on freshwater suspended particulate matter (SPM) collections from the German ESB to monitor fish communities through time. This analysis includes cryo-archived SPM samples from six riverine systems in Germany representing different conditions and fish communities. Another study used specimens of the zebra mussel (*Dreissena polymorpha*) collected over the last 25 years and stored at the same ESB [86]. The study investigates mussel diet through eDNA metabarcoding and the utility of mussels as eDNA filters of planktonic organisms. Recently, the German ESB together with University Duisburg-Essen started the project 'TrendDNA', which includes the comprehensive analysis of ESB samples through molecular approaches (www.trenddna.de) also aiming to formulate guidelines for sample quality assurance and control (QA/QC) for the application of molecular approaches. However, the potential of ESB collections is still very far from being fully used. Barcoding, metabarcoding as well as metagenomic approaches can be applied to banked specimens, sample mixtures or non-biological samples to investigate biodiversity, population dynamics or diet composition over long-term periods, assessing the influence of natural and human-made environmental changes through time [2, 12, 18, 50, 60].

To ensure the integrity of DNA and ideally even RNA molecules, environmental samples need to be stored under defined constant and controlled conditions which should ideally be standardized [32, 39, 74, 88]. Centralized repositories warrant consistent storage quality and cater to the needs of individual research institutes or individual researchers, who often have only limited, short-term storage capacities, and are not specialized on the task. Subsequent to analysis, remaining DNA extracts should be professionally archived to save resources and to warrant reproducibility of research results. Each extract, depending on isolation method, is unique. In addition, over the course of the years, often additional markers (up to meta-/genomes) are added to data sets that started out based on the analysis of a single gene. Long-term storage of DNA isolates can happen directly at ESBs. However, dedicated Biodiversity Biobanks (BBBs, see [3] and [24] focus specifically on archiving and handling DNA and RNA. As research collections, BBBs are directly in touch with biodiversity research groups and the user communities. In addition to the isolated biomolecules, they hold fixed tissues-sometimes even viable tissues (live cells)-that are linked to the respective species. Not uncommonly, BBBs are housed at natural history collections, which enables them to archive entire organisms as reference specimens and to make their holdings publicly visible (while implementing digital rights in accordance with depositor wishes). Currently more than 100 BBBs and associated initiatives have joined forces to form the

Global Genome Biodiversity Network [24]. GGBN.org offers a unified platform to internationally find and access samples suitable to molecular biodiversity research. BBBs see themselves as information brokers regarding the analytical data associated with their samples. For instance, they enrich their samples by linking them with publications and with the databases of the International Nucleotide Sequence Database Collaboration (e.g., ENA or NCBI GenBank, with the Barcode of Life Data systems (BOLD [67]), or others).

One exemplary instance of the described cooperation between an ESB and a BBB exists in Germany, where the German Environmental Specimen Bank collaborates closely for long-term storage of extracted DNA with the Leibniz Institute for the Analysis of Biodiversity Change (LIB) at Museum Koenig, Bonn. The LIB Biobank extends the offer to the ESB community and to metabarcoding projects to deposit environmental DNA or RNA extracts in its currently expanded cryofacility (contact through last author), making them available for future reference and potential sequencing of additional molecular markers.

Challenges

Storage conditions

Inadequate temperature or pH, exposure to degrading compounds or to light all compromise DNA and RNA quality. These and other factors have to be considered during storage [1, 70], upon which depends the success of molecular biodiversity assessment. Ideally, DNA isolation from substrate should be conducted as soon as possible after collection to maximize DNA quality and quantity. However, due to the nature of workflows or limited resources, this is not always an option. For not yet isolated DNA samples, conservation will vary according to its medium: DNA attached to soil particles, for instance, will persist considerably longer than free DNA [70, 80]. The time interval from field collecting until storage depends on sample type, aim of analysis and technical possibilities during sampling [58, 64, 68, 71]. Optimal conditions include the immediate freezing (the colder the better) of samples and the maintenance of cold chains. Storage can also be initiated by drying the (ideally cooled) material. Nevertheless, DNA extraction for conventional metabarcoding purposes (sequencing of individual genes) is still possible from samples exposed to ambient temperature up to several weeks, if feasible fixation is applied [6, 23, 37]. Long-term storage of tissue samples or small organisms should be conducted in fixative (e.g., 96% non-denatured ethanol) with no light exposure and cold or ultra-cold condition counteracting DNA degradation [52, 87]. Storage of isolated or amplified DNA is recommended buffered (for long-term archival most often in Tris EDTA or Tris low EDTA) or-depending on planned application-sometimes in water at −80 °C [42] down to −190 °C in liquid nitrogen storage tanks [7, 29], alternatively dried and sealed [16]. While −20 °C is not an optimal storage temperature, extracts immediately stored at −20 °C will likely lend themselves to DNA analysis for up to decades, if multiple freeze–thaw cycles are avoided and when a high initial DNA concentration is given (NB: DNA will gradually decay during this period at −20 °C). More careful processing is necessary if samples are to be used for RNA analysis. Due to the high instability of this biomolecule, cold chains should be maintained right from the moment of sampling (dry ice, liquid nitrogen-based vapor shippers, etc.) and kept at least at −80 °C at any time if molecules are not transferred to a specific preservation medium [56, 59, 73]. Additional concepts for RNA handling and storage have been developed as, e.g., RNA desiccation in RNAstable [9] and the subsequent storage at room temperature for up to 1 year [35, 54, 73]. However, while the RNA analysis of banked samples in combination with chemical measurements could provide interesting insights about ecotoxicogenomics and gene expression under the influence of anthropogenic stressors [76, 85], the processing of RNA from long-term stored substrate is largely unexplored and its application to banked samples needs to be further tested.

Contamination

DNA/RNA-based approaches are extremely susceptible to contamination with non-target molecules caused by free-circulating aerosols or cross-contamination between samples [74]. Since many applications aim to detect extremely low concentrated molecules from substrates, even the slightest contamination can skew analysis and depict erroneous results for species composition. Sampling of substrates to be screened via molecular analysis should, therefore, include intensive cleaning and sterilization of equipment between samples and quality assurance and quality control measures (QA/QC) covering the sampling process but also storage and reanalysis need to be implemented and integrated in standard operating procedures (SOPs). Sodium hypochlorite bleach is extensively used as a decontaminating solution in laboratory processes. The application of at least 2% sodium hypochlorite solution (exposure time 10 min) is recommended to remove extraneous DNA [30, 41, 89]. However, commercial bleach is a hazardous substance potentially corroding material and affecting fine-tuned chemical analysis. Where this has to be avoided, the decontamination of material through UV radiation or better the usage of single-use equipment should be considered to minimize as much as possible the transfer of DNA/RNA traces among samples [13, 30]. With respect

to QC, negative controls/field blanks should routinely be integrated in field sampling and laboratory processes to recognize potential contaminations in downstream analyses [30, 74].

Data accessibility

To increase visibility and accessibility of ESB samples (as for BBB samples through GGBN), information on these should be publicly available including relevant metadata such as storage protocols used, generated results and ideally even studies performed so far with the samples and based on FAIR (findability, accessibility, interoperability, reusability) data principles [44]. Data provision has already been initiated (https://www.umweltbundesamt.de/en/topics/chemicals/international-environmental-specimen-bank-group). A comprehensive, updated overview needs to be compiled giving detailed information on ESB samples and indicating their availability to the scientific community. This could potentially include a combined web interface that aggregates results generated from ESB samples around the globe. Such a tool would enable cross-linking and exploring available data and identifying and addressing global environmental concerns [19, 47, 62].

New sample types

Several biodiversity monitoring approaches rely on analyses of trapped arthropods to assess biodiversity change. Typical methods include Malaise traps for flying insects or pitfall traps for 'crawlers'. Since the advent of metabarcoding, the number of projects and studies employing arthropod traps has been rapidly increasing [37, 49, 52, 77], and with them the number of available community samples. Sometimes, caught arthropods are homogenized (ground up) prior to analysis, but often, they are preserved for additional biodiversity studies and only the killing and preservation fluid (ethanol, propylene glycol, etc.) is used for DNA extraction. While homogenized samples are relatively easy to store in ESBs or Biodiversity Biobanks due to small size, warranting cold storage for large numbers of entire jars of arthropods in ethanol is considerably more challenging. The existing frozen repositories typically do not and to date usually cannot focus on such samples. This leads to the situation that biologically very valuable trap samples are amassing rapidly without the perspective of long-term storage. Considerable funding goes into the underlying biomonitoring surveys and we urgently encourage the research community and policy makers to devise strategies and to work towards new infrastructures able to hold large numbers of non-homogenized trapped arthropod specimens.

Outlook and recommendations

With standard procedures from sampling to storage, Environmental Specimen Banks play an important role among the biomonitoring infrastructures. The combination of collections condensing the results of up to 40 years of field sampling with the rapidly developing molecular techniques for biodiversity assessment and for chemical pollutant analysis, holds the key for a new level of environmental research. It must now be examined in detail to what extent these standards are already sufficient to be able to use the samples as extensively as possible for genetic analysis and, if necessary, to harmonize optimized protocols for this purpose. Recently, first metabarcoding studies and projects were launched that already include ESB samples into molecular approaches. Tapping into this resource opens up high-quality, well-documented sample collections that allow easily adding a retrospective component to biomonitoring projects. For a fruitful synergy, biomonitoring research should be aware of ESBs as convenient sample sources and archives, while the ESB community should embrace biodiversity analyses as a new and highly relevant use of its collections. Thus, ESBs should cater increasingly also to the needs of the biomonitoring community, ideally in partnership with Biodiversity Biobanks. With further transparency of ESBs and the standardized publication of data, ESBs can become the basis for a wide array of interconnected scientific studies that allow scientists, natural resource managers and policy-makers an informed look back in time from an integrated biological and chemical perspective.

Abbreviations

BBB: Biodiversity Biobank; BOLD: Barcode of Life data system; DNA: Desoxyribonucleic acid; eDNA: Environmental desoxyribonucleic acid; ENA: European Nucleotide Archive; ESB: Environmental Specimen Bank; GGBN: Global Genome Biodiversity Network; HTS: High-throughput sequencing; IESB: International Environmental Specimen Bank Group; LIB: Leibniz Institute for the Analysis of Biodiversity Change; mRNA: Messenger ribonucleic acid; rRNA: Ribosomal ribonucleic acid; SPM: Suspended particulate matter.

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