

Forum

**The reuse of avian samples: opportunities, pitfalls, and a solution**

VOJTĚCH BRLÍK,<sup>1,2\*</sup> PAVEL PIPEK,<sup>2,3</sup> KATE BRANDIS,<sup>4</sup> NIKITA CHERNETSOV,<sup>5</sup> FÁBIO J. V. COSTA,<sup>6</sup> L. GERARDO HERRERA M.,<sup>7</sup> YOSEF KIAT,<sup>8</sup> RICHARD B. LANCTOT,<sup>9</sup> PETER P. MARRA,<sup>10</sup> D. RYAN NORRIS,<sup>11</sup> CHIMA J. NWAOGU,<sup>12,13</sup> PETRA QUILLFELDT,<sup>14</sup> SARAH T. SAALFELD,<sup>9</sup> CRAIG A. STRICKER,<sup>15</sup> ROBERT L. THOMSON,<sup>13</sup> TIANHAO ZHAO<sup>16</sup> & PETR PROCHÁZKA<sup>1</sup>

<sup>1</sup>Czech Academy of Sciences, Institute of Vertebrate Biology, Czech Republic

<sup>2</sup>Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic

<sup>3</sup>Czech Academy of Sciences, Institute of Botany, Czech Republic

<sup>4</sup>Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Australia

<sup>5</sup>Ornithology Lab, Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia

<sup>6</sup>Instituto Nacional de Criminalística, Polícia Federal, Brazil

<sup>7</sup>Estación de Biología Chamela, Instituto de Biología, Universidad Nacional Autónoma de México, México

<sup>8</sup>Israeli Bird Ringing Center, Israel Ornithological Center, Society for the Protection of Nature in Israel, Israel

<sup>9</sup>U.S. Fish and Wildlife Service, Migratory Bird Management, USA

<sup>10</sup>Department of Biology and the McCourt School of Public Policy, Georgetown University, USA

<sup>11</sup>Department of Integrative Biology, University of Guelph, Canada

<sup>12</sup>A.P. Leventis Ornithological Research Institute, University of Jos Biological Conservatory, Nigeria

<sup>13</sup>FitzPatrick Institute of African Ornithology, DSI-NRF Centre of Excellence, University of Cape Town, South Africa

<sup>14</sup>Department of Animal Ecology & Systematics, Justus Liebig University, Giessen, Germany

\*Corresponding author.  
Email: vojtech.brlik@gmail.com  
Twitter: @VojtechBrlik

<sup>15</sup>U.S. Geological Survey, Fort Collins Science Center, USA

<sup>16</sup>Groningen Institute for Evolutionary Life Sciences, University of Groningen, Netherlands

Tissue samples are frequently collected to study various aspects of avian biology, but in many cases these samples are not used in their entirety and are stored by the collector. The already collected samples provide a largely overlooked opportunity because they can be used by different researchers in different biological fields. Broad reuse of samples could result in multispecies or large-scale studies, interdisciplinary collaborations, and the generation of new ideas, thereby increasing the quality and impact of research. Sample reuse could also reduce the number of new samples needed for a study, which is especially pertinent to endangered species where sample collection is necessarily limited. Importantly, reusing samples may be mutually beneficial for both the researchers providing samples and those reusing them. Here, we identify the benefits of sample reuse, describe currently available sources of already collected samples and their limitations, and highlight the wide range of potential applications in a single research field – avian isotopic ecology. To facilitate the reuse of avian samples worldwide and across research fields, we introduce the AviSample Network metadata repository. The main aims of this metadata repository are to collate and provide access to descriptions of available avian tissue samples. We contend that the creation of the AviSample Network metadata repository will provide the opportunity for new collaborations and studies. Moreover, we believe that this will help create research connections between ornithologists across the globe and encourage sample reuse in other fields.

**Keywords:** birds, database, metadata, sample, tissue, transparency.

Millions of avian tissue samples have been collected worldwide. Typically, parts of the samples are analysed or archived for future use. However, already collected samples have unrealized potential to answer a broad range of other biological and ecological questions if they could be located, made available and reused for other purposes.

Already collected samples offer a wide range of potential uses for new projects, especially given the many recently developed analytical methods, or for addressing a question from a different perspective (Smith *et al.* 2003). Collaboration among people from different fields could increase the potential for new ideas with broad application, boosting research impact (Okamura 2019). Such collaborations would be mutually

beneficial for both researchers collecting the samples and those with ideas for reuse. Moreover, sampling avian tissues requires financial and human resources and is, by definition, invasive, making the reuse of such samples economically efficient and less stressful to study subjects. Sample reuse would also facilitate research of endangered species where an assessment of available samples could reduce the number of newly sampled animals, helping researchers to gain ethics clearance approval. Finally, an extensive reuse of samples might result in multispecies, long-term or broad-scale studies that would otherwise be hard to manage. Currently, effective and broad range sample reuse is limited due to a variety of limitations we describe below.

Museum collections of specimens are the largest source of already collected samples available for reuse. These institutional collections host enormous, and still growing, numbers of specimens (Winker 2004), often collected over long periods of time (Møller & Hochachka 2019). Thus, museum specimens have considerable potential for comparative studies across time (Suarez & Tsutsui 2004). Museum databases, usually publicly available, are now becoming globally integrated (<https://www.idigbio.org/>), which serves as one tool for searching for already collected samples of interest. However, museum online databases are also frequently incomplete due to the demand for digitization of specimen metadata. Moreover, a unified policy on the use of museum specimens is lacking and scientific projects applying destructive analytical methods or using large amounts of biological material might be rejected.

In addition to museum collections, there are large-scale scientific projects that involve the public, volunteers or hunters who help collect avian tissues. For example, the U.S. Fish and Wildlife Service's Wingbee project (<https://www.fws.gov/birds/news/160202wingbee.php>) organizes annual collection of large numbers of wings and tail feathers from hunted ducks, geese and other migratory game birds across North America. Species, sex and age of sampled individuals are documented and the information is used to estimate harvests, geographical distributions of species or population structure (Saunders *et al.* 2019). Similarly, the Feather Map of Australia project (Brandis *et al.* 2021) engages the broader public to collect feathers of waterbird species across Australian wetlands. These samples help inform about water bird movements, habitat use and wetland importance for targeted management actions (<https://feathermap.ansto.gov.au/ResultsOverview/index.htm>). Finally, using a continent-wide network of bird ringers collecting tissue samples, Bird Genoscape (<https://www.birdgenoscape.org/>) uses molecular markers to describe population identity of individuals. This information is then used to distinguish population-specific breeding, passage and non-breeding regions and link region-specific environmental conditions with breeding

population trends (Bay *et al.* 2018). Finding already collected samples of interest and requesting them from coordinators of such large-scale projects would be straightforward. However, there are only few such large-scale projects and the number of already collected samples held in their collections is rather limited.

Each year, many researchers collect thousands of avian tissue samples for a variety of projects worldwide. The reuse of these samples could benefit science and might overcome geographical or taxonomic biases. However, researchers looking for existing samples would face two situations that hamper their effective reuse. First, researchers may find that parts of the samples of interest have already been analysed and the results published. In this case, a researcher could find details about the study design and contact the authors with a request for reusing the available parts of the samples. However, a direct search for specific samples would be very difficult due to a lack of standard query tools specifying sample details and the enormous number of published papers (Bornmann & Mutz 2015). These circumstances would force researchers to focus on papers most relevant to their field of expertise, increasing the probability of overlooking a study with relevant samples as the divergence of the study topics increases. Luckily, scientific journals increasingly require authors to make the data underlying their results publicly available. Subsequent reuse of the open-access datasets stored in repositories has been shown to be beneficial for science (Piwowar *et al.* 2011) and the number of these repositories is growing steadily (Pinfield *et al.* 2014). The public availability of a large number of datasets has also great potential for sample reuse. Searchable repositories would help identify already collected samples of interest, locate them and facilitate their reuse. However, the absence of clear guidelines for reporting sample metadata (see AviSample Network metadata repository section) by both scientific journals and data repositories hampers effective searches for the already collected samples and their broad-scale reuse.

Secondly, a more difficult situation may arise when the information on sample collection is not published. This might happen due to a change in research plans, cuts in funding that prevent sample analysis, failure to collect a sufficient number of samples needed to achieve project goals, or difficulty in publishing null results. There are various ways to locate these samples, including contacting principal investigators, or advertising a request through social media and website forums. However, the effectiveness and reach of these communication channels can be severely restricted. Research networks mostly include people from a particular field, or people studying a similar group of species. Disseminating the request outside one's field of expertise may also be unrewarding, as people may be less inclined to respond to a general request, or when they do not recognize the researcher's name and institution. Thus,

locating unpublished samples and their owners for sample reuse can be challenging, time-consuming and often unsuccessful.

To conclude, there are multiple ways to locate and potentially reuse already collected samples of interest that would benefit studies in avian biology. However, searches for such samples would be inefficient, incomplete and would probably miss an enormous number of samples collected by individual researchers. These difficulties might lead to geographical, taxonomic and temporal biases. We illustrate the potential use of already collected samples in a single research field – avian isotopic ecology – as isotopes are ubiquitous in avian tissues and have a wide range of ecological applications. Finally, to facilitate wide reuse of avian samples worldwide and across research fields, we introduce the AviSample Network metadata repository with the main aim to collate and provide access to sample descriptions stored by researchers worldwide. We believe that this tool will provide an inclusive bridge between distinct fields of avian biology.

## APPLICATIONS OF STABLE ISOTOPIC COMPOSITION OF TISSUES

Natural stable isotopic compositions of tissues can be used as intrinsic markers and have a broad range of applications in avian biology (Hobson 2011, Hobson & Wassenaar 2018). Stable isotopes occur in all tissues and reflect spatial variation in the environment (West *et al.* 2010), as well as differences between habitats or diet (Michener & Lajtha 2007). Here, we present a brief overview of these applications to illustrate the potential of sample reuse in a single research field.

Water isotopes ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) in precipitation show predictable global geographical patterns mainly due to isotopic fractionation between vapour and precipitation (Dansgaard 1964, Bowen & Revenaugh 2003). Therefore, water isotopes from tissues are frequently used to infer the geographical origin of individuals (Norris *et al.* 2006), to study moulting patterns (Neto *et al.* 2006) and to reveal altitudinal migration (Boyle *et al.* 2011, Newsome *et al.* 2015).

Stable carbon ( $\delta^{13}\text{C}$ ) isotopes differ between plants with distinct photosynthetic pathways (Ehleringer & Cerling 2014). The bimodal pattern of  $\delta^{13}\text{C}$  values can then be used as a marker of diet switches and its timing (Jouta *et al.* 2017), diet composition (Phillips & Gregg 2001, Li *et al.* 2013), feeding strategy (Ambrose & DeNiro 1986), and vegetation structure and conditions (Marra *et al.* 1998, Bearhop *et al.* 2004, Norris *et al.* 2004). Distinct growth limits of  $\text{C}_3$  and  $\text{C}_4$  plants (Ehleringer *et al.* 1997) can be used to predict mean plant  $\delta^{13}\text{C}$  values on large scales (Still *et al.* 2003, Powell *et al.* 2012), which are applicable in geographical assignments (Wunder & Norris 2008).

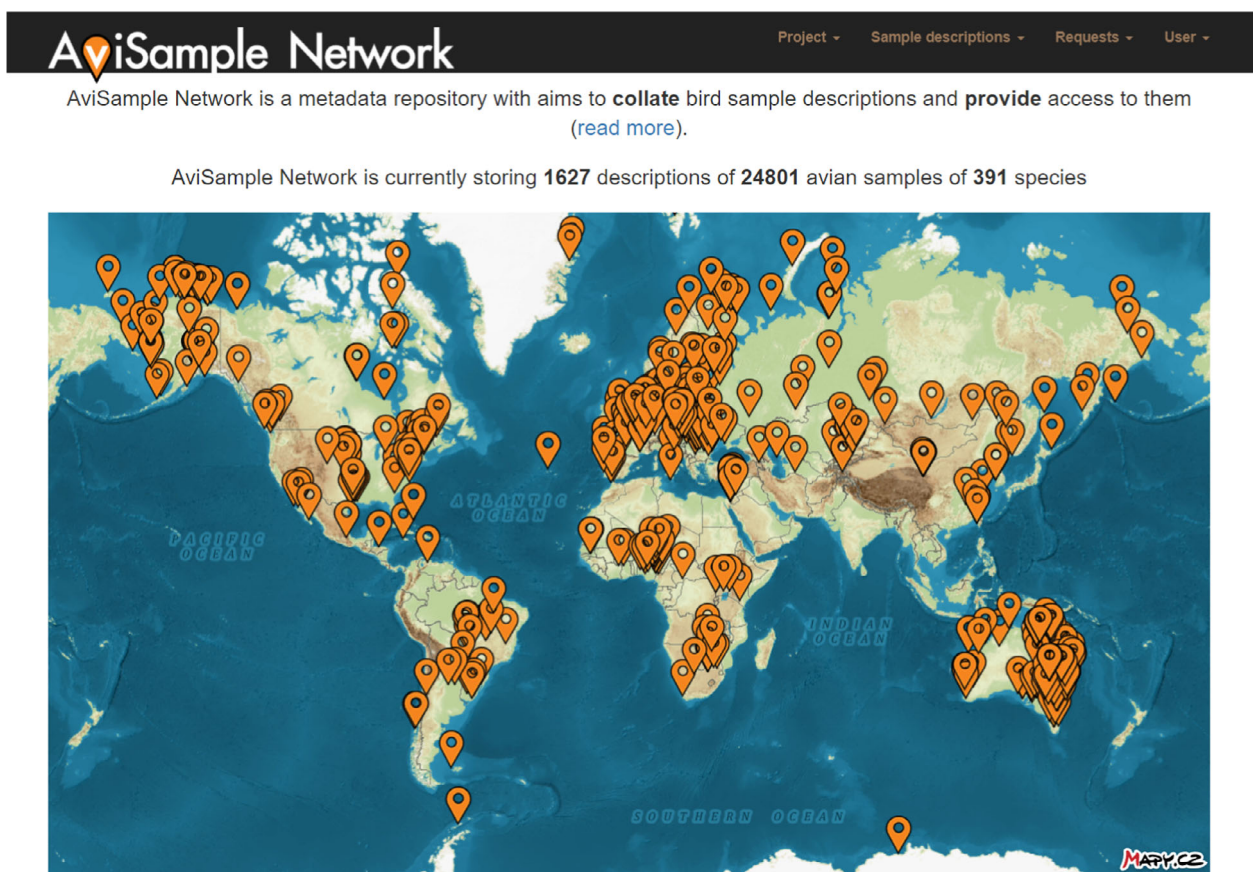
Stable nitrogen ( $\delta^{15}\text{N}$ ) isotopes are most frequently used to estimate trophic position in a food web (DeNiro & Epstein 1981, Vander Zanden *et al.* 2010), diet switches (Thompson *et al.* 1995) or the physiological condition of an individual (Hobson *et al.* 1993). In addition, bulk  $\delta^{15}\text{N}$  values show large-scale spatial gradients (Amundson *et al.* 2003, Sena-Souza *et al.* 2020), influenced by the amount of precipitation (Amundson *et al.* 2003). Finally,  $\delta^{15}\text{N}$  values differ between marine–freshwater/terrestrial or benthic–pelagic habitats, making them useful for inferring food web connections (reviewed in Hobson 1999, Quillfeldt *et al.* 2010).

Other stable isotopes are used less frequently due to the lack of information on how they relate to environmental variables and measurements at large geographical scales. A couple of previous studies have shown a strong relationship between sea spray transport and stable sulphur ( $\delta^{34}\text{S}$ ) values in organic tissues (Wadleigh & Blake 1999, Zazzo *et al.* 2011) and have uncovered spatial patterns of  $\delta^{34}\text{S}$  isotopes (Valenzuela *et al.* 2011, Kabalika *et al.* 2020). However, there is little evidence that this pattern holds at large continental scales (Valenzuela *et al.* 2011, Procházka *et al.* 2018). Similarly, there have been few studies on the spatial distribution of radiogenic strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ; Bataille *et al.* 2020), despite the evidence of a strong link between  $^{87}\text{Sr}/^{86}\text{Sr}$  and bedrock, which could provide an informative geographical marker of tissue origin (Sellick *et al.* 2009).

Collectively, an isotopic composition of bird tissue can provide useful information from a mixture of intrinsic markers reflecting diet, physiological conditions, habitat use, geographical origin and other environmental conditions during tissue growth. Therefore, multi-isotope composition can be viewed as an alternative measure of an individual or population's ecological niche, the so-called isotopic niche, describing space and resource use (Newsome *et al.* 2007, Shipley & Match 2020). The application of stable isotopic signatures in avian biology is thus manifold. However, it relies on our knowledge of spatial distributions and relationships with environmental variables where broad reuse of already collected samples could yield major improvements.

## AVISAMPLE NETWORK METADATA REPOSITORY

Sample reuse in avian biology could increase research impact, ease collaborations generating new ideas and unconventional interpretations, facilitate studies of endangered species, and be mutually beneficial for both researchers providing and reusing the samples. However, an efficient reuse of samples collected by researchers is currently hampered by the absence of a tool to locate samples of interest. To facilitate reuse of avian samples in ornithological research, we introduce the AviSample



**Figure 1.** A screenshot of the geographical distribution and number of sample descriptions stored in the AviSample Network metadata repository on 12 June 2021 (<https://avisample.net>).

Network metadata repository (<https://avisample.net>, Fig. 1).

The AviSample Network metadata repository is designed for storing sample metadata from a wide range of research fields. The main aims of the AviSample Network metadata repository are, first, to advertise and store information about the presence of bird tissues sampled worldwide and, secondly, to help researchers find and contact owners of potentially relevant samples. By connecting avian researchers across the globe, the potential of avian sample reuse across disciplines can be maximized. To ensure broad participation, the database submission includes only the basic and most important metadata during database entry. The AviSample Network metadata repository does not aim to archive samples, results or raw data.

Data submission requires each of the following variables for each set of samples – *sample description*:

- Sampling date or period in a year of sample collection.
- Coordinates of the sampling location or centre of the sampling area.
- Species common or scientific name.
- Tissue type (including specification of feather types).
- Preservation method.
- Geographical origin of sample (unknown or known; method of geographical location estimation).
- Status of the sampled individuals (wild or captive).
- Sex (if available) and age-class of sampled individuals (multiple choice allowed).
- Number of samples.
- Sample description already published (no or citation including DOI).
- Notes – including additional information such as approximate remaining amount of the sample material, current storage location, analytical methods applied or research plan (optional).

A unique and permanent identification number is assigned to each sample description submitted to the database. Although additional edits of sample

descriptions are allowed, the history of all changes will be stored and publicly available for all users to increase transparency. Database management, quality assurance and quality control will be done by the core team members of AviSample Network (<https://avisample.net/site/team>).

Researchers interested in finding already collected samples can search the database using the Search tool, which allows combining specifications and requesting multiple sample descriptions meeting focal criteria. A brief project description will be required to accompany the automatic request message sent to owners of selected sample descriptions. The owners will then be notified and asked to grant or reject individual requests. Granting the sample description request will automatically provide the requesting researcher with an e-mail address of the sample description owner to ease communication, but does not guarantee access to samples. Detailed tutorials on registration, submission, search and request of sample descriptions are provided at <https://avisample.net/site/tutorials>. We also provide the first version of machine-readable Application Programming Interface (API), which will be further developed (<https://avisample.net/site/api>). The API will allow simple exchange of data between software applications.

We believe that the straightforward entry of the sample descriptions into the AviSample Network metadata repository will facilitate sample reuse in a wide range of studies, including multispecies comparisons and broad-scale avian projects. This database will also help create a research network among ornithologists across the globe and with different fields of expertise, and, hopefully, inspire sample reuse in other fields.

We would like to thank Robert Robinson for a thorough review that significantly helped improve an earlier version of the manuscript. We also thank Paul Cryan, Dominic McCafferty, Michael B. Wunder, Leonard I. Wassenaar, Ondřej Belfín and Steffen Hahn for critical comments on the manuscript and the AviSample Network metadata repository. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

## FUNDING

This work was supported by the Czech Science Foundation (grant no. 20-00648S to P. Pr.).

## AUTHOR CONTRIBUTIONS

**Vojtěch Brlík:** Conceptualization (lead); Investigation (lead); Methodology (lead); Project administration (lead); Writing-original draft (lead); Writing-review & editing (lead). **Pavel Pipek:** Conceptualization (equal);

Methodology (equal); Project administration (equal); Software (lead); Writing-review & editing (equal). **Kate Brandis:** Methodology (supporting); Writing-review & editing (equal). **Nikita Chernetsov:** Methodology (supporting); Writing-review & editing (equal). **Fábio J. V. Costa:** Methodology (supporting); Writing-review & editing (equal). **L. Gerardo Herrera M.:** Methodology (supporting); Writing-review & editing (equal). **Yosef Kiat:** Methodology (supporting); Writing-review & editing (equal). **Richard B. Lanctot:** Methodology (supporting); Writing-review & editing (equal). **Peter P. Marra:** Methodology (supporting); Writing-review & editing (equal). **D. Ryan Norris:** Methodology (supporting); Writing-review & editing (equal). **Chima J. Nwaogu:** Methodology (supporting); Writing-review & editing (equal). **Petra Quillfeldt:** Methodology (supporting); Writing-review & editing (equal). **Sarah T. Saalfeld:** Methodology (supporting); Writing-review & editing (equal). **Craig A. Stricker:** Methodology (supporting); Writing-review & editing (equal). **Robert L. Thomson:** Methodology (supporting); Writing-review & editing (equal). **Tianhao Zhao:** Methodology (supporting); Writing-review & editing (equal). **Petr Procházka:** Conceptualization (supporting); Funding acquisition (lead); Methodology (equal); Supervision (lead); Writing-review & editing (equal).

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Received 11 January 2021;  
Revision 18 June 2021;  
revision accepted 24 June 2021.