Preserving avian blood and DNA sampled in the wild: a survey of personal experiences

Authors

Irene Di Lecce[1]*, Joanna Sudyka[2], David F. Westneat[3] and Marta Szulkin[1]

[1] Centre of New Technologies, University of Warsaw, Poland

[2] Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

[3] Department of Biology, University of Kentucky, Lexington, Kentucky, USA

*Correspondence: Centre of New Technologies, University of Warsaw, ul. S. Banacha 2c, 02-097 Warsaw, Poland; e-mail: irene.dileccce1@gmail.com

Abstract

Collecting and storing biological material from wild animals in a way that does not deteriorate data quality for analyses using DNA is instrumental for research in ecology and evolution. Our aims were to collect methods commonly used by researchers for the field collection and long-term storage of blood samples and DNA extracts from wild birds and gather reports on their effectiveness. Personal experiences were collected with an online survey targeted specifically at researchers sampling wild birds. Many researchers experienced problems with blood sample storage but not with DNA extract storage. Storage issues generated problems with obtaining adequate DNA quality and sufficient DNA quantity for the targeted molecular analyses, but were not related to season of blood sampling, access to equipment, transporting samples, temperature and method of blood storage. Final DNA quality and quantity were also not affected by storage time before DNA extraction or the methods used to extract DNA. We discuss practical aspects of field collection and storage and provide some general recommendations, with a list of pros and cons of different preservation methods of avian blood samples and DNA extracts.

Keywords

birds, blood sampling, long-term storage, DNA, molecular ecology

Introduction

Ecological and evolutionary processes shaping natural animal populations typically occur over multiple years or decades at least. Consequently, research in ecology and evolutionary biology often requires data extending over long periods of time and the accumulation of records of individuals over their entire lifespans (Clutton-Brock and Sheldon, 2010). Long-term individual-based studies have proven extremely valuable in gaining insight into the demographic and life-history traits of wild populations, for instance by making sense of aspects of breeding, survival, mate choice and lifetime reproduction (Bouwhuis, 2018; Mills et al., 2016). Over the past three decades, molecular tools have become easier to use and widely applied to multiple disciplines such as population ecology/biology (Deyoung & Honeycutt, 2005), biogeography (Riddle et al., 2008), conservation genetics (Primmer, 2009) or behavioural ecology (Bengston et al., 2018) and have led to the emergence of new fields such as landscape genetics, molecular quantitative genetics and population genomics (Black IV et al., 2001; Jensen et al., 2014; Manel et al., 2002). These techniques add to the size and richness of biological archives in recent years, spanning several decades and thousands of individuals. The wide array of cost-efficient molecular tools available nowadays and the increasing computational power able to handle large amounts of data allow researchers to reliably perform a variety of analyses on biological material from wild populations. However, sample storage conditions remain a key issue, and can limit the ability to generate high quality genetic data from animal blood or other tissues. At the time of logistical planning of a long-term study, an informed decision concerning sample preservation has to be made. Inadequate preservation might compromise sample quality and research scope, for example leading to the degradation of nucleic acids (Conrad et al., 2000; Kilpatrick, 2002; Seutin et al., 1991; Zimmermann et al., 2008). In this light, *storage method* and *temperature* are fundamental aspects of sample preservation. Because of their interactive influence on final sample quality, their effects – and limitations - have to be simultaneously taken into consideration before undertaking sampling for a specific project and in light of future applications that may arise with the progression of the study.

Storage method

Direct sample freezing is viewed as the method of choice for long-term storage, since enzymatic and other chemical activities decrease with lower temperatures (Wong et al., 2012). However, other storage methods which require a liquid preservative or physical support (e.g. paper-based substrate) might provide advantages over freezing, such as a reduction in space and energy consumption, no power outage risks, lower long-term costs, and easier transfer and shipping of samples. For example, blood can be stored in either 95-100% ethanol, in lysis buffers such as Queen's buffer and Longmire's buffer, or dried on filter paper, such as FTA® cards (Longmire et al., 1997; Seutin et al., 1991; Smith and Burgoyne, 2004). Such storage methods can differentially impact molecular assays that will be performed on the biological samples. For instance, in a PCR diagnostics study for avian and human malaria, lower accuracy of the test was associated with samples stored in a lysis buffer (containing sodium dodecyl sulfate or SDS) compared to a buffer lacking SDS (Freed and Cann, 2006). SDS may have been the cause since it releases endonucleases and creates extra cellular debris. There is also evidence that relative telomere length (RTL) measurements differ significantly depending on storage method: Reichert et al. (2017) showed how RTL of samples stored on FTA® cards at room temperature was significantly shorter than in samples preserved as frozen whole blood or frozen DNA. Different storage methods have also been shown to affect stable isotope signatures: in a study by Bugoni et al. (2008), a significant enrichment in δ^{13}C was detected in ethanol-preserved blood samples in comparison with dry and frozen blood samples. Samples preserved in ethanol also showed variation in δ^{13}C values according to the brand and even the batch of preservative.

Storage temperature

When directly freezing samples, temperatures of -80°C, or as low as possible, are recommended to maximise DNA preservation (Jackson et al., 2012; Wong et al., 2012). In case of biological material collected from birds, storage at -20°C was deemed sufficient to prevent DNA degradation, since avian red blood cell nuclei were considered metabolically inactive (Seutin et al., 1991). However, recent evidence (Stier et al., 2013) reports that avian blood cells do have functional mitochondria involved in respiratory cellular metabolism. Consequently, lower storage temperature (e.g. -80°C) may still offer better preservation conditions than freezing at -20°C. When deep-cold storage is not feasible or practical, ethanol, lysis buffer and filter paper form a valuable alternative, since they are routinely stored at room temperature (Kilpatrick, 2002; Seutin et al., 1991; Smith and Burgoyne, 2004). However, multiple studies have reported poor stability of DNA extracted from whole blood samples stored at room temperature and better DNA yields from samples kept at +4°C or lower (Madisen et al., 1987; Richardson et al., 2006; Visvikis et al., 1998). Moreover, filter paper is known to generate different DNA yields depending on storage temperature: Hollegaard et al. (2011) showed how storing dried blood spots (DBS) samples, also known as Guthrie cards, at +4°C negatively affected DNA concentration, which increased when samples were stored at -20°C. Mei et al. (2011) reported similar results on DBS tested for Toxo-specific immunoglobulin-M: reduced recovery was observed in DBS stored at room temperature compared with specimens stored at -20°C.

Additional factors influencing sample stability

Technical assessments of specific protocols (Kilpatrick 2002; Michaud and Foran, 2011; Seutin et al., 1991; Zimmermann et al., 2008) have considerable value but may be limited due to specific focus and carefully controlled conditions. The diversity of conditions under which samples are collected and the variety of purposes for which they have been used across many researchers may reveal additional limitations. Indeed, some specific constraints of field collection and long-term storage of biological material might have an impact on sample quality and research outcomes that is not necessarily captured in laboratory assessments of DNA degradation. For instance, collecting samples in spring or summer means that biological material may be challenged by high temperatures, with negative consequences on DNA quality/integrity. Access to equipment in the field, such as a fridge or a freezer, might have a positive effect, ensuring sample stability before the long-term storage in the laboratory. Different ways of transferring samples from the field to the laboratory might also affect sample integrity if samples are exposed to high temperatures during transportation or experience delays in shipping. Moreover, storing samples as blood or DNA extracts might have different outcomes on DNA integrity in the long run, so during logistical planning it might be necessary to take into consideration storage time before DNA extraction. In this light, we asked scientists directly about what experiences have informed their decisions regarding the long-term storage of blood and DNA, and about their perceived assessment of the efficacy of their storage procedures. Importantly, personal and first-hand experiences may shed light on how often problems that arise from sample collection or storage can affect DNA quality, thus impacting the final sample size and the quality of published research.

Here, we present the results from an online survey designed for researchers involved in the collection and storage of blood and DNA from wild birds. We circulated the survey among ecologists and field biologists in order to (i) review practices commonly used for field collection and storage of avian blood and DNA extracts, (ii) assess if any procedural or methodological gaps exist in current knowledge of avian blood and DNA storage and (iii) improve existing guidelines for the long-term storage of avian blood and DNA.

Methods

Survey

An online survey entitled "Preserving avian DNA from the wild: Your experience of blood sampling, DNA extraction and storage" was created on the Survey Monkey platform (Supporting Information 1). It comprised 3 sections (blood sample collection, blood storage, and DNA extraction and storage) with 52 questions, mostly multiple choice. 14 of these were open questions, where more detailed answers were required. Out of the 52 questions, 25% (n=13) were designed with a Likert scale, here a five-point rating scale which allows respondents to express how much they agree or disagree with a particular statement (Derrick and White, 2017). The survey was disseminated from summer 2018 until early spring 2019, specifically targeting researchers working with wild birds in the fields of evolution, ecology, and conservation biology. The survey was advertised on social media using Twitter, by email to colleagues known to have collected avian samples with kind requests for further forwarding, on evolution and ecology international and national mailing lists and dissemination websites (EvolDir, EvolFrance, the Ornithological Societies of North American newsletter, and zmihor.blogspot.com) and during conferences (i.e. International Society for Behavioural Ecology 2018, International Ornithology Conference 2018 and Polish Evolutionary Conference 2018). Participation in the survey was anonymous, but respondents could leave their contact information.

Data analysis

Data analysis was carried out in R 4.1.0 (R Core Team, 2021). Chi-squared tests of independence were used to test the relationship between having experienced storage issues and problems with obtaining adequate DNA quality (yes/no) or quantity (yes/no). Storage issues (i.e. problems with storage which might have negative consequences on DNA integrity) were coded as a yes/no variable. Fisher's exact and chi-squared tests of independence were used to test the relationship between storage issues and having changed storage method for blood samples (yes/no) or DNA extracts (yes/no). Because it is known that filter paper leads to DNA degradation, a Fisher's exact test was run to specifically investigate this, by creating an additional variable from the open answers regarding DNA degradation for the different storage methods. Fisher's and chi-squared tests were also used to investigate the relationship between problems with obtaining adequate DNA quality (yes/no) or quantity (yes/no) and several aspects of sample collection and preservation. The investigated aspects were: season of blood sampling (tested as two separate explanatory variables with a separate test: either coded in 4 categories -Spring, Summer, Autumn, Winter- or coded as 2 categories -Dry and Wet season- as respondents could choose only one option), access to equipment in the field (yes/no), means of transportation of blood samples to the laboratory (6 categories: Airplane, Car, Courier, Boat, Train, Other), storage temperature of blood samples (5 categories: Room temperature, +4°C, -20°C, -80°C, Other), type of molecular analysis performed on the samples (twelve categories: Gene expression, Methylation assay, MHC characterization, Microsatellite assay, Molecular sexing, mtDNA analysis, Parasite DNA analysis, RAD sequencing, SNP chip, SNP genotyping, Telomere length, Whole genome sequencing) and DNA extraction method (6 categories: Ammonium acetate, Chelex, Commercial column kit, In-house protocol, Phenol-chloroform, Other). To test whether some storage methods of blood samples were more likely to be associated with DNA quality/quantity problems further downstream, generalized linear models assuming quasibinomial error distribution (to correct for overdispersion) were employed. Occurrence of problems with obtaining adequate DNA quality (yes/no) or quantity (yes/no) were fitted as response variables and storage method for blood samples (7 categories: Ethanol, Lysis buffer, Direct freezing, Filter paper, TE buffer, RNA*later*, Other) as fixed categorical explanatory variable. Similar models were run to test whether storage time before DNA extraction mattered, with occurrence of problems with obtaining adequate DNA quality (yes/no) or quantity (yes/no) as response variables and storage time (4 categories: Up to 6 months, Up to 1 year, More than 1 year, No standard time frame) as fixed categorical explanatory variable. Generalized linear models were employed, instead of mixed models accounting for respondent id as random effect, due to lack of convergence of the mixed models (only 7% of responses were not independent, since the same respondents filled the survey twice or more times).

Results

Blood sample collection

A total of 219 responses to the survey were collected. All anonymized answers are available in Supporting Information 2. 209 responses on wild birds and molecular analyses were kept. Overall, researchers taking part in the survey worked on 123 species of wild birds, encompassing 53 families and 20 orders, with blue tit (*Cyanistes caeruleus*) and great tit (*Parus major*) being the most represented (Table S1-S3 in Supporting Information 3). Of the 155 responses on affiliation, 85% (n=131) were research institutions located in North America and Europe, with the greatest contribution from the USA, France, UK and Poland, in decreasing order (Figure 1A). However, the experience of respondents in field blood sampling covered 53 countries and territories across the globe, with half of responses from the USA, France, Canada, Spain, Poland and Sweden, in decreasing order (Figure 1B).

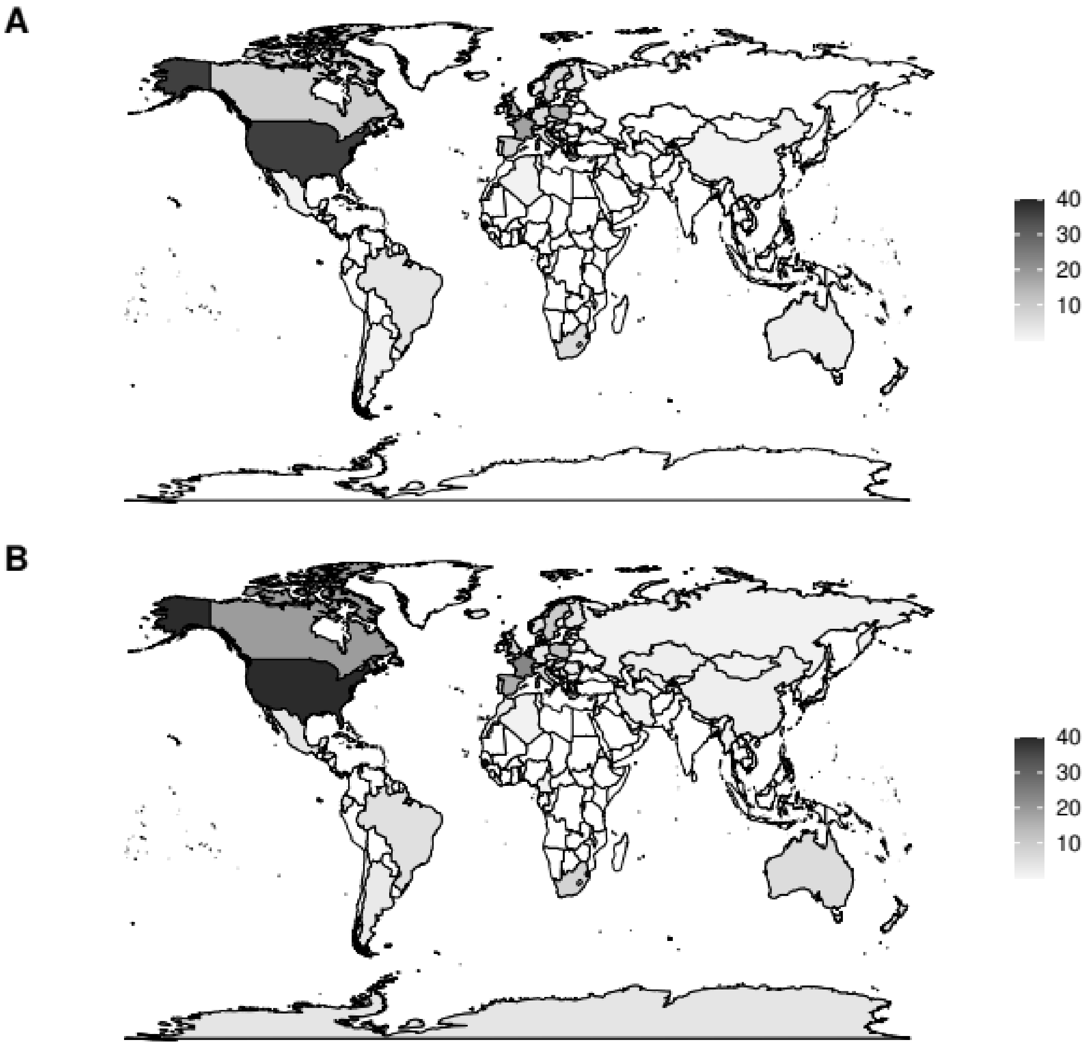

Figure 1 – World map reporting (A) countries of affiliation when researchers responded and (B) countries where fieldwork and blood sampling occurred.

Spring and summer were reported in 80% (165/203) of responses to the question "When are blood samples on this project most often collected?" (Figure 2A). Access to equipment in the field, such as a centrifuge, a fridge or a freezer, was reported in 68% (132/195) of responses (Figure 2B shows number of responses, with percentage of the total, for the different types of equipment).

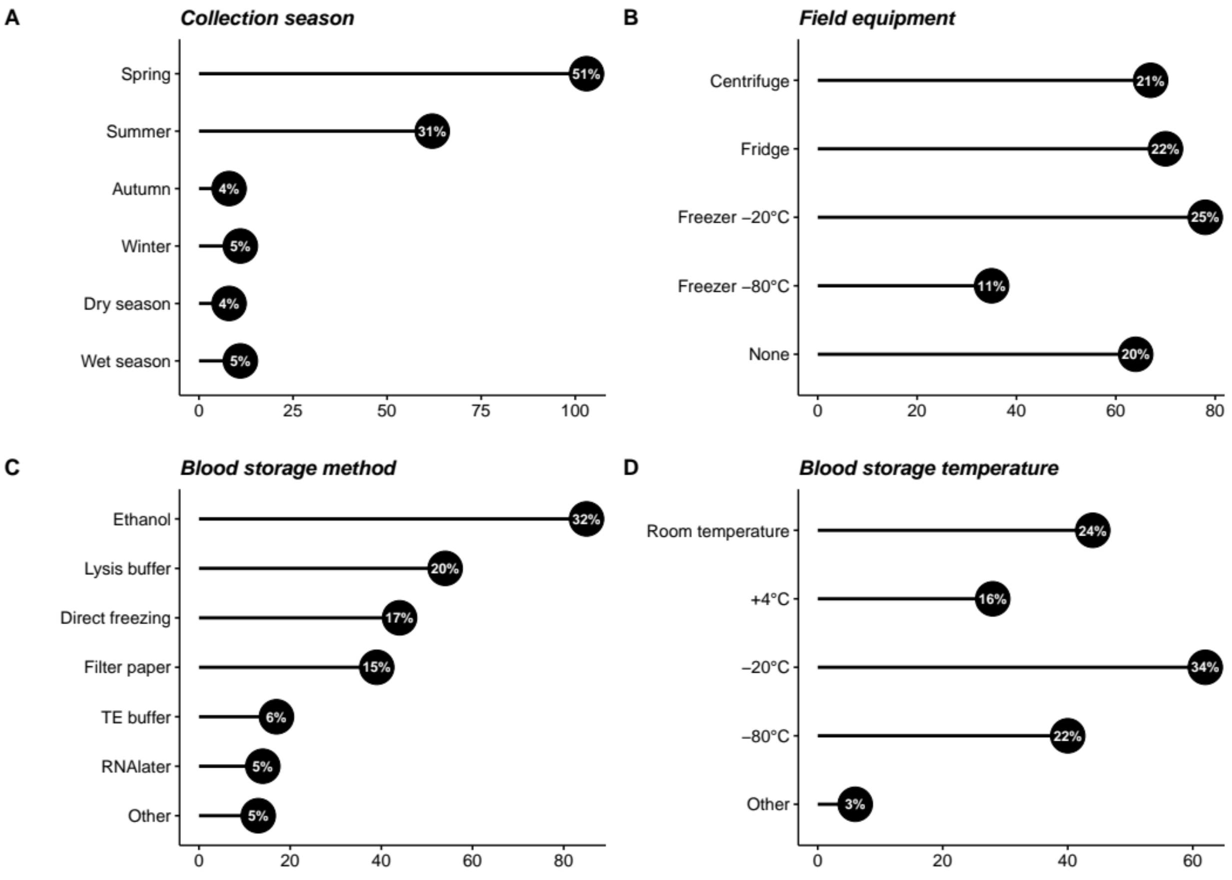

Figure 2 - Overview of aspects of collection and preservation of blood samples: season of blood sampling (A), equipment available in the field (B), storage method (C) and storage temperature for blood samples (D). On the x axis, number of responses, with percentages of the total, are shown; note that in (B) and (C) more than one response could be given. "Other" in (C) comprises: EDTA (3 responses), PBS (1), EDTA + PBS (1), a glycerol-based buffer (1), NBS buffer (1), TNE buffer (2), commercial buffer (3) and heparin buffered tubes (1). "Other" in (D) comprises: -35°C (1 response), -40°C (1), -50°C (1), -70°C (1) and liquid nitrogen (1).

Of the 284 responses to "How do you move blood samples from the field site to the permanent laboratory on this project?", 53% (n=151) were by car, followed by airplane (23%; n=64), courier service (11%; n=32), train (6%; n=17), boat (3%; n=9), on foot (2%; n=5), bicycle (1%; n=3), bus (1%; n=2) and one response by helicopter. Of the 204 responses to the question "How large is your sample database on this project?", 45% (n=91) were between 100 and 1,000 blood samples, followed by 34% (n=69) for 1,000-10,000 and 8% (n=17) for more than 10,000. Only 13% (n=27) of responses were for small sample sizes such as less than 100 samples.

Methods of blood storage used by field biologists

Ethanol, lysis buffer, direct freezing and filter paper (in decreasing order) were the methods of choice used to store blood samples (Figure 2C). TE buffer, RNA*later* and other mediums were used in the remaining 16% (44/266) of cases (Figure 2C). In terms of storage temperature, blood samples were stored at -20°C in 34% (62/180) of responses, followed by room temperature, -80°C and +4°C (Figure 2D). Table 1A shows storage methods for blood samples categorized by storage temperature, as reported in the survey.

Table 1 - Number of responses for each storage method by storage temperature for blood samples (A) and DNA extracts (B). Percentages within each storage method are shown in brackets. For blood samples, more than one response could be given. "RT" indicates Room temperature. "Other" comprises: EDTA (3 responses), PBS (1), EDTA + PBS (1), a glycerol-based buffer (1), NBS buffer (1), TNE buffer (2), commercial buffer (3) and heparin buffered tubes (1).

A. BLOOD SAMPLES						
	RT	**+ 4° C**	**- 20° C**	**- 80° C**	**Other**	**Total**
Ethanol	23 (29)	15 (19)	27 (34)	11 (14)	3 (4)	79
Lysis buffer	14 (29)	11 (22)	12 (24)	9 (18)	3 (6)	49
Direct freezing	0 (0)	0 (0)	18 (43)	22 (52)	2 (5)	42
Filter paper	16 (46)	5 (14)	5 (14)	7 (20)	2 (6)	35
TE buffer	1 (7)	3 (20)	7 (47)	4 (27)	0 (0)	15
RNA*later*	1 (7)	2 (14)	6 (43)	3 (21)	2 (14)	14
Other	0 (0)	2 (17)	3 (25)	6 (50)	1 (8)	12
Total	55	38	78	62	13	246

B. DNA EXTRACTS						
	RT	**+ 4°C**	**- 20°C**	**- 80°C**	**Other**	**Total**
TE	0 (0)	2 (3)	60 (80)	13 (17)	0 (0)	75
Water	0 (0)	3 (9)	21 (66)	8 (25)	0 (0)	32
Kit buffer	0 (0)	2 (10)	11 (52)	8 (38)	0 (0)	21

Tris	0 (0)	1 (5)	17 (77)	4 (18)	0 (0)	22
Total	0	8	109	33	0	150

Methods of DNA storage used by field biologists

Of the 165 responses to the question “How long after collecting blood samples do you usually extract DNA?”, 26% (n=43) were within 6 months of collection, 19% (n=32) within 1 year of collection and 19% (n=32) after 1 year. 35% (n=58) of responses were for “I don’t have a standard time frame”. Regarding DNA extraction, 57% (121/214) of responses were for commercial column kit, followed by phenol-chloroform, ammonium acetate, in-house protocol and other methods (Figure 3A). To preserve DNA extracts, TE was most frequently used, followed by water, a kit buffer and Tris (Figure 3B). DNA samples were most frequently archived at -20°C, followed by -80°C and rarely at +4°C (Figure 3C). Table 1B shows storage method categorized by storage temperature for DNA extracts.

Molecular analyses following DNA extraction

Of the 170 responses to the question “How long after DNA extraction do you usually perform analyses?”, 41% (n=70) were within 6 months of collection, 14% (n=24) within 1 year of collection and 10% (n=17) after 1 year. 35% (n=59) of responses were for “I don’t have a standard time frame”. Respondents performed a wide variety of analyses on the collected samples (Figure 3D). Microsatellite assay, parasite DNA screening, mtDNA analysis and RAD sequencing, in decreasing order, comprised 70% (286/410) of responses. The rest of the answers, in decreasing order, were: SNP chip, telomere length measurement, molecular sexing, gene expression analysis, methylation assay, sequencing, whole genome sequencing, MHC characterization and SNP genotyping (Figure 3D).

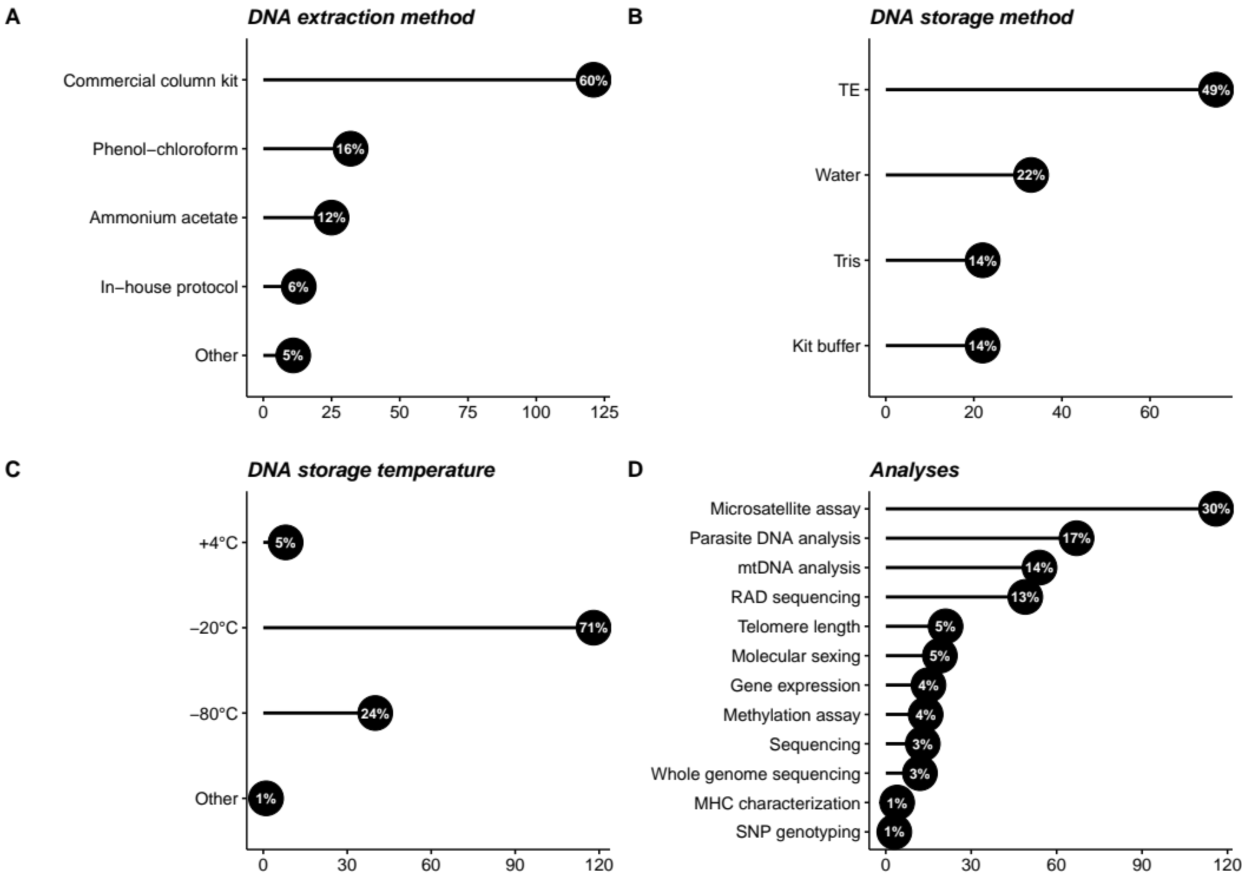

Figure 3 - Graphical summary of responses related to: DNA extraction method (A), storage method (B) and storage temperature for DNA extracts (C) and molecular analyses performed by respondents (D). On the x axis, numbers of responses, with percentages of the total, are shown; note that in (D) each respondent could provide multiple answers. “Other” in (A) comprises: Commercial magnetic bead kit (3 responses), salt extraction (3), CTAB (1), other types of commercial kits (3) and soda (1). “Other” in (C) refers to -50°C (1 response).

Table 2 shows type of molecular analyses performed depending on the type of storage method of blood (A) and DNA (B) samples.

Table 2 – Type of analyses for blood (A) and DNA (B) by sample storage method. Percentages within each storage method are shown in brackets. More than one response could be given.

A. BLOOD SAMPLES

	Microsatellite assay	SNP chip	RAD sequencing	Gene expression	Methylation assay	Parasite DNA analysis	mtDNA analysis	Molecular sexing	Sequencing	Telomere length	Whole genome sequencing	SNP genotyping	MHC characterization	Total
Ethanol	53 (27)	12 (6)	25 (13)	4 (2)	5 (3)	35 (18)	29 (15)	10 (5)	6 (3)	10 (5)	6 (3)	1 (1)	1 (1)	197
Lysis buffer	44 (34)	5 (4)	24 (19)	4 (3)	3 (2)	14 (11)	17 (13)	3 (2)	5 (4)	3 (2)	5 (4)	1 (1)	1 (1)	129
Filter paper	26 (29)	4 (4)	15 (16)	1 (1)	3 (3)	9 (10)	19 (21)	6 (7)	2 (2)	1 (1)	3 (3)	1 (1)	1 (1)	91
Direct freezing	15 (19)	3 (4)	8 (10)	2 (2)	3 (4)	17 (21)	9 (11)	6 (8)	3 (4)	10 (12)	2 (2)	2 (2)	0 (0)	80
RNA*later*	8 (20)	3 (7)	3 (7)	5 (12)	1 (2)	9 (22)	6 (15)	0 (0)	1 (2)	2 (5)	1 (2)	0 (0)	2 (5)	41
TE buffer	7 (23)	2 (6)	2 (6)	4 (13)	2 (6)	6 (19)	3 (10)	0 (0)	1 (3)	1 (3)	1 (3)	1 (3)	1 (3)	31
Other	8 (26)	1 (3)	4 (13)	2 (6)	1 (3)	5 (16)	4 (13)	1 (3)	1 (3)	3 (10)	1 (3)	0 (0)	0 (0)	31
Total	161	30	81	22	18	95	87	26	19	30	19	6	6	600
B. DNA SAMPLES														
TE	44 (27)	28 (17)	23 (14)	17 (11)	11 (7)	6 (4)	5 (3)	5 (3)	8 (5)	6 (4)	4 (2)	3 (2)	1 (1)	161
Water	18 (23)	15 (19)	14 (18)	13 (16)	0 (0)	5 (6)	2 (3)	1 (1)	3 (4)	2 (3)	5 (6)	0 (0)	1 (1)	79
Kit buffer	12 (28)	5 (12)	6 (14)	4 (9)	4 (9)	3 (7)	3 (7)	4 (9)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	43
Tris	13 (30)	9 (21)	2 (5)	7 (16)	2 (5)	2 (5)	4 (9)	2 (5)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)	43
Total	87	57	45	41	17	16	14	12	11	10	10	4	2	326

Storage issues can result in low DNA quality and quantity

Of the 199 responses to the question on experiencing issues with sample storage, 31% (n=61) reported problems: 21% (n=41) were problems with storage of blood samples, one with DNA storage and 2% (n=5) with storage of both blood and DNA samples. 7% (n=14) of responses were from researchers who experienced problems with sample storage but could not identify the issue (Figure 4A). 44 open responses explained what was the storage issue researchers faced, as follows: (i) use of anticoagulant (possibly due to an overuse of heparin) in collection devices interfering with PCR (5%; n=2), (ii) difficulties with DNA extraction due to lysis buffer, either because of too much blood for the amount of buffer or because of long storage time or lysis buffer interfering with telomere length assay (27%; n=12); (iii) DNA degradation when blood was stored on filter paper, especially in case of long storage time (23%; n=10); (iv) evaporation of ethanol from tubes and ethanol not good enough for PacBio sequencing (20%; n=9), (v) DNA degradation in TE buffer (9%; n=4), (vi) freezer failure (5%; n=2), (vii) misidentification of samples (2%; n=1), (viii) sample shipping (7%; n=3), (ix) DNA extraction (2%; n=1). DNA degradation, as reported in the open answers, was not more likely to occur in any of the four storage methods for blood samples indicated by respondents (filter paper, ethanol, lysis buffer, TE buffer; two-tailed Fisher's exact test, p-value = 0.136). Of the 61 responses reporting problems with sample storage, 84% (n=51) also reported a reduction in sample size of the project (Figure 4B), which for example led to a reduction in the geographic range of the sampling, exclusion of some target species, reduction in statistical power and left holes in paternity analyses.

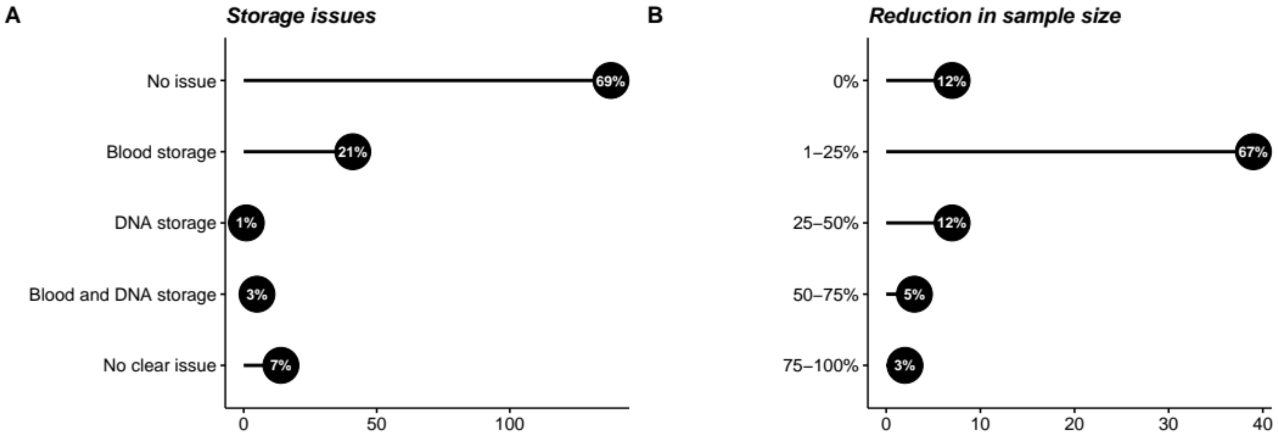

Figure 4 – Overview of problems with storage encountered by respondents (A) and reduction in sample size of the project due to storage issues (B). On the x axis, numbers of responses, with percentages of the total, are shown.

Consequently, 18% (n=11) of projects were not published and 8% (n=5) were published in a less prestigious journal. Storage issues were related to problems with obtaining adequate DNA quality (χ^2_1 = 28.596, p-value = 8.915e-08) and sufficient DNA quantity (χ^2_1 = 6.139, p-value = 0.013; Table 3).

Table 3 – Number of responses for problems with sample storage (both blood samples and DNA extracts) with respect to problems with obtaining adequate DNA quality and sufficient DNA quantity. Percentages within rows are shown in brackets.

	Problems with DNA quality			**Problems with DNA quantity**			
Storage problems	**Yes**	**No**	**Total**	**Yes**	**No**	**Not measured**	**Total**
Yes	27 (48)	29 (52)	56	19 (35)	32 (59)	3 (6)	54
No	12 (11)	101 (89)	113	18 (16)	82 (73)	13 (11)	113
Total	39	130	169	37	114	16	167

No aspect of sample collection and storage influences DNA quality and quantity

The survey did not identify any association between season when blood samples were collected and problems with obtaining either adequate DNA quality (categories for season: spring, summer, autumn, winter: two-tailed Fisher's exact test, p-value = 0.219; categories for season: dry vs wet season: two-tailed Fisher's exact test, p-value = 0.319) or sufficient DNA quantity (categories for season: spring, summer, autumn, winter: two-tailed Fisher's exact test, p-value = 0.524; categories for season: dry vs wet season: two-tailed Fisher's exact test, p-value = 1). Having access to equipment in the field was not associated with problems with obtaining adequate DNA quality (χ^2_1 = 0.368, p-value = 0.544) or sufficient DNA quantity (χ^2_1 = 0.076, p-value = 0.783). There was also no association between way of transferring samples from field to laboratory and problems with obtaining adequate DNA quality (two-tailed Fisher's exact test, p-value = 0.160) or sufficient DNA quantity (two-tailed Fisher's exact test, p-value = 0.282). No storage method for blood samples was more likely than others to generate problems with obtaining either adequate DNA quality (Table 4A) or sufficient DNA quantity (Table 4B).

Table 4 - Binomial generalised linear models explaining problems with obtaining adequate DNA quality (A) or sufficient DNA quantity (B) based on blood storage method. “Direct freezing” is the reference for parameter estimates.

Variable		**χ^2**	**df**	**Pr(> χ^2)**	**Estimate ± SE**
A. DNA quality		5.703	5, 119	0.3362	
	Ethanol				0.821 ± 0.735
	Filter paper				0.415 ± 1.006
	Lysis buffer				0.128 ± 0.889
	Other				0.174 ± 1.262
	TE buffer				1.897 ± 0.930
B. DNA quantity		3.292	5, 105	0.655	
	Ethanol				0.938 ± 0.758
	Filter paper				1.226 ± 0.883
	Lysis buffer				0.379 ± 0.844
	Other				0.245 ± 1.277
	TE buffer				0.091 ± 1.267

When researchers were asked whether they changed storage method, a higher number of responses (23%; 43/191) were collected for blood samples compared to DNA extracts (8%; 13/170). Experiencing storage issues was associated with having changed storage method for blood samples (χ^2_1 = 10.424, p-value = 0.001). Half (n=8) of the responses explaining why researchers changed storage method for blood samples indicated an increase in DNA yields and the other half (n=8) logistical reasons, either because of space constraints in the laboratory or because of issues during sample transport. There was no association between storage temperature of blood samples and problems with obtaining adequate DNA quality (two-tailed

Fisher's exact test, p-value = 0.482) or sufficient DNA quantity (two-tailed Fisher's exact test, p-value = 0.423). Storage time of blood samples before DNA extraction did not influence either obtaining adequate DNA quality (Table 5A) or sufficient DNA quantity (Table 5B).

Table 5 – Analysis of storage time of blood samples before extraction on problems with obtaining adequate DNA quality (A) or sufficient DNA quantity (B). Analysis used binomial generalized linear models and the category "Up to 6 months" was the reference for parameter estimates.

Variable		**χ^2**	**df**	**Pr(> χ^2)**	**Estimate ± SE**
A. DNA quality		1.712	3, 158	0.634	
	Up to 1 year				-0.233 ± 0.588
	More than 1 year				0.405 ± 0.532
	No standard time frame				-0.215 ± 0.500
B. DNA quantity		2.514	3, 141	0.473	
	Up to 1 year				0.811 ± 0.609
	More than 1 year				0.310 ± 0.651
	No standard time frame				0.707 ± 0.550

DNA extraction method did not predict problems with obtaining either adequate DNA quality (two-tailed Fisher's exact test, p-value = 0.268) or sufficient DNA quantity (two-tailed Fisher's exact test, p-value = 0.614). However, respondents shared pros and cons of different extraction methods: for instance, phenol chloroform is more time consuming and more toxic than commercial column kits, but provides higher DNA yields and is less expensive. Salt precipitation is less toxic, faster, without risk of contaminating samples and gives comparable results in terms of DNA yields relative to phenol-chloroform. Respondents also suggested to extract DNA sooner after collection and expressed an interest in finding methods that might provide higher yields, for instance magnetic beads. In case of DNA extracts, there was no relationship between storage issues and having changed DNA storage method (two-tailed Fisher's exact test, p-value = 0.210). Among open answers given to explain the change, 4 reported a change from buffer to water, so the sample was easily concentrated in case of necessity, 1 a change from -20°C to -80°C for logistical reasons and 1 because of issues with ethanol.

It was not possible to test whether some molecular analysis goals are more sensitive to problems from some storage methods. No molecular analysis was more likely associated with problems with obtaining either adequate DNA quality (two-tailed Fisher's exact test, p-value = 0.154) or sufficient DNA quantity (two-tailed Fisher's exact test, p-value = 0.871) than others. However, respondents provided recommendations regarding specific storage methods and assays: for instance, they suggested to use RNA*later* or direct freezing for Pac BIO sequencing or other NGS techniques and direct freezing for telomere length measurement, while ethanol, lysis buffer or FTA® cards are suitable for microsatellites, mtDNAanalysis and other PCR-based assays. Table 6 reports some issues faced by respondents with specific methods of blood storage.

Table 6 – Examples of comments given by respondents explaining problems with some storage methods and assays.

Ethanol
"While good and sufficient for most things the DNA quality (average fragment size) is too small for optimal Pac Bio sequencing"
"We have never had any problems with genotyping, sex-typing etc but we now believe that storage of blood in ethanol has a progressive effect on the detection of telomeric sequence by qPCR."
"The blood was put into 100% ethanol and stored long term. These samples were originally collected in 2007 and the tubes must not have been air tight seals as the ethanol evaporated and the blood became dried scabs"
Lysis buffer
"It was more difficult to achieve the minimum concentration for RADseq and whole genome with blood samples stored in lysis buffer.... sometimes (not always), but increasing the lysis incubation time and eluting with less buffer often did the trick."

“Previous samples were collected into a lysis buffer and stored at RT. Over the years the DNA seems to be of lower quality than that collected recently and stored frozen.”
“Used lysis for several years because of ease of preservation, but switched back to freezing when it became apparent this wouldn't work for telomeres”
“Lysis buffers, including "Queen's buffer", have two serious problems: 1) DNA degrades quickly (potentially within months) if not extracted soon after collection; 2) freezing often creates a gel-like consistency that proteases cannot penetrate”
Direct freezing
“Our freezer failed overnight and so a small number of extracted DNA samples were damaged”
FTA® cards
“Storage of blood on paper filter during 5 years, sufficient for microsatellite analyses but too degraded for next-generation sequencing”
“We had issue to perform whole genome sequencing from blood samples stored on FTA cards. They generated significantly less DNA and less pair-ended reads (77 millions vs. up to 215 millions with blood preserved in ethanol). Furthermore, we were not able to generate mate pair libraries out of it because of the lack of DNA available.”
“Used to use FTA cards for microsat work, but have since switched to lysis buffer for whole genome and RAD sequencing as DNA quality is much higher in buffer compared to on filter paper”

Discussion

Proper archiving of biological samples collected in the wild is crucial for current and future research in ecology and evolution, as the way samples are collected and stored has implications for the outcome of the project in many different disciplines. Furthermore, proper archived material provides opportunities for subsequent and future investigations allowed by technical developments (Jackson et al., 2012; Wong et al., 2012). We report first-hand experiences of ecologists and field biologists regarding worldwide practices for the field collection of blood samples from wild birds and the long-term storage of blood samples and DNA extracts in the lab. In terms of DNA storage, recommendations available in the literature overlap with the storing conditions most often reported in the present survey (see Morin et al., 2010; Prendini et al., 2002). DNA is usually archived dry, or in a neutral pH buffer with chelating agents such as EDTA at low temperatures (e.g., -20°C). Respondents also suggested using aliquots of valuable samples to reduce time in the fridge and limit freeze-thaw cycles, provided space is not an issue. Some respondents suggested that FTA® cards might be used to store DNA, if this method would prove efficient. While no clear signal emerged from the survey in terms of co-variation between specific storage methods and DNA quality and quantity output in downstream lab work, a critical point identified by the survey is that the outcome of molecular analyses often depends on storing conditions of blood samples and less of DNA extracts, possibly because clean DNA is easier to store and/or more resilient to damage. We integrate personal experiences of respondents and the available literature to provide general recommendations for blood storage and to ameliorate blood storing practices (Table 7).

Table 7. Storage methods, pros and cons, and recommended best practices for storing blood samples.

Storage method	Pro	Con	Best practices
Ethanol	Relatively inexpensive, readily available and easy to handle (this study)	Difficult to transport with some shipping companies and evaporation in low-quality tubes, leading to DNA degradation (this study) Evidence of DNA degradation over long periods of time at room temperature (not specifically blood) (Kilpatrick, 2002)	Optimal concentration between 95-100% (this study; Wong et al., 2012) Shaking tubes right after collection improves DNA yield (this study) NGS and telomere length measurement are likely to be negatively affected (this study) Remove all ethanol before extraction (this study) Ethanol-tissue (not specifically blood) ratio at least 3:1 (Wong et al., 2012).
Lysis buffer e.g. Longmire's, Queen's buffer	Relatively inexpensive, easy to handle and transport (this study)	Evidence of DNA degradation over long periods of time at room temperature (this study; Kilpatrick, 2002)	Avoid refrigeration (this study), despite recommendations for storage at +4°C or -20°C (Longmire et al., 1997; Seutin et al., 1991) NGS and telomere length measurement are likely to be negatively affected (this study) Lysis buffer is sensitive to changes in storage temperature (this study) Control amount of blood going into each tube of lysis buffer and collect duplicate tubes (this study). Blood to buffer ratio of 1:10 for Longmire's and Queen's buffer (Longmire et al., 1997; Seutin et al., 1991).
Freezing	-20°C, -80°C or liquid nitrogen provide minimal DNA degradation over long periods of time (Kim et al., 2011)	Difficult access to freezers, dry ice, or liquid nitrogen in remote field locations; difficult shipping of frozen samples; high costs and power consumption; high space requirements and chance of power loss and freeze-thaw cycles (this study)	Works well with all kinds of assays (this study) Setting ULT freezers at -70°C is energy saving compared to -80°C (https://www.freezerchallenge.org/resources.html).
Filter paper	Easy to handle and transport and minimal space requirements (this study)	Relatively expensive (this study) Routinely kept at room temperature, leading to DNA degradation (this study; Carpentieri et al., 2021; Hollegaard et al., 2011)	Long-term storage should be in a freezer (avoid fridge for risk of developing mildew) (this study; Carpentieri et al., 2021; Hollegaard et al., 2011) NGS and telomere length measurement are likely to be negatively affected (this study) Extract soon after collection (this study) up to 500 µL maximum total volume/card for Whatman® FTA® card technology (https://www.sigmaaldrich.com/NL/en/substance/whatmanftacardtechnology1234598765?context=product).

Overall, the responses collected by the survey (see examples in Table 6) are in agreement with the information currently available in the literature. However, respondents reported that "blood in lysis buffer annoyingly clogs up, whether storing in fridge or freezer" and "freezing often creates a gel-like consistency that proteases cannot penetrate" (Table 6). By contrast, previous literature recommends storing lysis buffer at +4°C or -20°C (Longmire et al., 1997; Seutin et al., 1991). These recommendations are targeted to facilitate avoiding problems with sample storage while setting up or introducing changes in biological sample libraries. This is of crucial importance, because, as shown by the personal experience of the surveyed researchers, sample storage issues were related to problems with obtaining adequate DNA quality and sufficient DNA quantity for the intended molecular analyses. Overall, 31% (61/199; Figure 4A) of the collected responses reported problems with storage of blood samples and/or DNA extracts, which, for the majority, led to a reduction in sample size of the project. Responses indicated that the blood storage issues and resulting DNA quality/quantity problems were not related to: (i) season of blood sampling, (ii) access to equipment in the field, (iii) means of sample transportation, (iv) storage time before DNA extraction and (v) DNA extraction methods. There was also no indication of storage method or temperature to affect DNA quality and/or quantity, despite previous studies reporting the opposite, similarly to first-hand accounts of respondents (Table 6). For instance, storing blood samples on FTA® cards at room temperature was reported to affect RTL measurements compared to frozen blood or DNA (Reichert et al., 2017); storing blood on Guthrie cards at +4°C affected DNA concentration relative to samples stored at -20°C (Hollegaard et al., 2011) and storing blood in ethanol was related to changes in stable isotope signatures (Bugoni et al., 2008). According to our survey, the effectiveness of a preservation method is not the only factor that should be considered when choosing how to collect and store biological material. Our respondents' experiences show that planned or possible future analyses, storage time, logistics in the field, storage space and sample storage costs all contribute to influencing the choice of sample storage conditions. Respondents of the survey also reported that in most cases it is necessary to find cost-efficient solutions, often compromising between convenience in the field, storage space in the laboratory and costs for expensive preservation methods or for the maintenance of fridges and freezers. Consequently, logistical or funding reasons might force researchers to adopt more convenient methods that still provide reasonable sample quality for the specific goal which they originally planned. The choices of storage methods are also often based on historical practices, therefore long-established methods might often be unsuitable for specific needs, especially for targets (e.g., telomeres) whose importance has emerged more recently. Accordingly, researchers planning new research goals that involve archived samples should make sure that the current storage method and temperature are suitable for the specific target assay, also considering new goals that may emerge later. For instance, according to respondents, the quality of DNA extracted from blood samples stored on filter paper were suitable for microsatellites but not for whole genome sequencing or RAD sequencing, which require higher quality DNA. For the latter analyses, blood samples should be stored in lysis buffer or ethanol, or even better, frozen or stored in RNA*later*. Moreover, while storing samples in lysis buffer is more affordable and logistically easier than freezing samples, based on the experience of respondents, it might compromise results of telomere length assays. Lysis buffer samples are also quite sensitive to the quantity of blood used; typically, 20 µl of blood in 1 mL of buffer is a good target, and if more blood is available, placing it in duplicate tubes is preferable. Some of the compromises regarding blood storage may be less harmful if DNA is extracted as soon as possible, as some respondents reported DNA quality declining with time for some storage methods (lysis buffer or filter paper).

Other promising techniques

Blood sampling has been the preferred source of DNA from wild birds because of the presence of nucleated erythrocytes, which provide high yields of good quality DNA suitable for a wide array of molecular applications (McDonald and Griffith, 2011). Recent evidence suggests that a growing number of less invasive techniques might yield good enough DNA to address a wide variety of questions in the fields of ecology and evolution (Beja-Pereira et al., 2009). We did not survey respondents about these techniques, but a brief review of the literature is instructive as there are pros and cons to these alternatives. For instance, feathers are an alternative source of DNA (Harvey et al., 2006; Vilstrup et al., 2018). However, the presence of potential PCR inhibitors such as the proteins melanin and keratin (McDonald and Griffith, 2011) result in a lower quality of genetic data compared to blood (Harvey et al., 2006; Maurer et al., 2010; Sacchi et al., 2004). Also, considerable variation occurs in DNA quantity and quality between plucked and shed feathers (Yannic et al., 2011) and feather sampling is not possible for very young nestlings with no feathers (Seki, 2003; Wellbrock et al., 2012). Another alternative to blood sampling is buccal swabs, which are proving to be a reliable source of DNA (Brubaker et al., 2011; Bush et al., 2005; Handel et al., 2006; Wellbrock et al., 2012). Buccal swabs provide several advantages over blood sampling: they are less invasive, they require minimal training of personnel and the chance of injuring birds is very low (Vilstrup et al., 2018). Also, handling time for buccal swabs is shorter and minimizing disturbance from the experimenter is important, for instance when studying breeding success, behaviour and survival and when birds might be more susceptible from stress, such as during cold weather or molt (Handel et al., 2006). Furthermore, studies of paternity and sex ratios might benefit from using buccal swabs because of the ease of sampling very young nestlings thanks to their gaping behaviour (Handel et al., 2006). Nevertheless, not all assays or study questions allow switching from drawing blood (for example to assess immune components, hormones in blood or molecular markers related to this tissue type having a particular cellular turnover rate, e.g. for telomere length measurements). Future investigations will determine whether buccal sampling is able to yield suitable amount and quality of genomic DNA for high-throughput technologies or the latest molecular technological advancements and result in comparable quality of genetic data as blood sampling.

Conclusions

There is a clear consensus on the need of preserving the biological material that has been collected from wild animals as successfully as possible. The experiences of respondents show that choosing storage method and temperature has the potential to generate considerable variation in DNA quality and/or quantity, with possible non-trivial consequences for research outcomes. We found no perfect method, and the collective wisdom of the avian researchers' community indicates that multiple factors must be considered when choosing storage conditions. Depending on research aims, an optimal preservation method should be able to guarantee adequate quality and enough DNA required by the planned assay, but also be flexible enough to offer suitable material for future possible technological developments, as in the case of telomere length measurements. We recommend that researchers setting up or planning to introduce changes in long-term biological archives carefully take into consideration the effectiveness of currently available preservation methods, together with funding opportunities and logistic limitations.

References

Beja-Pereira, A., Oliveira, R., Alves, P. C., Schwartz, M. K., & Luikart, G. (2009). Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources*, *9*(5), 1279–1301. https://doi.org/10.1111/j.1755-0998.2009.02699.x

Bengston, S. E., Dahan, R. A., Donaldson, Z., Phelps, S. M., van Oers, K., Sih, A., & Bell, A. M. (2018). Genomic tools for behavioural ecologists to understand repeatable individual differences in behaviour. *Nature Ecology & Evolution*, *2*(6), 944–955. https://doi.org/10.1038/s41559-017-0411-4

Black IV, W. C., Baer, C. F., Antolin, M. F., & DuTeau, N. M. (2001). Population genomics: Genome-wide sampling of insect populations. *Annual Review of Entomology*, *46*(1), 441–469. https://doi.org/10.1146/annurev.ento.46.1.441

Bouwhuis, S. (2018). On the ecological insights provided by a long-term study on an even longer-lived bird. *Journal of Animal Ecology*, *87*(4), 891–892. https://doi.org/10.1111/1365-2656.12836

Brubaker, J. L., Karouna-Renier, N. K., Chen, Y., Jenko, K., Sprague, D. T., & Henry, P. F. P. (2011). A noninvasive, direct real-time PCR method for sex determination in multiple avian species. *Molecular Ecology Resources*, *11*(2), 415–417. https://doi.org/10.1111/j.1755-0998.2010.02951.x

Bugoni, L., McGill, R. A. R., & Furness, R. W. (2008). Effects of preservation methods on stable isotope signatures in bird tissues. *Rapid Communications in Mass Spectrometry*, *22*(16), 2457–2462. https://doi.org/10.1002/rcm.3633

Bush, K. L., Vinsky, M. D., Aldridge, C. L., & Paszkowski, C. A. (2005). A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of Greater Sage-Grouse (*Centrocercus uropihasianus*). *Conservation Genetics*, *6*(5), 867–870. https://doi.org/10.1007/s10592-005-9040-6

Carpentieri, D., Colvard, A., Petersen, J., Marsh, W., David-Dirgo, V., Huentelman, M., Pirrotte, P., & Sivakumaran, T. A. (2021). Mind the quality gap when banking on dry blood spots. *Biopreservation and Biobanking*, *19*(2), 136–142. https://doi.org/10.1089/bio.2020.0131

Clutton-Brock, T., & Sheldon, B. C. (2010). Individuals and populations: The role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution*, *25*(10), 562–573. https://doi.org/10.1016/j.tree.2010.08.002

Conrad, K. F., Robertson, R. J., & Boag, P. T. (2000). Difficulties storing and preserving Tyrant Flycatcher blood samples used for genetic analyses. *The Condor*, *102*(1), 191–193. https://doi.org/10.1093/condor/102.1.191

Derrick, B., & White, P. (2017). Comparing two samples from an individual Likert question. *International Journal of Mathematics and Statistics*, *18*, 1–13.

Deyoung, R. W., & Honeycutt, R. L. (2005). The molecular toolbox: Genetic techniques in wildlife ecology and management. *Journal of Wildlife Management*, *69*(4), 1362–1384. https://doi.org/10.2193/0022-541X(2005)69[1362:TMTGTI]2.0.CO;2

Freed, L. A., & Cann, R. L. (2006). DNA quality and accuracy of avian malaria PCR diagnostics: A review. *The Condor*, *108*(2), 459–473. https://doi.org/10.1650/0010-5422(2006)108[459:DQAAOA]2.0.CO;2

Freezer challenge. https://www.freezerchallenge.org/resources.html (accessed 18.11.2021)

Handel, C. M., Pajot, L. M., Talbot, S. L., & Sage, G. K. (2006). Use of buccal swabs for sampling DNA from nestling and adult birds. *Wildlife Society Bulletin*, *34*(4), 1094–1100. https://doi.org/10.2193/0091-7648(2006)34[1094:UOBSFS]2.0.CO;2

Harvey, M. G., Bonter, D. N., Stenzler, L. M., & Lovette, I. J. (2006). A comparison of plucked feathers versus blood samples as DNA sources for molecular sexing. *Journal of Field Ornithology*, *77*(2), 136–140. https://doi.org/10.1111/j.1557-9263.2006.00033.x

Hollegaard, M. V., Grove, J., Grauholm, J., Kreiner-Møller, E., Bønnelykke, K., Nørgaard, M., Benfield, T. L., Nørgaard-Pedersen, B., Mortensen, P. B., Mors, O., Sørensen, H. T., Harboe, Z. B., Børglum, A. D., Demontis, D., Ørntoft, T. F., Bisgaard, H., & Hougaard, D. M. (2011). Robustness of genome-wide scanning using archived dried blood spot samples as a DNA source. *BMC Genetics*, *12*, 58. https://doi.org/10.1186/1471-2156-12-58

Illumina Sequencing Sample Requirements. https://rtsf.natsci.msu.edu/genomics/sample-requirements/illumina-sequencing-sample-requirements (accessed 18.11.2021)

Jackson, J. A., Laikre, L., Baker, C. S., Kendall, K. C., & The Genetic Monitoring Working Group. (2012). Guidelines for collecting and maintaining archives for genetic monitoring. *Conservation Genetics Resources*, *4*(2), 527–536. https://doi.org/10.1007/s12686-011-9545-x

Jensen, H., Szulkin, M., & Slate, J. (2014). *Molecular quantitative genetics. In: „Wild Quantitative Genetics" pp. 209-227 (Charmantier, Garant & Kruuk eds.), Oxford University Press.*

Kilpatrick, C. W. (2002). Noncryogenic preservation of mammalian tissues for DNA extraction: An assessment of storage methods. *Biochemical Genetics*, 10. https://doi.org/10.1023/A:1014541222816

Kim, Y.-T., Choi, E.-H., Son, B.-K., Seo, E.-H., Lee, E.-K., Ryu, J.-K., Ha, G.-W., Kim, J.-S., Kwon, M.-R., Nam, J.-H., Kim, Y.-J., & Lee, K.-R. (2011). Effects of storage buffer and temperature on the integrity of human DNA. *Korean Journal of Clinical Laboratory Science*, *44*, 24–30.

Longmire, J., Maltbie, M., & Baker, R. J. (1997). *Use of "Lysis Buffer" in DNA isolation and its implication for museum collections*. *163*. https://doi.org/10.5962/bhl.title.143318

Madisen, L., Hoar, D. I., Holroyd, C. D., Crisp, M., Hodes, M. E., & Reynolds, J. F. (1987). The effects of storage of blood and isolated DNA on the integrity of DNA. *American Journal of Medical Genetics*, *27*(2), 379–390. https://doi.org/10.1002/ajmg.1320270216

Manel, S., Berthier, P., & Luikart, G. (2002). Detecting wildlife poaching: Identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology*, *16*(3), 650–659. https://doi.org/10.1046/j.1523-1739.2002.00576.x

Maurer, G., Beck, N., & Double, M. C. (2010). A 'feather-trap' for collecting DNA samples from birds. *Molecular Ecology Resources*, *10*(1), 129–134. https://doi.org/10.1111/j.1755-0998.2009.02711.x

McDonald, P. G., & Griffith, S. C. (2011). To pluck or not to pluck: The hidden ethical and scientific costs of relying on feathers as a primary source of DNA. *Journal of Avian Biology*, *42*(3), 197–203. https://doi.org/10.1111/j.1600-048X.2011.05365.x

Mei, J. V., Li, L., Rasmussen, S. A., Collier, S., Frias, J. L., Honein, M. A., Shaw, G. M., Lorey, F., Meyer, R., Chaing, S., Canfield, M. A., Jones, J., & Hannon, W. H. (2011). Effect of specimen storage conditions on newborn dried blood spots used to assess *Toxoplasma gondii* immunoglobulin M (IgM). *Clinica Chimica Acta*, *412*(5–6), 455–459. https://doi.org/10.1016/j.cca.2010.11.028

Michaud, C. L., & Foran, D. R. (2011). Simplified field preservation of tissues for subsequent DNA analyses. *Journal of Forensic Sciences*, *56*(4), 846–852. https://doi.org/10.1111/j.1556-4029.2011.01771.x

Mills, J. A., Teplitsky, C., Arroyo, B., Charmantier, A., Becker, Peter. H., Birkhead, T. R., Bize, P., Blumstein, D. T., Bonenfant, C., Boutin, S., Bushuev, A., Cam, E., Cockburn, A., Côté, S. D., Coulson, J. C., Daunt, F., Dingemanse, N. J., Doligez, B., Drummond, H., … Zedrosser, A. (2015). Archiving primary data: Solutions for long-term studies. *Trends in Ecology & Evolution*, *30*(10), 581–589. https://doi.org/10.1016/j.tree.2015.07.006

Morin, P. A., Martien, K. K., Archer, F. I., Cipriano, F., Steel, D., Jackson, J., & Taylor, B. L. (2010). Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. *Journal of Heredity*, *101*(1), 1–10. https://doi.org/10.1093/jhered/esp107

Prendini, L., Hanner, R., & DeSalle, R. (2002). Obtaining, storing and archiving specimens and tissue samples for use in molecular studies. In R. DeSalle, G. Giribet, & W. Wheeler (Eds.), *Techniques in Molecular Systematics and Evolution* (pp. 176–248). Birkhäuser. https://doi.org/10.1007/978-3-0348-8125-8_11

Primmer, C. R. (2009). From conservation genetics to conservation genomics. *Annals of the New York Academy of Sciences*, *1162*(1), 357–368. https://doi.org/10.1111/j.1749-6632.2009.04444.x

R Core Team. (2021). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reichert, S., Froy, H., Boner, W., Burg, T. M., Daunt, F., Gillespie, R., Griffiths, K., Lewis, S., Phillips, R. A., Nussey, D. H., & Monaghan, P. (2017). Telomere length measurement by qPCR in birds is affected by storage method of blood samples. *Oecologia*, *184*(2), 341–350. https://doi.org/10.1007/s00442-017-3887-3

Richardson, A. J., Narendran, N., Guymer, R. H., Vu, H., & Baird, P. N. (2006). Blood storage at 4°C—factors involved in DNA yield and quality. *Journal of Laboratory and Clinical Medicine*, *147*(6), 290–294. https://doi.org/10.1016/j.lab.2006.01.005

Riddle, B. R., Dawson, M. N., Hadly, E. A., Hafner, D. J., Hickerson, M. J., Mantooth, S. J., & Yoder, A. D. (2008). The role of molecular genetics in sculpting the future of integrative biogeography. *Progress in Physical Geography: Earth and Environment*, *32*(2), 173–202. https://doi.org/10.1177/0309133308093822

Sacchi, P., Soglia, D., Maione, S., Meneguz, G., Campora, M., & Rasero, R. (2004). A non-invasive test for sex identification in Short-toed Eagle (*Circaetus gallicus*). *Molecular and Cellular Probes*, *18*(3), 193–196. https://doi.org/10.1016/j.mcp.2004.01.002

Seki, S.-I. (2003). Molecular sexing of individual Ryukyu Robins *Erithacus komadori* using buccal cells as a non-invasive source of DNA. *Ornithological Science*, *2*(2), 135–137. https://doi.org/10.2326/osj.2.135

Seutin, G., White, B. N., & Boag, P. T. (1991). Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, *69*(1), 82–90. https://doi.org/10.1139/z91-013

Smith, L. M., & Burgoyne, L. A. (2004). Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. *BMC Ecology*, *4*, 4. https://doi.org/10.1186/1472-6785-4-4

Stier, A., Bize, P., Schull, Q., Zoll, J., Singh, F., Geny, B., Gros, F., Royer, C., Massemin, S., & Criscuolo, F. (2013). Avian erythrocytes have functional mitochondria, opening novel perspectives for birds as animal models in the study of ageing. *Frontiers in Zoology*, *10*(1), 33. https://doi.org/10.1186/1742-9994-10-33

Vilstrup, J. T., Mullins, T. D., Miller, M. P., McDearman, W., Walters, J. R., & Haig, S. M. (2018). A simplified field protocol for genetic sampling of birds using buccal swabs. *The Wilson Journal of Ornithology*, *130*(1), 326–334. https://doi.org/10.1676/16-105.1

Visvikis, S., Schlenck, A., & Maurice, M. (1998). DNA extraction and stability for epidemiological studies. *Clinical Chemistry and Laboratory Medicine*, *36*(8). https://doi.org/10.1515/CCLM.1998.094

Wellbrock, A. H. J., Bauch, C., Rozman, J., & Witte, K. (2012). Buccal swabs as a reliable source of DNA for sexing young and adult Common Swifts (*Apus apus*). *Journal of Ornithology*, *153*(3), 991–994. https://doi.org/10.1007/s10336-012-0843-1

Whatman® FTA® card technology. https://www.sigmaaldrich.com/NL/en/substance/whatmanftacardtechnology1234598765?context=product (accessed 18.11.2021)

Wong, P. B., Wiley, E. O., Johnson, W. E., Ryder, O. A., O'Brien, S. J., Haussler, D., Koepfli, K.-P., Houck, M. L., Perelman, P., Mastromonaco, G., Bentley, A. C., Venkatesh, B., Zhang, Y., Murphy, R. W., & G10KCOS. (2012). Tissue sampling methods and standards for vertebrate genomics. *GigaScience*, *1*(1). https://doi.org/10.1186/2047-217X-1-8

Yannic, G., Sermier, R., Aebischer, A., Gavrilo, M. V., Gilg, O., Miljeteig, C., Sabard, B., Strøm, H., Pouivé, E., & Broquet, T. (2011). Description of microsatellite markers and genotyping performances using feathers and buccal swabs for the Ivory gull (*Pagophila eburnea*). *Molecular Ecology Resources*, *11*(5), 877–889. https://doi.org/10.1111/j.1755-0998.2011.03015.x

Zimmermann, J., Hajibabaei, M., Blackburn, D. C., Hanken, J., Cantin, E., Posfai, J., & Evans, T. C. (2008). DNA damage in preserved specimens and tissue samples: A molecular assessment. *Frontiers in Zoology*, *5*(1), 18. https://doi.org/10.1186/1742-9994-5-18

Data Accessibility

Supporting Information and data are available via Figshare at 10.6084/m9.figshare.17281793.

Competing interests

The authors declare no conflict of interests.

Author contributions

Irene Di Lecce: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Methodology (equal); Writing-original draft (lead); Writing-review & editing (equal). Joanna Sudyka: Conceptualization (equal); Methodology (equal); Writing-review & editing (equal). David F. Westneat: Conceptualization (equal); Methodology (equal); Writing-review & editing (supporting). Marta Szulkin: Conceptualization (equal); Methodology (equal); Writing-review & editing (equal).

Acknowledgements

Funding: Polish National Science Centre grant no. 2016/21/B/NZ8/03082 awarded to Marta Szulkin.

Preserving avian DNA from the wild: Your experience of blood sampling, DNA extraction and storage

Consent

You are invited to participate in a research study entitledPreserving avian DNA from the wild: Your experience of blood sampling, DNA extraction and storage. This study is carried out by Irene Di Lecce, Joanna Sudyka and Marta Szulkin from the University of Warsaw, Poland, and by David F. Westneat from the University of Kentucky, USA.

The purpose of this survey is to assess common practices used in field collection and storage of avian blood samples and DNA extracts.

This online survey is split into 3 sections, and will take you approximately 15 minutes to complete. Your participation in this study is entirely voluntary and you can withdraw at any time by emailing Irene at *irene.dilecce@cent.uw.edu.pl*. You do not have to answer any questions you do not want to.

If you would like to take the survey multiple times to share your experience from multiple projects, please re-take the survey - such an option will be possible as soon as you finish any round of replies.

We believe there are no known risks associated with this research study; however, as with any online related activity the risk of a breach is always possible. Your responses will be kept strictly confidential, and digital data will be stored in secure computer files. Any report of this research that is made available to the public during seminars, conferences or in publications will not include your name or any other individual information by which you could be identified. In other words, compiled results will be entirely anonymous. If you have questions, fell free to contact Irene at the email address provided above.

Completing the questionnaire below indicates that you are 18 years of age or older, and indicates your consent to participate in this survey.

1. Blood sample collection

Please answer questions <u>specifically in the context of one research project you choose to assess in this survey</u>. If you would like to assess multiple research projects you were involved in during your career, you are very welcome to do so: simply re-take the survey - such an option will be possible as soon as you finish any round of replies.

1. What species of bird is the focus of the project you will be reporting on here?

2. Where is the fieldwork on this project carried out? Please indicate location and country.

3. When are blood samples on this project most often collected? Select only one.

○ Wet season
○ Dry season
○ Spring
○ Summer
○ Autumn
○ Winter

4. How are blood samples on this project collected? Select all that apply.

☐ Capillary
☐ Syringe
☐ Microvette
☐ Other (please specify)

5. Which laboratory equipment do you use daily during fieldwork for handling blood samples on this project? Select all that apply.

☐ Fridge

☐ Centrifuge

☐ Freezer -20°C

☐ Freezer -80°C

☐ None

☐ Other (please specify)

6. How do you move blood samples from the fieldsite to the permanent laboratory on this project? Select all that apply.

☐ Car

☐ Train

☐ Airplane

☐ Courier

☐ Other (please specify)

7. Does your shipping strategy (Q6) influence your method of blood sample collection?

○ Yes

○ No

8. How large is your sample database on this project?

○ <100 blood samples

○ 101-1,000

○ 1,001-10,000

○ > 10,000

9. Which genetic analyses are performed on the collected samples? Select all that apply.

☐ Microsatellite assay

☐ SNP chip

☐ RAD sequencing

☐ Gene expression

☐ Methylation assay

☐ Parasite DNA analysis

☐ mtDNA analysis

☐ Other or more than one (please specify)

10. Have you ever experienced problems in generating data during genetic analyses (Q9) for this project because of storage issues?

○ Yes, because of blood storage issues

○ Yes, because of DNA storage issues

○ Yes, because of both blood and DNA storage issues

○ Yes, but not sure what was the issue

○ No. You can proceed to Q17

11. If you had problems, what was the issue with a particular method of blood or DNA storage?

12. Did these problems ever reduce the quality of your biological conclusions for the project?

○ Yes

○ Maybe

○ No. You can proceed to Q14

13. How did these problems reduce your biological conclusions?

14. How much did these problems reduce your sample size for analysis? Select one response.

○ 0%

○ 1-25%

○ 25-50%

○ 50-75%

○ 75-100%

15. Did these problems ever lower the quality of the target journal to which you submitted, or plan to submit, your work on this project?

○ Yes

○ Maybe

○ No

16. Did these problems ever prevent you, or might prevent you, from publishing an aspect of this project?

○ Yes

○ Maybe

○ No

17. Thank you for completing section 1 of this survey! Do you have any further comments?

2. Blood storage

Please answer questions <u>specifically in the context of one research project you choose to assess in this survey</u>. If you would like to assess multiple research projects you were involved in during your career, you are very welcome to do so: simply re-take the survey - such an option will be possible as soon as you finish any round of replies.

18. Which of the following do you use for genetic analyses (Q9) on this project? Select one.

○ Whole blood
○ Red blood cells

19. In what medium do you preserve blood samples for this project? Select all that apply.

☐ Ethanol
☐ Queen's lysis buffer
☐ FTA cards or other filter paper
☐ TE buffer
☐ RNAlater
☐ Direct freezing with no buffer (e.g. in freezer, liquid nitrogen, or dry ice)
☐ Other (please specify)

20. Do you use different preservation methods (Q19) for different types of genetic analysis (Q9)?

○ Yes
○ No. You can proceed to Q22

21. Please explain the different preservation methods.

22. At what temperature do you store blood samples? Select one response.

○ Room temperature

○ + 4° C

○ - 20° C

○ - 80° C

○ Other (please specify)

☐

23. Overall, are you satisfied with your current method of preserving blood samples for this project?

○ Very satisfied

○ Satisfied

○ Neither satisfied nor dissatisfied

○ Dissatisfied

○ Very dissatisfied

24. Have you ever changed storage method of blood samples during this project?

○ Yes

○ No. You can proceed to Q26

25. For what reason?

☐

26. Are you currently thinking about changing your storage method for this project?

○ Yes

○ Maybe

○ No. You can proceed to Q28

27. Which storage method are you considering?

☐

28. Would you do something different regarding sample collection or storage method if you had to start your research project all over again?

○ Yes

○ Maybe

○ No. You can proceed to Q30

29. Please explain.

30. Thank you for completing section 2 of this survey! Do you have any further comments?

3. DNA extraction and storage (final section!)

Please answer questions <u>specifically in the context of the research project you choose to assess in this survey</u>. If you would like to assess multiple research projects you were involved in during your career, you are very welcome to do so: simply re-take the survey - such an option will be possible as soon as you finish any round of replies.

31. In which medium do you store extracted DNA for this project?

○ Tris

○ TE

○ Water

○ Other (please specify)

32. At what temperature do you store extracted DNA? Select one response.

○ + 4° C

○ - 20° C

○ - 80° C

○ Other (please specify)

33. Overall, are you satisfied with your current method of preserving extracted DNA for this project?

○ Very satisfied

○ Satisfied

○ Neither satisfied nor dissatisfied

○ Dissatisfied

○ Very dissatisfied

34. Have you ever changed storage method of extracted DNA during this project?

○ Yes

○ No. You can proceed to Q36

35. For what reason?

36. Are you currently thinking about changing storage method of extracted DNA for this project?

○ Yes

○ Maybe

○ No. You can proceed to Q38

37. Which storage method are you considering?

38. Have you ever encountered difficulties in obtaining adequate DNA extracts in terms of **quality** because of storage issues?

○ Yes

○ No. You can proceed to Q40

39. What kind of difficulties? Select all that apply.

☐ Degraded DNA

☐ Impure DNA (poor 260/280 Nanodrop ratio)

☐ Impure DNA (poor 230/260 Nanodrop ratio)

☐ Impure DNA (poor 260/280 and 230/260 Nanodrop ratios)

☐ Other (please specify)

40. Have you ever encountered difficulties in obtaining adequate DNA extracts in terms of **quantity** because of storage issues on this project?

○ Yes

○ No

○ DNA quantity was not measured

41. What kind of DNA extraction protocol do you use for this project?

☐ Commercial kit

☐ Commercial kit with modified protocol

☐ In-house protocol

☐ Other (please specify)

42. What kind of DNA extraction method do you use?

☐ Chelex

☐ Phenol-chloroform

☐ Ammonium acetate

☐ Other (please specify)

43. Overall, are you satisfied with your current extraction method for this project?

○ Very satisfied

○ Satisfied

○ Neither satisfied nor dissatisfied

○ Dissatisfied

○ Very dissatisfied

44. How long after collecting blood samples do you usually extract DNA?

☐ Up to 6 months

☐ Up to 1 year

☐ More than 1 year

☐ I don't have a standard time frame

45. How long after DNA extraction do you usually perfom analyses?

☐ Up to 6 months

☐ Up to 1 year

☐ More than 1 year

☐ I don't have a standard time frame

46. Would you do something different regarding the extraction method if you had to start your research project all over again?

○ Yes

○ Maybe

○ No. You can proceed to Q48

47. Please explain.

48. Would you do something different regarding the storage method of extracted DNA if you had to start your research project all over again?

○ Yes

○ Maybe

○ No. You can proceed to Q50

49. Please explain.

50. Based on your experience, did different methods of blood sample storage influence DNA quality or quantity during extraction?

○ Yes, DNA quality

○ Yes, DNA quantity

○ Don't know. You can proceed to Q52

○ No. You can proceed to Q52

51. Can you say how?

52. Thank you for completing section 3 of this survey! Do you have any further comments?

THANK YOU for participating!

53. Basic information about you

Name & Surname

Research Institution

City/Town

Country

Work Email Address

You are done! Thank you for participating - we believe that a global assessment of how avian researchers store their blood samples and DNA extracts, and an account of the adversities they have faced when extracting DNA for genetic analyses is greatly needed. We hope that quantitative and qualitative data derived from this survey can allow us to identify whether any procedural or methodological gaps exist - an information that is often anecdotal when the experiences of only a few researchers from molecular ecology labs are compared.

We hope that the results of this survey and the experiences shared by its participants will help improving existing guidelines relevant for the long-term storage of biological data - we will aim to share our conclusions from this survey as soon as possible.

Sincerely,
Irene Di Lecce, Joanna Sudyka, David F. Westneat and Marta Szulkin

Supporting Information 3 for "Preserving avian blood and DNA sampled in the wild: a survey of personal experiences"

Table S1 – List of species sampled by respondents

	Common name	Latin/Scientific name	Number of responses
1	Blue tit	*Cyanistes caeruleus*	17
2	Great tit	*Parus major*	15
3	Rock dove	*Columba livia*	6
4	Black-legged kittiwake	*Rissa tridactyla*	4
5	Collared flycatcher	*Ficedula albicollis*	4
6	Common starling	*Sturnus vulgaris*	4
7	House sparrow	*Passer domesticus*	4
8	Mallard	*Anas platyrhynchos*	4
9	Pied flycatcher	*Ficedula hypoleuca*	4
10	Tree swallow	*Tachycineta bicolor*	4
11	Adélie penguin	*Pygoscelis adeliae*	3
12	Black-capped chickadee	*Poecile atricapillus*	3
13	Eurasian blackcap	*Sylvia atricapilla*	3
14	European blackbird	*Turdus merula*	3
15	Red-winged blackbird	*Agelaius phoeniceus*	3
16	Thick-billed murre	*Uria lomvia*	3
17	Alpine swift	*Tachymarptis melba*	2
18	Barn swallow	*Hirundo rustica*	2
19	Berthelot's pipit	*Anthus berthelotii*	2
20	Blue-black grassquit	*Volatinia jacarina*	2
21	Blue-footed booby	*Sula nebouxii*	2
22	Carolina chickadee	*Poecile carolinensis*	2
23	Dark-eyed junco	*Junco hyemalis*	2
24	Eastern bluebird	*Sialia sialis*	2
25	Little auk	*Alle alle*	2
26	Mountain chickadee	*Poecile gambeli*	2
27	Peregrine falcon	*Falco peregrinus*	2
28	Red kite	*Milvus milvus*	2
29	Red-backed fairywren	*Malurus melanocephalus*	2
30	Rufous-collared sparrow	*Zonotrichia capensis*	2
31	Savannah sparrow	*Passerculus sandwichensis*	2
32	Seychelles warbler	*Acrocephalus sechellensis*	2
33	Spotless starling	*Sturnus unicolor*	2
34	White-throated sparrow	*Zonotrichia albicollis*	2
35	Zebra finch	*Taeniopygia guttata*	2
36	African penguin	*Spheniscus demersus*	1
37	American kestrel	*Falco sparverius*	1
38	Andean condor	*Vultur gryphus*	1
39	Asian Houbara bustard	*Chlamydotis macqueenii*	1
40	Bachman's sparrow	*Peucaea aestivalis*	1
41	Bahama oriole	*Icterus northropi*	1
42	Bar-tailed godwit	*Limosa lapponica baueri*	1
43	Barred owl	*Strix varia*	1
44	Bearded reedling	*Panurus biarmicus*	1
45	Black grouse	*Lyrurus tetrix*	1
46	Black guillemot	*Cepphus grylle*	1
47	Black sparrowhawk	*Accipiter melanoleucus*	1
48	Blue crane	*Grus paradisea*	1
49	Brown booby	*Sula leucogaster*	1

50	Bulwer's petrel	*Bulweria bulwerii*	1
51	Cape vulture	*Gyps coprotheres*	1
52	Chinstrap penguin	*Pygoscelis antarcticus*	1
53	Cliff swallow	*Petrochelidon pyrrhonota*	1
54	Common linnet	*Linaria cannabina*	1
55	Common murre	*Uria aalge*	1
56	Common redstart	*Phoenicurus phoenicurus*	1
57	Common whitethroat	*Sylvia communis*	1
58	Corncrake	*Crex crex*	1
59	Cory's shearwater	*Calonectris borealis*	1
60	Dunnock	*Prunella modularis*	1
61	Eurasian reed warbler	*Acrocephalus scirpaceus*	1
62	Eurasian stone-curlew	*Burhinus oedicnemus*	1
63	European turtle dove	*Streptopelia turtur*	1
64	European woodcock	*Scolopax rusticola*	1
65	Field sparrow	*Spizella pusilla*	1
66	Florida scrub jay	*Aphelocoma coerulescens*	1
67	Galapagos mockingbird	*Mimus parvulus*	1
68	Gentoo penguin	*Pygoscelis papua*	1
69	Great dusky swift	*Cypseloides senex*	1
70	Great-tailed grackle	*Quiscalus mexicanus*	1
71	Greater ani	*Crotophaga major*	1
72	Greater flamingo	*Phoenicopterus roseus*	1
73	Grey crowned crane	*Balearica regulorum*	1
74	Gyrfalcon	*Falco rusticolus*	1
75	Hawaiian goose	*Branta sandvicensis*	1
76	Herring gull	*Larus argentatus*	1
77	Hihi	*Notiomystis cincta*	1
78	House finch	*Haemorhous mexicanus*	1
79	Ivory gull	*Pagophila eburnea*	1
80	King penguin	*Aptenodytes patagonicus*	1
81	Lappet-faced vulture	*Torgos tracheliotos*	1
82	Leach's storm petrel	*Oceanodroma leuchoroa*	1
83	Magpie	*Pica pica*	1
84	Marsh tit	*Poecile palustris*	1
85	Mauritius parakeet	*Psittacula eques*	1
86	Monteiro's storm petrel	*Oceanodroma monteiroi*	1
87	North African Houbara bustard	*Chlamydotis undulata undulata*	1
88	Northern cardinal	*Cardinalis cardinalis*	1
89	Northern flicker	*Colaptes auratus*	1
90	Ocellated turkey	*Meleagris ocellata*	1
91	Red-eyed vireo	*Vireo olivaceus*	1
92	Red-throated caracara	*Ibycter americanus*	1
93	Saltmarsh sparrow	*Ammospiza caudacuta*	1
94	Sand martin	*Riparia riparia*	1
95	Seaside sparrow	*Ammospiza maritima*	1
96	Sedge warbler	*Acrocephalus schoenobaenus*	1
97	Silvereye	*Zosterops lateralis*	1
98	Song sparrow	*Melospiza melodia*	1
99	Sooty swift	*Cypseloides fumigatus*	1
100	Southern crested caracara	*Caracara plancus*	1
101	Southern dunlin	*Calidris alpina schinzii*	1
102	Southern pied babbler	*Turdoides bicolor*	1
103	Spotted owl	*Strix occidentalis*	1
104	Sun parakeet	*Aratinga solstitialis*	1
105	Superb fairywren	*Malurus cyaneus*	1

106	Swainson's hawk	*Buteo swainsoni*	1
107	Tawny owl	*Strix aluco*	1
108	Wattled crane	*Grus carunculata*	1
109	Western bluebird	*Sialia mexicana*	1
110	Western capercaillie	*Tetrao urogallus*	1
111	Western sandpiper	*Calidris mauri*	1
112	Whimbrel	*Numenius phaeopus*	1
113	Whinchat	*Saxicola rubetra*	1
114	Whiskered tern	*Chlidonias hybrida*	1
115	White stork	*Ciconia ciconia*	1
116	White-backed vulture	*Gyps africanus*	1
117	White-browed sparrow-weaver	*Plocepasser mahali*	1
118	White-collared swift	*Streptoprocne zonaris*	1
119	White-winged snowfinch	*Montifringilla nivalis*	1
120	Wire-tailed manakin	*Pipra filicauda*	1
121	Wood duck	*Aix sponsa*	1
122	Wood warbler	*Phylloscopus sibilatrix*	1
123	Yellow-legged gull	*Larus michahellis*	1

Table S2 – List of families sampled by respondents

	Family name	Number of responses
1	Paridae	41
2	Passerellidae	13
3	Muscicapidae	10
4	Accipitridae	9
5	Hirundinidae	9
6	Laridae	9
7	Alcidae	7
8	Columbidae	7
9	Falconidae	7
10	Spheniscidae	7
11	Anatidae	6
12	Passeridae	6
13	Scolopacidae	6
14	Sturnidae	6
15	Turdidae	6
16	Acrocephalidae	5
17	Apodidae	5
18	Icteridae	5
19	Maluridae	4
20	Sylviidae	4
21	Gruidae	3
22	Phasianidae	3
23	Strigidae	3
24	Sulidae	3
25	Thraupidae	3
26	Cathartidae	2
27	Corvidae	2
28	Estrildidae	2
29	Fringillidae	2
30	Hydrobatidae	2
31	Motacillidae	2
32	Otididae	2
33	Procellaridae	2
34	Zosteropidae	2
35	Burhinidae	1
36	Cardinalidae	1
37	Charadriidae	1
38	Ciconiidae	1
39	Cuculidae	1
40	Leiothrichidae	1
41	Mimidae	1
42	Notiomystidae	1
43	Panuridae	1
44	Phoenicopteridae	1
45	Phylloscopidae	1
46	Picidae	1

47	Pipridae	1
48	Ploceidae	1
49	Prunellidae	1
50	Psittacidae	1
51	Psittaculidae	1
52	Rallidae	1
53	Vireonidae	1

Table S3 – List of orders sampled by respondents

	Order name	Number of responses
1	Passeriformes	144
2	Charadriiformes	26
3	Accipitriformes	10
4	Columbiformes	8
5	Falconiformes	8
6	Sphenisciformes	7
7	Anseriformes	6
8	Galliformes	5
9	Apodiformes	4
10	Procellariformes	4
11	Gruiformes	3
12	Strigiformes	3
13	Suliformes	3
14	Otidiformes	2
15	Psittaciformes	2
16	Cathartiformes	1
17	Ciconiiformes	1
18	Cuculiformes	1
19	Phoenicopteriformes	1
20	Piciformes	1