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Eggs is eggs: A case study in destructive sampling and analysis of museum natural history specimens

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Abstract
Where destructive sampling of museum natural history collections is proposed, the needs of current research must be balanced against preservation for future use of a finite resource. This paper presents a case study of an interaction between researchers at the University of York and curators at York Museums Trust (YMT) regarding a request by the former for destructive sampling from YMT’s historic bird egg collection. We draw attention to reasons for success and share an approach to managing a destructive sampling request in a regional museum useful to both researchers (in preparing requests) and curators (in assessing and acting upon these).

Keywords: Birds; Museum egg collections; Biomolecular archaeology; Destructive sampling

Introduction
This case study represents an interaction between researchers at the University of York and curators at York Museums Trust (YMT) surrounding a request for destructive sampling from YMT’s historic bird egg collection. The paper summarises key questions raised and shares an approach to managing destructive sampling from the perspective of both curator and researcher.

Collecting the eggs of wild birds was a popular pastime during late Victorian and Edwardian eras, when many collections numbered in the thousands (Manson-Bahr, 1959; Lightman, 2000). However, with introduction of the Protection for Birds Act 1954 this practice became illegal. Museums now represent the best accessible source of material for researchers wishing to study the eggs of non-domestic species (Russell, et al., 2010).

The last few decades have seen significant development in scientific research techniques, and the rate of progress in molecular technology has been particularly advanced in recent years. These new developments have opened up exciting new scientific possibilities, leading to a concomitant increase in requests for destructive analysis of museum specimens. These possibilities enhance still further the existing long-term value of natural history collections. However, factors driving research and requirements to destroy a specimen for analysis, in whole or part, are often not easily compatible with rigorous curatorial care of collections and their preservation for the future.

In particular, a desire for high-profile publication in the competitive environment of professional science can lead to a bias in requests for destructive analysis of the most high-impact and irreplaceable specimens, including of extinct or endangered species. Whilst large samples may be required from specimens before analytical techniques are refined, advances in technology over relatively short timescales can facilitate analysis of much smaller samples. For example, it is now possible to recognise morphologically indeterminate bones and other materials using protein sequencing and selecting these for DNA analyses (Buckley, et al., 2009; Richter, et al., 2011; van Doom, et al., 2011).

Background to the case study

The aim of the researchers’ project was to produce a proteomics-based system for species identification of archaeological eggshell fragments, based on highly sensitive mass spectrometry and peptide mass fingerprinting. Eggshell is common on many archaeological sites but the large volume of material often found, combined with an inability to rapidly identify it, has previously precluded its systematic archaeological interpretation (Stewart, et al., 2013). The interaction between the research project and YMT arose due to the need for a reference database representing the eggs of as many species as possible.

The first step in the process was addressing a number of key questions prior to submitting a sampling request, as follows:

What does the YMT egg collection contain – can it support the proposed research?

As for many museums, incomplete documentation of this historic collection presented an immediate barrier to exploring this question in detail. The nucleus of YMT’s egg collection, a taxonomically arranged collection of specimens made by the Yorkshire Philosophical Society, plus a large historical collection made by collector William Cooper, had previously been accessioned and catalogued to specimen or clutch level. However a series of collections in small cabinets or boxes, made by individual collectors or containing specimens of mixed provenance, remained largely undocumented. To overcome this, the researchers were supported in creating an ‘Excel’ catalogue of the undocumented collection (approximately 4,000 individual egg specimens) for curatorial review and import to the Museum’s computerised object management system (Adlib). Unaccessioned specimens were not accessioned at this stage, so that curators could consider whether some specimens should be separated into a new destructive sampling collection or be put forward for disposal, following Russell, et al., (2010).

What is meant by ‘destructive’ analysis in this context? Nature, size and extent of sampling

The research team invested time testing and refining analytical techniques using commercially available eggs to establish the smallest possible amount of material required for robust results (Stewart, et al., 2013). The protein content of eggshell is high, and sufficient concentrations could be recovered from very small (<1mg) pieces of shell. In addition to the size of individual sample per specimen, the number of samples needed per specimen and number of specimens needed per species were considered. Based on analyses of domestic species, it was found that proteomic content is remarkably consistent both between and within the eggshell of any given species. This minimises the number of specimens required; for specimens not common in the collection, and for which only very limited sampling is possible, a single sample from a single specimen will suffice. However, sampling from two or three specimens taken by different collectors was preferred as a cross-check on taxonomic identification, which relied on the specimen’s label.

What type and resolution of data are required for the research?

For this research only taxonomic identification was required, to the level of species.

Is specimen condition important?

The physical condition of the specimens was not important for this research. This included eggs affected by ‘Byne’s disease’ - a chemical reaction which degrades the eggshell, caused by an acidic environment plus high relative humidity and characterised by a crystalline surface efflorescence (Carter, 2000). Whilst damaging to some research and display functions, the researchers ascertained that this degradation did not affect the preservation and recovery of the intra-crystalline proteins (preserved within calcium carbonate biominerals of the eggshell) required for this project.

Formalising and processing the request

Request form, policy and procedure

To capture and assess the request, a policy document and a destructive and invasive sampling request form were developed for internal museum use, based on advice from colleagues in other museums and guidelines in Carter & Walker (1999). These are shared in Appendix 1 and 2.

The request form is divided into a number of parts:

Part 1 Details of people and places/institutions involved.

Part 2 Project details:

- a project outline helps summarise and advocate the project internally/externally and assess the strength and significance of the research question.
- asking justification for sampling helps question why collections, and your collections in particular, are required (non-destructive alternatives may be possible).
- detail of sampling methodology and analysis, including proven success of the technique and the researcher’s experience, enable ex-
tent of destruction and likelihood of success to be assessed (new techniques may be approved depending on strength of research); the curator should feel able to ask for evidence in the form of papers, descriptions or photographs if unfamiliar with techniques.

- maximising sample use for future research where this is not destroyed.

Part 3 Specimen details – plus a framework of issues to consider per specimen

Part 4 Terms and conditions: it is important to clarify these in writing to ensure expectations are addressed at the start of a request and that maximum benefit is obtained from the research and for the collection.

The completed request form was initially assessed by the curator with final approval from the Collections Management team.

Assessing which specimens are suitable for destructive analysis

Published precedence exists for a method of physically pre-filtering egg collections to aid requests for destructive sampling at the Natural History Museum, London (Russell, et al., 2010), who ranked specimens according to their associated data:

Class I: taxonomic identification plus field collection data, notably date and location - accessioned into the main research collection.
Class II: taxonomic identification only - not accessioned but retained as a destructive research resource.
Class III: neither of the above - disposal.

Within the above system Class I eggs are potentially available for sampling using minimally destructive techniques, for example where the case for research is very strong and no other material is available. However, these techniques would be developed on Class II material, which is also used for any research involving more than minimal damage.

Physically sorting YMT’s undocumented collection using this approach was outside the scope and resource of this project. Instead, the decision-tree that Russell, et al. (2010) used to assign specimens into different classes was used as a framework for interrogating the collection catalogue to help identify specimens of requested species for destructive sampling. This framework was extended for the regional museum setting, where Class II or III specimens may be considered of value due to display or learning potential or local links. Class II specimens with additional evidence of provenance, such as those within an original collector’s cabinet, were considered of greater potential cultural or educational value than those without. These were ranked against each other, taking into account aesthetic value, condition and completeness. Effectively this meant that a series of original collector’s cabinets or single specimens with handwritten labels but only Class II data was highlighted as of value, for example in revealing the story of egg collecting as a popular historic pastime. Class II specimens at the other end of this spectrum were prioritised for sampling for this research project, and might in future be amalgamated to a taxonomically-arranged unaccessioned destructive sampling collection. Class III specimens were not considered in depth as they were not useful to this research project.

In practice, answers to initial key questions greatly facilitated this ‘bottom-up’ approach to identifying specimens suitable for destructive analysis using the collection catalogue. The curator could identify the least data-rich specimens in worst condition (cracked, broken or affected by Byne’s disease) for each species when considering which to sample from, and subsequently consider factors including rarity or cultural value. Above all, the small sample size required for this project opened up a larger portion of the collection for research.

Due to small sample size required, and curatorial recognition that being attached to this research increased the value of individual specimens, which also offered the potential for re-testing results, all specimens sampled were accessioned into the main collection for their scientific value, despite some being poor in data or condition.

Sampling

Sampling technique was refined using non-collection eggshell before taking samples from specimens in the collection, which because of their age could be delicate and brittle. Very fine scissors or dental tools were used to remove small amounts of shell from around the original collector’s hole or from broken edges of damaged specimens. Care was taken to check that no collector data or marks which might be present near a blow-hole were compromised, and if in doubt sampling was not undertaken. In some specimens the blow-hole was covered over, which prevented sampling. All specimens sampled were photographed before and after.

Research Results

The researchers obtained material for 56 species of bird in 13 orders from YMT’s historic collections, aiding development of a new analytical technique for rapid taxonomic identification of eggshell in the archaeological record by peptide mass fingerprinting (Stewart, et al., 2013).

Development of this tool will facilitate new insights into patterns of use of the eggs of non-domestic bird species by people in the past. Bird eggs have been a significant resource for people through time. They are highly nutritious as foodstuffs, have symbolic value in many cultures (for example of fertility or rebirth), and their various components have been used to make, for example, containers, jewellery or paint (Stewart, et al., 2014). However there have been large gaps in knowledge about use of
wild bird eggs, a potentially important interaction between many cultures and the ecosystems in which they lived, in part due to lack of a taxonomic identification tool (Stewart, et al., 2014).

Initial research by the team involved with this project, focusing on two sites in Anglo-Scandinavian York, has revealed both an apparent lack of exploitation of the eggs of wild birds, even though these were presumably readily available, and that relative prevalence of goose eggshell may become a useful indicator of status (Stewart, et al., 2014).

Whilst a recent study has urged caution in using museum eggshell for proteomics research because some proteins present in modern eggshell were not recovered from museum specimens (Portugal, et al., 2010), the success of our analysis, for which the full suite of eggshell proteins was not required, highlights that museum collections are an invaluable resource to this field.

Conclusions
Museums receive ever increasing numbers of requests for destructive analysis of specimens, often using novel technologies that possess limited track records. At the same time cuts in resourcing for natural sciences collections mean that museum staff have limited resources to process and interrogate these. Where technology is novel, there is also a risk that the outcomes may be limited.

This project highlights the value of early and open dialogue between curatorial staff and research scientists, which enables hard questions to be asked on the science: how many, how much, what will we learn? It also highlights the willingness of researchers to directly contribute to the cataloguing of collections if the curator is able to support this. Close working helped promote a shared understanding and led to a streamlined request that was for the most part approved.

The researchers’ drive to invest time early on to refine analytical technique and establish the smallest amount of material required for robust results reduced the ‘destructive’ nature of the request and opened up more of the collection for testing, and this research to reduce required sample size is still on-going. One outcome of this research is a web-based software tool www.thermal-age.eu which attempts to estimate the level of molecular destruction in bone samples based upon the thermal history of the sample.

The value of retaining data-poor egg collections and / or specimens in poor condition is also highlighted (cf Russell et al., 2010). Ultimately this research will continue to generate stories of past human interactions with our natural world that would not be possible without museum collections. This is of value both in helping to engage audiences and in advocating the scientific value of natural sciences collections even where data or condition are poor. By working together we can help push the boundaries of scientific research on museum natural history collections whilst ensuring this precious resource is preserved for the future.

Acknowledgements
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References
APPENDIX 1.

YORK MUSEUMS TRUST
DESTRUCTIVE AND INVASIVE SAMPLING POLICY – NATURAL SCIENCES

1. YMT actively encourages non-destructive research on its natural science collections.

2. YMT will consider all requests for destructive and invasive research including all forms of sampling on a case-by-case basis and reserves the right to refuse permission to any request.

3. For all forms of research a request form including details of research justification, a researcher profile, a methodology statement and statement of dissemination will be required in advance prior to YMT’s consideration.

4. YMT will only grant permission for destructive research once it has been convinced that the scientific justifications for the removal of samples from specimens are robust and worthwhile and the research question(s) cannot be addressed using non-destructive techniques.

5. Particular justification will be required for sampling from type or figured specimens; CITES specimens; extinct, endangered or historic specimens. Specimen uniqueness; preservation state (poorly preserved specimens will be targeted first); strength and feasibility of research; evidence of sufficient lab facilities, experience and proven track record of analytical technique will all contribute to YMT’s decision on whether to approve a sampling request.

6. YMT will ensure that all activity conforms to legal and ethical constraints and to professional codes of practice, e.g. CITES legislation.

7. All sampling should be fully documented by the Curator so future researchers will know what has been taken. Documentation should link to any publication produced as a result of specimen sampling.

8. The specimen sampled should be fully recorded and measured by the Curator prior to sampling. Under some circumstances (for example if the specimen is intended for museum display or further metric work might be compromised) consideration should be given to producing a cast of parts that will be damaged or destroyed.

9. The Curator will advise researchers on how to house, label and document any residual samples if these are removed by the Researcher.

10. YMT will place all research on record in a publicly accessible research register. This will include project name, research objectives, date of research, outputs – publications and data holdings, research involving sampling, sample location and size, the sampling process and eventually the full records of the results of analysis.

11. YMT will retain any material removed but not destroyed during analysis in its collection.

12. YMT will devise a research framework in conjunction with recognised experts for its natural science collections.

All requests for access to research the natural science collections should be made to the Curator of Natural Science, York Museums Trust, Yorkshire Museum, Museum Gardens, YORK, Y01 7FR.
APPENDIX 2.

Destructive and invasive sampling request form developed for the Natural Sciences collections at York Museums Trust (YMT)

Based on templates shared by the Natural History Museum, London; York Bones Forum; Bristol Museum & Art Gallery and guidelines in Carter & Walker (1999).

YORK MUSEUMS TRUST
DESTRUCTIVE AND INVASIVE SAMPLING REQUEST FORM – NATURAL SCIENCES

YMT actively encourages research on its natural science collections. We also have a duty to care for our collections and preserve them for future generations.

Destructive or invasive research will be considered on a case by case basis according to YMT’s sampling policy.

To enable us to process your request, please complete this form and return to:

Curator of Natural Science
Email: Post: Yorkshire Museum, Museum Gardens, York, YO1 7FR

Processing of requests by the Curator and Collections Management team will take approximately 6 – 8 weeks. (This can change at the discretion of YMT.)

Informal initial enquiries to determine which specimens are available in the collections should be directed to the Curator of Natural Science.

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| 1.2 DETAILS OF SUPERVISOR OR HOST        |
| Please note – requests from non-permanent staff (e.g. students or visiting researchers) must be accompanied by a letter of support from your supervisor or host, who accepts full responsibility to comply with the terms of agreement. |
| Name                                     |
| Position                                 |
| Institutional address                    |
| Email                                    |
| Telephone number                         |
### 1.3 DETAILS OF ANALYTICAL LAB TO BE USED

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### PART 2: PROJECT DETAILS

**Project title**

**Project outline:** Please include aims, significance, outcomes and plans for dissemination.

**Sampling justification:** Why is material from YMT’s collections important to this research?

**Sampling methodology:** How will the sample be taken; size of sample; location of sample on specimen; is the least destructive method possible being used? *Include photographs of sampling equipment & illustration of proposed sampling site.*

**Analysis:** Brief outline of methodology; examples with references of previous studies evidencing competence of investigator and success of the particular technique used.

**Maximising sample use:** Is the method of analysis destructive or non-destructive of the sample taken? If non-destructive, please indicate potential to share or re-use samples with future researchers.

**Duration of project (months):**

**Date sample(s) required by:**

### PART 3: SPECIMENS TO BE USED

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**Additional specifications (if appropriate):** geographical region / country; field collection date(s); sex; storage time; preparation or preservation conditions.

**Accession numbers (if known):**
1. Destructive and invasive sampling requests are approved on a case by case basis at the discretion of the Curator and Collections Management Team.

2. YMT reserves the right to refuse permission for any destructive and invasive sampling request.

3. Particular justification is required for sampling from type or figured specimens; CITES specimens; extinct, endangered or historic specimens.

4. Applicants must provide any additional information requested by YMT in relation to legislation, e.g. CITES, before an application is approved.

5. Applications from non-permanent staff (e.g. students or visiting researchers) must be supported in writing by a supervisor, host or head of laboratory, who also takes full responsibility for adhering to terms and conditions.

6. Any costs associated with sampling will be borne by the applicant or host institution. Sampling and analysis are solely for the non-commercial academic research purposes outlined in this form.

7. Access to specimens will only be allowed under supervision of appropriate Museum staff.

8. If handling of material by the Researcher is approved by the Curator this must be undertaken in an appropriate manner. The Researcher will be required to wear relevant personal protective equipment to ensure good standards of care for themselves and the collection.
9. Specimen accession numbers must be detailed in any publications and attached to any data stored in the public domain.

10. YMT retains all rights to all material sampled from its specimens.

11. Residual samples must be returned to YMT within a maximum period of one year (longer periods must be agreed in advance).

12. Applicants must follow guidance from YMT staff for the housing, numbering and labelling of specimens once sampled.

13. If the whole specimen is destroyed during the process then the Researcher must inform YMT.

14. Two copies of all publications resulting from research and copies of any images taken by the Researcher must be provided to YMT.

15. Researchers must provide feedback to YMT if analysis is not successful, detailing reasons why.

16. YMT will place a record of all research in a publicly accessible research register.

17. Use of specimen images must be approved by YMT’s image use request procedure. Enquiries should be directed to the Curator of Natural Science.

18. Loan of material for sampling must be approved by YMT’s loan request procedure. Enquiries should be directed to the Curator of Natural Science. Researchers must not remove any material from the collections without express permission.

SIGNATURES
I have read, understood and agree to abide by the statements above:

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