“SNARGE” DNA SAMPLING OPTIMISATION

Duncan Macdougall , *Rolls-Royce PLC, Derby, United Kingdom.*

Andrea Waeschenbach , *Natural History Museum, London, United Kingdom.*

Claire Griffin , *Natural History Museum, London, United Kingdom.*

Clare Pool , *Rolls-Royce PLC, Derby, United Kingdom.*

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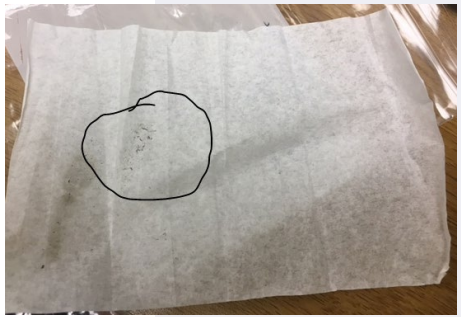
Background

Bird remains collection at RR

- We record around 300 events per year, and try to gather remains from as many of these as possible.
- Any remains that we can import (assuming no restrictions owing to bird flu) are processed at the Natural History Museum, in London.
- The quality of the remains varies:



8108

8548

8701

8965

- Depending on the sample, we decide whether to identify the species using either feather or DNA analysis.
- Sometimes, remains can be quite old prior to being sampled. To maximise the chance of success, are we following the best practice for DNA analysis?

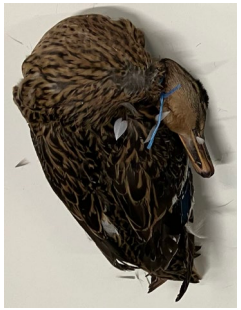
Motivation

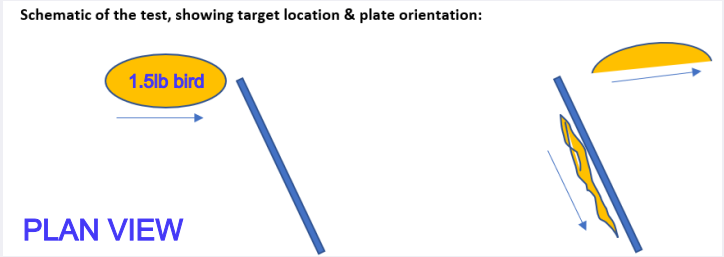
Motivation for this research

- RR advice is to use wet wipes, but not specifically alcohol / water based.
- FAA (AC 150/5200-32B): advises the use of alcohol-based wipes, and not to use water-based wipes.
- Smithsonian Bird guidance: Advises alcohol -based wipes, or 70% alcohol spray + paper towel.
- Australian ATSB report (2006) # exposed bird material to different environments:
  - Most damaging to DNA samples was either:
    - high temperature (120 °C) + pressure or
    - just leaving samples at room temperature for >7 days.
  - Drying or heating (to 90 °C) did not appear to significantly damage DNA.
- Questions:
  - Does DNA degrade over time? Do certain techniques become less effective for old remains?
  - Which solvent is best? Alcohol, Quaternary Ammonium Detergent, or Water.
  - Should the swab be allowed to dry before being bagged?
- Decided to explore these questions experimentally…

"Forensic Identification of Aviation Bird Strikes in Australia", Christidis *et al*, Australian Transport Safety Bureau, Research and Analysis Report B2005/0117,

Introduction





- Two bird impact tests carried out at Event Horizon (Somerset, U.K.) in November 2024.
- Birds were “sliced” by the plates, mimicking ingestion into a fan. The normal component of the velocity is similar to that experienced in an engine at high power during take -off.
- 1.5lb ducks (Mallard) launched at plates inclined at 60 degrees, ~150m/s, birds targeted 10mm back from plate leading edge.
- Both tests used fresh birds, which had been refrigerated but not frozen.

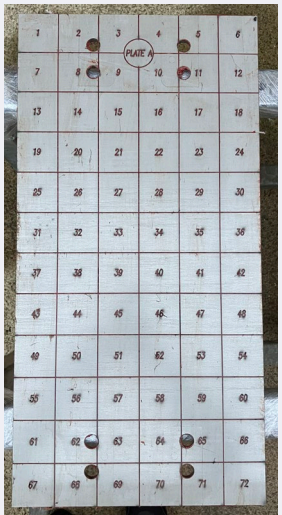
Results – plate A

Results

1. Postimpact

2. Smearing process

3. Smeared plate



4. Heating for 20 minutes

Plate storage & Schedule

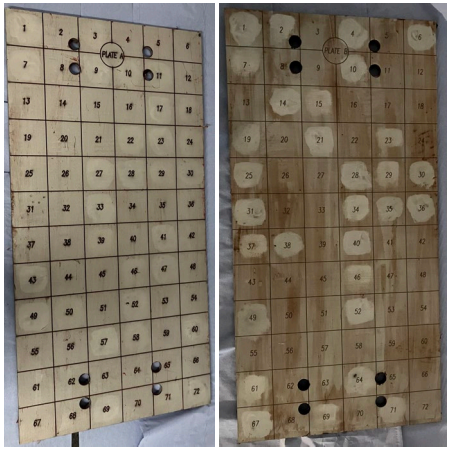


Plate A Plate B

Smearing & swabbing aren't an exact science!

Schedule of taking samples

- Nov 2024, on Day 1 (about an hour after the test)
- July 2025, after 8 Months
- Final set of samples planned for August 2026, after 22 Months

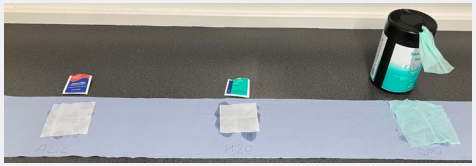
Storage of plates

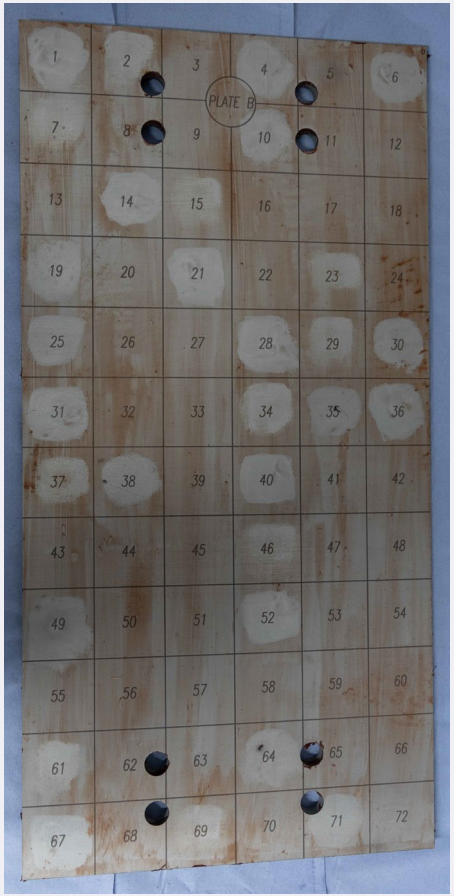
- After being allowed to dry for a day, the plate was wrapped in plastic (but not air-tight), with baffles to prevent the plastic from touching the impacted side of the plate. The intent is to prevent attracting flies, etc and prevent contamination from dust / debris.
- Stored plates in a ventilated wooden crate at outside temperature & humidity (in Somerset, so may see-5oC in winter occasionally; and 30oC in the summer occasionally)
- Not in direct sunlight

"Day 1" samples

Day 1 samples

Samples taken on Day 1 [60 samples taken]			
Swab solvent	H_2O Water (with 0.9% NaCl)	ALC 75% Iso-propyl Alcohol	QAD Quaternary Ammonium Detergent
Bagged Wet or Dry	Half of the samples air-dried prior to being bagged; half bagged wet.		
Maturation of samples	Half of the samples "matured" for 2 weeks; half for 4 weeks (each sample had half the time at RT, half in fridge)		
Plate A and B	Samples taken evenly from plates A & B		





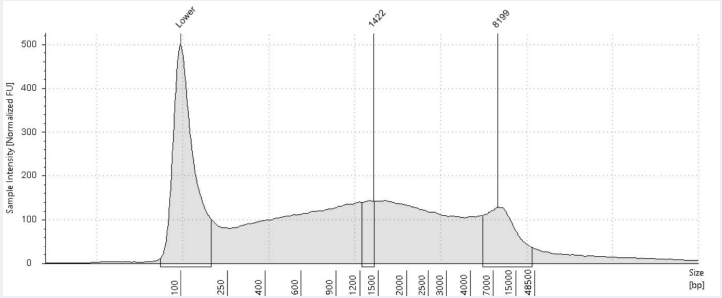
Molecular methodology

DNA quantity

- Invitrogen Qubit Fluorometer & Agilent Tapestation
- Data: DNA concentrations

DNA integrity

- Agilent Tapestation
- Data: Intensity-weighted mean/median DNA fragment sizes

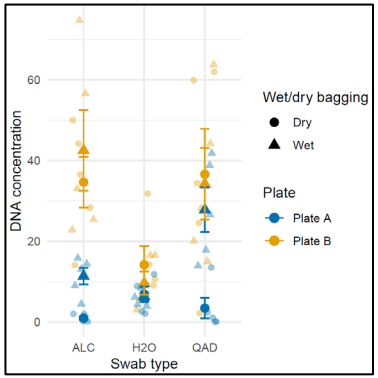


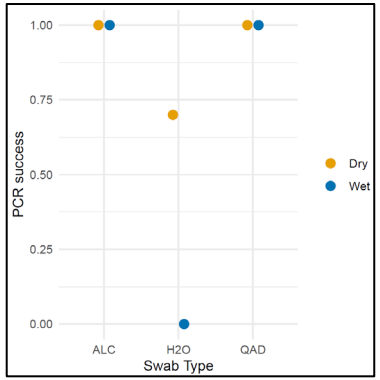
Positive identification via PCR

- Mitochondrial gene NAD2 amplifications and sequences
- Data: Binary (0/1)

"Day 1" results

Day 1 results





Qubit & Tapestation: DNA concentration

PCR success (i.e. identification of species)

	Day 1	
Minor influence	Swab solvent	H_2O poor; ALC & QAD both 100%
Higher DNA conc for Wet (for ALC & QAD)	Bagged Wet or Dry	Drying helps for H_2O
✗	Maturation of samples	✗
DNA conc for B >> A	Plate A and B	✗

✗ = no significant influence

- ALC or QAD solvents were much more successful than H_2O.
- Bagging samples wet was <u>beneficial</u> if solvent was a preservative (ALC or QAD); it was <u>detrimental</u> if H_2O was used.
- As expected, quantity of DNA on plate B >> A.

“8 Month” samples

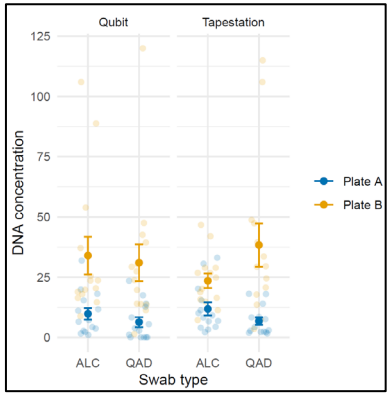
8 Month samples

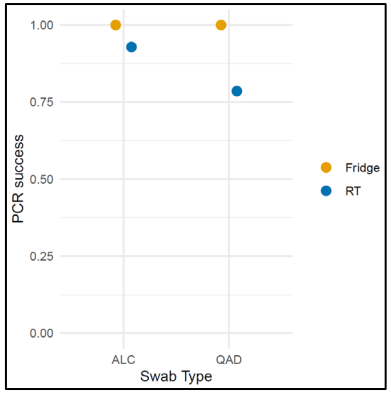
→ For second set of samples after 8 Months:
H_2O was dropped; all samples bagged wet.

Samples taken after 8 Months	[56 samples taken]		
Swab solvent		ALC 75% Iso-propyl Alcohol	QAD Quaternary Ammonium Detergent
Bagged Wet or Dry	All bagged wet.		
Maturation of samples	Half of the samples matured for 2 weeks; half for 8 weeks.		
Temperature during Maturation	Half stored at RT; half stored in fridge.		
Plate A and B	Samples taken evenly from plates A & B		

"8 Month" results

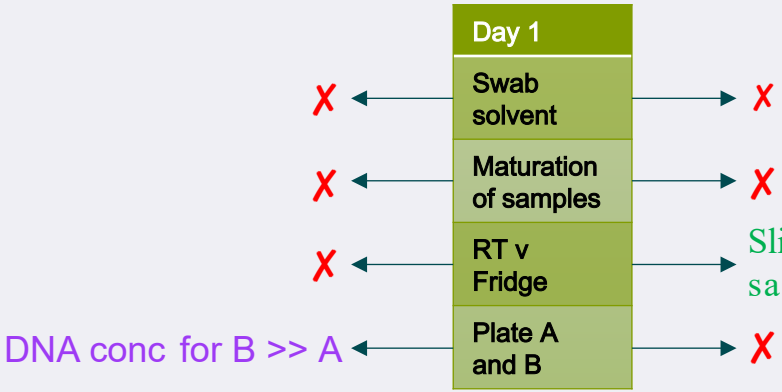
8 Month results





Qubit & Tapestation:
DNA concentration

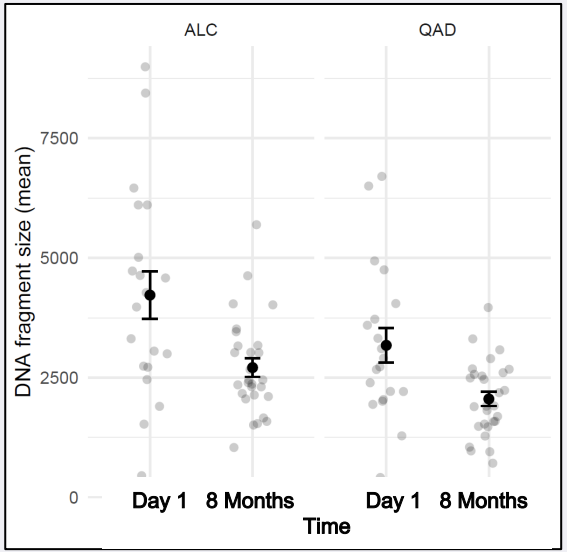
PCR success
(i.e. identification of species)

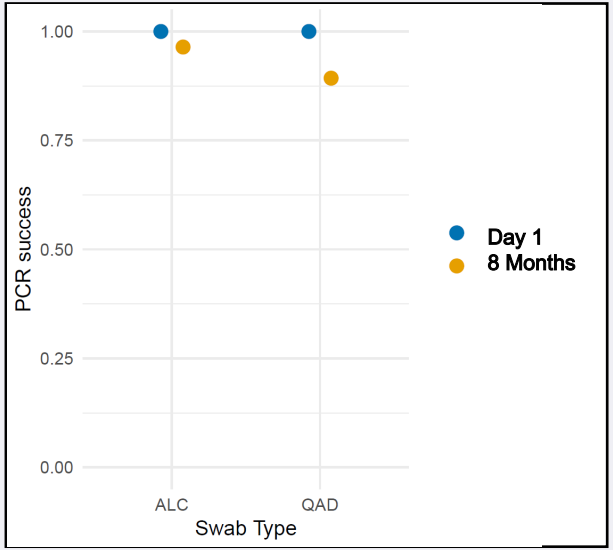


- Both ALC or QAD swab solvent performed well in DNA concentration and PCR.
- Majority of samples had a successful PCR; some failures for samples stored at RT
  -> better to store samples in fridge
- As expected, quantity of DNA on plate B >> A.

Influence of age of bird remains: Comparison of "Day 1" and "8 Month" results

Age of bird remains





- Age of bird remains did not influence the DNA concentration.
- But, greater age increases the degree of DNA fragmentation.
- ALC seems a bit better than QAD at suppressing fragmentation.
- PCR success dropped slightly with increasing age of bird remains.

→ A third (and final) set of samples planned for August/September 2026, at about 22 Months: Same as second set, to explore Age effect further.

Future work

DNA quantity

- Invitrogen Qubit Fluorometer & Agilent Tapestation
- Data: DNA concentrations

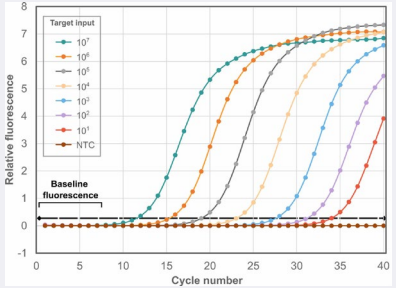
DNA integrity

- Agilent Tapestation
- Data: Intensity-weighted mean DNA fragment sizes

Measures **total DNA** (incl. fungal & bacterial DNA)

Quantitative PCR

- Measures only **target DNA** (duck)
- Data: Cycle threshold [Ct] values
- Another way to assess DNA quantity and integrity



Conclusions

- DNA of bird remains degrades over time. But even after 8 months, most samples produced a successful PCR test.
- Quaternary Ammonium Detergent tried as a solvent. Generally successful, but alcohol solvent performed slightly better (and, much easier to obtain!).

- Sample collection best-practice:
  - Alcohol-based solvent is best (avoid water as a solvent)
  - Collect remains & bag up samples “wet”
  - Store remains in fridge (if possible)

Future work

- Take third set of samples at ~22 months.
- Process all 3 sets of samples using Quantitative PCR.