How to measure eggshell thickness

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Background

Eggshell thinning is considered a good proxy indictor for DDT exposure of the sampled population; especially DDT and its degradation products have been identified as a key group of POPs responsible for the causing eggshell thinning leading to widespread reduction in breeding success and subsequent population decline in the several top predators (Hickey 1969; Newton, 1979; Cade et al., 1988). Shell thickness has recovered in parts of Europe and is "back to normal" 30 years after banning DTT usage (Wegner et al. 2005, Odsjö & Sondell 2014, Andreasen et al. 2018). But although evidence is still limited, some other, more recently introduced compounds (e.g. PBDEs, a group of flame retardants) may also potentially affect the shells (Guigueno & Fernie 2017).

Since collecting eggshell fragments from hatched eggs is an easy accessible and non-invasive sampling method, ERBF recommends they be sampled opportunistically during nest visits for ringing and other controls. Likewise, unhatched addled eggs offer samples for eggshell thickness measurements as well as contaminant analyses of the egg contents.

Sampling for measuring eggshell thickness

Fragments from eggs hatched (in particular in stick nests) can become hard to find, so sampling as early after hatching increases the chance of obtaining enough material. Since eggshell thickness varies within the egg (typically thicker at the ends) there is a risk that too few samples may bias the results, so make all effort to sample as many measurable fragments as possible – and at least 20 pieces (Odsjö and Sondell 1982). During a nest visit, carefully turn over the nest linings and any dirt/sand (on cliff ledges) and use a pair of tweezers to pick even tiny (around 2x2 mm) fragments; do not bother whether the fragments have the (inner) shell membrane attached to them. Store in a hard container to avoid further breaking; since no contaminant analyses are (normally) planned, there are no specific requirements for containers; and mark with all relevant information on species, date, location etc.

If whole addled eggs are present, they can, of course, also be included in measurements of shell thickness; for sampling procedures of whole eggs see elsewhere in the ERB Advice Hub. For measuring shells from whole eggs, ensure they are dried (at room temperature for several months, or in laboratory drying cabinet). Measurements should be taken at the equator of the egg, so easiest if the eggs are opened by cutting along the equator line.

Measuring shell thickness

There are many methods for measuring shell thickness and new approaches continue to emerge, including non-destructive measurement on whole living eggs (e.g. Khaliduzzaman et al. 2020).

The most widespread, simple and cheap method is a *Micrometer* (see picture panel), preferably a digital model and linked direct to a computer for capturing the measurement straight into a spreadsheet (one example is Mitutoyo 293-240-30 External Micrometer). Fasten (glue) a small stainless steel ball to the rotating jaw of the Micrometer in order to fit the inner curved surface of the eggshells. It is recommended to fix the Micrometer in some sort of laboratory stand so the Micrometer jaws are horizontal (rotating jaw pointing up).

Preparing samples

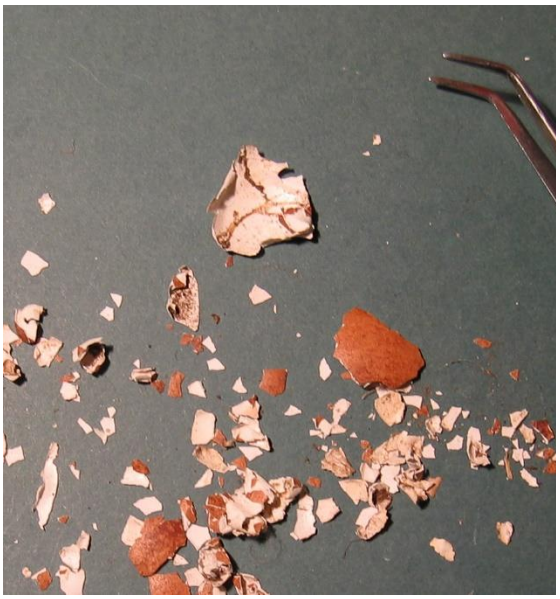
- Open the container and pour the fragments on a dish or large piece of paper; check if the fragments are dry, to the point where any eggshell membranes are 'crispy' instead of humid and soft (often smelly). Dry if needed.
- When dry, sort the sample of fragments and blow or brush clean the surfaces of each fragment.

- Check if the thick 'inner membrane' of the egg is attached – and if so, firmly attached, not loose/cracking off. Most often, smaller fragments don't have the membrane.

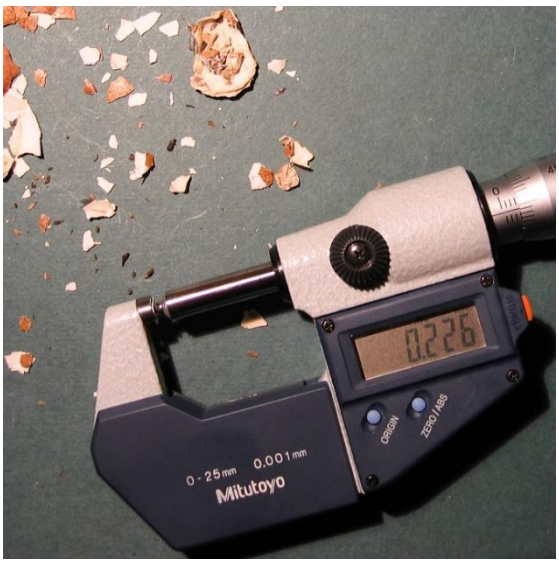
Measuring

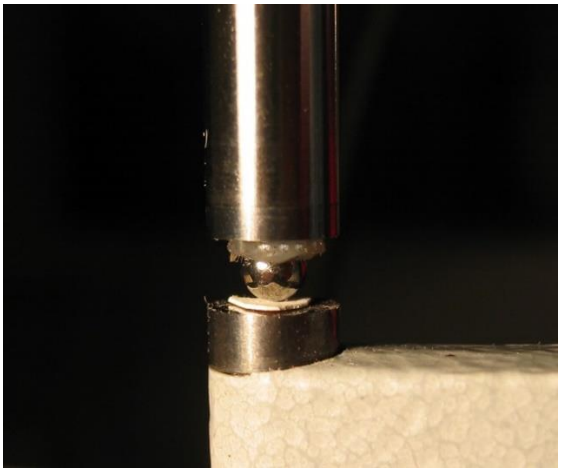
- Calibrate Micrometer to 0, and practise how to apply a "standardised" pressure, e.g. rotate the Micrometer handle slowly to contact and then continue "three clicks" on the rotating handle; also test with a piece of eggshell to see consistent measuring values (practise a number of times, and for each person to take measurements).
- Place the eggshell fragment on the fixed jaw, inner side of egg facing up towards the stainless steel ball on the rotating jaw.
- Rotate the Micromenter jaw slowly to contact with the inner fragment and then apply your standardised "three click" pressure.
- Record the value (3 decimals) and note if each measurement is *with* or *without* membrane.
- On larger pieces with some membranes attached, take measurements of adjacent shell areas with and without membranes and in your data sheet mark those 'pairwise records'. This is to gather data for establishing a 'membrane correction value' for your study species so you can use all fragments, and compare with values from whole eggs in collections that typically are measured *with* membrane. For instance, the membrane correction value for Peregrines is around 0.07 mm (Falk et al. 2006 [0.071 mm], Court et al. 1990 [0.069 mm], Nygård 1983 [0.07 mm]).
- Once the membrane correction value has been well established, it is easiest to work with the fragment without membrane since it can be difficult to ensure the membrane is firmly fixed to the shell and, hence, it is harder to trust the measurement.

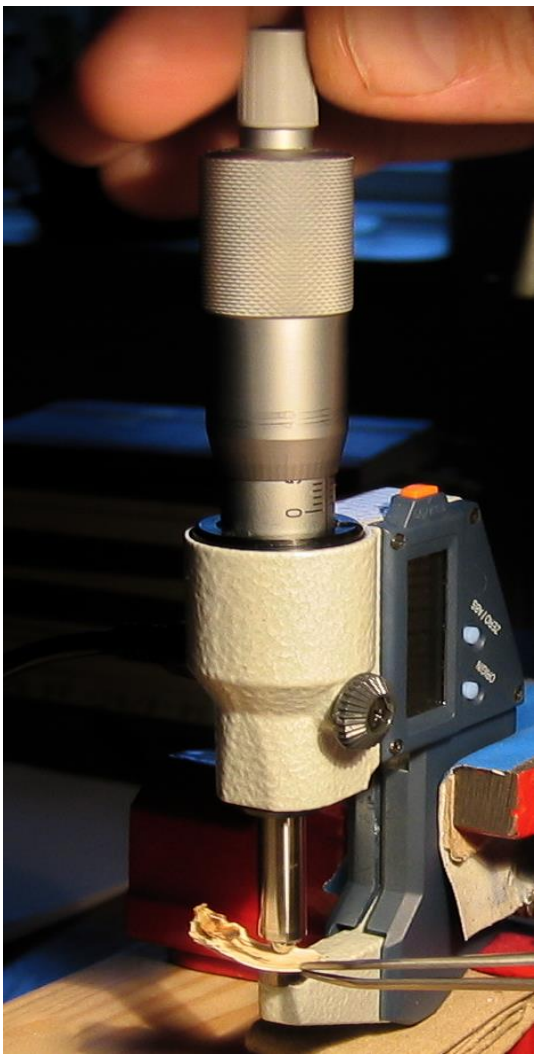
Collecting fragments in peregrine falcon nest (*all photos: www.vandrefalk.dk*)



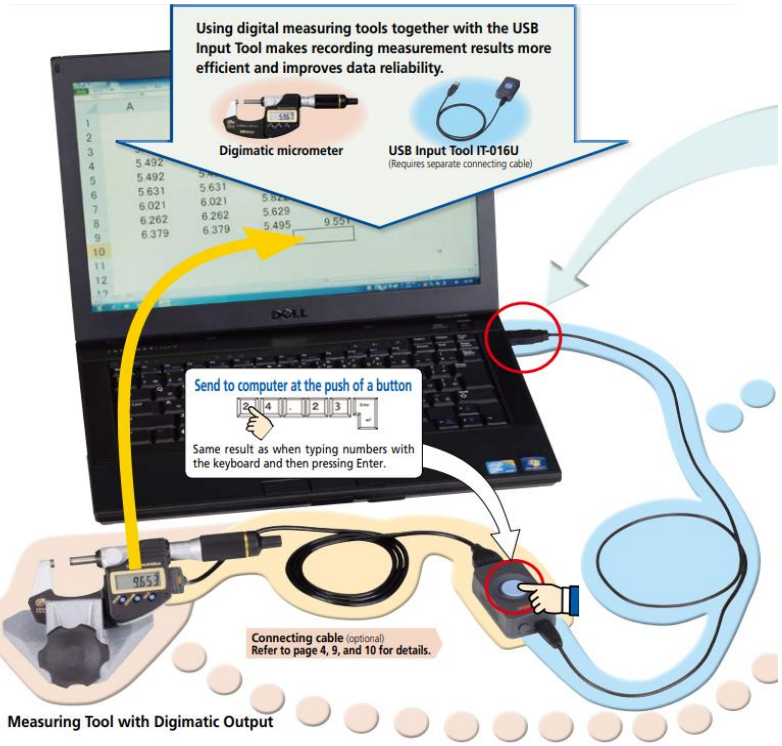
A good sample with more than 20 fragments; note the dried membrane on some fragments, in some places peeling off



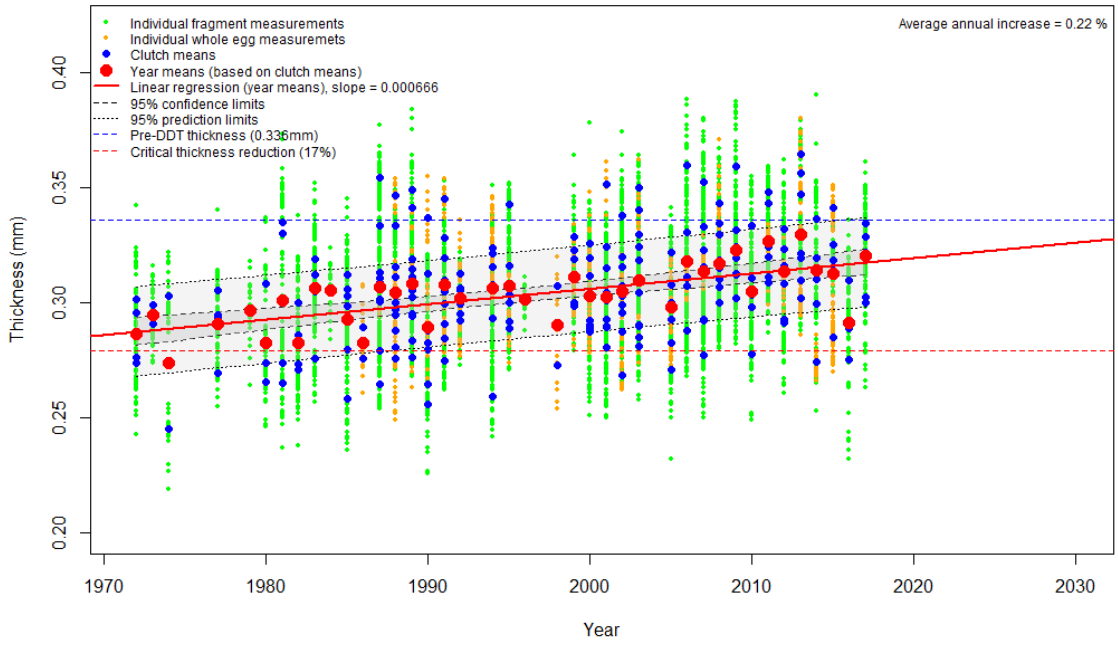




Measuring small fragments with Micrometer fixed in vertical position; note the stainless steel ball on the upper (rotating) jaw to fit the inside of a slightly curved eggshells. Right picture: measuring adjacent points *with* and *without* membrane.



An example of linking a Micrometer to a computer for direct recording of measurements (from https://www.mitutoyo.co.jp/eng/support/service/catalog/10/E12007.pdf)



An example of shell thickness trend analysis based on whole egg and shell fragment measurements (re-analysis of Falk et al. 2018)

Analysis

- Use all the pairwise measurement with and without membrane to establish the 'membrane correction value'; add the value to all measurements without membrane.
- Calculate clutch means – average thickness of all fragments from one nest (= one clutch); if eggshell fragments as well as whole eggs have been measured, calculate separate means and test if the two means differ significantly.
- Calculate the mean of the clutch means for each sampling year.

You are now in a position to compare with reference collections for your study species, or start looking for time trends in shell thickness (see figure for example).

Some studies suggest that embryo development, absorbing calcium from the egg shell, may affect shell density and/or thickness (e.g. for Peregrine, see Ratcliffe 1970, Bunck et al. 1985, Bennett 1995, Castilla et al. 2010) which may challenge comparisons between the two types of samples. For Peregrines, Falk et al. (2006, 2018) did not find any difference in shell thickness identified in fragments vs. whole eggs but the effect could differ between study species. Nevertheless, while the potential error introduced by embryo calcium absorption from the shell may influence the recorded *value* of the shell thickness, estimates of *trends* remain unaffected.

References

Andreasen NP, Falk K, Møller S (2018). The Danish Peregrine Falcon population: Reestablishment and eggshell thinning. Ornis Hungarica 26(2):159–163. DOI: 10.1515/orhu-2018-0024.

Bennett RS (1995). Relative sensitivity of several measures of eggshell quality to the stage of embryonic development. Bull. Environ. Contam. Toxicol. 54:428–431. doi:10.1007/BF00195116

Bunck, C.M.; Spann, J.W.; Pattee, O.H.; Fleming, W.J. (1985). Changes in eggshell thickness during incubation: implications for evaluating the impact of organochlorine contaminants on productivity. Bull. Environ. Contam. Toxicol. 35:173–182. doi:10.1007/BF01636496

Cade TJ, Enderson JH, White CM, Thelander CG (1988). Peregrine Falcon Populations: Their Management and Recovery. Peregrine Fund.

Castilla AM, Herrel A, Robles H, Malone J, Negro JJ (2010). The effect of developmental stage on eggshell thickness variation in endangered falcons. Zoology 113:184–188. doi:10.1016/j.zool.2009.10.002

Court G, Gates C, Boag D, MacNeil J, Bradley D, Fesser A, Patterson J, Stenhouse G, Oliphant L (1990). A toxicological assessment of Peregrine Falcons, *Falco peregrinus tundrius*, breeding in the Keewatin, District of the Northwest Territories, Canada. Can. F. Nat. 104:255–272.

Falk K, Møller S, Mattox WG (2006). A long-term increase in eggshell thickness of Greenlandic Peregrine Falcons *Falco peregrinus tundrius*. The Science of the Total Environment 355:127-134. DOI: 10.1016/j.scitotenv.2005.02.024

Guigueno, MF, Fernie, KJ (2017). Birds and flame retardants: A review of the toxic effects on birds of historical and novel flame retardants. Environmental Research 154:398-424. https://doi.org/10.1016/j.envres.2016.12.033.

Hickey JJ (1969). Peregrine Falcon Populations, their Biology and Decline. Univ. Wisconsin Press, Madison & London.

Khaliduzzaman A, Konagaya K, Suzuki T, et al. (2020). A nondestructive eggshell thickness measurement technique using terahertz waves. Sci Rep 10, 1052. https://doi.org/10.1038/s41598-020-57774-5

Newton, I (1979). Population ecology of raptors. T. & A. D. Poyser, Berkhamstead.

Nygård T (1983). Pesticide Residues and Shell Thinning in Eggs of Peregrines in Norway. Ornis Scand. 14:161–166.

Odsjö T, Sondell J (1982). Eggshell thinning and DDT, PCB and mercury in eggs of osprey (*Pandion haliaetus* (L)) in Sweden and their relations to breeding success, in: Odsjö T. (Ed.) Eggshell Thickness and Levels of DDT, PCB and Mercury in Eggs of Osprey (*Pandion Haliaetus* (L.)) and Marsh Harrier (*Circus Aeruginosus* (L)) in Relation to Their Breeding Success and Population Status in Sweden. GOTAB, Stockholm.

Odsjö T, Sondell J (2014). Eggshell thinning of osprey (*Pandion haliaetus*) breeding in Sweden and its significance for egg breakage and breeding outcome. The Science of the Total Environment 470-471: 1023–1029. doi:10.1016/j.scitotenv.2013.10.051

Ratcliffe DA (1970). Changes Attributable to Pesticides in Egg Breakage Frequency and Eggshell Thickness in Some British Birds. J. Appl. Ecol. 7, 67–115. doi:10.2307/2401613

Wegner P, Kleinstäuber G, Baum F, Schilling F (2005). Long-term investigation of the degree of exposure of German peregrine falcons (*Falco peregrinus*) to damaging chemicals from the environment. J. Ornithol. 146:34-54. DOI 10.1007/s10336-004-0053-6