



# How to measure eggshell thickness

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## Background

Eggshell thinning is considered a good proxy indicator for DDT exposure of the sampled population; especially DDT and its degradation products have been identified as a key group of POPs responsible for causing the 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 DDT usage (Wegner et al. 2005, Odsjö & Sondell 2014, Andreassen 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 ERBF 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 IP65; item # 293-240-30). In order to fit the inner curved surface of the eggshells, fit either a Mitutoyo “ball attachment” (# 101468M) or glue a 3-5 mm diameter stainless steel ball, to the rotating jaw of the Micrometer. It is recommended to fix the Micrometer in some sort of laboratory stand so the Micrometer jaws are horizontal (rotating pointing handle 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.

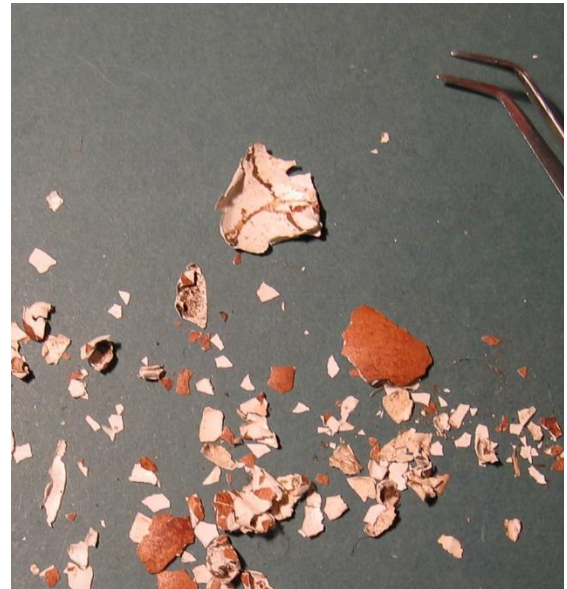
- Check if the thick ‘inner membrane’ of the egg is attached – and if so, firmly attached, not loose/cracking off. Most often, smaller fragments don’t have the membrane.

### Measuring

- Calibrate Micrometer to 0, and practise how to apply a very soft measuring technique, e.g., rotate the Micrometer handle slowly so you just *feel the contact*, but do *not apply pressure* (do not let the rotating handle “click”<sup>1</sup>). 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 rounded ball on the rotating jaw.
- Rotate the Micrometer jaw slowly until you feel the contact point.
- 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 (and your measuring method) 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 have been reported as around 0.07 mm (Falk et al. 2006 [0.071 mm], Court et al. 1990 [0.069 mm], Nygård 1983 [0.07 mm]) – but a later analysis, applying the “no pressure” measurement technique described above, gave a lower value of 0.06 mm (Falk & Lindberg in press).
- 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.

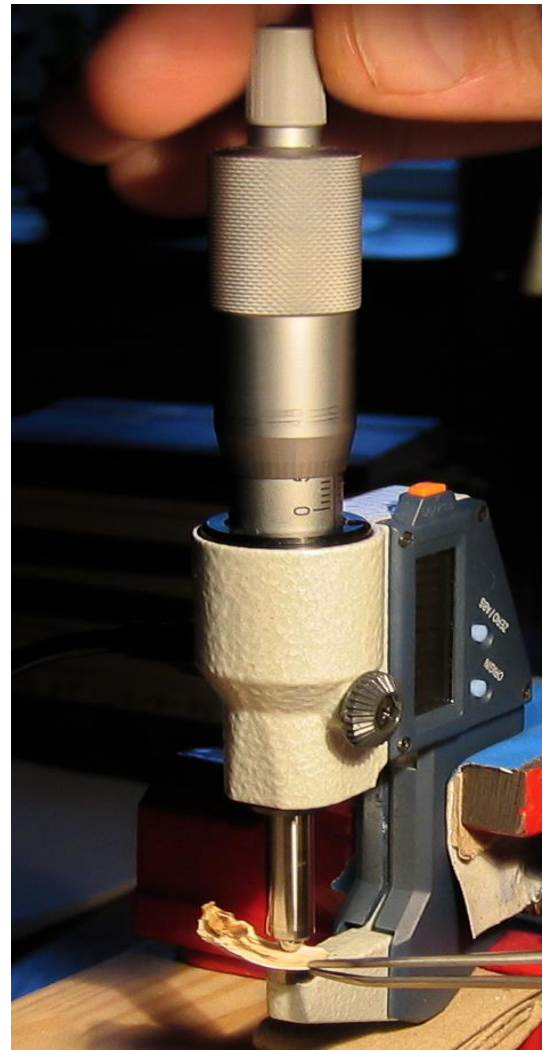
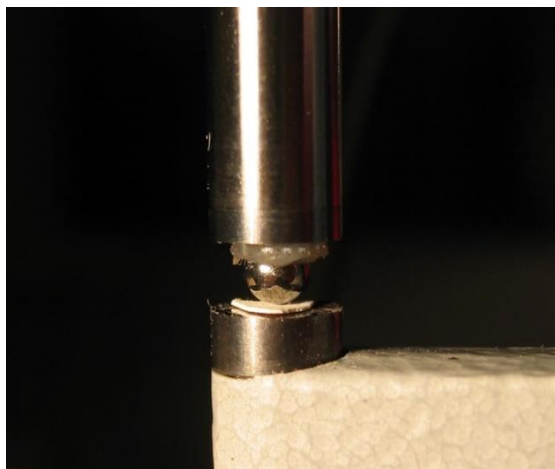


Collecting fragments in peregrine falcon nest (all photos: [www.vandrefalk.dk](http://www.vandrefalk.dk))

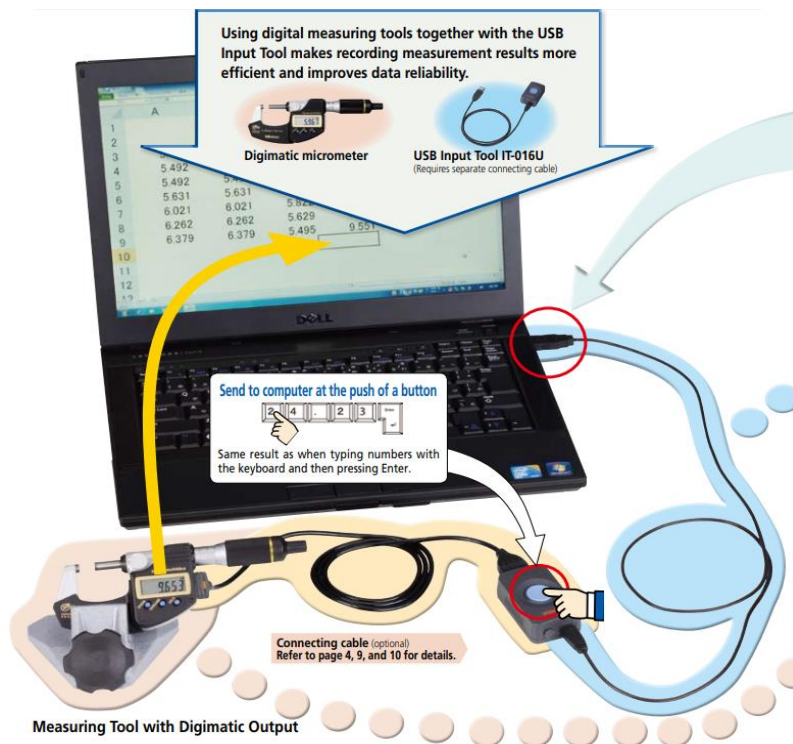


A good sample with more than 20 fragments; note the dried membrane on some fragments, in some places peeling off

<sup>1</sup> An earlier version of this guide recommended to apply a standardised “3 clicks” pressure but since the steel ball – at least in newer models of the Micrometers available – exerts a high pressure, it will compress the membrane or even break small/thin eggshell fragments. So for consistent measurements the lowest possible pressure is recommended.

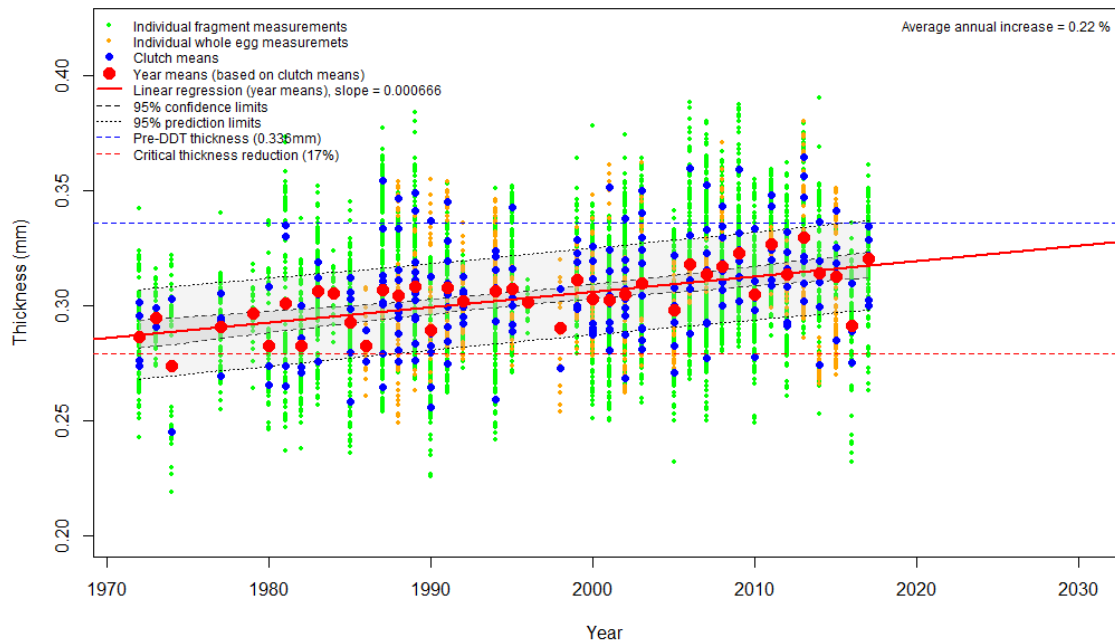


Measuring small fragments with Micrometer fixed in vertical position; note the stainless steel ball on the upper (rotating) jaw to fit the inside of a slightly curved eggshells. Right picture: measuring adjacent points *with* and *without* membrane.



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.



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