

# PGCCDBS Minimum Sampling Protocol for Age Calibration

Version 1

2–6 March 2009

Montpellier, France

## **Minimum Sampling Protocol for Age Calibration**

---

A minimum sampling protocol for age calibration has been developed based on the EFAN/TACADAR outcome (see PGCCDBS 2006 report).

### **1. Written Protocol**

1.1. Develop a written protocol for each type of Calcified Structure (CS) preparation and species.

### **2. Fish Sampling**

2.1. Define measurements, e.g. total length to 0.5 cm below, whole weight +/- 5g.

2.2. Specify all the required additional information, e.g. species, area, date, fishing gear, sex, maturity, etc. (minimum = species, area and date of capture).

### **3. Selection of CS**

3.1. Determine which calcified structures are to be used e.g., otoliths, illicia.

3.2. Identify the preferred method of otolith removal for the fish species.

### **4. Collecting CS Samples**

4.1. Specify cleaning method, e.g. removing blood/tissue before drying.

4.2. Transport and storage must prevent damage and deterioration.

4.3. Moisture content should be controlled, e.g. store in a cool dry place.

### **5. CS Preparation**

5.1. Identify the most appropriate preparation, e.g. sectioning, burning/staining.

### **6. Equipment Maintenance and Set Up**

6.1. Ensure equipment is serviced regularly and correctly maintained.

6.2. Set up microscope for each individual reader before age reading.

6.3. Ensure work position is comfortable and there is sufficient time read the CS.

### **7. Calibrated Image of CS**

7.1. Use a computer connected to a digital camera fitted on a binocular microscope.

7.2. Define a standard set-up for each species. Make sure light settings, magnification and equipment are standardised to the highest degree possible.

7.3. Prepare images for each otolith and for each viewing method used (using reflected light and/or transmitted light).

7.4. Calibrate each image by adding a scale bar (e.g. 2mm for *Pollachius virens* otoliths) and save the image using the unique CS sample ID number in the file name.

## 8. Age Reading

- 8.1. Log on to the database if using electronic data storage.
- 8.2. Follow the protocol. Check sample ID and otolith ID.
- 8.3. Define growth rings (translucent or opaque) and reading axes.
- 8.4. Apply criteria for rejection of CS, e.g. badly damaged or crystalline otoliths.
- 8.5. Apply criteria for the identification of false rings, e.g., juvenile growth.
- 8.6. Apply criteria for counting the valid annual rings (growth zones).
- 8.7. Apply birthday criteria for estimating age, usually 1 January.
- 8.8. Apply criteria to for the interpretation of *annuli* in relation to the 'birthday' of a fish (e.g. quarter 3 'pre-birthday' *annuli* in young fish, missing *annulus* in first quarter if protocol requires counting of opaque bands).
- 8.9. Apply criteria to recognise incomplete growth rings in older fish.
- 8.10. Consider an initial 'blind' reading before looking at the biological data, (e.g. length, sex, maturity, etc.). This may help to increase age reader precision.
- 8.11. Record the age, otolith edge growth and confidence in the age reading.
- 8.12. The integrity of the links between the data and original CS material must be maintained. Data edits must be backed-up and traceable. Keep original records.
- 8.13. Annotate the calibrated images with the positions of the *annuli*.

## Quality Assurance

- Develop a written protocol.
- Ensure age readers follow the written protocol.
- Allow adequate time for readings and re-readings.
- Provide advice on other potential age reading problems.
- Provide advice on using length, weight & maturity when reading CS.
- Use a glossary (e.g. EFAN/TACADAR).
- Develop and implement a training programme.
- Back up all electronic data and edits. Keep all paper records.

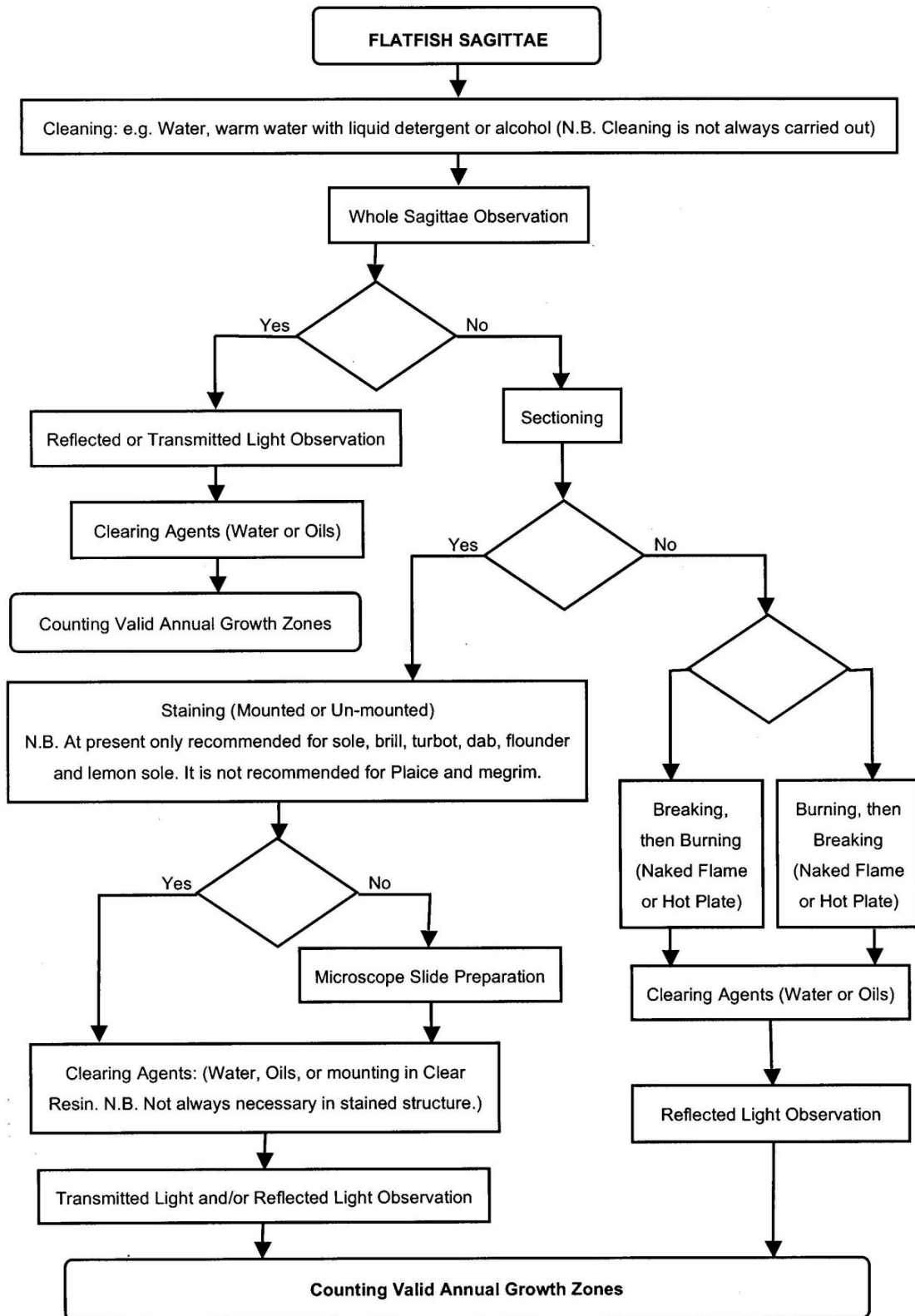


Figure 1. Methods used to prepare and observe the otoliths of flatfish species (Pleuronectiformes) for age reading (Reference: fig. WG2-7 from the final TACADAR Report)

## Quality Control

- Good conservation of CS is essential: Some CS will be re-read at intervals to monitor age reader precision. If the condition of the CS has deteriorated significantly compared with a previous reading, the two readings cannot be compared to measure change in age reader precision.
- Monitor age reading precision. If there is only one reader for the species, he should as a minimum, monitor changes in their own precision by regularly re-reading a sample of the CS. Return statistics on precision to age readers.
- Material of known age (usually from mark/recapture experiments) is rare. Ensure that age readers have the opportunity to take part in CS exchanges and Age Calibration Workshops (ACWK).
- Revise the written protocol as new information becomes available, e.g. mark and recapture information from new research, or experience gained at an ACWK.

Review methodology: A wide variety of techniques are used across European institutes for the preparation and age reading of otoliths of the same fish species. Fig. WG2-7 from the final TACADAR Report (Anon. 2006) shows some of the methods used to prepare and observe the otoliths of flatfish species (Pleuronectiformes), for age reading (Figure 1).