

# PGCCDBS Guidelines for Workshops on Maturity Staging

Version 3

## Version history

Version	Author	Date	Changes
Version 3	ICES PGCCDBS	4 March 2010	<b>Changes based on WKMSSPDF. Topics to consider when preparing a Workshop</b> f) modified and i) added. <b>Topics to consider during the Workshop</b> e)added. b)ii)modified Guidelines for collecting maturity data and histological analyses for maturity workshops 8) modified

## Introduction

The main objectives of a maturity staging workshop are: i) to agree on a common maturity scale for the species/stock of concern across laboratories, based on a comparison of existing scales and standardization of maturity determination criteria; ii) to establish correspondence between old and new scales so that time series of previous data can be converted; iii) to reduce sources of error in maturity determination by validating macroscopic staging, and iv) to propose an optimal sampling strategy to estimate accurate maturity ogives.

## Topics to consider when preparing a Workshop

- a) Identify sources of data that, at present, are used to collect maturity data and their current sampling protocols.
- b) Gather information on the reproductive biology and ecology of the species / stock of concern with emphasis on the timing of the different stages of the reproductive cycle, particularly spawning time, delimitating clearly its duration.
- c) Studies are required on spawning synchronicity among individuals within a stock, as low synchronicity will mean there is temporal overlap of different stages (developing, spawning, spent and/or resting).
- d) The organization for the collection of the samples and the methods for histological analysis need to be decided amongst the experts but guidance can be found below (Guidelines for collecting maturity data).
- e) Maintain contact with participating countries to ensure adequate sample coverage is obtained prior to the workshop's analyses of samples. In this sense the following should be ensured:
  - Laboratories participating in stock assessment or data collection of the stock of concern should participate even if they do not collect routinely maturity data.
  - However, there are practical limits to the number of participants; in this case each laboratory will need to ensure that only the most suitable people attend.
  - Experts on histology, maturation process and the reproductive ecology/biology of the species of concern or at least a related species should participate in the workshop.
- f) Ideally, a fresh sample should be provided during the workshops. This needs to be taken into account when setting the timing of the meeting. The best time of year to do a workshop on maturity staging is when the diversity in maturity stages is high.
- g) Identify the metadata that are needed to accompany samples collected for analyses and specify it in the sampling protocols (see guidelines below).

- h) Provide detailed protocols on collecting images of the gonads sampled, including at least a precise description of the quality of images (set-up of camera and format) and image calibration. Additionally, in case of histologically images, agree on the histological protocol and microscope set-up (see guidelines for histological process below).
- i) Use images as a tool for calibration prior to a workshop.
- j) Gather information on how the data are, or could be used, in the assessment process.
- k) Put in place arrangements for histological analyses of collected material taking into account that all participants may not have facilities or resources to meet this requirement. Arranging for centrally located analyses has proved effective in the past and has ensured that adequate samples are validated. Consider bi-lateral agreements to cover the cost of such work.
- l) Each laboratory should carry out investigations into potential discrepancies in maturity staging between scientists within the laboratory. They should consider macroscopic staging and, if available, microscopic staging. If possible provide statistical analysis of precision and accuracy within the laboratory. Potential causes for lack of precision and accuracy should also be analyzed.
- m) Prepare a full set of reference material covering both the spatial and temporal aspect of the species/stock of concern. These consist of pictures of all maturity stages together with their histology report.
- n) The meeting should be held in an institute with suitable wet laboratory facilities and ideally with histological facilities. If not histological facilities are not available at least with sufficiently high quality research microscopes with attached high definition cameras.

### **Topics to consider during the Workshop**

- a) Provide information on participating laboratory procedures, including sampling procedures, macroscopic maturity determination process, maturity scale definitions and if applicable gonad preservation and histological methods, and protocols used to determine microscopic maturity.
- b) Resolve interpretation differences between readers and laboratories both at macroscopic and microscopic scales. Differences may arise from:
  - i) Using different maturity scales
  - ii) Different interpretation of the same macroscopic stages (terminology and precise definition of stages are critical issues)
  - iii) Different sampling protocols, e.g. timing and/or gear selectivity or availability, see guidelines for collecting maturity data below.
  - iv) Different interpretation of gonad structures and gamete development in histological slides. This should not be an issue, so experts on gametogenesis should be involved in workshops.
- c) Agree and create a single maturity scale. Consider the following aspects:
  - i) Keep the scale as simple and efficient as possible. Not everything can be extracted from a maturity scale and a complex maturity scale may introduce more errors than relevant information (See WKMAT report)
  - ii) Describe the stages precisely avoiding ambiguity and overly subjective description (like colour descriptions), for example, give measurements instead of saying “bigger”.
  - iii) If two stages are hard to distinguish macroscopically, they should normally be merged. This often occurs with resting and/or mature inactive stages that are confused with immature or developing (at early stages).

- iv) In these cases, histology must be used to separate the merged maturity stage into the different real stages. It is necessary to define the minimum number of samples to be collected, the timing of the sampling, how they should be histologically processed, and what criteria should be used to distinguish between stages, and if possible define a reference lab (see below).
- d) As a calibration exercise, each participant should classify the workshop sample collection using the agreed maturity scale. This will provide a test of the new scale and any discrepancies in interpretation should be identified and resolved.
- e) Based on the experiences e.g. of the WKMSSPDF (22-26.02.2010) it is recommended to set the maximum fish to stage in one session to 120. However, the total numbers to stage should also take into account the species and any sample size requirements for statistical comparisons. This applies to fresh samples as well as pictures.
- f) The results from the calibration exercise should be recorded to provide data for statistical analysis. If you want to measure improvements in agreement due to the workshop then ideally a different set of samples should be used, not the ones already staged earlier in the workshop.
- g) Provide a statistical report comparing observed maturity stage with validated histological stage for the workshop participants to consider.
- h) Differences in staging between laboratories should be statistically analyzed in terms of precision and accuracy; sources of discrepancies should also be analyzed.
- i) Try to use standard terminology (Murua and Saborido-Rey, 2003; Brown-Peterson *et al.*, 2007) during the workshop and in the report. Try to keep the recommended maturity scale as similar to the standard as possible.
- j) When a new agreed maturity scale is proposed the impact on maturity historical series should be evaluated
- k) Produce an agreed reference collection of preserved gonads, histological slides and images that should be stored in a reference lab and always available for the scientific community. Copies of histological slides can be made and distributed with referenced images of these slides.
- l) A reference laboratory should be defined, for each species, with experience and equipments to define, with precision, maturity stages and to “solve problems”.
- m) The minimum output from species-specific workshops should be an illustrated manual.
- n) Provide recommendations to stock assessment Working Groups and Benchmarks on relevant issues derived from maturity stage studies, such as timing of sampling, changes on maturity time series, spatial differences on maturity, differential sex maturation, etc.