Guidelines for collecting maturity data and histological analyses for maturity workshops

These guidelines are partly taken from Workshop on sexual maturity staging of Cod, Whiting, Haddock and Saithe (WKMSCWHS). The guidelines should be regularly evaluated based on research developments and the experience from maturity staging workshops.

1) Sampling has to be conducted by cooperation between the participating laboratories.

2) The number of samples by length range, sex and location has to be clearly defined considering number of countries involved, timing, and spatial overlap of sampling.

3) Preferably, the sampling procedure should be executed several times during a year to follow the reproductive cycle and development of the gonads. At least 4 times at year, or more frequent depending on species.

4) However, cruises are normally not conducted each quarter or several times at year at the same location and hence limitations in sampling capacity are recognised. Commercial fleet samples (e.g., from observers onboard) can be used to complete sampling if gonads are properly preserved and observers properly trained for maturity staging.

5) Sampling at landing should generally be avoided as in most occasions gonads have already undergone lyses. Sampling at landing can only be used if a known catch has occurred recently before landing and the location of the catch is known.

6) For data collection and histology samples, each specimen should be given a fish ID including the following information: Country, station, date and fish number

7) For each specimen the following information should be collected:

- Procedures made to collect maturity data
- Location of sample collection
- Date of sample collection
- Fish total length
- Sex
- Maturity (as noted at time of collection)
- Fish total weight
- Gonad weight
- Fish gutted weight
- Age if available
- Additionally, other parameters should be taken if demonstrated to be relevant to assess temporal patterns in gonad development, like liver weight.

8) A series of photographs of the fish and gonad including the identification number should be taken during the sampling process. The WKMSSPDF 2010 clearly showed that staging from pictures is more difficult than staging from fresh materials. Generic comments were that some of the stage descriptions were only suitable for fresh samples and the characteristics were not visible on the pictures. There is a need for clear descriptions on pictures to be taken. When staging from pictures, it is necessary to standardise the way the pictures are taken. There have to be stringent procedures even down to equipment and/or settings used. General marks for staging from pictures:

- pictures have to be taken on fresh fish,
- add at least sampling time, area, unique sampling number, fish length and species in the picture,
- take care that the samples should be clean/tidy, preferable without intestines,
- take at least six pictures, in case of flat fish, four 4 in case of round fish. The differentiation between dorsal and ventral side is necessary only in case of flatfish:

    - from the dorsal side: overview of the fish on a measuring board, with the gonads visible in the fish; the ability to look at the whole fish with the gonads intact is vital to get the ratio of gonads to body length
    - from the dorsal side: detail of picture 1, zoomed in on the gonads; show the pressure characteristic on the picture to see if fish is running
    - from the ventral side: overview of the fish on a measuring board, with the gonads visible in the fish; the ability to look at the whole fish with the gonad intact is vital to get the ratio of gonad to body length
    - from the ventral side: detail of picture 3, zoomed in on the gonads; show the pressure characteristic on the picture to see if fish is running
    - picture of gonads outside the fish, placed on a measuring board, allowing to view the gonad in more details
    - picture of longitudinally cut gonad
  - for the best results is there a certain time when the photos are not that useful or not required however, getting as many different stages is useful as an educational tool
  - the pictures needs a lot of free space on the PC and the PC system operating very slow when many participants try to use it simultaneously
  - when organising a maturity workshop, where staging from pictures will be done, a server prepared for this purpose has to be used - the WebGR tool (REF) might be the right application to support maturity staging workshops,
  - in addition, a table including biological and sampling information should be available.

9) The gonad or sub-samples of the gonad tissue has to be preserved immediately after collection. If only pieces of gonads are collected, these should be representative of the entire gonad (for example from the anterior, middle and posterior part of the organ). The sampled tissue has to be preserved in buffered 4% formaldehyde.

10) Histological process has to be done in similar manner across laboratories or a single laboratory selected to process the samples.

11) Pieces of tissue should be embedded in wax or resin, but agreement on the location of the tissue within the gonad is very important, as differences in oocyte development across the gonad may bias the results. There is not an *a priori* preferred location, which should be investigated for each species.

12) Thickness of histological section is not critical but should not exceed 5 microns.

13) Staining protocol is a key aspect to be considered as differences in histological section interpretation may occur due to this, especially for cortical alveoli, postovulatory follicles and atresia. Haematoxylin-Eosin is a standard, but experts should advice on this. In any case the same protocol across laboratories should be used.

14) Slides should be used at the meeting, but images should also be taken for discussions and dissemination. Previous agreement is required on microscope set-up (illumination and numerical aperture is critical for microscopic image definition), setup of camera, image format (size and compression) and image calibration.