# ICES WKMATCH REPORT 2012

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# Report of the Workshop for maturity staging chairs (WKMATCH)

11–15 June 2012

Split, Croatia

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# Contents

## Executive summary

The recorded maturity stage at the time of observation is an important biological parameter to be used in the calculation of maturity ogives (and therefore of Spawning Stock Biomass), for the definition of the spawning season of a species, for the monitoring of long-term changes in the spawning cycle, and for many other research needs regarding the biology of species. Thus, maturity data are fundamental part of the stock assessment process and hence a vast effort is put on validating the macroscopic inspection of gonads. In the last decade a series of workshops addressed the maturity staging of different species with the objective of developing common maturity scales, decreasing discrepancies between laboratories and validating maturity staging through microscopic evaluation.

A total of 11 of those workshops on species-specific maturity staging were revised here. These workshops have analysed 20 teleosts, elasmobranchs as a whole subclass, three orders of cephalopods and four crustacean species. The WKMAT 2007, and later WKMSCWHS 2007, proposed a six point maturity scale for both males and females that have been used as a reference in the different maturity workshops to develop and adopt a common scale between laboratories for each species. A notable effort has been made by all workshop participants to standardize the existing maturity scales and accommodate the standard scale proposed. All workshops acknowledged the biological differences between the reference scale stages. All workshops proposed new scales that although were generally consistent with WKMAT scale, showed several differences. As a result, the four stage scale proposed in WKMAT has generally not adopted, partially because such scale does not allow reflecting specific particularities, useful for a number of species.

To overcome this, we propose the use a single scale of 4+2 stages or divisions which is believed to be universal, that is, it can be used for the majority of species, although viviparous and hermaphrodites may need some adaptation. This 6 stage codes and names should be used for all species and both sexes without exception; species-specific particularities should be reflected creating subdivisions and never modifying the 6 main stages. Using this proposed coding system, particularities of species and stocks can be addressed by each workshop (subdivisions), without losing consistency and traceability (divisions). In this way the code number of the divisions or main stages has the same biological meaning across species and laboratories. Some potential subdivisions are proposed as well, for its facultative use in some batch spawners an in viviparous species. The merging of different stages should be avoided and instead a combined code should be used. In this manner the consistency of stages definition and codes is maintained across species.

The definition of each stage needs to be linked to biological phases and incorporate into its description species-specific aspects relevant for an easy identification of each stage. The use of the terminology for maturity stages considers a general scheme of the reproduction that can be applied to all male and female elasmobranchs and teleost fishes, including hermaphrodites and livebearers. A full glossary of terminology was compiled. Training (of the observers) is the major issue for maturity staging, and it should be strengthen within the umbrella of ICES.

When staging maturity macroscopically timing of the sampling is critical to obtain reliable results. To define this period it is important to know the timing of the reproductive cycle, as this is species specific. If maturity staging outside the optimal periods is required, this should be based on histological information. However, we

suggest that whole mounts preparations are useful to validate macroscopic staging of ovaries being particularly useful to separate between early developing and developing specimens, immature and regressing/regenerating specimens, or even specimens that have just completed a spawning season from those have not yet entered sexual maturity. Nevertheless, it is recommended that the whole-mounts method is carefully calibrated before taken into practical use.

The maturity Workshops should discuss the new and general scale in their respective WKs by e-mail to assess the correspondence with the agreed scale, and evaluate the uncertainties and the problems this new general scale may cause. At the same time, ICES should ensure an appropriate attendance and a required level of basic knowledge, both on maturity studies and on the species targeted by the Workshop. Beyond of experts in the matter, the participants should be trained people, this can be achieved by training courses in ICES. We have revised and updated the Guidelines for Workshops on Maturity Staging, and provided general recommendations for future workshops.

We reviewed a total of 148 stocks of 53 species from 8 ecoregions from which ICES provides some type of advice. In 88 stocks (59%) maturity data are not used or are used improperly. This includes the use of time invariant maturity ogives when annual ogives can be available. In 39 stocks (26%) the assessment uses a proper maturity ogive, but over a limited time period. Finally in only 21 stocks 14% of the total advised stocks the maturity ogive has been estimated on regular basis and in these cases they are used properly in the assessment. Therefore, lack of data and/or poor quality is the main causes of maturity not being used. However, there is a general lack of information in the reports on how the maturity data was collected, ogive estimated, quality control and other relevant information. There is a need to determine what maturity data are required for assessment purposes, including how phenomena such as skipping spawning should be included in assessments. In spite of the effort on collecting maturity data, almost in 100% of the cases sex-specific ogives are combined without analysing the impact of this.

Expert groups should provide comprehensive reports on how the maturity data is used, and more specifically, at least: the method used to estimate maturity, in which sex and how a sex-specific maturity ogive is used in the assessment, source of data (survey, commercial sampling), the time of the year when the sampling was conducted, and years of proper estimation. The impact on the assessment of combining sex-specific maturity ogives should be analysed.

# 1 Overview

The Workshop for maturity staging chairs (WKMATCH), chaired by Fran Saborido-Rey, Spain, was held in Split, Croatia, 11-15 June 2012. The list of participants is included in Annex 1.

The workshop participants want to express gratitude to Barbara Zorica and Vanja Kec, extended to Institute of Oceanography and Fisheries, for acting as local organizers providing all the support needed for the successful completion of the workshop.

## 1.1 Background

The recorded maturity stage at the time of observation is an important biological parameter to be used in the calculation of maturity ogives (and therefore of Spawning Stock Biomass), for the definition of the spawning season of a species, for the monitoring of long-term changes in the spawning cycle, and for many other research needs regarding the biology of species. Thus, maturity data are fundamental part of the stock assessment process and hence a vast effort is put on validating the macroscopic inspection of gonads. In the last decade a series of workshops addressed the maturity staging of different species with the objective of developing common maturity scales, decreasing discrepancies between laboratories and validating maturity staging through microscopic evaluation. However, the appropriate sampling design for estimating accurate maturity ogives (catchability issue) together with the interpretation of the observed maturation pattern is species specific and thereby depends on its reproductive biology. Cooperation between assessment scientists and experts on reproductive biology including maturity schemes is therefore urgently needed in order to define the optimal use of maturity data in stock assessment.

Chairs of the different Workshops on maturity held since 2007 were asked to participate, together with experts on maturation and the use of biological information in stock assessment. In view of its relevance to the Data Collection Framework (DCF), the Workshop was expected to attract wide interest from ICES Member States involved in stock assessment. Most Workshop participants (10) were experts on reproductive biology, while four have previously been chairs of Workshops on sexual maturity staging. There was no participation by stock assessment working group chairs, although several at the present Workshop participants had been involved in such work, although not yet as chairs.

## 1.2 Terms of Reference

The terms of reference for WKMATCH were as follows:

a ) Revising and, if necessary, enhancing consistency in the currently adopted methods;

b ) Analyse, verify and agree methods and protocols for an accurate maturity staging;

c ) Develop standard protocols for quality control and tools to analyse error and bias;

d ) Evaluate the impact of a newly developed common scales on historical databases;

e ) Update the Guidelines for collecting maturity data and developing maturity;

f ) Advise on the best way to incorporate newly collected data into assessment.

## 2 Adoption of the agenda

The agenda adopted is found in Annex 2. ToRs a), b), e), and f) were fully addressed during the meeting while ToRs c) and d) were only discussed. Addressing ToR c) was considered too premature as there was insufficient background information to discuss the development of these protocols. The WebGr tool was presented and the participants acknowledged its usefulness without further assessment. All the participants agreed that the evaluation of the impact of a newly developed common scale on historical databases (ToR d) has to be conducted by each WKs with experts from each species and laboratories involved in data collection and no further discussion on this ToR was undertaken.

# 3 Consistency in the currently adopted methods

## 3.1 Review of results of workshops

A total of 11 workshops on species-specific maturity staging were considered here (Annex 3; Table 3-1). These workshops have analysed 20 teleosts, elasmobranchs as a whole subclass, three orders of cephalopods and four crustacean species. WKMSSPDF has met twice and the results of both meetings are considered here. After this WKMATCH meeting, and while compiling this report, WKMSEL met again and its results have been incorporated here as well. In 2012 WKMSEL expanded the species analysed from 44 to 64.

The WKMAT 2007 proposed a five point maturity scale for both males and females. Afterwards, the WKMSCWHS 2007 proposed to add an extra scale for abnormal gonads. These two scales (Table 3-1) have been used as a reference in the different maturity workshops to develop and adopt a common scale between laboratories for each species. Nevertheless, the reference maturity scale (WKMAT or WKMSCWHS) is actually a 4 stage scale within the normal reproductive cycle, as the last two stages (skip spawning and abnormal) are exceptional stages. This should be considered when comparing the adopted scales by each Workshop. Additionally, the *standardized* terminology proposed by Brown-Peterson *et al.*, (2011) has been used as reference in some WKs (Table 3-1). The two reference scales (WKMAT and standardized) are very similar in conception. Both are composed of four major stages or phases, with the same code number (1-4) representing the same biologically event. The main difference is that *spawning* stage in WKMAT corresponds to two stages or phases in the *standardized* scale: *spawning capable* and *actively spawning* to allow for the peculiarities of batch spawners to be reflected in the scale. Besides, the terminology differs between the two scales (see Table 3-1, section 3.4 and Brown-Peterson *et al.*, 2011 for more details).

### 3.1.1 Material and methods used in the workshops

A notable effort has been made by all workshop participants to standardize the existing maturity scales and accommodate the standard scale proposed by WKMAT. For the majority of the WKs the main task was to agree on a common scale to be used across laboratories, and so other topics were considered secondary. Thus, for example, the national or DCF sampling protocols were not revised, except in WKMSCWHS. However, five WKs (WKMSCWHS, WKMSSPDF, WKMSTB, WKMSREGH, WKMSEL) agreed and reported a standardized sampling strategy to optimize maturity staging, while the other six WKs do not. However, one important constrain in this analysis is that the ToRs were not uniform across WKs: the adoption of a common maturity scale for each species analysed was the only common ToR across WKs, but important aspects such as the calibration among maturity observers or the sampling strategy were not.

All workshops acknowledged the biological differences between the proposed WKMAT stages and proposed new scales that were generally consistent with WKMAT scale. However, several differences were detected, as explained below. Generally the biology of species, i.e. the reproductive cycle and the different events of the gametogenesis, were considered in the discussions but irregularly reported by each WK, making difficult to assess to what extent the agreed scales reflect the species biology. However, considering that the vast majority of the participants were experts on reproductive biology, this aspect likely has been always considered. Nev-

ertheless, two key aspects on reproductive biology have not generally considered, or at least reported: reproductive timing and population synchronicity of developmental stages within the breeding season. Both aspects determine the optimal time to estimate maturity, which depends on studying variable (estimation of length at first maturity, etc.). In species with asynchrony, the inherent difficulty on assessing maturity should be considered when interpreting the adopted scales. There is an unavoidable link between the definition of the maturity scale and its use on data collection routine that has to be analysed. In this respect, considerable effort was made to compare the existing national/laboratory scales for each species producing and calibrating a new common scale for each species. However, for some workshops the participation was limited and the universality of the adopted scales was not guaranteed. Similarly, in many occasions the data collection and monitoring responsible for each laboratory/country do not participate in the workshops, so the transfer of the adopted scales to the data collection routines was not guaranteed either.

The understanding of species biology it is critical when using the maturity scale. When staging a species, it is important to have an understanding of the biology of the species and its reproductive cycle in the sampling area. This helps to distinguish the transitions between the stages that most frequently are not easily to distinguish. It is recommended that institutes carry out in-house workshops on the reproductive biology of the species and maturity staging (WKMSSPDF, 2010).

Seven of the Workshops (WKMSCWHS, WKMSHM, WKMSSPDF, WKMSHS, WKMSTB, WKMSREGH and WKMSCEPH) used gonads collected previous to the workshop that were pictured and then histologically processed. As a consequence, the discussions were based on staging different types of material. The calibration exercises were performed between unprocessed gonads (fresh, preserved or images) and histologically processed. This approach is the ideal to correct bias and errors in maturity staging. In contrast, in the rest of WKs samples were not histologically processed and gonads were not microscopically staged and assessed. Among these only in two (WKMSMAC, WKSPMAT) fresh material that was collected *in situ* or frozen was used.

Nevertheless, only in hake (WKMSHM) was a basic statistical analysis comparing maturity staging among participants conducted. In spite of these limitations differences among laboratories were solved in all Workshops and they reach agreements for all species, so common adopted maturity scales were defined for each species.

### 3.1.2 Differences among adopted maturity scales

A summary of the adopted scales are shown in Table 3-1 and Table 3-2 for females and males respectively. For the majority of the species analysed it was defined a maturity scale for both males and females, except for crustaceans, Greenland halibut and redfish. However, the effort performed in males has been considerably smaller than in females. The biological meaning of each stage of WKMAT and/or *standardized* scales were contrasted and compared within each species analysed in the 11 WKs. Except for *Recovery*/*Regenerating*/*Resting* stages (see below) the four main biological distinct stages were adopted without definition errors. The finally adopted scales are now more similar among them than when using the myriad of national or laboratory scales. Nevertheless, the 29 adopted scales still differ largely between them, being far from universal or standardized, mainly due to three factors: i) differences between taxa; ii) the manner on how these stages were adopted, coded and termed; iii) the merging of several stages; and iv) the definition of more stages than the main four

proposed. These differences may still produce errors and bias in the maturity scale and hence in the maturity ogive, especially for females.

All workshops defined the stage *immature* or *virgin* appropriately and accordingly to the definition of WKMAT. However, for three species in both sexes (hake, anchovy and sardine; Table 3.1 and Table 3-2) this stage was merged with the *resting* stage based on the difficulty in distinguishing macroscopically *immature* from *resting* females. A *resting* stage was not specifically defined by WKMAT, but likely it should be assimilated within the *recovery* or *regenerating* stage. The same difficulty in distinguishing macroscopically stages lead to the merger of *resting* and *skip of spawning* stages in ten species for females (cod, whiting, haddock, saithe, sole, plaice, dab, flounder, herring and sprat; Table 3.1) and in eight for males (cod, whiting, haddock, saithe, plaice, dab, flounder and sprat; Table 3-2).

In the case of mackerel and horse mackerel the old 6 stage scale was not abandoned and its use was still recommended (WKMSMAC, 2007). There was a clear correspondence between the majority of the maturity scales for these two species and the WKMAT scale. WKMSMAC recommended that scales used that were less detailed should be abandoned in favour of the WKMAT standard scale. However, it was recommended that more detailed scale, such as Walsh scale mostly used for mackerel and horse mackerel, should be retained (Table 3-1 and Table 3-2). In other cases, as in hake, the four stage scale was adopted, but with the definition of sub stages (3a and 3b) in females. However, the same Workshop proposed for monkfish a five stages scale, where two stages of *developing* were defined, *early* (stage 2) and *late* (stage 3 or pre-spawning), and additionally stage 2 was merged with *resting*. Thus, the same workshop took two different approaches to adopt a common scale.

Similarly, for anchovy and sardine three different stages (3, 4 and 5) were defined for spawning activity in both sexes. These are rather coincident with the three stages used for horse mackerel and mackerel (Walsh scale). For sprat, on the contrary, spawning was divided in two substages (3a and 3b). For these pelagic species, as well for hake, there is a clear need on identifying with precision the spawning activity in females for its use in egg production methods. The use of these substages in males is, thus, more questionable. For these species, WKMAT scale does not seem to be adequate.

In the same way, viviparous species have the need on accounting the ovulation and embryogenesis stages. For elasmobranchs, these stages were pooled in stage 4, with the definition of substages (4a to 4c), allowing, somehow, a correspondence with WKMAT scale. This was not followed for redfish where five stage scale was suggested.The Brown-Peterson *et al.*, (2011) terminology was initially adopted by WKMSEL for oviparous and viviparous elasmobranchs. Thus, four major stages were defined. Stage 3 (spawning) was divided in two sub-stages in oviparous species (3a *spawning capable* and 3b *actively spawning*) and in three in viviparous (4a, b and c: *early*, *mid* and *late pregnancy* respectively); in both cases there was a clear correspondence with the reference scale (WKMAT) as the code and terminology was equivalent (stage 3, *spawning*). Similarly, stage 4 is considered as a single stage maintaining the equivalency with WKMAT, but was divided in two sub-stages (4a. *regressing* and 4b. *regenerating*) for both reproductive strategies. However, in the 2012 WKMSEL meeting, the names of two stages of oviparous fish were modified, although the essence of the scale was maintained. However, a major change was taken in viviparous fish, where the former stage 3b, c and d are now coded as stage 4, and the former stage 4 is now divided in two different stages (5 and 6) named *post-partum* and *regenerating*, respec-

tively. For males WKMSEL produced the same and more simple 4-stage scale for both viviparous and oviparous.

In the case of Crustaceans and Cephalopods gametogenesis was divided in two substages: *developing* and *maturing*. This division makes sense given the particularities of these two taxa. However, while in cephalopods there is a single stage 2 subdivided in 2a and 2b, i.e. maintaining the equivalence to the WKMAT scale; in crustaceans they are two stages, 2 and 3. And stage 2 is merged with *recovering* stage. In both taxa the meaning of *developing* (2 and 2a) is the same and concordant with the stage, and term, used for fish. However, the stage *maturing* (3 and 2b) is highly confusing. First because of the name used (see Terminology in section 3.4), and secondly because the definition seems to correspond with the spawning capable, i.e. more connected with spawning activity than with vitellogenesis, but this should be evaluated by the respective Workshop. Similarly the use of *mature* (stage 4 and 3a respectively) is inappropriate (see Terminology in section 3.4) and both represent the spawning event in these taxa.

A notable case is Greenland halibut. This species has been documented to have a reproductive cycle longer than a year and this particularity has to be considered in the estimation of the maturity ogive. It is for this reason that the first stage after maturation is *mature primiparous, functionally immature*. While the fish is biologically mature (presence of secondary growth oocytes), it will not contribute to spawning stock until next year, at least. The long vitellogenesis justifies the split in several substages. Again, however, the equivalence with the WKMAT scale is lost in this manner.

Another difficulty when interpreting the scale and adopting common scales refer to stages after the end of spawning and before the start of the next breeding season (See Annex 5 for terminology). The length of this period is variable among species. Gonadas short after the end of spawning are easily identifiable and mostly termed as spent (*post-spawning* in hake and monk, *cessation* in sprat and *regressing* in redfish, Greenland halibut and elasmobranchs). However, after this stage a general disagreement exists. In nine species (eight in males) a distinct *resting* stage is defined but merged with skip spawning. The difference between *resting* and *recovery* (WKMAT stage) is unclear, but apparently means the same thing for many workshops as either *recovering* (or *regenerating*) or *resting*, or even both, are not defined and thus not used as stage. However, for herring *recovering* and *resting* are defined as two distinct substages in females, the latest merged with *skip spawning*. *Recovering* is merged with *developing* in female crustaceans, and *resting* is merged with *immature* stage in hake, sardine and anchovy and with *developing* in monk. In summary, there is not a single pattern for these stages; being it the consequence of the fact they are macroscopically hard to be distinguished.

In general there is a correspondence of the four main stages among species (highlighted in green in Table 3-1 and Table 3-2), although the codes and terms largely differ. In a number of cases, however, there is not such correspondence, mostly due to the merging of two different stages, as commented above (highlighted in brown). Finally, stages not properly defined in WKMAT, such as those related with spawning activity and embryogenesis, have been considered in different ways. Thus, the species-specific particularities regarding the WKMAT standard scale has been treated differently among workshops resulting in again different scales that although more harmonized than before still are not standardized. Thus, the four stage scale proposed in WKMAT has generally not adopted, partially because such scale does not allow reflecting specific particularities, useful for a number of species.

**Table 3-1. Overview of the female maturity scales proposed by each of the ICES maturity staging Workshops since 2007.**

| WK | SPECIES | CODES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **WKMAT** | General | 1. Virgin | 2. Maturing | | 3. Spawning | | 4. Spent/ Recovery | 5. Omitted spawning | |
| **Standardized** | | 1. Immature | 2. Developing | 3b. Spawning capable | 3a. Actively Spawning | | 4. Regressing/ Regenerating | | |
| **WKMSCWHS** | Cod | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/ Skip Spawning | 6. Abnormal |
| **WKMSCWHS** | Whiting | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/ Skip Spawning | 6. Abnormal |
| **WKMSCWHS** | Haddock | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/ Skip Spawning | 6. Abnormal |
| **WKMSCWHS** | Saithe | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSHM** | Hake | 1. Immature/ Resting | 2. Developing/ Maturing | | 3a. Hydrated Spawning | 3b. Partly spawning | 4. Post-spawning | | |
| **WKMSHM** | Monk | 1. Immature | 2. Developing/ Resting | 3. Pre-Spawning | 4. Spawning | | 5. Post-spawning | | |
| **WKMSMAC** | Mackerel | 1. Immature | 2. Early ripening | 3. Late ripening | 4. Ripe | 5. Partly spent | 6. Spent/ Recovery | | |
| **WKMSMAC** | Horse mackerel | 1. Immature | 2. Early ripening | 3. Late ripening | 4. Ripe | 5. Partly spent | 6. Spent/ Recovery | | |
| **WKSPMAT** | Sardine | 1. Immature/ Resting | 2. Developing | 3. Imminent spawning | 4. Spawning | 5. Partial post-spawning | 6. Spent | | |
| **WKSPMAT** | Anchovy | 1. Immature/ Resting | 2. Developing | 3. Imminent spawning | 4. Spawning | 5. Partial post-spawning | 6. Spent | | |
| **WKMSSPDF** | Sole | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSSPDF** | Plaice | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSSPDF** | Dab | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSSPDF** | Flounder | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSHS** | Herring | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5a. Recovering<br>5b. Resting/SS | 6. Abnormal |
| **WKMSHS** | Sprat | 1. Immature | 2. Maturing | 3a. Spawning Inactive | 3b. Spawning Active | 4. Cessation | 5a. Recovering | 5b.Resting/ Skip Spawning | 6. Abnormal |

| WK | Species | Codes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **WKMSTB** | Turbot | 1. Immature | 2. Maturing | | 3. Spawning | 4. Spent | 5. Skip Spawning | 6. Abnormal |
| **WKMSTB** | Brill | 1. Immature | 2. Maturing | | 3. Spawning | 4. Spent | 5. Skip Spawning | 6. Abnormal |
| **WKMSREGH** | Greenland halibut | 1. Immature | 2. mature, functionally immature | 3. Mature | 4. Mature late | 5. Spawning | 6. Regressing / Regenerating | |
| **WKMSREGH** | Redfish | 1. Immature | 2.1. Skip spawning | 2.2. Maturing | 3. Mature/ Fertilized | 4. Parturition | 5. Regressing / Regenerating | |
| **WKMSEL** | Elasmobr. Viviparous | 1. Immature | 2. Developing | 3. Capable to Reproduce | 4a. Early<br>4b. Mid pregn. | 4c. Late pregnancy | 5. Post-partum | 6. Regenerating |
| **WKMSEL** | Elasmobr. Oviparous | 1. Immature | 2. Developing | 3a. Capable to Reproduce | 3b. Egg-laying | 4a. Post-laying<br>4b. Regenerating | | |
| **WKMSCEPH** | Octopoda | 1. Immature | 2a. Developing | 2b. Maturing | 3a. Mature/ Spawning | 3b. Spent | | |
| **WKMSCEPH** | Teuthida | 1. Immature | 2a. Developing | 2b. Maturing | 3a. Mature/ Spawning | 3b. Spent | | |
| **WKMSCEPH** | Sepiida | 1. Immature | 2a. Developing | 2b. Maturing | 3a. Mature/ Spawning | 3b. Spent | | |
| **WKMSC** | *Aristeus antennatus* | 1. Immature | 2. Developing/ Recovering | 3. Maturing | 4. Mature (imminent spawning) | 5. Spent | | |
| **WKMSC** | *Aristaeomorpha foliacea* | 1. Immature | 2. Developing/ Recovering | 3. Maturing | 4. Mature (imminent spawning) | 5. Spent | | |
| **WKMSC** | *Parapenaeus longirostris* | 1. Immature | 2. Developing/ Recovering | 3. Maturing | 4. Mature (imminent spawning) | 5. Spent | | |
| **WKMSC** | *Nephrops norvegicus* | 1. Immature | 2. Developing/ Recovering | 3. Maturing | 4. Mature (imminent spawning) | 5. Spent | | Berried females |

**Table 3-2. Overview of the male maturity scales proposed by each of the ICES maturity staging Workshops since 2007.**

| WK | Species | Codes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **WKMAT** | General | 1. Virgin | 2. Maturing | | 3. Spawning | | 4. Spent/ Recovery | 5. Omitted spawning | |
| **Standardized** | | 1. Immature | 2. Developing | 3b. Spawning capable | 3a. Actively Spawning | | 4. Regressing/ Regenerating | | |
| **WKMSCWHS** | Cod | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSCWHS** | Whiting | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSCWHS** | Haddock | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSCWHS** | Saithe | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSHM** | Hake | 1. Immature/ Resting | 2. Developing/ Maturing | | 3. Spawning | | 4. Post-spawning | | |
| **WKMSHM** | Monk | 1. Immature | 2. Developing/ Resting | 3. Pre-Spawning | 4. Spawning | | 5. Post-spawning | | |
| **WKMSMAC** | Mackerel | 1. Immature | 2. Early ripening | 3. Late ripening | 4. Ripe | 5. Partly spent | 6. Spent/Recovery | | |
| **WKMSMAC** | Horse mackerel | 1. Immature | 2. Early ripening | 3. Late ripening | 4. Ripe | 5. Partly spent | 6. Spent/Recovery | | |
| **WKSPMAT** | Sardine | 1. Immature/ Resting | 2. Developing | 3. Imminent spawning | 4. Spawning | 5. Partial post-spawning | 6. Spent | | |
| **WKSPMAT** | Anchovy | 1. Immature/ Resting | 2. Developing | 3. Imminent spawning | 4. Spawning | 5. Partial post-spawning | 6. Spent | | |
| **WKMSSPDF** | Sole | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting | 6. Abnormal |
| **WKMSSPDF** | Plaice | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSSPDF** | Dab | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSSPDF** | Flounder | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | 5. Resting/Skip Spawning | 6. Abnormal |
| **WKMSHS** | Herring | 1. Immature | 2. Maturing | | 3. Spawning | | 4. Spent | | |
| **WKMSHS** | Sprat | 1. Immature | 2. Maturing | 3a. Spawning Inactive | 3b. Spawning Active | 4. Cessation | 5a. Recovering | 5b.Resting/Skip Spawning | 6. Abnormal |

| WK | Species | Codes | | | | | |
|---|---|---|---|---|---|---|---|
| **WKMSTB** | Turbot | 1. Immature | 2. Maturing | | 3. Spawning | 4. Spent | 6. Abnormal |
| **WKMSTB** | Brill | 1. Immature | 2. Maturing | | 3. Spawning | 4. Spent | 6. Abnormal |
| **WKMSREGH** | Greenland halibut | Not defined | | | | | |
| **WKMSREGH** | Redfish | Not defined | | | | | |
| **WKMSEL** | Elasmobr. Viviparous | 1. Immature | 2. Developing | 3a. Capable to Reproduce | 3b. Active | 4. Regressing | |
| **WKMSEL** | Elasmobr. Oviparous | 1. Immature | 2. Developing | 3a. Capable to Reproduce | 3b. Active | 4. Regressing | |
| **WKMSCEPH** | Octopoda | 1. Immature | 2a. Developing | 2b. Maturing | 3a. Mature/Spawning | 3b. Spent | |
| **WKMSCEPH** | Teuthida | 1. Immature | 2a. Developing | 2b. Maturing | 3a. Mature/Spawning | 3b. Spent | |
| **WKMSCEPH** | Sepiida | 1. Immature | 2a. Developing | 2b. Maturing | 3a. Mature/Spawning | 3b. Spent | |
| **WKMSC** | *Aristeus antennatus* | Not defined | | | | | |
| **WKMSC** | *Aristaeomorpha foliacea* | Not defined | | | | | |
| **WKMSC** | *Parapenaeus longirostris* | Not defined | | | | | |
| **WKMSC** | *Nephrops norvegicus* | Not defined | | | | | |

## 3.2 A common maturity scale

The main reason for having a common and standardized scale is because it provides and improves consistency. A wide usage will allow comparisons among laboratories and along time within species, but also comparisons across species, as well the possibility for non-specialists for that species to evaluate the data and the comparison of maturity data collected. The maturity scale should reflect the biology of the species rather than the preferences of the observer, thus easy to be transposed to different uses such as assessment, advice, management, general biology or ecology. The proposed scale tries also to overcome species-specific particularities, without losing the consistency and standardization across species.

The maturity scales proposed for 24 species and five orders in 11 workshops differed, but some consistencies across scales allowed us to define a single scale that it is believed is universal (at least fish, cephalopods and commercial crustaceans). It can be used for the majority of species, although viviparous and hermaphrodites may need some adaptation. As shown in a previous section there is a clear need for using different numbers of maturity and gonadal developmental stages in each species. To overcome this problem we propose the use of a scale with 4+2 general stages or divisions, which can be subdivided. The 4+2 divisions or main stages should be used for all species, whilst the sub-divisions can be species-specific.

The proposed common scale, valid for both sexes, is shown in Table 3-3. Detailed definitions for each division and subdivisions are found in Annex 4.

It is **important to keep the same 6 codes/stages for all species**. Using this coding system, particularities of species and stocks can be addressed by each workshop (subdivisions), without losing consistency and traceability (divisions). In this way the code number of the divisions or main stages has the same biological meaning across species and laboratories. These **stages can be subdivided** for different purposes, but not necessarily in each species. It can be used as many subdivisions as needed, but they should be kept to a minimum to obtain the maximum efficiency i.e. getting the required precision without increasing noise and errors.

**Table 3-3 Proposed common scale for maturity staging**

| | CODE | NAME |
|---|---|---|
| Sexually immature | 1 | Immature |
| Sexually mature | 2 | Developing |
| | | 2a. Developing but functionally immature |
| | | 2b. Developing and functionally mature |
| | 3 | Spawning |
| | | 3a. Actively spawning |
| | | 3b. Spawning capable |
| | 4 | Regressing/Regenerating |
| | | 4a. Regressing |
| | | 4b. Regenerating |
| | 5 | Omitted spawning |
| | 6 | Abnormal |

The terminology proposed here is in some cases relatively new compared with previous uses (WKMAT). It tries to avoid the confusion generated from traditional terminology used in older maturity scales, such as *maturing*, *spent* or *resting* (see Terminology in section 3.4). The proposed terminology is based on developmental biology of gonads.

**In summary, we recommend using the 6 stage codes and names for all species and both sexes without exception. Species-specific particularities should be reflected in the subdivisions without ever modifying the 6 main stages.**

The subdivisions suggested here (2a, 3a, 3b, 4a, 4b) are the most commonly used across the different adopted scales. The use of these sub-divisions is optional, and their use should be evaluated and agreed for each species. As mentioned above other species-specific sub-divisions can be used. And, of course, if no sub-divisions are necessary, the scale should be restricted to the 6 main divisions.

In some cases the *developing* stage is subdivided into early and late developing given the temporal length of this stage (months in some species). However, in terms of maturity, egg production, spawning stock biomass and other biological features related to stock assessment and management, the division between early and late developing (vitellogenesis in females) makes no difference, but may introduce errors and noise. This applies especially for males where there are not clear macroscopic indicators of the different stages of spermatogenesis. Thus, we do not recommend the use of subdivisions for early and late developing. Of course in studies focusing reproductive ecology the distinction between early and late vitellogenesis is important and useful, but it should be addressed histologically and it is out of the scope of the maturity scale use that is being analysed here.

The suggested subdivisions 2a and 2b are very particular case for species with a reproductive cycle longer than a year (only female Greenland halibut among those analysed here). For these species the stage of vitellogenesis in the first year after maturation means that while the fish is biologically mature will not contribute to the SSB that year, but likely the next, being thus functionally immature. Because the proportion of young primiparous fish can be significant, it is relevant to quantify them using subdivision 2a.

For the particular case of cephalopods and crustaceans the former stages 2b and 3 should be included within stage 3, as spawning capable. Former stages 3a and 4 also pertain to stage 3, but as actively spawning. These correspondence should be evaluated by their respectively Workshop.

In some batch spawners there is a need to quantify the females producing a batch within a certain period, for example to estimate spawning frequency. In these cases it is necessary to distinguish females releasing eggs, normally hydrated eggs, i.e. *actively spawning* (3a) from females simply within spawning season, i.e. *spawning capable* (3b). The term 'spent' or 'partially spent' should be avoided. The use of these subdivisions in males is questionable and probably unnecessary.

Stage 4 is present in all species analysed, and it is believed to be universal in all taxa considered, included semelparous species. However, if subdivisions 4a and 4b are used, it should be considered that 4b is not present in semelparous species (some fishes and the vast majority of cephalopods).

The term *capable to reproduce* used for elasmobranchs should be avoided. Because a fish is capable of reproducing once it is mature, this term does not reflect the cyclic process of breeding and spawning. WKMSEL define this stage for female oviparous

elasmobranchs as fish with "follicles ready to be ovulated", i.e. spawning capable, so this term is preferable. The same situation applies to males. For the case of viviparous female fish (elasmobranchs and teleost) WKMATCH acknowledge that the term spawning maybe is not fully adequate. However, spawning capable means fish is able to ovulate imminently, producing an egg that is released (spawn in oviparous fish), or retained (internally fertilized in viviparous fish). Therefore, although viviparous fish do not spawn[1], spawning is an analogous term to ovulation and can be used for maturity scale purpose in viviparous fish. In the case of viviparous males, there are no differences with oviparous males.

In viviparous fish, the egg is retained after ovulation and fertilized internally to be released (parturition) later as an embryo or juvenile. Embryogenesis, thus, takes place within female and such stages should be accounted for in the maturity scale. Although the homologous stages in oviparous fish occur outside female, there is analogy between spawning and embryogenesis. Both can be considered as the latest developmental stages within the female reproductive organs prior to the offspring release (egg or embryo). In this sense the maternal stages (defined by WKMSEL for viviparous elasmobranchs) and the gestation stages (defined for redfish by WKMSREGH) can be assigned to spawning stage and included as subdivisions (Table 3-4).

**Table 3-4. Proposed subdivisions for viviparous fish (based on WKMSEL and WKMSREGH)**

| | ELASMOBRANCHS | REDFISH |
|---|---|---|
| 3. Spawning | 3a. Spawning Capable | 3a. Spawning Capable |
| | 3b. Early pregnancy | 3b. Early embryogenesis |
| | 3c. Mid pregnancy | 3c. Parturition |
| | 3d. Late pregnancy | |

The merging of different stages should be avoided since they can be confused macroscopically. For some species it has been suggested that merging stages such as *immature/recovering* or *recovering/skip spawning*, because even during the optimal sampling time there are difficulties in distinguishing these stages. For these species it was recommended that histological analyses should be undertaken on subsamples to assess the proportion of each stage. We strongly endorse this recommendation. However, we recommend not merging different stages and in these cases a combined code should be used, e.g. 1/4b, 4b/5. In this manner the consistency of stages definition and codes is maintained across species and still stages 1 and 4b can be used if distinguishable.

The majority of the Workshops have defined a stage 0 referring to undetermined sex. However, this is not a maturity stage, but a sex code, corresponding to a different variable. When determining sex, 0, 1 and 2 can be used. But once sex has been determined, then maturity should be able to be staged with precision. If there is hesitation about staging use combining codes instead: 1/4b, 4b/5, if there is doubt about the sex of an individual, then obviously maturity should not be staged.

[1] The Oxford dictionary defines Spawn (verb) as the act of release or depositing eggs. Thus, although traditionally spawn has been used as the release of eggs from the ovary to the environment for their fertilization, it is not completely inadequate using it as synonymous of ovulation.

The definition of each stage needs to be linked to biological phases and incorporated into its relevant species-specific description for an easy identification of each stage. However, it is important to avoid ambiguity, as for example the use of colour as a diagnostic feature.

Previous maturity Workshops participants should discuss the new and general scale in their respective WKs by e-mail to assess the correspondence with the agreed scale, and evaluate the uncertainties and the problems this new general scale may cause.

The term *omitted spawning* is not universal as it has not been demonstrated that this phenomenon occurs in all species, rather it may be restricted to cold water species with determinate fecundity and long vitellogenesis. Although non-annual spawning may also occur in male fishes, it has been reported considerably more often in females (Rideout & Tomkiewicz 2011). It is important to highlight that this stage in females includes two types:

i) An individual developing oocytes for first time (primiparous) and ceasing the development and hence remaining as pubescent and having never contributed to the egg production. It is debatable if these individuals are mature or remain as immature, but because oocytes develop into vitellogenesis, macroscopically they cannot be staged as immature.
ii) An individual that has previously spawned but will skip the current spawning season, i.e. skipped spawning (see Annex 5). It involves an earlier termination of the reproductive cycle of a mature fish prior to release of any gametes. Therefore the reproductive cycle does not culminate in spawning.

Macroscopically is difficult to distinguish *omitted spawning* from other normal maturity stages. Thus, at early stages of spawning omission, ovaries resemble a normal developing female; once the resorption is massive it may easily confused with a regressing ovary or even a late spawning capable. Finally, at advanced *omitted spawning* stage the ovary looks like regenerating and even immature. Only in species with strong synchronicity of the reproductive cycle at population level and short spawning season this stage can be identified, e.g. an ovary resembling early developing stage during the spawning season, or resembling regressing during the pre-spawning period. Nevertheless, given the inherent difficulty on distinguishing macroscopically this stage it is strongly recommended the use of histology for its assessment.

The stage *abnormal* should be used with caution. It is allowed to have it as stage but be careful not using it as "wild card or trash can". This should not be used for an individual that cannot be staged. This designation should only be used for clear problems in the normal development of the gonad such as necrosis, sclerosis, intersex in gonochoristic fishes or where part of the gonad looks healthy but not the majority or part of it. Intersex is an abnormal variation in sex characteristics (including gonads) that do not allow an individual to be definitively identified as male or female. Therefore, it is not a hermaphrodite that is one of the several sexual patterns observed in fish and normal in many species.

## 3.3 The use of the common maturity scale data for the construction of maturity ogives

It is not the intention of this section to lay out how maturity ogives should be estimated given the available data on the stock as this needs to consider many other fac-

tors including the temporal and areal coverage of the sampling and appropriate weighting factors for the input data (see for further details WKMOG, 2008). Rather, here we explain what data are to be utilised and what the output will mean at the stock level.

Currently the maturity ogives are used to estimate the Spawning Stock Biomass (SSB) from the total numbers of individuals in the stock. In essence this has not changed, however, the new category 5 (*omission of spawning*) allows one to estimate the total mature population and the mature population contributing to spawning in a given year. There was a certain amount of confusion in the past as in some cases individuals undertaking spawning omission (young individuals not contributing at their first potential spawning or skipped spawners) were included in the estimation of SSB either deliberately or inadvertently as 'non-mature individual' in the maturity ogives. This applies also to the stage 2a defined for Greenland halibut and potentially for oviparous elasmobranchs (see above), which are biologically mature although have never spawned and not contributing to SSB in the current season.

To estimate the total mature stock (which will be the subject of a fishery) then stages 2 to 5 should be included in the maturity ogive calculation as 'mature individuals' (Table 3-5). If the intention is to consider stock 'productivity', stock-recruitment relationships, Total Egg Production (TEP) estimates or reference points then only stages 2-4 should be considered in the estimate of the breeding population. To go further than TEP into estimates of Stock Reproductive Potential (SRP) then other considerations need to be taken in to account e.g. the effect of first and repeat spawners but these factors are also beyond the scope of this Workshop.

An important question discussed is how to use *omitted spawning* in stock assessment. This question also affects stage 2a. We agreed that for egg production estimation and for stock recruitment analysis, they should be excluded from the mature population, as these fish are not contributing to the spawning stock in that particular year. However, they should be considered as mature to estimate SSB as fishable stock and for spatial and temporal analysis of the maturity ogive (Table 3-5). Stage 6 should never be used for these estimations.

WKMSEL proposed a 4 stages scale for oviparous and male viviparous, and 6 stage scale for females viviparous. Only stage 1 is defined as *immature* (stage name) in these scales. However, in the definition of "maturity", stages 1 and 2 (*developing*) are considered as immature. This is highly confusing and reasons for it are not explained in the report. Biologically the *developing* phase defines a mature fish and once fish enter in this phase will never return to immature phase. Therefore, *developing* is a sexually mature fish (Table 3-5). This fish may not be able to copulate or to spawn, but yet is a sexually mature fish. However, as with *omitted spawning* or *developing but functionally immature*, this stage may be included in the maturity ogive calculation as 'mature fish' or as 'immature fish' depending on the use of the ogive. How to use the common maturity scale data for the construction of maturity ogives in elasmobranchs should be clarified by WKMSEL.

**Table 3-5 Proposal on how to consider maturity scale for the construction of maturity ogives. SSB for the purposes of estimating the annual egg production (TEP) and the numbers or biomass contributing to the immediate next generation of offspring. SSB for the purposes of calculating the total numbers or biomass of sexually mature individuals (those that could spawn either in the present or subsequent years) or the length (L50) or age (A50) at the midway point of the maturity ogive.**

| | | Ogive for SSB (TEP) | Ogive for SSB (L50 & Total mature stock) |
|---|---|---|---|
| Sexually immature | Immature | 0 | 0 |
| Sexually mature | Developing | 1 | 1 |
| | 2a. Developing but functionally immature | 0 | 1 |
| | Spawning | 1 | 1 |
| | 3a. Spawning capable or another term | 1 | 1 |
| | 3b. Actively spawning | 1 | 1 |
| | Regressing/Regenerating | 1 | 1 |
| | 4a. Regressing | 1 | 1 |
| | 4b. Regenerating | 1 | 1 |
| | Omitted spawning | 0 | 1 |
| | Abnormal | - | - |

## 3.4 Terminology

To interpret correctly the suggested terminology it is important to consider the proposed reproductive cycle (Figure 3-1) as a general scheme that can be applied to all male and female elasmobranchs and teleost fishes, including hermaphrodites and livebearers, cephalopods and some crustaceans[2].

The terminology proposed here is the consequence of the discussions held during the workshop and mostly based on previous work (Murua and Saborido-Rey 2003; Rideout and Tomkiewicz 2011; Brown-Peterson *et al.*, 2011; Lowerre-Barbieri *et al.*, 2011). A summary of the terminology is given in Table 3-6 and definition details in Annex 5.

The use of the following terms should be avoided:

- 'inactive' or 'resting': the gonad is never dormant; the cellular processes are always on-going and are extremely complex
- the maturity stage 'mature' as it can be confused with 'sexually mature'
- the maturity stage 'maturing' as it can be confused with 'maturation' which refers either to the ontogenic sexual maturation or to the oocyte germinal vesicle migration and or breakdown
- the maturity stage 'spent' (completion of spawning) as many maturity scales in the literature include 'partially spent' (which could be better named as 'partially spawned') for species shedding many egg batches labelling de facto 'oocytes' as 'eggs'

[2] A comprehensive review of the reproductive biology of Crustaceans has not been performed here, but only those species analysed in WKMSC.

**Figure 3-1 Scheme of the reproductive cycle and associated terminology**

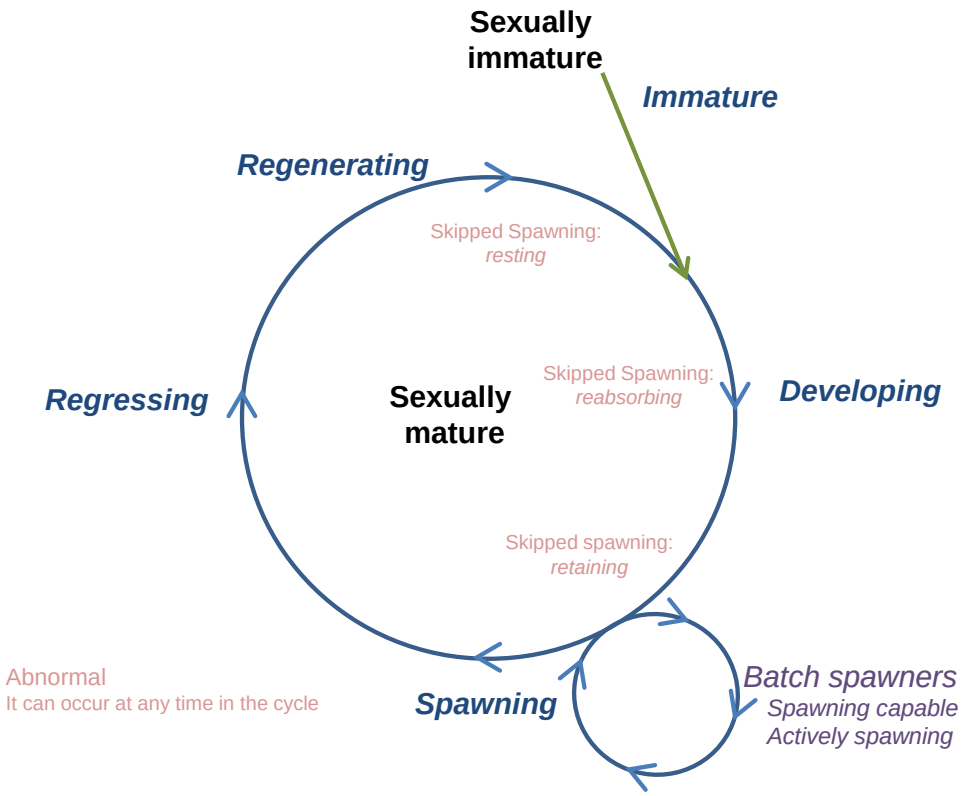

**Table 3-6 List of recommended terms with their short definition. For further details see Annex 5.**

| TERM | DEFINITION |
|---|---|
| Sexual maturity | The status of an individual in relation to achieved sexual capability (i.e. initiation of sex hormone production and activation of associated receptors) |
| Sexually immature | Sexual incompetence i.e. unable to produce offspring |
| Puberty | The transition between sexual incompetence and sexual capability |
| Sexually mature | The individual has the capability to enter, either regularly or continuously, the gonadotropin-dependent reproductive cycle with the resulting production of sex steroids and activation of related hormonal receptors |
| Sexual maturation | The processes of moving from a sexually immature to a sexually mature state |
| Egg | The sex cell following ovulation (secondary meiosis/release of secondary body), i.e. female gamete |
| Oocyte | Sex cell in first meiosis |
| Reproductive cycle | The formation of complete sex cells all the way from oogonia or spermatogonia to gamete production |
| Maturity stage/phase | Refers to the reproductive status at a specific part of the reproductive cycle. This division is based on overall characteristics possible to judge both macroscopically and microscopically. |
| Immature | A maturity phase; the initial part of the reproductive cycle. An individual is at this stage when it is sexually immature. Therefore, once it matures it will never return to this stage. |
| Developing | A maturity phase; the sex cells have entered the gonadotropin-dependent part of the reproductive cycle: production of follicle-stimulation hormone (FSH) and subsequent estradiol in females. The corresponding sex hormone in males is testosterone |
| Spawning | A maturity phase during which gametes are produced and released.<br>After completion of developing phase individual becomes developmentally and physiologically able to spawn in this phase, but does not spawn or release gametes continuously. For this reason this phase can be referred as spawning capable.<br>The period within this phase when individuals are truly releasing gametes can be referred as actively spawning. |
| Oocyte Maturation | A stage in oocyte development that initiates at the point in time when the nuclear material moves from the centre towards the animal pole and ends with the ovulation. |
| Macroscopic criterion | Possible to see by the naked eye |
| Microscopic criterion | Can only be detected by the aid of magnification tools |
| Whole mounts | The cell (e.g oocyte) is intact in shape, i.e. unsectioned, and typically looked at fresh or in the fixative under the microscope. |
| Indeterminate spawner | An individual in which the production of developing oocytes continues into the spawning period |
| Determinate spawners | An individual in which the production of developing oocytes is finalised prior to the onset spawning period |
| Breeding season | The period of time within the reproductive cycle during which a individual undergoes a major reproductive activity involving energetic allocation to reproduction, as vitellogenesis and spawning.<br>Spawning, therefore is part of the breeding season |

| TERM | DEFINITION |
|---|---|
| Spawning event or batch | It refers to each of the single episodes of releasing the eggs and sperm. |
| Spawning period | The time during which an individual liberates sex gametes, i.e. produce batches. |
| Spawning season | The calendar period in which a population liberates gametes |
| Spawning frequency | It refers to the number of spawning events within a spawning period (for an individual) or the spawning season (for the population). |
| Mass/Massive atresia | The majority of oocytes are in atretic condition.<br>If happening before spawning then, no eggs are released due to resorption of developing oocytes (see omission of spawning below)<br>If happening after the end of spawning indicates the regression of the ovary |
| Skipped spawning | It refers to the earlier termination of the reproductive cycle of a mature* individual prior to release of any gametes. Therefore the reproductive cycle does not culminate in spawning.<br>* Therefore 'Skipping' requires that the individual has spawned previously. |

# 4 Methods and protocols for an accurate maturity staging

It was out of the scope of this workshop to analyse and verify in deep the different methods and protocols for an accurate maturity staging. There is not sufficient information about the laboratories practices to stage maturity to conduct such a review. However, three aspects were discussed during the workshop: the definition of optimal sampling for maturity staging, when the use of histology is recommended and the potential use of whole mounts as a fast and cheap approach to assess maturity.

For a detailed review of methods and protocols for an accurate maturity staging see the recently produced *Handbook of applied fisheries reproductive biology for stock assessment and management* (Dominguez-Petit *et al*., eds, 2014). Important information can be also found in the reports of WKMAT and WKMSCWHS.

## 4.1 Sampling timing

There are two types of sampling where timing is relevant: sampling for the maturity workshops and sampling for the routine estimation of maturity. These two types of sampling have very different requirements and should not be mixed.

Sampling for Workshops is included in the Guidelines for Workshops on Maturity Staging (Annex 7). One of the main goals of the workshops is to conduct calibration exercises to assess the differences between laboratories, to improve procedures and to estimate misclassifications on maturity staging. For these purposes the sampling should be conducted when misleading stages are more probable to find in each species.

There are basically two sources for collecting biological data for estimation of critical parameters for target species: a scientific survey that attempts to sample individuals that represent the actual population of the target species in the ocean and surveys that sample individual from selected commercial catches. For scientific surveys, the researcher has more or less complete control over how the sampled individual are collected, while the sampling of commercial catches is often driven by the dynamics of the fishery. Sampling for maturity is often limited to the periods of the year when a survey is conducted or a fishery develops. We will not discuss here a full sampling design protocol or methodological approach that can be found elsewhere (e.g. Stransky *et al*., in press), but a quick consideration on the sampling timing in relation with the reproductive biology.

When staging maturity macroscopically the most reliable results are obtained when sampling is conducted at the time of the year when the possibility on confusing stages is minimised, i.e. when the differences between stages is either sufficiently large or some of the stages simply are not present. To define this period it is important to know the timing of the reproductive cycle, as this is species specific. In particular three aspects are important to know: the duration of the vitellogenesis, the duration of the spawning season and the synchronicity of spawning activity:

- In species with long vitellogenesis (slow oocyte developmental rates), a restricted spawning period and a relative short spawning season (due to high synchronicity of spawning periods among individuals) the optimal time is during prespawning, i.e. at late vitellogenesis. During this period is unlikely to find females at early development or at regenerating stages that can be misidentified as immature. Sampling during the spawning season is

also optimal, as the eventual regressing females occurring by the end of the season are rarely mistaken by immature. However, females omitting spawning may have degenerated ovary sufficiently to resemble immature.

- In species with short duration of vitellogenesis, i.e. few weeks, but yet a relative short spawning season (due to the synchronicity of spawning periods among individuals) only the peak of spawning season is suitable for a reliable estimation of maturity. Prespawning period is normally too short and many females in early developing may be mistaken as immature. Because the regressing/regenerating phase can be also a fast process, the end of the spawning season is not an adequate period as these females may resemble immature.
- Finally, in species with a protracted spawning season resulting from a low synchronicity of spawning periods among individuals, although the peak (or peaks) of the spawning season is the best choice, microscopically assessment/calibration of each stage should be performed. Because of the asynchronicity mentioned even during the spawning season early development and regenerating females can be found and mistaken as immature.

If maturity staging outside these respective periods is required, this should be based on histological information.

## 4.2 Histology

Although the proposed common scale tries to minimize the sources of errors on staging maturity, there are still uncertainties when staging macroscopically, especially when this is conducted outside the optimal sampling time (see previous section). These uncertainties are considerably reduced using histology for an accurate maturity staging.

The importance of histology in maturity staging is acknowledged in many previous ICES workshops and it has been referred as the only tool that allows an accurate identification of the maturity stage (Saborido-Rey & Junquera 1998; Murua *et al*., 2003; Vitale *et al.*, 2006; Lowerre-Barbieri, Brown-Peterson *et al*., 2011). However, in this context histology has been mainly used for validating macroscopic staging and mostly focused on females.

When maturity is macroscopically estimated during the optimal period (see above) and for populations with synchronized spawning seasons, results are likely comparable to the use of histology. However, if these conditions are not met, the use of histology is strongly recommended, and at least histology should be used to assess stages with highest uncertainties, i.e. immature-regressing/regenerating-omitted spawning. Histology should be compulsory to assess stages than have been merged. During the regenerating phase within the reproductive cycle only histology produce reliable results and not without difficulties (Junquera & Saborido-Rey 1996).

Nevertheless, it is recommended to conduct regularly validation exercises comparing macro- and microscopically estimated ogives, even if these have been estimated during the optimal period. Histological techniques applied to estimation of maturity have been described in numerous studies ((Tomkiewicz *et al.*, 2003; Lowerre-Barbieri, Brown-Peterson, *et al*., 2011; Alonso-Fernández *et al.*, 2011).

## 4.3 Whole mounts (females)

In this technique the biological material of interest is directly examined under the light microscope or stereomicroscope (Figure 4-1). This material might either be fresh or fixed but in both cases all cells should be intact and seen in their true 3-D dimension. As this is a simple method (Kjesbu 1991) it has been applied since the early days of marine biology research (West 1990). Logically this fast and cheap approach means that further insight might in some cases depend on other methods, in particular histology. However, one main advantage in relation to the last mentioned method is that the size of objects (such as cells) in whole mounts (wm) can be measured without any systematic bias as well as the counting of these objects is independent of their size. Hence small and large objects have an equal chance of being observed provide the applied magnification is adequate for the purpose. Nevertheless, the fact that each object is only observed from its outside means that internal processes cannot be described explicitly. Fortunately, in many cases there is a close relationship between object size and object microstructure as demonstrated for marine fish oocytes (Óskarsson *et al.*, 2002). Also new staining methods open up for the possibility to recognize different types of objects, such as post-ovulatory follicles (POFs), based not on only on their appearance (size, shape and contrast) but also on their ability to take colour and the type of colour expressed (Witthames *et al.*, 2009). Separation between normal and atretic cells also seems possible based on considerations of shape factor and trends in transparency; an atretic oocyte will be more irregular in shape and display a peripheral band of high transparency (Óskarsson *et al.*, 2002). So this traditional technique has a great underutilised potential, especially when applied in connection with image analysis to automatically measure objects (Thorsen & Kjesbu 2001; Alonso-Fernández *et al.*, 2009). Despite this, costly histological examinations are often conducted without any prior examination if the same study could have been undertaken by the wm method. With the present installation of computers nearly everywhere and the free access to image analysis programs on the internet the wm method should be considered as an option for further exploration, when relevant.

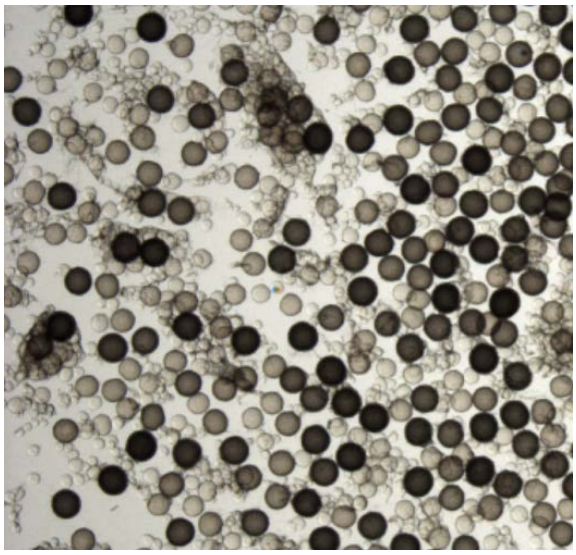

**Figure 4-1. A developing cod ovary showing both transparent previtellogenic oocytes, weakly semi-transparent cortical alveoli oocytes and non-transparent oocytes in different phases of vitellogenesis. Maturity staging: possibilities and limitations**

The wm method can be applied both in the laboratory/fish processing plant as well at sea where there is a suitable working place. Wm preparations are useful to validate macroscopic staging of ovaries, provided the wm protocol as such is successfully calibrated in advance with histological results through parallel examinations. A central practise is to measure the size of the largest oocytes (leading cohort (LC)) in the wm sample and use this is a guide when assessing the maturity stage under the microscope (Kjesbu 1991). As variation in LC diameter is often small, only a few oocytes (≈ 5) normally need to be reported. In many cases it is simply enough to cast a glance at the size because other supporting factors provide enough information to do the right staging. In addition to measured size, transparency is an important criterion for proper oocyte staging and thereby maturity staging. Here previtellogenic oocytes and hydrated oocytes are highly transparent, while cortical alveoli oocytes and early vitellogenic oocytes are semi-transparent and mid and late vitellogenic oocytes are fully non transparent.

In the case of fixed tissue a couple of weeks should be allowed for the oocyte size to settle prior to any measurements. Fresh tissue should be kept in isotonic sea water (around 1.07%). This is easily made by mixing 1/3 of sea water with 2/3 of fresh water. To mention only, in brackish water areas less fresh water should, of course, be added. The ovarian tissue is spread gently on the bottom of a petri dish (using if needed a brush) and the material thereafter observed under the microscope at a moderate level of magnification. Light settings appear important (Thorsen & Kjesbu 2001). Fresh samples kept in isotonic water are normally thrown away after the job is done due to likely swelling of the oocytes if they are kept overnight, or due to dilution of the storage fluid if the sample is poured into a glass with fixative.

The wm method is in particular useful to separate between early developing and developing specimens, i.e. in cases where the latter category show oocytes in the cortical alveoli stage or oocytes in early vitellogenesis, a situation which is not detectable by the naked eye. The wm method also has a potential for separating between immature and regressing/regenerating specimens, in particular when the sample contains 'left-over eggs' or vitellogenic/atretic oocytes. In more advanced studies the LC diameter of the previtellogenic oocytes can be measured as these are typically smaller in specimens that have just completed a spawning season than in specimens that have not yet entered sexual maturity (Witthames *et al.*, 2010). There are few benefits in using the wm method on well-developed gonads as the maturity stage can be safely assessed externally. However, if the aim is to track oocyte growth then the wm method also will most appropriate in this situation.

Recommendation

It is suggested that the wm method is carefully calibrated before taken into practical use (cf. establishing proper ICES workshops).

# 5 Maturity data and stock assessment

We reviewed a total of 148 stocks of 53 species from 8 ecoregions from which ICES provides some type of advice (Annex 6). In 56 stocks (38%) there is no analytical assessment and maturity is not even mentioned in the reports. In 4 stocks (3%) with analytical assessment, maturity is not mentioned in the report and assumed that it is not used at all. Finally in 88 stocks (59%) there is some kind of description of how maturity is used in the assessment.

Within those 88 stocks our analysis revealed that:

- In 12 (14%) maturity is not used at all (due to several reasons)
- In 13 (15%) a single knife edge ogive is used
- In 3 ( 3%) the ogive is modelled
- In 39 (44%) the maturity ogive is based in real observations but over a short period
- In 21 (24%) the maturity ogive is based on real observations over a sufficient period of time

Therefore in 60 stocks maturity data based on biological estimation is being used. Nevertheless in 88 stocks, out of 148 where ICES provide advice, maturity is not used or is used improperly.

Within those 60 stocks using real maturity data, the majority, 39, uses a single maturity ogive across years or very few years of data are used, i.e. virtually there is no variation in maturity incorporated into the assessment. And finally in only 21 stocks (14% of the total advised stocks) the maturity ogive has been estimated on regular basis. The period where maturity was estimated ranges for 23-65 years covering between 21 and 100% of the assessment period (Table 5-1).

Out of the 60 stocks where some kind of biologically sound maturity data are used, in only twelve the maturity stage method is reported (in all cases was visual or macroscopic staging). In the vast majority, 58 stocks, it is not reported if ogives are sex-specific or simply maturity ogives are combined. In the other two stocks, in one female only maturity ogive is used (Greenland halibut in Subareas I and II) and in the other one single sex-specific maturity ogive is used for all years in the assessment (Plaice in Division VIIa, Irish Sea).

EGs should provide comprehensive reports on how the maturity data being used, and more specifically, at least: the method used to estimate maturity, in which sex and how a sex-specific maturity ogive is used in the assessment, source of data (survey, commercial sampling), the time of the year when the sampling was conducted, and years of proper estimation.

**Table 5-1. The 21 stocks where maturity is estimated on regular basis with the indication of the first year considered in the assessment, the first year when maturity was estimated, the number of years with maturity data and the proportion of overlap between the two periods (All stocks referred to the 2011 advice)**

| SPECIES | STOCK | ASSESSMENT | REAL OGIVE | | % |
|---|---|---|---|---|---|
| Anchovy | Division IXa | 1988 | 1988 | 23 | 100.0 |
| Cod | Division IIIa East (Kattegat) | 1971 | 1989 | 40 | 55.0 |
| | Division Va (Icelandic cod) | 1955 | 1955 | 56 | 100.0 |
| | Subareas I and II (Northeast Arctic cod) | 1946 | 1946 | 65 | 100.0 |
| | Subdivision Vb1 (Faroe Plateau) | 1961 | 1983 | 50 | 56.0 |
| | Subdivisions 22–24 | 1970 | 1991 | 41 | 48.8 |
| Greenland Halibut | Subareas I and II | 1964 | 1984 | 47 | 57.4 |
| Haddock | Division Va | 1979 | 1984 | 32 | 84.4 |
| | Division Vb | 1957 | 1982 | 54 | 53.7 |
| | Subareas I and II (Northeast Arctic) | 1950 | 1980 | 61 | 50.8 |
| Hake | Divisions VIIIc and IXa (Southern stock) | 1982 | 1982 | 29 | 100.0 |
| Herring | Division Via (North) | 1957 | 1991 | 54 | 37.0 |
| | Division VIIa North of 52º 30 N (Irish Sea) | 1972 | 2003 | 39 | 20.5 |
| | Subarea IV and Divisions IIIa and VIId (North Sea autumn spawners) | 1988 | 1988 | 23 | 100.0 |
| | Subdivision 30, Bothnian Sea | 1973 | 1983 | 38 | 73.7 |
| | Subdivision 31, Bothnian Bay | 1980 | 1983 | 31 | 90.3 |
| | Northeast Atlantic (Norwegian spring-spawning herring) | 1950 | 1950 | 61 | 100.0 |
| Saithe | Division Va (Icelandic saithe) | 1985 | 1985 | 26 | 100.0 |
| | Division Vb | 1962 | 1983 | 49 | 57.1 |
| Sandeel | Division IIIa and Subarea IV | 1983 | 2005 | 28 | 21.4 |
| Sardine | Divisions VIIIc and Ixa | 1978 | 1988 | 33 | 69.7 |

# 6 Updating guidelines

The proposed guidelines are included in Annex 7. Changes proposed by WKMATCH are highlighted in red. Following is the rationale for the changes proposed:

PGCCDBS recommended creating of the **European Fish Maturity Stagers Forum** (similar to the European Age Readers Forum[3]) in tandem with the **WebGR** tool (http://webgr.wiki.azti.es/doku.php) to streamline the preparation and the implementation of maturity staging exercises and workshops. WKMATCH is of the opinion that although this forum can be created, it is not sufficient as maturity staging is not comparable to age determination. Age readers tend to be the same people over time, while maturity data is collected by observers and/or participants in surveys that not necessarily are the same persons. In this sense maturity stage is consider as fish length and weight, i.e. it is collected by the "sampler". Therefore, beyond the forum proposed for experts, WKMATCH considers that training (of the observers) is the major issue for staging maturity, and it should be strengthen within the umbrella of ICES.

The current guidelines indicate: "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." We instead prose that experts on histology, maturation process and the reproductive ecology/biology as well as those in charge in collecting process of the species of concern or at least a related species should participate in the workshop.

We acknowledge the importance of having fresh samples during the workshop, so the timing of the meeting should be carefully decided. However, for many species the duration of each phase/stage within the reproductive cycle is long enough to prevent obtaining fresh samples on a variety of stages during the meeting. Also the most interesting timing for holding the meeting is not when many maturity stages can be found, but when the stages are more difficult to be distinguished, as it is normally the main source of errors. Thus, the reproductive biology of the species should be carefully considered for selecting the workshop dates.

The use of both histological slides and images as a tool for calibration prior to a workshop is highly recommended. We consider especially important to conduct such calibration exchange **before** the workshop because results will point out possible discrepancies between labs and that has to be addressed during the workshop. A statistical report of the exchange results should be provided **before** the workshop. It must include a comparison of the observed maturity stage with validated histological stage, as well differences in staging between laboratories in terms of precision and accuracy; sources of discrepancies should also be analysed.

We consider that accuracy within and between laboratories can be improved by means of whole-mounts (see section 4.3).

We strongly recommend using the proposed 4+2 maturity scale, creating, only if necessary, appropriating subdivisions. But it is critical to keep the scale as simple and efficient as possible.

[3] http://groupnet.ices.dk/AgeForum/default.aspx

The merging of different stages should not occur, even if they can be confused macroscopically. For some species it has been suggested merging stages such as immature/recovering or recovering/skip spawning, because even during the optimal sampling time there are difficulties on distinguishing these stages. For these species it was recommended that histological analyses of subsamples should be undertaken to assess the proportion of each stage. However, we recommend not merging different stages and in these cases a combined code should be used, e.g. 1/4b, 4b/5. In this manner the consistency of stages definition and codes is maintained across species and still stages 1 and 4b can be used if distinguishable. We support the recommendation that histology should be conducted in subsamples of these stages.

# 7 Conclusions and general recommendations

## 7.1 Conclusions

- A single scale of 4+2 stages is defined which is believed to be universal, that is, it can be used for the majority of species, although viviparous and hermaphrodites may need some adaptation. Species-specific particularities can be reflected creating subdivisions and never modifying the 6 main stages.
- The definition of each stage needs to be linked to biological phases and incorporate into its description species-specific aspects relevant for an easy identification of each stage.
- The use of the terminology for maturity stages considers a general scheme of the reproduction that can be applied to all male and female elasmobranchs and teleost fishes, including hermaphrodites and livebearers. A full glossary of terminology was compiled.
- When staging maturity macroscopically timing of the sampling is critical to obtain reliable results. To define this period it is important to know the timing of the reproductive cycle, as this is species specific. If maturity staging outside the optimal periods is required, this should be based on histological information.
- Whole mounts preparations are useful to validate macroscopic staging of ovaries being particularly useful to separate between early developing and developing specimens, immature and regressing/regenerating specimens, or even specimens that have just completed a spawning season from those have not yet entered sexual maturity.
- Generally, maturity data are not used or are used improperly in stock assessment. This includes the use of time invariant maturity ogives when annual ogives can be available. Only in 14% of the total advised stocks the maturity ogive has been estimated on regular basis and in these cases they are used properly in the assessment. Therefore, lack of data and/or poor quality is the main causes of maturity not being used.
- There is a general lack of information in the reports on how the maturity data was collected, ogive estimated, quality control and other relevant information. There is a need to determine what maturity data are required for assessment purposes, including how phenomena such as skipping spawning should be included in assessments. In spite of the effort on collecting maturity data, almost in 100% of the cases sex-specific ogives are combined without analysing the impact of this.

## 7.2 Recommendations

1) Maturity staging
   a) The 6 stage codes and names should be used for all species and both sexes without exception; species-specific particularities should be reflected in the subdivisions and never modifying the 6 main stages.
   b) The merging of different stages should be avoided and instead a combined code should be used, e.g. 1/4b, 4b/5. In this manner the consistency of stages definition and codes is maintained across species.

c) The stages Omitted spawning and Abnormal (codes 5 and 6) are not fully universal as they are not present in all species, so they should be used with caution. In particular Abnormal should not be used for specimens that cannot be staged.
d) The stage 0 referring to undetermined sex should not be used within a maturity scale, as it is not a maturity stage, but a sex code, corresponding to a different variable. If there is doubt about the sex of an individual, then obviously maturity should not be staged.
e) Several traditionally used terms in relation to reproduction should be avoided and adopt the terminology proposed for further use.
f) Training (of the observers) is the major issue for maturity staging, and it should be strengthen within the umbrella of ICES.
g) The understanding of species biology it is critical when using the maturity scale. When staging a species, it is important to have an understanding of the biology of the species and its reproductive cycle in the sampling area. This helps to distinguish the transitions between the stages that most frequently are not easily to distinguish. It is recommended that institutes carry out in-house workshops on the reproductive biology of the species and maturity staging.
h) It is suggested that the whole-mounts method is carefully calibrated before taken into practical use.

2) Maturity Workshops
a) Workshops should discuss the new and general scale in their respective WKs by e-mail to assess the correspondence with the agreed scale, and evaluate the uncertainties and the problems this new general scale may cause.
b) The most important aspect in a Workshop are the participants. ICES should ensure an appropriate attendance and a required level of basic knowledge, both on maturity studies and on the species targeted by the Workshop. Beyond of experts in the matter, the participants should be trained people, this can be achieved by training courses in ICES.
c) Before having second versions of previous workshops, an exchange should exist. The exchange should follow a training program that should have been established during the workshop, but optionally can be developed later. But it is important to consider that maturity staging requires training (as otolith reading does), and without it staging discrepancies can be simple derived from lack of experience of some readers, rather than in a criteria itself.
d) The use of both histological slides and images as a tool for calibration prior to a workshop is highly recommended.
e) Future WKs should start to investigate in the use of Whole mounts for maturity staging.
f) Before organizing a maturity workshop on new species, ICES should ensure a critical mass of participants is achieved and a real need exists. The targeted species has to be routinely staged in, at least, three different laboratories. If a species is not routinely staged, i.e. there is no historical data series, then the proposed maturity scale should be applied from the beginning. Otherwise the single laboratory owing the historical data series should compare and calibrate the maturity scales. Also, before implementing a new Workshop an exchange should be done (following the guidelines of WKMATCH) to ascertain if discrepancies exists.

g) WKMATCH has not found a new species for which a workshop should be developed. However, given the importance of hermaphroditic species on developing European fisheries (sea breams, wrasses, groupers,…) and the lack of a proper maturity scale for this reproductive strategy, WKMATCH recommend ICES to establish a Workshop on maturity stage of hermaphroditic species.
h) WKMATCH acknowledge that maturity staging of males has been addressed in most of the maturity WKs. However, in general only a few people/laboratories have adequate knowledge of male maturation and reproductive cycles, and very few experts on this matter have attended WKs. A maturity scale should have strong and clear correspondence with individual biology, and hence male biology should be better considered. As with females a validation based on histology is required, and therefore it is required experts on male histology to attend WKs.

3) A number of aspects need to be addressed by the Assessment Expert Groups.
   a) How to use omitted spawning in stock assessment needs further discussion. For example when estimating egg production skippers should be removed, but for SSB (the whole sexually mature stock) as fishable stock skippers should be included.
   b) EGs should provide comprehensive reports on how the maturity data is used, and more specifically, at least: the method used to estimate maturity, in which sex and how a sex-specific maturity ogive is used in the assessment, source of data (survey, commercial sampling), the time of the year when the sampling was conducted, and years of proper estimation.
   c) The impact on the assessment of combining sex-specific maturity ogives should be analysed.

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# Annex 1: List of participants

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# Annex 2: Agenda

**Monday 11th June**

| | |
|---|---|
| Morning (9:00-13:00) | Welcome and adoption of the agenda |
| | Local organization and logistics (Barbara Zorica and Vanja Kec) |
| | Sharepoint<br>General considerations about the Workshop. Maturation and maturity staging. Maturity ogive. Sampling strategy. Terminology. ICES Guidelines. (Fran Saborido-Rey)<br>General Discussion |
| | Coffee break |
| | ToR a) Revising and enhancing consistency in the currently adopted methods<br>Presentations of Maturity Workshop results (15 min each): Gualtiero Basilone (WKSPMAT), Paola Belcari (WKMSCEPH), Fran Saborido-Rey (WKMSREGH and WKMSCWHS) |
| 13:00-14:30 | Lunch |
| Afternoon (14:30-18:00) | ToR a) Cont.<br>Consistency across workshops.<br>Update Table WKMAT<br>Methods and agreements in Workshops.<br>ICES guidelines. General discussion |
| | Coffee break |
| | ToR a) Cont. |

**Tuesday 12th June**

| | |
|---|---|
| Morning (9:00-13:00) | ToR b) Analyse, verify and agree methods and protocols for an accurate maturity staging<br>ICES Guidelines<br>Optimal time for sampling<br>Histological analyses- Manuals and details on literature.<br>Macroscopic and microscopic criteria |
| | Coffee break |
| | ToR b) Cont.<br>Reproductive strategies<br>Is a single scale possible? |
| 13:00-14:30 | Lunch |
| Afternoon (14:30-18:00) | ToR b) Cont.<br>General discussion. Proposal of methods and protocols. |
| | Coffee break |
| | ToR b) Cont. |

**Wednesday 13th June**

| | |
|---|---|
| Morning (9:00-13:00) | ToR c) Develop standard protocols for quality control and tools to analyse error and bias<br>Histological analyses<br>Terminology<br>Macroscopic and microscopic criteria<br>WebGr |
| | Coffee break |
| | ToR c) Cont. |
| 13:00-14:30 | Lunch |
| Afternoon (14:30-18:00) | ToR d) Evaluate the impact of a newly developed common scales on historical databases<br>Does the new scale differ from old ones in terms of maturity? |
| | Coffee break |
| | ToR d) Cont. |

**Thursday 14th June**

| | |
|---|---|
| Morning (9:00-13:00) | ToR e ) Update the Guidelines for collecting maturity data and developing maturity<br>PGCCDBS Guidelines (see sharepoint)<br>Guidelines for Work-shops on Maturity Staging<br>Guidelines for collecting maturity data and histological analyses for maturity workshops |
| | Coffee break |
| | ToR e) cont.<br>Guidelines for collecting maturity data and matur-ity ogive estimation for stock assessment purpose |
| 13:00-14:30 | Lunch |
| Afternoon (14:30-18:00) | ToR f) Advise on the best way to incorporate newly collected data into assessment<br>Revision of maturity data use by assessment working groups<br>Availability of maturity data |
| | Coffee break |
| | ToR f) Cont.<br>Reasons preventing the use of maturity data. Quality and quantity<br>Does the new information on maturity affect assessment: source of uncertainty<br>Advise the best way on incorporating the new information into assessment |

# Annex 3: ICES Maturity staging Workshops

| ACRONYM | WORKSHOP | YEAR |
|---|---|---|
| WKMAT | Workshop on Sexual Maturity Sampling | 2007 |
| WKMSCWHS | Workshop on Sexual Maturity Staging of Cod, Whiting, Haddock and Saithe | 2007 |
| WKMSHM | Workshop on Sexual Maturity staging of Hake and Monk | 2007 |
| WKMSMAC | Workshop on Sexual Maturity Staging of Mackerel and Horse Mackerel | 2007 |
| WKSPMAT | Workshop on Small Pelagics (Sardina Pilchardus, Engraulis Encrasicolus) Maturity Stages | 2008 |
| WKMSC | Workshop on crustaceans (Aristeus antennatus, Aristaeomorpha foliacea, Parapenaeus longirostris, Nephrops norvegicus) maturity stages | 2009 |
| WKMSCEPH | Workshop on Sexual Maturity Staging of Cephalopods | 2010 |
| WKHERMAT | Workshop on estimation of maturity ogive in Norwegian spring spawning herring | 2010 |
| WKMSEL | Workshop on Sexual Maturity Staging of Elasmobranchs | 2010, 2012 |
| WKMSSPDF | Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder | 2010, 2012 |
| WKMSREGH | Workshop on Sexual Maturity Staging of Redfish and Greenland Halibut | 2011 |
| WKMSTB | Workshop on Sexual Maturity Staging of Turbot and Brill | 2012 |

# Annex 4: Maturity scale

## Females

| MATURITY | CODE/NAME | DESCRIPTION |
|---|---|---|
| Sexually immature | **1. Immature** | Individuals that have never reproduce before. The ovary is barely discernible and it contains no developed oocytes. |
| Sexually mature | **2. Developing** | In the vast majority of the species this is a single and clear stage spanning from the beginning of the breeding season to the imminence of spawning. Thus, this corresponds to the oocyte development period from cortical alveoli until some (in batch spawners) or all (in total spawners) oocytes reach the full development. |
| | 2a. Developing but functionally immature | Pubescent. Only for species with reproductive cycle longer than one year. In some species as Greenland halibut, a subdivision is recommended because during the year after maturation fish is sexually mature showing developing oocytes, but those oocytes will not be spawn that particular year, so not contributing to spawning stock, thus they are functionally immature. |
| | 2b. Developing but functionally mature | In species with reproductive cycle longer than one year, fishes at this stage will spawn within the year, and hence contributing to spawning stock. Vitellogenic oocytes are in more advanced stage than in 2a. |
| | 3. **Spawning** | Spawning generally means the female is clearly ovulating or releasing eggs, i.e. with the presence of hydrated eggs or equivalent structure. This stage thus helps to define the spawning season.<br>However, in some species is possible, and even convenient, to identify individuals that are within spawning season but in the period between batches, or very close to the spawning season, i.e. the spawning capable phase. Then, this stage can be divided in: |
| | 3a. Spawning capable | There are evidences of the imminence of spawning or that the specimen has already initiated the spawning (at least one batch has been produced in batch spawner species). Thus in batch spawners this stage correspond to the period between batches. In total spawners this is a short stage right before spawning.<br>This stage became macroscopically more obvious in the second half of the spawning season. |
| | 3b. Actively spawning | The ovulation of the oocyte is being produced. This means that hydrated oocytes are observed in species where hydration occurs. Other signals of ovulation can be detected as the presence of fertilized eggs/embryos in viviparous fish, the presence of oocytes in the oviducts in cephalopods or visible eggs in crustaceans. |
| | | Other subdivisions related with spawning activity can be defined if necessary. |

| MATURITY | CODE/NAME | DESCRIPTION |
|---|---|---|
| | **4. Regressing/ Regenerating** | This is a long stage for most of the species representing the whole period between the end of the spawning and the start of the next breeding season. In iteroparous species during this phase, the ovary is reorganized and prepared for next cycle. In semelparous species is short as the individual die soon after the end of the spawning.<br>This stage includes both the regressing and recovering phases, but in the majority of the cases can be considered as a single stage without the need on subdividing it.<br>However, while regressing has features that allow to be identified macroscopically, in some species the regenerating phase can be confused with immature or omitted spawning fish. In these cases two subdivisions can be used. |
| | 4a. Regressing | Regressing: returning to a former state. Ovary reabsorbs material from previous activity. There are important metabolic and physiological activity within the ovary and macroscopically the ovary shown clear evidences of prior spawning activity, but clearly egg production is finished. |
| | 4b. Regenerating | Regenerating: returning to a normal state. Ovary reaches a normal state and prepare for next breeding season, therefore present only in iteroparous species. There are metabolic and physiological activity affecting primarily PG oocytes, but these are not evident and macroscopically the ovary returns to a state resembling an immature or early developing ovary. |
| | **5. Omitted spawning** | Includes both, i) individuals developing oocytes for first time and cancelling the development and hence remaining as pubescent and having never contributed to the egg production, and ii) specimens that have previously spawn but will skip the current spawning season. Macroscopically cannot be distinguished i and ii. But timing of the process in relation to normal development may help (this issue will be further explained in the report). |
| | **6. Abnormal** | This stage has to be always clearly defined. It is not related with the presence of massive atresia, is not something difficult to classify or indeterminate. It reflects clear problems in the normal development of the gonad. It may happen that part of the gonad looks healthy but not the majority or part of it. Necrosis, sclerosis, intersex in gonochoristic species. It is allowed to have it as stage but be careful not using it as "wild card-trash can". |

## Males

| MATURITY | CODE/NAME | DESCRIPTION |
|---|---|---|
| Sexually immature | **1. Immature** | Individuals that have never reproduce before. The testes are small, barely discernible, threadlike and often clear or translucent. |
| Sexually mature | **2. Developing** | In the vast majority of the species this is a single and clear stage spanning from the beginning of the breeding season to the imminence of spawning. Thus, this corresponds to the active spermatogenesis period. Small testes but easily identified, blood vessels more prominent, empty transparent spermatoducts. |
| | **3. Spawning** | Spawning generally means the male is developmentally and physiologically able to spawn in this cycle, i.e. with the presence of milt in lumen of lobules (that can be extruded under pressure) and/or sperm ducts (flows freely). This stage thus helps to define the spawning season. Large and firm testes |
| | **4. Regressing/ Regenerating** | This is a long stage for most of the species representing the whole period between the end of the spawning and the start of the next breeding season. During this phase, the testes is reorganized and prepared for next cycle.<br>This stage includes both the regressing and recovering phases, but in the majority of the cases can be considered as a single stage without the need on subdividing it.<br>However, while regressing has features that allow to be identified macroscopically, in some species the regenerating phase can be confused with immature or omitted spawning fish. In these cases two subdivisions can be used. |
| | 4a. Regressing | Regressing: returning to a former state. Small, contracted, and flaccid testes, no milt release with pressure. |
| | 4b. Regenerating | Regenerating: returning to a normal state. Testes reach a normal state resembling immature or early developing testes and prepare for next breeding season. Small testes, often threadlike. |
| | **5. Omitted spawning** | Macroscopically there are serious difficulties in identifying this phenomenon in males due to the small size of spermatogenic cell stages, so histological analyses is advised to assess. Generally testes at this stage resemble early developing or spent stages during a period they should not be present. |
| | **6. Abnormal** | This stage has to be always clearly defined. It is not something difficult to classify or indeterminate. It reflects clear problems in the normal development of the gonad. It may happen that part of the gonad looks healthy but not the majority or part of it. Necrosis, sclerosis, intersex in gonochoristic fishes. It is allowed to have it as stage but be careful not using it as “wild card-trash can”. |

# Annex 5: Terminology

| TERM | DEFINITION | REMARKS |
|---|---|---|
| Sexual maturity | The status of an individual in relation to achieved sexual capability (i.e. initiation of sex hormone production and activation of associated receptors) | This is an overall concept which can be subdivided into specific periods (ages) in the life of an individual |
| Sexually immature | Sexual incompetence i.e. unable to produce offspring | An individual is sexually immature only once in its life |
| Puberty | The transition between sexual incompetence and sexual capability | Often called 'the adolescent phase' or in some cases also 'dummy runs' |
| Sexually mature | The individual has the capability to enter, either regularly or continuously, the gonadotropin-dependent reproductive cycle with the resulting production of sex steroids and activation of related hormonal receptors | When an animal becomes sexually mature it remains so for the rest of its life |
| Sexual maturation | The processes of moving from a sexually immature to a sexually mature state | It refers exclusively to the ontogenetic process that occurs once in the lifetime of an individual. To describe the development of the reproductive status of an individual during a reproductive cycle, one should use "ripening" |
| Egg | The sex cell following ovulation (secondary meiosis/release of secondary body), i.e. female gamete | In many cases corresponding to the point when the sex cell is released into the water to be fertilized |
| Oocyte | Sex cell in first meiosis | Typically sex cells located in the ovary |
| Reproductive cycle | The formation of complete sex cells all the way from oogonia or spermatogonia to gamete production | Stem cell/primordial cell formation is not normally considered as a part of the reproductive cycle as such.<br>The term is analogous to oogenesis in females and spermatogenesis in males, or the sum of all defined maturity stages. |
| Maturity stage/phase | Refers to the reproductive status at a specific part of the reproductive cycle. This division is based on overall characteristics possible to judge both macroscopically and microscopically. | According to some proposal (Brown-Peterson *et al.*, 2011) to avoid confusion, phase should refer to specific period of the reproductive cycle when the whole gonad is assessed; stage thus exclusively referring to each period of the oocyte development. |
| Immature | A maturity phase; the initial part of the reproductive cycle. An individual is at this stage when it is sexually immature. Therefore, once it matures it will never return to this stage. | In females the most advanced oocytes are still in the primary growth (PG) stage, or spermatogonia in males. The individual do not have the capability to produce gametes during the current reproductive cycle. |

| TERM | DEFINITION | REMARKS |
|---|---|---|
| Developing | A maturity phase; the sex cells have entered the gonadotropin-dependent part of the reproductive cycle: production of follicle-stimulation hormone (FSH) and subsequent estradiol in females. The corresponding sex hormone in males is testosterone (11-keto-). | A maturity phase characterized by a marked increase in oocyte size, and a marked reduction in male sex cells. The first sign is the detection of cortical alveoli (CA) oocytes and production of spermatocytes in females and males respectively.<br>For females, confirmation is given by the appearance of vitellogenin in blood plasma or yolk granules in oocyte cytoplasm, i.e. the presence of vitellogenic oocytes in the ovary. The presence of CA oocytes to define the onset of this phase should be treated with caution as an absolute criterion without previous investigation. |
| Spawning | A maturity phase during which gametes are produced and released.<br>After completion of developing phase individual becomes developmentally and physiologically able to spawn in this phase, but does not spawn or release gametes continuously. For this reason this phase can be referred as spawning capable.<br>The period within this phase when individuals are truly releasing gametes can be referred as actively spawning. | Gonadotropin production switches from FSH to luteinizing hormone (LH) and the sex steroids falls into the class of maturation-inducing steroids (MIS). Oocytes enter successively 'oocyte maturation', oocyte 'hydration' (with exceptions) ('clear eggs') and 'ovulation' ('running eggs').<br>Spermatozoa is present in lumen of lobules and/or sperm ducts. All stages of spermatogenesis (Sg2, Sc, St, Sz) can be present. Spermatocysts are visible throughout testis, with an active spermatogenesis. |
| Oocyte Maturation | A stage in oocyte development that initiates at the point in time when the nuclear material moves from the centre towards the animal pole and ends with the ovulation. | Refers specifically to oocyte maturation or 'final oocyte maturation', which are synonymous to germinal vesicle migration, 'nuclear migration' or just 'the migratory stage". In many species it involves also the oocyte hydration. |
| Macroscopic criterion | Possible to see by the naked eye | Usually used for the rapid classification of stages of the ovarian development, i.e. phases. |
| Microscopic criterion | Can only be detected by the aid of magnification tools | Refers normally to the use of optical microscopy |
| Indeterminate spawner | An individual in which the production of developing oocytes continues into the spawning period | Typically seen in warm water/sub-temperate species with long spawning periods |
| Determinate spawners | An individual in which the production of developing oocytes is finalised prior to the onset spawning period | Typically seen in temperate/arcto-boreal species with restricted spawning periods |
| Breeding season | The period of time within the reproductive cycle during which a individual undergoes a major reproductive activity involving energetic allocation to reproduction, as vitellogenesis and spawning.<br>Spawning, therefore is part of the breeding season | Usually a breeding season occurs annually; however, some species may show several breeding seasons in a year, while others may have prolonged breeding seasons, i.e. two years or more. The latest is not equivalent to skipped spawning; it simply involves a long oocyte developing. |

| TERM | DEFINITION | REMARKS |
|---|---|---|
| Spawning event or batch | It refers to each of the single episodes of releasing the eggs and sperm. | In some species there is a unique spawning event during the reproductive cycle (total spawners), while other species produce two or more batches (batch spawners). |
| Spawning period | The time during which an individual liberates sex gametes, i.e. produce batches. | Should be kept separate from 'spawning season' which refers to the whole spawning population.<br>Within this period there are days during which a batch is produced, while the time period between spawning events, is referred as spawning interval. There is a single spawning period within a reproductive cycle. |
| Spawning season | The calendar period in which a population liberates gametes | It is therefore a term defined at the population level. Spawning seasonality varies in terms of its duration (restricted or extended); the degree of synchronization among individual spawning periods; and the season of occurrence (e.g., fall–winter or spring–summer). |
| Spawning frequency | It refers to the number of spawning events within a spawning period (for an individual) or the spawning season (for the population). | We use "spawning fraction" to indicate the proportion of mature females spawning daily. "Spawning interval" is used to refer to the time period between spawning events and at the population level is estimated as the reciprocal of the spawning fraction. |
| Mass/Massive atresia | The majority of oocytes are in atretic condition.<br>If happening before spawning then, no eggs are released due to resorption of developing oocytes (see omission of spawning below)<br><br>If happening after the end of spawning indicates the regression of the ovary | The targeted sex cells are normally vitellogenic oocytes where at least 50% are undergoing resorption at the time of examination.<br>This term is exclusively used for females and oocytes (cf. sperm apoptosis in males which operates differently but which also might have significant negative effects on the production). |

| TERM | DEFINITION | REMARKS |
|---|---|---|
| Skipped spawning | It refers to the earlier termination of the reproductive cycle of a mature* individual prior to release of any gametes. Therefore the reproductive cycle does not culminate in spawning.<br>* Therefore 'Skipping' requires that the individual has spawned previously. | The phenomenon can appear during different phases of the reproductive cycle. In females it can occur i) before the onset of vitellogenesis, i.e. individual will not develop yolk oocytes and thus will skip the entire breeding season (Resting type); ii) it can be the consequence of massive atresia of yolked oocytes (and/or CA oocytes), thus females although have initiated the developing phase will not progress further and will skip the subsequent spawning period (Reabsorbing type); or will complete the developing phase but will never release the gametes (Retaining type).<br>It has been described in determinate spawners, but its presence in indeterminate spawners is uncertain. |
| Whole mounts | Complete (i.e. un-sectioned) cells/tissue preparations | Typically examined under a stereo microscopy. Either fresh or fixed (e.g. in formaldehyde) |

# Annex 6: Stocks analysed

| WG | ECOREGION | SPECIES | STOCK |
|---|---|---|---|
| WGBFAS | North Sea | Cod | Division IIIa East (Kattegat) |
| | | Sole | Division IIIa and Subdivision 22-24 (Skagerrak, Kattegat and the Belts) |
| WGBFAS | Baltic | Cod | Subdivisions 22–24 |
| | | | Subdivisions 25–32 |
| | | Dab | Subdivisions 22–32 (Baltic Sea) |
| | | Brill | Subdivisions 22–32 (Baltic Sea) |
| | | Flounder | Subdivisions 22–32 (Baltic Sea) |
| | | Herring | Subdivision 28.1 (Gulf of Riga) |
| | | | Subdivision 30, Bothnian Sea |
| | | | Subdivision 31, Bothnian Bay |
| HAWG | Baltic | Herring | Subdivisions 22–24 and Division IIIa (Western Baltic spring spawners) |
| WGBFAS | Baltic | Herring | Subdivisions 25–29 and 32 (excluding Gulf of Riga herring) |
| | | Plaice | Subdivisions 22 32 (Baltic Sea) |
| | | Sea Trout | Subdivisions 22–32 (Baltic Sea) |
| | | Sprat | Subdivisions 22–32 (Baltic Sea) |
| | | Turbot | Subdivisions 22–32 (Baltic Sea) |
| | | Salmon | Subdivisions 22–31 (Main Basin and Gulf of Bothnia) |
| WGBAST | Baltic | Salmon | Subdivision 32 (Gulf of Finland) |
| AFWG | Barents Sea and Norwegian Sea | Capelin | Subareas I and II, excluding Division IIa west of 5°W (Barents Sea capelin) |
| | | Cod | Subareas I and II (Northeast Arctic cod) |
| | | | Subareas I and II (Norwegian coastal waters) |
| | | Greenland Halibut | Subareas I and II |
| | | Haddock | Subareas I and II (Northeast Arctic) |
| | | Golden redfish | Subareas I and II |
| | | Beaked redfish | Subareas I and II |
| | | Saithe | Subareas I and II (Northeast Arctic) |
| WGANSA | Bay of Biscay and Atlantic Iberian waters | Anchovy | Division IXa |
| | | | Subarea VIII (Bay of Biscay) |
| | | Blue jack mackerel | Subdivision Xa2 (Azores) |
| | | Horse mackerel | Division IXa (Southern stock) |
| | | Sardine | Divisions VIIIc and Ixa |
| WGHMM | Bay of Biscay and Atlantic Iberian waters | Black-bellied anglerfish | Divisions VIIIc and IXa (Lophius budegassa) |
| | | Four-spot megrim | Divisions VIIIc and IX |
| | | Hake | Divisions VIIIc and IXa (Southern stock) |

| WG | ECOREGION | SPECIES | STOCK |
|---|---|---|---|
| WGHMM | Bay of Biscay and Atlantic Iberian waters | Megrim | Divisions VIIIc and Ixa |
| | | Nephrops | Division Ixa |
| | | | Division VIIIc (North Galicia and Cantabrian Sea, FU 25 and 31) |
| | | | in Division VIIIab (Bay of Biscay, FU 23-24) |
| | | Plaice | Subarea VIII and Division IXa (Bay of Biscay and Iberian waters) |
| | | Pollack | Subarea VIII and Division IXa (Bay of Biscay and Iberian coast) |
| | | Sole | Divisions VIIIa,b (Bay of Biscay) |
| | | Sole | Divisions VIIIc and IXa (Iberian waters) |
| | | White anglerfish | Divisions VIIIc and Ixa |
| | | Whiting | Subarea VIII and Division Ixa (Bay of Biscay and Iberian waters) |
| WGHMM | Celtic Sea and West of Scotland | Anglerfishes | Divisions VIIb-k and VIIIa,b,d |
| WGCSE | | Anglerfishes | Divisions Iia, IIIa, Subareas IV, and VI |
| | | Cod | Division VIa (West of Scotland) |
| | | | Division Vib (Rockall) |
| | | | Division VIIa (Irish Sea) |
| | | | Divisions VIIe-k |
| | | Haddock | Division VIa (West of Scotland) |
| | | | Division VIb (Rockall) |
| | | | Division VIIa (Irish Sea) |
| | | | Divisions VIIb-k |
| | | Megrim | Divisions IVa and Via |
| | | | ICES Division VIb (Rockall) |
| WGHMM | | Megrim | Divisions VIIb,c,e-k and VIIIa,b,d |
| WGNSSK | Celtic Sea and West of Scotland | Nephrops | Division Via |
| WGCSE | | Nephrops | Subarea VII |
| | | Norway pout | Division VIa |
| | | Plaice | Division VIIa (Irish Sea) |
| | | | Division VIIe (Western Channel) |
| | | | Divisions VIIb,c (West of Ireland) |
| | | | Divisions VIIf and g (Celtic Sea) |
| | | | Divisions VIIh-k |
| | | Pollack | Subareas VI and VII (Celtic Sea and West of Scotland) |
| WGNSSK | Celtic Sea and West of Scotland | Saithe | Subarea IV (North Sea), Division IIIa (Skagerrak), and Subarea VI (West of Scotland and Rockall) |
| WGCSE | Celtic Sea and West of Scotland | Sandeel | Division VIa |
| | | Sole | Division VIIa (Irish Sea) |
| | | | Division VIIe (Western Channel) |
| | | | Division VIIh–k (Southwest of Ireland) |
| | | | Divisions VIIb,c (West of Ireland) |
| | | | Divisions VIIf and g (Celtic Sea) |

| WG | ECOREGION | SPECIES | STOCK |
|---|---|---|---|
| WGCSE | Celtic Sea and West of Scotland | Whiting | Division VIa (West of Scotland) |
| | | | Division Vib (Rockall) |
| | | | Division VIIa (Irish Sea) |
| | | | Divisions VIIe-k |
| HAWG | Celtic Sea and West of Scotland | Herring | Division Via (North) |
| | | | Division Via (South) and VIIb, c |
| | | | Division VIIa North of 52º 30 N (Irish Sea) |
| | | | Division VIIa South of 52° 30′ N and VIIg,h,j,k (Celtic Sea and South of Ireland) |
| | | Sprat | Divisions VIId, e |
| | | | Subarea VI and Divisions VIIa-c and f-k (Celtic Sea and West of Scotland) |
| | North Sea | Sprat | Division IIIa (Skagerrak – Kattegat) |
| | | | Subarea IV (North Sea) |
| | | Herring | Subarea IV and Divisions IIIa and VIId (North Sea autumn spawners) |
| WGNEW | North Sea | Brill | Subarea IV, Subdivision IIIa and VIId,e |
| | | Dab | Subarea IV and Division IIIa |
| | | Flounder | Division IIIa and Subarea IV |
| | | Lemon sole | Subarea IV, Division IIIa and VIId |
| | | Turbot | Subarea IV and Division IIIa |
| | | Witch | Subarea IV, Division IIIa and VIId |
| WGNSSK | North Sea | Cod | Subarea IV (North Sea), Division VIId (Eastern Channel) and Division IIIa (Skagerrak) |
| | | Haddock | Subarea IV (North Sea) and Division IIIa (Skagerrak-Kattegat) |
| | | Norway pout | Subarea IV (North Sea) and Division IIIa (Skagerrak- Kattegat), June |
| | | Plaice | Division IIIa (Skagerrak-Kattegat) |
| | | Plaice | Division VIId (Eastern Channel) |
| | | Plaice | Subarea IV (North Sea) |
| | | Pollack | Subarea IV and Division IIIa |
| | | Saithe | Subarea IV (North Sea), Division IIIa (Skagerrak), and Subarea VI (West of Scotland and Rockall) |
| WGNSSK | North Sea | Sandeel | Division IIIa and Subarea IV |
| | | Sole | Division VIId (Eastern Channel) |
| | | | Subarea IV (North Sea) |
| | | Whiting | Division IIIa (Skagerrak – Kattegat) |
| | | | Subarea IV (North Sea) and Division VIId (Eastern Channel) |
| | | Nephrops | Division IIIa |
| | | | Subarea IV (North Sea) |
| NIPAG | North Sea | Northern shrimp | Division IVa (Fladen Ground) |
| | | | Divisions IIIa and IVa East (Skagerrak and Norwegian Deep) |
| NWWG | Faroe Plateau | Cod | Subdivision Vb1 (Faroe Plateau) |

| WG | ECOREGION | SPECIES | STOCK |
|---|---|---|---|
| | | | Subdivision Vb2 (Faroe Bank) |
| NWWG | Faroe Plateau | Haddock | Division Vb |
| | | Saithe | Division Vb |
| | Iceland and East Greenland | Beaked Redfish | Division Va and Subarea XIV (Icelandic Slope stock) |
| | | | Subarea XIVb (Demersal) |
| | | | Subareas V, XII, and XIV and NAFO Subareas 1+2 (Deep pelagic stock > 500 m) |
| | | | Subareas V, XII, and XIV and NAFO Subareas 1+2 (Shallow pelagic stock < 500 m) |
| | | Capelin | the Iceland-East Greenland-Jan Mayen area (Subareas V and XIV and Division Iia |
| | | Cod | Division Va (Icelandic cod) |
| | | | ICES Subarea XIV and NAFO Subarea 1 (Greenland cod) |
| | | Golden redfish | Subareas V, VI, XII and XIV |
| | | Greenland Halibut | Subareas V, VI, XII and XIV |
| | | Haddock | Division Va |
| | | Herring | Division Va (Icelandic summer-spawning herring) |
| | | Saithe | Division Va (Icelandic saithe) |
| WGDEEP | Widely distributed and migratory stocks | Alfonsinos/Golden eye perch | the Northeast Atlantic |
| | | Black scabbardfish | the Northeast Atlantic |
| | | Blue ling | all areas in the Northeast Atlantic |
| | | Blue whiting | Subareas I–IX, XII, and XIV (Combined stock) |
| | | Boarfish | the Northeast Atlantic |
| | | European eel | all areas |
| | | European seabass | the Northeast Atlantic |
| | | Greater forkbeard | the Northeast Atlantic |
| | | Greater silver smelt | all areas |
| | | Grey gurnard | the Northeast Atlantic |
| WGHMM | Widely distributed and migratory stocks | Hake | Division IIIa, Subareas IV, VI, and VII, and Divisions VIIIa,b,d (Northern stock) |
| WGWIDE | | Horse mackerel | Divisions IIIa, IVb,c, and VIId (North Sea stock) |
| | | Horse mackerel | Divisions IIa, IVa, Vb, VIa, VIIa–c,e–k, and VIIIa–e (Western stock) |
| | | Herring | the Northeast Atlantic (Norwegian spring-spawning herring) |
| | | Ling | all areas in the Northeast Atlantic |
| | | Mackerel | the Northeast Atlantic (combined Southern, Western, and North Sea spawning components) |

| WG | ECOREGION | SPECIES | STOCK |
|---|---|---|---|
| WGWIDE | Widely distributed and migratory stocks | Orange roughy | all areas |
| | | Red seabream | the Northeast Atlantic |
| | | Red gurnard | the Northeast Atlantic |
| | | Roundnose grenadier | the Northeast Atlantic |
| | | Spurdog | the North-East Atlantic |
| | | Striped red mullet | the Northeast Atlantic |
| | | Tusk | all areas |

# Annex 7: Guidelines for Workshops on Maturity Staging

## Version history

| VERSION | AUTHOR | DATE | CHANGES |
|---|---|---|---|
| Version 3 | ICES PGCCDBS | 4 March 2010 | Changes based on WKMSSPDF. Topics to consider when preparing a Workshop<br>f) modified and i) added.<br>Topics to consider during the Workshop<br>e)added.<br>b)ii)modified<br>Guidelines for collecting maturity data and histological analyses for maturity workshops<br>8) modified |
| Version 4 | ICES PGCCDBS | 02 February 2012 | Changes based on WKMSSPDF2012: recommendation to create European Fish Maturity Stagers Forum added |
| Version 5 | ICES PGCCDBS | June 2014 | Changes based on WKMATCH recommendations |

## 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.

PGCCDBS recommends creating of the **European Fish Maturity Stagers Forum** (like the European Age Readers Forum, http://groupnet.ices.dk/AgeForum/default.aspx) in tandem with the **WebGR** tool (http://webgr.wiki.azti.es/doku.php) to streamline the preparation and the implementation of maturity staging exercises and workshops.

WKMATCH recommends the establishment of regular training courses on maturity staging targeting observers normally collecting biological data and the laboratory responsible of this data collection.

## 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 may participate even if they do not collect routinely maturity data.
- Experts on histology, maturation process and the reproductive ecology/biology as well as those in charge in collecting process of the species of concern or at least a related species should participate in the workshop.

f) Ideally, fresh samples should be provided during the workshops. This needs to be taken into account when setting the timing of the meeting according to the species reproductive biology.

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 histological slides and images as a tool for calibration prior to a workshop. This is especially important because results from the calibration exchange will point out possible discrepancies between labs. They should be address during the 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. Accuracy may be estimated by means of whole-mounts (see guidelines for whole-mounts analysis protocol). They should also consider, 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 analysed.

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) Illustrated and validated manuals will be developed in order to enhance accuracy in maturity staging among laboratories.

~~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) **Provide a statistical report of exchange comparing observed maturity stage with** validated histological stage for the workshop participants to consider. Differences in staging between laboratories should be statistically analysed in terms of precision and accuracy; sources of discrepancies should also be analysed.

c) **Resolve interpretation differences between readers and laboratories both** at macroscopic and microscopic scales. Differences may arise from:

- Using different maturity scales
- Different interpretation of the same macroscopic stages (terminology and precise definition of stages are critical issues)
- Different sampling protocols, e.g. timing and/or gear selectivity or availability, see guidelines for collecting maturity data below.
- 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.

d) Agree and create a single maturity scale. Consider the following aspects:

- Follow the general maturity scale proposed by WKMATCH.
- If subdivision of scale is needed, 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)
- Describe the stages precisely avoiding ambiguity and overly subjective description (like colour descriptions), for example, give measurements instead of saying "bigger".
- If two stages are hard to distinguish macroscopically, they should be both indicated. This often occurs with resting and/or mature inactive stages that are confused with immature or developing (at early stages). In these cases, histology must be used to confirm the maturity stage.

  ~~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).~~

e) **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.

~~The process of trial discussion retrial should based on fresh gonads samples and at least two staging sessions on fresh materials have to be done during future workshop.~~

f) 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.

g) Participants should indicate the level of experience on the determination of the maturity staging. This will help the on the analysis of the results calibration exercise.

h) The results from the calibration exercise should be recorded to provide data for statistical analysis.

i) Improvements in agreement due to the workshop should be analysed. Ideally a different set of samples should be used, not the ones already staged earlier in the workshop. Discrepancies of maturity staging between participants should be statistically analysed in terms of precision and accuracy.

j) Try to use standard terminology from the WKMATCH 2012.

k) When a new agreed maturity scale is proposed the impact on maturity historical series should be evaluated.

l) Produce an agreed reference collection of preserved gonads, histological slides and images that should be stored in a reference lab (defined by the Workshop) and always available for the scientific community. Copies of histological slides can be made and distributed with referenced images of these slides.

~~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.

# Annex 8: Recommendations

| | RECOMMENDATION | ADRESSED TO |
|---|---|---|
| 1. | Adopt the WKMATCH proposed maturity scale for all species and both sexes, including the guidelines on how to use it and the proposed terminology. | ACOM<br>PGCCDBS |
| 2. | Maturity staging Workshops should discuss the WKMATCH proposed maturity scale in their respective WKs by e-mail to assess the correspondence with the respective WK agreed scale, and evaluate the uncertainties and the problems this new general scale may cause | PGCCDBS |
| 3. | Update guidelines for Workshops on Maturity Staging with the WKMATCH recommendations | PGCCDBS |
| 4. | To establish a Workshop on Sexual Maturity Staging of hermaphroditic species | PGCCDBS |
| 5. | EGs should provide comprehensive reports on how the maturity data is used, and more specifically, at least: the method used to estimate maturity, in which sex and how a sex-specific maturity ogive is used in the assessment, source of data (survey, commercial sampling), the time of the year when the sampling was conducted, and years of proper estimation. | ACOM<br>EGs |
| 6. | The impact on the assessment of combining sex-specific maturity ogives should be analysed | ACOM<br>EGs |
| 7. | Proposal for ICES training course on maturity staging | PGCCDBS<br>WGBIOP |