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Report of the Workshop on sexual maturity staging of cod, whiting, haddock, saithe and hake (WKMSGAD)

4–8 November 2013

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Contents

Executive summary

The Workshop on sexual maturity staging of cod, whiting, haddock, saithe and hake (WKMSGAD) met on 4–8 November 2013 in San Sebastian, Spain. In total 15 participants from 7 countries joined the meeting (List in Annex 1).

The meeting's purpose was to test the applicability of the maturity scales proposed by WKMSCWHS 2007 and WKMSHM 2007 and enhance the maturity stages' descriptions. A further aim was the validation of the macroscopic maturity scales, together with the identification of potential of staging errors, using histological investigations.

Evaluation of maturity scales

It was ascertained that the maturity scales, as proposed by the two WKs in 2007, have not been incorporated by all countries. Nevertheless most laboratories, succeeded in translating the national (or local) scale into the 2007 proposed scales.

Changes in the maturity scale descriptions

Stages' descriptions were evaluated and some changes were made in the assessing criteria, based on participants' expertise and experiences.

The baseline of the new proposed maturity scales was the universal scale (4+2), valid across species and time, developed during the Workshop for maturity staging chairs (WKMATCH) in 2012. The new scale adopts the standardized terminology for describing reproductive development in fish (Brown-Petersen *et al*., 2011) and introduces the term code instead of stage.

The macroscopic descriptors were also revised in order to make the scale universal, i.e. suitable for all stocks. Consequently all the characteristics based on subjectivity, such as colour, size and presence/absence of blood vessels, were avoided as considered stock specific. Only objective and validated criteria were chosen by the group as stage descriptors. Concerning hake the group agreed on maintaining the stock specific criteria as *indicative criteria*. The modifications of the maturity keys do not have any impact on the currently estimated maturity ogives or on historical national time-series.

Staging exercises

Three staging exercises were carried out:

1) using fresh fish;
2) using frozen gonads;
3) using pictures.

In fresh sample exercise conducted on hake, the group obtained 74% of agreement evidencing a decline in agreement compared to the 85.5% reached during the Workshop on Sexual Maturity Staging of hake and monk (WKMSHM) in 2007. The agreement between visual inspection and histology was also 74%, highlighting inaccuracies in macroscopic maturity stage identification. In frozen sample exercise, conducted on cod, 61% and 53% of agreement was respectively obtained for females and males. In WebGR exercise, the percentage of agreement based on agreed stage

(modal stage) was between 61% and 82% in all gadoid species. The histological validation revealed an accuracy between 66% and 75%, except for males in whiting, haddock and saithe where the agreement dropped to 51–55% evidencing inaccuracies in visual maturity stage assessment.

Next Meeting

Concerning hake, cod and saithe it is suggested to conduct an exchange (using pictures through WebGR) to test the validity of the new proposed scales but also for a calibration purpose in order to evaluate the need of a follow-up workshop. Institutes are also strongly encouraged to conduct calibration exercises on a regular basis to monitor a possible interannual variability. Concerning whiting and haddock WKMSGAD did not include enough participants from ICES countries involved in the maturity staging of these species. Thus the possibility of a follow up workshop needs to be further evaluated.

1 Introduction

WKMSGAD met 4-8 November 2013 in San Sebastian, Spain. 15 participants from 7 countries joined the meeting. The participant list is in Annex 1.

1.1 Terms of Reference

a) Evaluate the applicability of the 2007 proposed common maturity scales;
b) Assess maturity staging of all species using pictures with histology as ground-truth for determination of staging errors;
c) Validate macroscopic maturity determination with histological analysis;
d) Enhance the macroscopic and microscopic description of the characteristics of the stages of the 2007 scales and finalize the illustrated manuals initiated in 2007;
e) Evaluate the impact on the currently used maturity ogives if changes in the maturity staging are recommended.
f) Consider local training programs for scientists and technicians sampling gadoids;

ToR a, e and f were discussed in plenary. ToR b and c consisted in individual calibration exercises using fresh fish, frozen fish and pictures through WebGR as stage recording tool.

ToR d was dealt with in three subgroups (hake, whiting, and cod/saithe/haddock) and subsequently discussed in plenary. The ToRs are discussed in separate chapters.

1.2 Adoption of the agenda

The agenda addressed all ToRs and was adopted without changes. The agenda can be found in Annex 2.

2 Use of the common maturity scales proposed in 2007 (ToR a)

During the Workshop on Sexual Maturity Staging of Cod, Whiting, Haddock and Saithe (WKMSCWHS) in 2007 (ICES, 2007a) a common maturity scale, including six gonadal developmental stages, was developed (Annex 3, Figure A.3.1). The six stages are:

1) Juvenile/Immature;
2) Maturing;
3) Spawning;
4) Spent;
5) Resting/Skip of spawning;
6) Abnormal.

This 6-point scale has since 2007 replaced the 4-point scale historically used by ICES. The addition of two extra stages, namely resting and abnormal, has represented an important step forward for ensuring an accurate classification of the gonadal maturity status. In addition, fish that are omitting the spawning and those showing abnormal features can be considered important ecosystem indicators. Further changes introduced during the 2007 workshop concerned the stage 3 (Spawning). This stage was historically interpreted as synonymous of "running" thus only fish caught during the eggs/sperm release were included in this stage. However, as pointed out during WKMSCWHS 2007, catching a fish that is running is quite random, while the presence of hydrated oocytes, given that gadoids are batch spawners, characterizes an ongoing spawning season. Thus since 2007, fish with hydrated oocytes and fish that have recently released a batch are considered spawners and included in stage 3. This has no impact on the calculation of the Spawning Stock Biomass (SSB) but acquire importance in spatial/temporal maturity investigations.

The Workshop on Sexual Maturity Staging of hake and monk (WKMSHM) in 2007 standardized a maturity key for hake (ICES, 2007b and Annex 3, Figure A.3.2) which takes into account the reproductive cycle and includes 4 stages namely:

1) Immature/Resting;
2) Developing/Maturing;
3) Spawning;
4) Post-spawning.

It was decided to include immature and resting stages in a single stage as they are hardly discernible macroscopically and resting females do not contribute to the SSB for the current year. It was thus recommended to collect samples for the maturity ogives estimation during the peak of the spawning season, being the proportion of early developing/resting females lower than during the rest of the year and thus lowering the bias generated by stage misclassifications.

During WKMSGAD it was ascertained that the maturity scales proposed by WKMSCWHS and WKMSHM in 2007 have not been fully adopted by all countries (Table 2.1).

Table 2.1. Scales currently used by the different countries (represented at the meeting) for the different species. All scales are shown in detail in Annex 3 (Figures A3.1-A3.7).

Country	Hake	Cod/Whiting/Haddock/Saithe
France	WKMSHM 2007/Medits Scale	WKMSCWHS 2007
Spain	WKMSHM 2007	WKMSCWHS 2007
Norway	-	National Scale
Sweden	National Scale	National Scale
Germany	-	Tomkiewicz et al., 2002
Denmark	-	WKMSCWHS 2007
Italy	Medits Scale	-

Concerning cod, whiting, haddock and saithe, the use of national maturity scales by some countries is mainly due to a more detailed nature of those scales compared to the scale agreed upon during the 2007 workshop. Nevertheless local/national maturity scales are translated into the international staging key before data submission to ICES.

Similarly, the Medits scale (Annex 3, Figure A.3.3) is commonly used for the Mediterranean hake. This scale was consensually adopted by all countries involved in the MEDITS project (Medits-handbook, 2012) which was carried out for the Mediterranean basin. As in the case of the other gadoids species, Medits scale is translated into WKMSHM 2007 scale before data submission to JRC (Joint Research Centre, EU Commission).

During this session interesting presentations on own research were given by participants from COISPA (Italy), IEO (Spain) and IIM (Spain). The abstracts are included in Annex 5.

3 Assessing validated maturity staging (ToR b)

The determination of gonadal maturity in gadoids, both macroscopic and histological is extensively elucidated in WKMSCWHS' report (ICES, 2007a) and thus it was deemed redundant in the present report, being this workshop a follow up.

During WKMSGAD the macroscopic maturity staging was validated with histological analyses and tested through a series of different staging calibration exercises based on fresh fish, frozen fish and pictures. In all calibration exercises participants were asked to use the international maturity keys currently in use, i.e. WKMSCWHS 2007 and WKMSHM 2007.

Data analysis

Each observer was assigned a level of expertise (i.e. expert or trainee) and a Mann-U Whitney test, non-parametric, was used to detect potential statistically significant differences between only experts and the entire group. This test reveals as well the level of applicability of the used maturity scale.

In each calibration exercise, the first percent of agreement value was calculated using the most agreed (most frequent, modal) stages for each specimen. This allowed monitoring the level of precision reached by the observers, meaning how much observers' readings are close to each other.

Subsequently, the percent of agreement was validated using the histological analysis, when available. This value can be interpreted as an index of accuracy that allows ascertaining how much observers' readings are close to the true value.

A non-parametric Kruskal–Wallis test was used to assess the percent of agreement among observers in each histological stage and compare this among stages (within each sex) to see which stages were the more/less correctly identified and rank them accordingly (group a, b, c).

3.1 Fresh fish staging – Hake

The fresh fish staging was carried out on 50 fresh specimens of hake, 19 males and 31 females. Specimens were landed on 3 November 2013 (out of the main spawning season, as also recommended by WKMATCH, 2012) and kept on ice until the staging occurred two days after. The fish were cut open and the gonads were left in the fish. All participants, independently of their expertise, were involved in this exercise (Figure 3.1).

Figure 3.1. Hake individuals used in the fresh fish maturity staging exercise.

No differences in modal or histological staging were corroborated between the expert group and the entire group (Mann-Withney, p>0.05), thus the whole group was thereafter considered for the data analysis.

The number of observations by agreed (modal) maturity stage in shown in Table 3.1.

The overall percent of agreement based on modal stage (precision index) is 74% for both sexes for the whole group. This result evidences a decline compared to the 85.5% reached during WKMSHM in 2007.

According to the histological analysis, conducted on females, only stages 1 and 2 were present in the sample and could be validated. However those stages were sometimes mistakenly interpreted by some observers through visual inspection as stages 3 and 4, leading to a misclassification rate of 19% (i.e. 81% agreement) for stage 1 and 32% (i.e. 68% agreement) for stage 2. The overall percent of agreement for females based on histological stages (accuracy index) is also 74%.

Table 3.1. Fresh samples. Number of observations by agreed maturity stage.

Stage	OBS1	OBS2	OBS3	OBS4	OBS5	OBS6	OBS7	OBS8	OBS9	OBS10	OBS11	OBS12	OBS13
1	22	19	28	3	3	19	24	18	29	17	15	25	17
2	15	17	21	43	43	19	16	27	13	17	27	22	29
3	3	0	0	1	1	1	1	1	4	4	0	1	0
4	10	14	1	3	3	11	9	4	4	11	8	2	4
5	0	0	0	0	0	0	0	0	0	1	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0
1 to 6	50	50	50	50	50	50	50	50	50	50	50	50	50

3.2 Whole mount staging (females) – Hake

During the maturity calibration exercise using fresh samples, a pipette sample of the gonad was taken from each female and preserved in Nunc tubes filled with 4% formaldehyde. This allowed the validation of macroscopic maturity observations, both through an immediate whole mount examination and through histological analysis after the workshop.

The whole mount method is a quick and easy exercise that helps to enhance maturity determination by light microscopy or stereomicroscopy (Kjesbu, 1991). The female gonad is cut longitudinally with a sharp scalpel and some of the oocytes are scraped out of the gonad. The observation can either be conducted immediately on fresh ma-

terial kept in isotonic water (i.e. 1/3 seawater and 2/3 freshwater) or on fixed material, as long as the oocytes remain intact.

The main issue here was to discriminate between ovaries in stage 1 and 2, characterized by oocytes in different stages. Through this method, previtellogenic oocytes appear highly transparent, cortical alveoli oocytes and early vitellogenic oocytes semi-transparent and mid and late vitellogenic oocytes fully dark. The intention was also to distinguish, when possible, between immature and resting ovaries, due to the presence in the latter of spawning markers (i.e. POFs, atresia, etc.).

Results, also confirmed by histology, show that a third of the ovaries within (agreed) stage 1 and all ovaries in agreed stage 4 were in fact in stage 2 when observed on whole mounts (Figure 3.2). The actual stage 2 was always correctly identified.

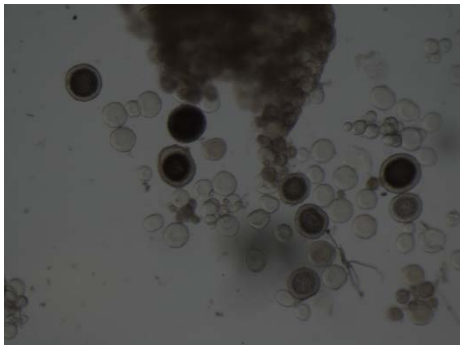

Figure 3.2. Whole mounts on individual mistakenly classified as stage 1 (agreed stage).

As mentioned above, no ovaries judged to be in stage 3 and 4 were confirmed through whole mount or histological analysis as they were all in stage 2. These results are extremely relevant because of their direct impact on the maturity ogives (see section 6).

3.3 Frozen ovary staging – Cod

Before the meeting participants were asked to collect samples for histological analysis. Two of the institutes collecting cod, belonging to the Spanish Institute of Oceanography (IEO), namely A Coruña and Vigo, preserved a half of the gonad for histological analyses while freezing the other half. The IEO kindly made this frozen material available for a staging exercise during the workshop (Figure 3.3). Histology was available for almost all ovaries.

Figure 3.3. Cod frozen ovaries used for maturity stages exercise.

Albeit well recognized that maturity staging on frozen material is poorer than with fresh material, the second maturity staging calibration exercise was nonetheless carried out on those 30 thawed gonads. All participants, independently of their expertise, were involved in this exercise. No differences in modal or histological staging were corroborated between the expert group and the entire group (Mann-Withney, $p > 0.05$).

Considering the modal stage, it was found a 61% of agreement for females and 53% for males among observers (precision index). Misclassification occurred mainly on the stage agreed as 5. Although there was a relatively high agreement among stagers, almost all the gonads (9 out of 10) judged to be in stage 5 were instead histologically judged to be in stage 2 (one gonad was histologically judged as stage 1); only one ovary classified as stage 2 (agreed stage) was instead histologically in stage 5 (Table 3.2). The agreement between female macroscopic and histological inspection is shown in Table 3.3.

It is noteworthy that participants did not have the entire fish but just the gonad to base their judgment upon. Furthermore participants were informed only after the exercise that the gonads were collected outside the spawning period, and could re-evaluate the probability to find gonads in stage 5 (resting, spawning omission). This may have caused the high misclassification rate observed between those two stages while highlighting the importance of taking into consideration the sampling time in relation to the reproductive cycle.

The misclassification between stage 2 and stage 5 plays an important role as it may lead to an incorrect estimation of the SSB. No significant differences among stages were detected (Kruskal–Wallis test, $p=0.065$)

Table 3.2. Frozen samples. Maturity stage classification by observers and by agreed stage, sex combined. OBS: Observer; PA: Percent of Agreement.

Fish number	Sex	HISTOLOGY	OBS1	OBS2	OBS3	OBS4	OBS5	OBS6	OBS7	OBS8	OBS9	OBS10	OBS11	OBS12	Agreed stage	Frequency	PA
1	2	2	5	1	1	5	5	5	5	5	1	4	4	1	5	6	50%
2	1		5	4	2	2	2	4	2	2	4	5	4	4	4	5	42%
3	2	3	3	2	2	2	3	3	3	3	2	3	3	3	3	8	67%
4	2	2	5	3	1	2	2	2	5	5	1	2	2	1	2	5	42%
5	2	4	4	3	2	4	3	3	4	3	4	5	5	3	3	5	42%
6	2	2	5	5	5	2	2	2	5	5	1	5	5	5	5	8	67%
7	2	2	5	5	1	2	2	2	5	5	1	5	5	5	5	7	58%
8	2	2	5	5	1	2	2	2	5	5	1	5	5	5	5	7	58%
9	2	2	5	5	1	5	5	4	5	5	1	5	4	5	5	8	67%
10	2	2	4	4	5	5	5	5	5	5	1	5	5	5	5	9	75%
11	2		1	1	1	1	1	1	1	1	1	1	1	1	1	12	100%
12	2	3	4	2	2	3	3	3	3	3	2	3	3	3	3	8	67%
13	1	2	4	2	2	2	2	2	2	2	4	4	2	4	2	8	67%
14	2	2	5	5	5	2	2	5	5	5	1	2	5	5	5	8	67%
15	2	2	5	5	5	4	4	5	5	5	1	2	5	5	5	8	67%
16	2	2	4	5	5	2	2	2	5	2	1	2	2	1	2	6	50%
17	2	5	4	4	5	5	5	2	5	5	2	2	4	3	5	5	42%
18	2	2	4	2	5	2	2	5	5	5	1	5	5	2	5	6	50%
19	2		4	4	5	4	4	2	5	2	2	2	2	2	2	6	50%
20	2	2	6	4	1	6	6	6	6	6	1	6	6	6	6	9	75%
21	2		3	2	2	2	2	3	2	2	2	2	2	3	2	9	75%
22	2	1	4	2	5	5	5	3	2	5	2	5	5	1	5	6	50%
23	2		1	1	1	1	1	1	1	1	1	1	1	1	1	12	100%
24	1	2	4	1	1	2	2	4	1	2	4	4	4	4	4	6	50%
25	2	3	4	2	2	2	2	5	5	5	2	2	5	1	2	6	50%
26	2		5	2	2	4	4	3	3	3	2	3	3	3	3	6	50%
27	2	5	5	2	5	5	5	3	5	2	2	2	2	2	2	6	50%
28	2		4	4	5	4	4	3	4	5	3	4	4	2	4	7	58%
29	2		4	3	5	4	4	3	5	2	2	5	5	2	5	4	33%
30	2		4	1	1	1	1	1	1	1	1	1	1	1	1	11	92%
TOTAL OBS		21	30	30	30	30	30	30	30	30	30	30	30	30		ALL OBS	60%
																STAGE 1	97%
																STAGE 2	55%
																STAGE3	56%
																STAGE 4	50%
																STAGE 5	57%
																STAGE 6	75%

Table 3.3. Frozen samples. Percent of agreement between female macroscopic and histological inspection.

Histological stages	Agreement with visual inspection
1	8%
2	19%
3	44%
4	33%
5	42%
All	74%

3.4 Picture staging through WebGR– Cod, whiting, haddock, saithe, and hake

The last staging exercise was based on pictures and carried out using WebGR, a European project supporting studies of fish growth (age) and reproduction (maturity). WebGR is realized as web application and is published as open source software under a creative commons license. It promotes the use of online services to organize and perform calibration workshops, divided in one of more calibration exercises. The websites, currently hosted at www.webgr.azti.es, consists of a repository of images grouped or classified by workshop (species, date, area, etc.) and accessible to all workshop participants.

Although deemed really useful WebGR is still in its first version and had no further developments since 2010. WKMSGAD participants' feedback was mostly about pic-

tures. Pictures' quality (especially the close up) needs to be enhanced and an image of the gonad transversal cut is indispensable. Furthermore male gonads should be distended before taking the photo. Finally, there was a general opinion supporting the idea that this kind of online workshops should be carried out before the meeting and results discussed during meeting.

The pictures used during this exercise were taken by some of the participants during their sampling as a contribution to the workshop. All individuals' maturity stage was validated through histology.

Histology was an important tool to achieve a consensus on the maturity stage evaluation and to quantify the level of misclassification using visual gonadal evaluation.

All participants, independently of their expertise, were involved in these exercises; however each one was assigned a level of expertise and labelled either as expert or trainee. The online workshop consisted in five calibration exercises, one per species. The maturity stages were assigned according to the current internationally adopted maturity scales (Annex 3, Figures A.3.1 and A.3.2).

Unfortunately only few participants were familiar with whiting and haddock, thus the expertise for those species was lacking. Participants experienced in judging cod and saithe gonads were considered experts in the other two species (whiting and haddock) too.

The number of observations per species by agreed maturity stage is shown in Tables 3.4-3.8. The experts and trainees ratio was respectively 8:6 in haddock, whiting and saithe, 9:6 in cod and 7:7 in hake. However, according to the Mann-Withney test, results obtained by the entire group were not in any case significantly different from those obtained when only the expert group was considered. Consequently in each exercise the whole group of participants was considered for estimating the misclassification rates based on histology.

The Kruskal–Wallis test was used for testing the agreement among observers in each stage (based on histology), within sexes and comparing it among stages, i.e. recognize the more/less accurately identified stages and rank them (groups a/b/c) based on this (Tables 3-9-3.13).

3.4.1 Cod

In total, the maturity of 44 (20 females and 24 males) specimens was ascertained through histological examination of the gonadal tissue and the results compared with initial macroscopic assessments.

Females

Maturity stages of specimens ranged from 1 to 5, with the exception of stage 4.

Overall agreement among observers was 77% based on modal stage and 73% based on histology (Table 3.9), which implies that the maturity of 27% of females in the study group was misclassified through the macroscopic examination. This result shows an improvement compared to the misclassification rates of 36.8% achieved during WKMSCWHS 2007.

In general the first three stages for females show fairly high percent of agreement, ranging between 76% and 89%. Stage 5 was the stage less correctly identified in females (60%), thus ranked as a separate group, according to the Kruskal–Wallis test.

The observed misclassification between stage 2 and 5 reinforces what already observed during the calibration exercise conducted on frozen gonads.

Males

Maturity of specimens ranged from 1 to 5. Overall agreement among observers was 69% based on modal stage and 66% based on histology (Table 3.9), which implies that the maturity of 44% of males in the study group was misclassified through the macroscopic examination. This result shows an improvement compared to the misclassification rates of 53% for males reached during WKMSCWHS 2007. Stage 2 (24%), 4 (13%) and 5 (33%) resulted the less correctly identified, while stage 1 and 3 showed over 70% of accuracy.

3.4.2 Whiting

In total, the maturity of 25 (20 females and 5 males) specimens was ascertained through histological examination of the gonadal tissue and the results compared with initial macroscopic assessments.

Females

Maturity stages ranged from 1 to 3. Overall agreement among observers was 79% based on modal stage and 70% based on histology (Table 3.10), which implies that the maturity of 30% of females in the study group was misclassified through the macroscopic examination. The misclassification rate for females was approximately the same achieved during WKMSCWHS 2007 (i.e. 29%). Stage 1 and 3 had a high agreement, 93% and 87% respectively, while stage 2 was correctly identified in 55% of cases.

Males

Only maturity stages 1 and 2 were found in the sample. Overall agreement among observers was 70% based on modal stage and 51% based on histology (Table 3.10), which implies that the maturity of 49% of males in the study group was misclassified through the macroscopic examination. This result shows an improvement compared to the misclassification rates of 81% reached for males during WKMSCWHS 2007. Stage 1 showed 62% of agreement, while stage 1 was never correctly identified. Males were not tested using the Kruskal–Wallis, given the low sample size and the fact that stage 1 had 100% of misclassification.

3.4.3 Haddock

In total, the maturity of 44 (30 females and 14 males) specimens was ascertained through histological examination of the gonadal tissue and the results compared with initial macroscopic assessments.

Females

Maturity stages ranged from 1 to 6, with the exception of stage 5.

Overall agreement among observers was 82% based on modal stage and 73% based on histology (Table 3.11), which implies that the maturity of 27% of females in the study group was misclassified through the macroscopic examination. This result

shows an improvement compared to the misclassification rate of 46% for females achieved during WKMSCWHS 2007. The agreement between visual and histological inspection topped 80% for stage 1 and 3 while it was poorer for stage 2 (58%) and 4 (39%). The stage hardest to identify was stage 6, where the agreement between the two evaluation methods was only 7% yet based only on a single individual.

Males

Maturity stages ranged from 1 to 5 with the exception of stage 4.

Overall agreement among observers was 66% based on modal stage and 54% based on histology (Table 3.11), which implies that the maturity of 46% of males in the study group was misclassified through the macroscopic examination. This result shows a slight improvement compared to the misclassification rates of 48% for males achieved during WKMSCWHS 2007. The most misclassified stage was once more stage 5 where the agreement achieved between visual inspection and histology was 21%. Stage 1 and 2 showed an agreement of 64% and 75% respectively, while stage 3 with its 50% of agreement did not show statistical difference with any of the other stages according to the Kruskal–Wallis test, thus ranked as "ab".

3.4.4 Saithe

In total, the maturity of 34 (14 females and 20 males) specimens was ascertained through histological examination of the gonadal tissue and the results compared with initial macroscopic assessments.

Females

Maturity stages ranged from 1 to 3. Overall agreement among observers was 75% based on modal stage and 69% based on histology (Table 3.12), which means that the maturity of 31% of females in the study group was misclassified through the macroscopic examination. The obtained misclassification rate in females is higher than 22% attained during WKMSCWHS 2007. The most difficult stage to identify was stage 1, showing a 43% of accuracy. Stage 2 and 3 show instead a fairly high percent of agreement with histology, showing respectively 77% and 80% of accuracy.

Males

Maturity stages ranged from 1 to 3. Overall agreement among observers was 65% based on modal stage and 55% based on histology (Table 3.12), which means that the maturity of 45% of males in the study group was misclassified through the macroscopic examination. This result shows an improvement compared to the misclassification rates of 60% for males achieved during WKMSCWHS 2007. Stage 3 was the most correctly identified with an accuracy of 73%, while stage 1 and 2 where accurately recognized only around 30% of cases.

3.4.5 Hake

In total, the maturity of 66 (46 females and 20 males) specimens was ascertained through histological examination of the gonadal tissue and the results compared with initial macroscopic assessments.

Females

Specimens' maturity stages ranged from 1 to 3. Overall agreement among observers was 78% based on modal stage and 67% based on histology (Table 3.13), which means that the maturity of 33% of females in the study group was misclassified through the macroscopic examination.

Stage 1 was rather correctly identified showing an agreement between visual inspection and histology of 83%. Stage 2 was not easily recognized in view of the lower percentage of agreement (43%) while stage 3 was correctly detected in 63% of cases. The inaccuracy of stage 3 is probably due to the fact that translucent oocytes were not easily distinguished in pictures, where a transversal cut had helped to correctly identify this stage. Only one specimen was observed in stage 6 and only detected by the means of histology.

Males

Specimens' maturity stages ranged from 1 to 3. Overall agreement among observers was 75% based on modal stage and 61% based on histology (Table 3.13), which means that the maturity of 39% of males in the study group was misclassified through the macroscopic examination.

Similar to females, identification of stage 1 obtained the highest percentage of agreement to histology, i.e. always correctly identified. Stage 2 and 3 were correctly identified with a percentage of agreement around 60%.

All together this results evidence a decline compared to the 85.5% reached for combined sexes during WKMSHM in 2007.

Table 3.4. Cod. Number of observations by maturity stage.

Stage	OBS Trainee	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Trainee	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Expert	OBS Expert	OBS Expert
1	9	11	9	20	9	11	13	6	8	15	12	15	12	8	13
2	11	15	17	5	5	9	10	14	12	7	12	11	10	8	8
3	12	9	12	12	13	8	12	12	13	11	14	8	8	9	13
4	4	1	0	0	3	0	2	5	8	1	1	1	2	6	0
5	7	8	5	7	8	11	5	6	3	9	5	7	12	9	10
6	0	0	0	0	1	0	2	1	0	1	0	1	0	2	0
1 to 6	43	44	43	44	39	39	44	44	44	44	44	43	44	42	44

Table 3.5. Whiting. Number of observations by maturity stage.

Stage	OBS Trainee	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Trainee	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Expert
1	1	1	1	5	1	6	4	3	2	1	2	2	5	2
2	14	11	14	10	8	7	11	8	8	9	13	8	9	13
3	8	13	10	9	16	11	10	10	13	15	10	12	11	8
4	2	0	0	0	0	0	0	2	0	0	0	0	0	2
5	0	0	0	1	0	0	0	0	2	0	0	2	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 to 6	25	25	25	25	25	24	25	23	25	25	25	24	25	25

Table 3.6. Haddock. Number of observations by maturity stage.

Stage	OBS Trainee	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Trainee	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Expert
1	6	7	5	14	7	11	10	7	9	9	10	6	12	7
2	8	18	18	11	6	14	8	8	11	10	10	7	15	12
3	23	14	19	17	20	13	24	23	18	22	23	17	13	14
4	2	2	2	0	6	2	1	1	1	3	1	8	3	7
5	3	3	0	2	3	2	0	4	2	0	0	6	0	4
6	0	0	0	0	1	0	0	1	3	0	0	0	0	0
1 to 6	42	44	44	44	43	42	43	44	44	44	44	44	43	44

Table 3.7. Saithe. Number of observations by maturity stage.

Stage	OBS Trainee	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Trainee	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Expert
1	3	0	1	9	0	3	9	4	2	0	7	1	3	2
2	6	15	9	7	4	10	7	7	20	13	9	4	5	6
3	16	10	17	16	15	13	16	13	3	15	15	17	16	15
4	6	2	2	0	9	2	2	5	5	4	2	4	4	7
5	2	7	5	1	6	5	0	5	3	2	0	8	5	3
6	0	0	0	0	0	0	0	0	0	0	1	0	1	1
1 to 6	33	34	34	33	34	33	34	34	33	34	34	34	34	34

Table 3.8. Hake. Number of observations by maturity stage.

Stage	OBS Trainee	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Trainee	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Expert	OBS Trainee	OBS Trainee	OBS Expert
1	21	24	13	27	20	25	24	22	19	24	22	26	27	22
2	28	18	29	14	11	17	11	16	22	17	15	18	27	18
3	12	16	19	21	27	17	27	18	17	18	22	21	7	17
4	5	8	3	2	7	4	1	4	8	6	0	1	3	9
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	1	1	1	0	2	3	0	0	7	0	1	0
1 to 6	66	66	65	65	66	63	65	63	66	65	66	66	65	66

Table 3.9. Cod. Percent of agreement between visual inspection and histology per stage and sex. The ranking according to the Kruskal–Wallis test is shown by the letters beside the % value.

Histological Stage	Male	Female
1	71% a	89% a
2	24% b	76% a
3	76% a	77% a
4	13% b	-
5	33% b	60% b
6	-	-
All	66%	73%

Table 3.10. Whiting. Percent of agreement between visual inspection and histology per stage and sex. The ranking according to the Kruskal–Wallis test is shown by the letters beside the % value.

Histological Stage	Male	Female
1	0%	93% a
2	62%	55% b
3	-	87% a
4	-	-
5	-	-
6	-	-
All	51%	70%

Table 3.11. Haddock. Percent of agreement between visual inspection and histology per stage and sex. The ranking according to the Kruskal–Wallis test is shown by the letters beside the % value.

Histological Stage	Male	Female
1	64% a	82% a
2	75% a	58% b
3	50% ab	86% a
4	-	39% b
5	21% b	-
6	-	7%
All	54%	73%

Table 3.12. Saithe. Percent of agreement between visual inspection and histology per stage and sex. The ranking according to the Kruskal–Wallis test is shown by the letters beside the % value.

Histological Stage	Male	Female
1	32% b	43% b
2	33% b	77% a
3	73% a	80% a
4	-	-
5	-	-
6	-	-
All	55%	69%

Table 3.13. Hake. Percent of agreement between visual inspection and histology per stage and sex. The ranking according to the Kruskal–Wallis test is shown by the letters beside the % value.

Histological Stage	Male	Female
1	100% a	87% a
2	60% b	43% c
3	57% b	63% b
4	-	-
5	-	-
6	-	-
All	61%	67%

4 Sampling and histological analysis for validation (Tor c)

Prior to the workshop, participants were asked to collect samples for histological analysis during their own surveys, following a specific sampling protocol (Annex 4), describing the biometrical parameters as well as the photographic material to be collected.

Unfortunately it was eventually not possible to collect samples of all species throughout the entire latitudinal range (Tables 4.1, 4.2 and 4.3). Moreover, some species were less represented in the workshop than others.

Tables 4.1, 4.2 and 4.3. Location (upper table), timing (middle table) and number of samples (lower table) collected (females·males) by each country in preparation to the workshop.

Location

Species	Ireland	Norway	Spain	Sweden
Cod	ICES VIIa	ICES IIa, IVa	NAFO 3L	ICES IIIb
Whiting	ICES VIIa			ICES IIIb
Haddock	ICES VIIa	ICES IIa, IVa		ICES IIIb
Saithe		ICES IIa		ICES IIIb
Hake	ICES VIIa		ICES VIIIabc+Ixa; GFCM GSA 01,17	ICES IIIb

Season

Species	Ireland	Norway	Spain	Sweden
Cod		Prespawning and spawning	Post-spawning	spawning
Whiting				spawning
Haddock		Prespawning		spawning
Saithe		Spawning		spawning
Hake			Spawning; out of spawning	uncertain

Number

Species	Ireland	Norway	Spain	Sweden	Total
Cod	7·2	6·9	23·22	11·9	24·20
Whiting	10·0			10·5	20·5
Haddock	13·2	9·6		8·6	30·14
Saithe		11·14		3·6	14·20
Hake	5·5		177·79	4·5	186·89

5 Enhancement of 2007 scales' descriptors and manuals (ToR d)

The foundation of WKMSGAD discussion on potential enhancement of currently used maturity keys was the universal maturity scale, valid across species and time, developed during WKMATCH 2012. The importance of having a universal maturity scale is that too often, when combining data from different institutes/countries it is necessary to have knowledge of the historical local development of maturity coding. Adopting a generalized scale 4+2 (Table 5.1), as proposed by WKMATCH, where each stage can be subdivided at convenience, ensures a consistency across scales used by different countries and lessen the risk of bias when combining data. The correspondence between the currently used standard maturity scales for cod/whiting/haddock/saithe (WKMSCWHS, 2007) and for Hake (WKMSMH, 2007) and the newly proposed universal scale (WKMATCH 2012) is shown in Table 5.1. Moreover, the WKMATCH universal maturity scale adopts the standardized terminology for describing reproductive development in fish (Brown-Petersen *et al*., 2011, see WKMATCH report for a broader discussion) and introduces the term *code* instead of *stage*. It is essential to underline that this is a universal scale thus its applicability needs to be discussed on the species level.

Table 5.1. Correspondence between the currently used standard maturity scales for cod/whiting/haddock/saithe (WKMSCWHS, 2007) and for Hake (WKMSMH, 2007) and the universal scale proposed by WKMATCH 2012.

	Universal maturity scale - WKMATCH 2012	Standard maturity scale - WKMSCWHS 2007	Standard maturity scale - WKMSHM 2007
SEXUALLY IMMATURE	1.Immature	1.Juvenile/Immature	1.Immature/Resting
SEXUALLY MATURE	2.Developing	2.Maturing	2.Developing/Maturing
	2a. Developing but functionally immature		
	3.Spawning	3.Spawning	3.Spawning
	3a. Active Spawning		
	3b.Spawning capable		
	4.Regressing/Regenerating		
	4a.Regressing	4.Spent	4.Post-Spawning
	4b.Regenerating	5.Resting/	1.Immature/Resting
	5.Omitted spawning	Skip of spawning	
	5.Abnormal	6.Abnormal	

In the WKMATCH scale, code 1 is unchanged, including juvenile/immature individuals. Concerning codes 2 and 3, some subdivisions have been introduced. Distinguishing between developing females and females without hydrated eggs but within spawning season (3b) is difficult even with histology, but yet possible macroscopically for some species. The solution adopted here is combining (not merging) codes. The former stage *maturing* included all types of vitellogenesis, and unavoidably they should be code 2 in the new scale albeit some of them would be 3b. The observer should now annotate 2 for clear developing females; 3b when they think can be late developing or in the intervals between batches, and 3a for those with hydrated oocytes. However, the subdivision of this stage is species-specific.

The main novelty of the universal maturity scale is that the *regressing* (*spent*) and *regenerating* (*resting*) codes are now combined in the same stage, while *omitted spawning* (generally merged with *regenerating*/*resting*) has now a separate code. The first reason behind this change is that all species go through a *regressing (spent)* period followed by a *regenerating (resting)* phase but in many species this step is so short that no distinction is made, so having two stages is confusing. However, in species where it is possible and necessary to distinguish between *regressing (spent)* and *regenerating (resting)*, two (or more) subdivisions can be made, but still ensuring that it is always a single stage, i.e. 4. Thus code 4 has still the same unambiguous biological meaning in

both species. Moreover it is hard to correctly identify a *regenerating/resting* stage during spawning season. In this view a merged stage *regenerating(resting)/omitted spawning* makes no sense, while a single stage (4) for the entire period between end of spawning and onset of ripening (next season) has a biological meaning. Finally, finding a fish that shows sign of a previous spawning but still undeveloped while the spawning season is approaching is a sign of *omitted spawning*. Distinguishing *omitted spawning* by naked eye is also quite difficult, so one has to be careful when using this code.

WKMSGAD participants were divided in 2 teams, one working with cod/whiting/haddock/saithe and one with hake, for evaluating the applicability of the proposed universal scheme to the species in object.

5.1 Cod, whiting, haddock and saithe

The determination of gonadal maturity of gadoids is extensively described in the report from the previous workshop (ICES, 2007a). The WKMSCWHS report also include a broad overview of each species' reproductive strategy, existing information on maturity staging and histological criteria, thus this information is not replicated in the present report.

Because of a limited number of participants during WKMSGAD, each species could not be discussed separately. Furthermore there was a lack of expertise concerning two of the species, i.e. whiting and haddock. The team, dealing with the 4 species together, evaluated the applicability of the universal scale and agreed on the modification of the WKMSCWHS 2007 maturity key according to the WKMATCH universal maturity scale.

The macroscopic and histological criteria for the newly proposed maturity scale for females and males of cod, whiting, haddock and saithe are shown in Table 5.2a-b.

Table 5.2 (a) Suggested common maturity scale for female cod, whiting, haddock and saithe including macroscopic and histological characteristics of the 6 stages. PN: perinuclear oocytes, CNR: circumnuclear oocytes, CA: cortical alveoli stage, VT: vitellogenic oocytes, FM: final maturation stage, HYD: hydrated eggs, POF: Post ovulatory follicles.

CODE	Description of ovarian appearance	Histology
1	Juvenile/Immature	
	No sex determination: juvenile below a certain size, depending on species /stock, risk of mistaking gonads for bladder	Oogonia/PN
	Sex determination: Translucent or transparent ovaries with transparent ovary wall	PN/CNR
2	Developing	
	Firm. Transparent ovary wall. The ovary may show from tiny but recognizable oocytes to clearly visible yolked oocytes when cut transversally.	CA/VT
3	Spawning	
	Hydrated oocytes (from one to several) visible through the ovary wall or in a transversal cut. Occasionally running eggs.	FM/HYD/POF
4	Regressing/Regenerating	
	4a. Regressing* Lax and baggy ovary with abundant capillaries in the tissue. Thick whitish ovary wall. Single hydrated oocytes may be visible through the ovary wall or in a transversal cut.	POF/PN/CNR/possible atresia
	4b. Regenerating** Ovary has shrunken and fully or partly recovered its firmness and shape, without visible development. Whitish ovary wall in larger specimens. Observed during the spawning season or in post-spawning period.	PN/CNR/ possible atresia
5	Omitting spawning*** Ovary without visible development. Signs of previous spawning (whitish ovary wall) may occur. It is used for 4b observed in prespawning period.	PN/CNR/ possible atresia
6	Abnormal Hard parts (connective tissue), only one lobe developed, intersex, etc.	Variable

*** previously called spent**

**** previously called resting**

***** previously called skip of spawning**

Table 5.2. (b) Suggested common maturity scale for male cod, whiting, haddock and saithe including macroscopic and histological characteristics of the 6 stages. SG: Spermatogonia, SC1: Primary spermatocytes, SC2: secondary spermatocytes, ST: spermatides, SZ: spermatozoa.

CODE	Description of testis appearance	Histology
1	Juvenile/Immature	
	No sex determination: Juvenile below a certain size, depending on species /stock, gonads difficult to identify.	Germ cells/SG
	Sex determination: With developing (translucent or opaque) empty frills.	Germ cells/SG
2	Developing	
	Opaque reddish to whitish filled frills. Empty spermatoducts.	SC1/SC2/ST, non motile flagellate SZ
3	Spawning	
	Milt visible in wide spermatoducts, milt often flows at light pressure.	
4	Regressing/Regenerating	
	4a. Regressing * Lax testis with blood in the tissue. Mostly empty but some milt may still be present	Aligned ripe SZ proximally and spermatoduct, cyst, no lobule walls
	4b. Regenerating ** Testis has shrunk and fully or partly recovered its firmness, without a visible development. Distended spermatoduct compared to stage 1. Observed during the spawning season or in post-spawning season.	Migrating germ cells/SG, interlobular walls thickens, atretic spermatozoa
5	Omitted spawning *** Testes without a visible development. Relatively larger lobules than stage 1 and distended spermatoduct as in 4b. It is used for 4b, when observed in prespawning season.	Migrating germ cells/SG, resting cysts of SG and SC1
6	Abnormal Adipose tissue, only one lobe developed, intersex or other abnormalities.	Variable

*** previously called spent**

**** previously called resting**

***** previously called skip of spawning**

The macroscopic descriptors included in the WKMSWCHS 2007 maturity scale were revised in order to make the scale universal, i.e. suitable for all stocks. Consequently all the characteristics based on subjectivity, such as colour, size and presence/absence of blood vessels, were avoided as considered stock specific. Only objective and validated criteria were chosen by the group as stage descriptors.

In this new maturity scale, code 1, 2 and 3 were not modified. Code 3 in fact can be a single one for those four species and it is identified by the presence of hydrated oocytes. The identification of individuals in an inter-batches condition (i.e. 3b) is difficult for those species thus the separation of stage 3 in 3a-3b was not applied. Furthermore this separation would have had an impact on historical dataseries. Hence, specimens with advanced vitellogenic oocytes but no hydrated eggs are still

categorized as code 2. Individuals included in both codes (2 and 3) are considered mature (for ogives/SSB) and the identification of code 3 allows defining the spawning season.

Concerning code 4, the group decided to follow the suggestion proposed by WKMATCH and include the previous stage 5 (resting) in code 4 and classify it as 4b. In this way only individuals showing an *omitted spawning* (previously called skip of spawning) are now classified as code 5. Code 6 is still referring to fish showing abnormal gonadal development. The monitoring of the last 2 codes, i.e. *omitted spawning* and *abnormal*, can be used as ecosystem indicator.

As mentioned above the histological identification of cells development corresponding to the macroscopic maturity stages was comprehensively discussed during WKMSCWHS 2007 thus it remained unchanged.

The applied modifications on the WKMSCWHS 2007 scale do not have any impact on the translation of local scales and thus on historical national time-series.

5.2 Hake

The WKMSHM 2007 had developed a standard maturity key which takes into account the reproductive cycle of the species and includes histologically validated criteria. This maturity scale, broadly described in the workshop's report (ICES, 2007b), was considered a consistent and handy key widely used among different institutes. However, WKMSGAD ascertained that, unlike the other hake stocks, for the Mediterranean hake it is in same cases possible to discriminate between the stages immature and resting.

In view of this and following the recommended WKMATCH 6-stage scale few changes were proposed during this workshop. The applied modifications do not have any impact on the translation of local scales or on historical data. Basically, the main change is the use of the term "code" instead of "stage" and the adoption of the standard terminology (Brown-Petersen *et al*., 2011). In addition, two new stages are included in accordance with the universal 6-stage maturity key, i.e. "5: Omitted spawning" and "6: Abnormal". These two stages are not frequently observed in hake, but they are still important to be monitored.

In the new proposed scale (Table 5.3a-b) each gonadal stage is described using two types of criteria. Objective criteria mirror the unambiguous developmental characteristics and are free of subjectivity while the indicative criteria are not conclusive and may vary between stocks being influenced by the environment. Such indicative criteria, e.g. gonad's colour, gonad's size and blood vessels' size, may guide the observer taking a decision.

Table 5.3. (a) Suggested common maturity scale for female hake

PHASE	CODE		DESCRIPTION	
Immature	1-4b	1*	Objective criteria: Small translucent ovary. Oocytes not visible to the naked eye. The ovary has a firm consistency.	Indicative criteria: Ovary is shorter than 1/3 of the body cavity. Light orange/pink colour.
Regenerating**		4b*	Objective criteria: ovary not translucent. Oocytes are not visible to the naked eye. The ovary has firm consistency. Sometime the blood vessels are visible.	Indicative criteria: Ovary is variable in size, around ½ of body cavity. Visible thick wall in the ovary transversal section. Ovary pinkish-reddish to reddish-orange.
Developing	2		Objective criteria: Granulated texture of gonad. Opaque oocytes present, visible at naked eye trough the ovary wall. Transparent oocytes absent. The ovary has a firm consistency. Blood vessels are visible.	Indicative criteria: Ovary is 2/3 of the body cavity approximately. Thin blood vessels. The colour of ovary is homogeneous throughout a cross section. Ovary pink-yellow to orange.
Actively spawning	3a		Objective criteria: Gonad has a granulated texture. Transparent oocytes are clearly visible and are released under slight pressure. The ovary has firm consistency. Blood vessels are evident.	Indicative criteria: Ovary is from 2/3 to fully of the body cavity. Transparent oocytes may be concentrated in the central area of a cross section (lumen). The thickness of blood vessels is variable (thin and thick). Ovary pink or reddish orange.
Spawning capable	3b		Objective criteria: Gonad has a granulated texture. Opaque oocytes are abundant while transparent oocytes are not visible. The ovary is flaccid. Blood vessels are visible.	Indicative criteria: Ovary covers about 2/3 of the body cavity. The colour of ovary is darker in the central area when cross sectioned. Few transparent oocytes may be present. The size of blood vessels is variable (thin and thick). Opaque oocytes not tightly packed.. Ovary colour is pink or reddish orange.
Regressing***	4		Objective criteria: Residual opaque and/or transparent oocytes may be present. Ovary is shrunken and flaccid.	Indicative criteria: Ovary is variable in size (from 2/3 to ½ of length of the body cavity). Sometimes residues of blood are visible. Ovary colour is dark pink/orange or purple
Omitted spawning	5		Not observed in European hake	
Abnormal	6			

***Warning: The stage 4b could be confused with stage 1. When the distinction is not possible the gonad will be classified as 1-4b (Immature/Resting).**

**** Previous nomenclature: Resting, Recovering**

*****Previous nomenclature: Spent**

Table 5.3. (b)Suggested common maturity scale for male hake

PHASE	CODE	DESCRIPTION	
Immature	1	Objective criteria: Thin and transparent testis. Sperm absent, ribbon no sign of developing no curling	Indicative criteria: Shorter than 1/3 of the body cavity.
Developing	2	Objective criteria: Developing and curling. In a transversal cut the sperm is absent. The testis has firm consistency.	Indicative criteria: Testis whitish to creamy in colour. Testis is variable in size (from ½ to 2/3 of length of the body cavity). The width is about 1 cm
Spawning	3	Objective criteria: The sperm is expelled under light pressure and/or flows freely. In transversal cut the sperm is present. Testis forms large curling firm bands.	Indicative criteria: Colour is white-creamy. Size ranges from ½ to full length of the body cavity.
Regressing*	4	Objective criteria: Testis shrunken and flabby. Sometimes the anterior part (junction lobules) is empty and enlarged while the posterior part in the spermatoduct is very thin. Some sperm may still be visible. The blood vessels are visible. The testis is flaccid.	Indicative criteria: Size about ½ the length of the body cavity. Colour is reddish to light pink
Omitted spawning	5	Not observed in European hake	
Abnormal	6		

***Previous nomenclature: Spent**

Regenerating stage (4b): It is not possible until now define clear objective criteria for identifying unequivocally this stage

A correspondence table between the new proposed scale, WKMSMH 2007 scale and Medits scale is presented in Table 5.4.

Table 5.4. Correspondence table between the currently used macroscopic maturity scales and the new proposed common maturity key for female (upper table) and male (lower table) hake.

WKMSHM 2007		WKMSGAD 2013		Medits scale	
		FEMALE			
1	Immature/Resting	1	Immature	1	Immature/Virgin
				2a	Virgin Developing
2	Maturing	2	Developing	2c	Maturing
3a	Hydrated	3a	Actively spawning	3	Mature/Spawner
3b	Partial Spawning	3b	Spawning capable		
4	Post Spawning	4a	Regressing	4a	Spent
1	Immature/Resting	4b	Regenerating	4b	Resting
				2b	Recovering
		5	Omitting Spawning		
		6	Abnormal		

WKMSHM 2007		WKMSGAD 2013		Medits scale	
		MALE			
1	Immature	1	Immature	1	Immature/Virgin
				2a	Virgin Developing
2	Developing	2	Developing	2b	Recovering
				2c	Maturing
3	Spawning	3	Spawning capable	3	Mature/Spawner
4	Post spawning	4	Regressing	4a	Spent
				4b	Resting
		5	Omitting Spawning		
		6	Abnormal		

5.3 Manuals

During WKMSCWHS 2007, photographs of gonads and tissue were selected as basis for sexual maturity manuals. One illustrated manual for each species (cod, whiting, haddock and saithe) was thus drafted and uploaded on the PGCCDBS' repository (http://www.ices.dk/community/Pages/PGCCDBS-doc-repository.aspx). Unfortunately it was not possible to finalize those drafted manuals using the samples collected in preparation for WKMSGAD, as some of the developmental stages are still missing. Drafts will be updated and re-uploaded on the PGCCDBS' repository.

On the other hand, samples collected in preparation for WKMSGAD allowed creating a full illustrated manual for hake, which will also be uploaded on the same repository.

6 Impact on currently used maturity ogives (ToR e)

The new proposed scales (one for cod, whiting, haddock and saithe and one for hake) mainly represent an improvement of stages' descriptions and stage separation. The do not differ from the international scales currently in use and can easily be translated from the national scales. Hence there is no impact on currently used maturity ogives for none of the species. However the degree of stage misclassification, detected during the calibration exercise carried on during WKMSGAD, highlighted a potential risk of bias when calculating maturity ogives. Concerning cod, whiting haddock and saithe, the identified misclassification between stage 1: immature, 2: developing and stage 5: resting/skip of spawning (recoded as 1, 2 and 4b/5 during the present workshop), has the consequences of excluding specimens that have actually spawned (stage 4b) from the SSB when mistakenly judged as immature or including in the SSB specimens that omitted spawning when mistakenly judged as developing. This will lead to an incorrect estimation of the stock reproductive potential.

WKMSHM 2007 had already corroborated a misclassification occurring between immature and regenerating (resting) ovaries also for hake. In addition, during the present workshop misclassification of immature/ regenerating (resting) and early developing hake was detected when working with fresh gonads, confirmed both in whole mounts and in histological slides. This problem in gonads classification could have a significant effect on the estimation of maturity ogives. Moreover, the calibration exercise with cod frozen samples highlighted the misclassification between developing and spawning omission stages. It is noteworthy that the use of frozen samples is not optimal for assigning maturity thus results should be cautiously interpreted.

These results have an impact on the calculation of maturity ogives and lead to an underestimation the SSB. However it is important to stress that the accuracy of macroscopic maturity determination depends on the sampling time in relation to the spawning period.

Identifying the correct sampling time is also of a great help for minimizing the risk of misjudgement. The recommended optimal sampling time for accurately estimating maturity ogives varies with the reproductive strategy of the different species (see WKMATCH 2012).

WKMSWCHS 2007 recommended sampling just before the spawning season for cod, whiting, haddock and saithe, in order to properly identify fish in spawning omission and avoid mistaking them with regenerating (resting) specimens. WKMSHM 2007 recommended sampling hake during the main spawning season in order to reduce the probability to encounter samples in early developing and regenerating (resting) stage. WKMATCH 2012 and the present workshop agree to reinforce these previous recommendations (Annex 6). A clear spawning peak cannot be detected for Mediterranean hake (Recasens *et al*., 2008; Al-Absawy, 2010) thus monthly sampling is recommended in order to better calibrate the use of maturity data based on macroscopic evaluation in the estimation of ogives. However because of national logistic problems is not always possible to sample in the suggested period, thus alternatives should be proposed to minimize the misclassification risk and consequently the bias in maturity staging. The most accurate solution would be to use histology or whole mount, if not for analysing all the samples, at least for calculating a correction factor to be applied in each length class.

It is important to underline that the stages to be considered in the estimation of maturity ogives vary according to the purpose, whether it is to estimate a) the Spawning-stock biomass (SSB) from the total numbers of individuals in the stock or b) the total mature stock (subject to a fishery) or c) the stock 'productivity', stock-recruitment relationships, Total Egg Production (TEP) estimates or reference points. A comprehensive discussion on the use of common maturity scale data for the different purposes can be found in WKMATCH 2012 report.

7 Local training programs (ToR f)

Ensuring high quality biological data are a commitment that each sampling country has within the Data Collection Framework. Hence both age reading and maturity staging require routinely trained personnel. In principle the staff, either scientists or technicians, going out at sea and evaluating fish individual maturity stage is requested to have a basic knowledge of the biology of each species they are dealing with and to be fully trained in the recognitions of the different developmental phases.

Nevertheless, some countries have to deal with local issues and sending regular personnel out at sea seems to be a task difficult to fulfil. This is especially valid for those countries dealing with a large amount of stocks, mainly sampled by observers on commercial vessels that frequently spent several months at sea, working in different fishing grounds and dealing with several species from which take data and /or samples for several parameters other than maturity. Thus in contrast to age readers, maturity stagers working out at sea are often occasional and have to assimilate a huge quantity of information in relatively short time. The risk is that errors in maturity stage identification and the consequent bias in maturity ogives estimation may derive from a lack of expertise by the stager rather than from a problem in current assessing criteria. It is thus strongly recommended that new maturity stagers are not sent out at sea alone, rather always followed by at least one experienced stager. Furthermore the error produced by the lack of experience can be reduced with clear and unambiguous descriptions of the morphology of the different maturity stages.

As stressed above, knowledge of the biology of the species sampled for maturity needs to be included in the information provided to the staff, as stagers need to be aware that different maturity stages can be encountered during different periods of the reproductive cycle. This will increase the quality during data collection and minimize the risk of misjudgement.

Each institute should have an established local training routine program for updating and testing the quality of maturity staging made by regular personnel (scientists and/or technicians) working on board. Inter-calibration exercises should be carried on possibly using fresh sample but also frozen in case of impossibility of having fresh samples. A tool as WebGR, based on pictures, is also a useful help for calibration both within and between institutes. In this way the consistency in maturity staging is ensured, potential drift over the time by the stagers can be detected and the ability of the stagers will always be up to date.

Concerning the maturity workshops it is strongly recommended that each national laboratory guarantees the attendance of participants with an adequate level of knowledge and expertise. Competence and experience in biology and maturation pattern of the species dealt with during a workshop is a prerequisite for a successful and operational meeting.

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Annex 1: List of participants

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Annex 2: Agenda

Monday 4 November

09.00: Welcome by the organizers and housekeeping

Participants' introduction round

Terms of Reference and adoption of the agenda

9.45: (Tor a) Previous workshops outcomes (F. Vitale, M. Kruger and M. Korta)

General consideration about WKMSGAD

10.45: Coffee Break

11.15: Introduction on the different species: Assessment status, reproductive strategies, macroscopic and microscopic maturity determination and quality assurance of data (F. Vitale)

11.40: WKMATCH, 2012 outcomes (M. Korta)

12.00: Presentations by each Institute on own stocks, sampling, spawning time, maturity scales (ca 5 min each)

13.00: Lunch

14.00: Overview of maturity scales used by different institutes

14.30: Team work: Discussion of shortcomings and problems with existing scales and sampling schemes (time and frequency) depending on purpose of data.

15.45: Coffee Break

16.15: Report of the teamwork and discussion

18.00: End of day 1

Tuesday 5 November

09.00: Practical exercise: Hake fresh samples and cod frozen

10.45: Coffee Break

13.00: Lunch

14.00: Team work: Improvement of macroscopic stage descriptors and adoption of a new standard scale

15.45: Coffee Break

16.15: Team work (cont)

17.00: Summarize team work in plenary: Discussion of existing scales and Improvement of stage descriptors. Correspondence of new (WKMATCH and old scales (ICES, national) and evaluation of the potential impact.

18.00: End of day 2

Wednesday 6 November

09.00: Results of the practical exercise using fresh and frozen samples, validated with whole mount (fresh) and histology (frozen).

10.00: Hake maturation pattern in Mediterranean Seas (P. Carbonara)

10.45: Coffee Break

11.15: (ToR d) Teamwork: Improvement of microscopic descriptions in the 2007 scales' stages

13.00: Lunch

14.00: (ToR d) Teamwork (cont.)

15.45: Coffee Break

16.10: Discussion and summary of the teamwork

18.00: End of day 3

Thursday 7 November

09.00: Introduction to WebGR (Maria Korta)

09.30: Practical exercises using WebGR (macroscopic)

10.45: Coffee Break

11.15: Practical exercises using WebGR (macroscopic)

13.00: Lunch

14.00: (ToR b) Macroscopic and histological criteria:

Presentation on male gadoids (M. Kruger)

14.20: (Tor c) Results of the practical exercise using WebGR, after validation with histology. General discussion and identification of problems. Feedback on WebGR.

(ToR d) Teamwork: Improvement of microscopic descriptions in the 2007 scales' stages and finalization of the illustrated manuals initiated in 2007

15.45: Coffee Break

18.00: End of day 4

Social Dinner

Friday 8 November

09.00: (ToR e) Discussion on the impact on currently used maturity ogives and use of spawning proportion.

10.00: (ToR f) Local training programs for scientists and technicians sampling gadoids

10.45: Coffee Break

11.15: General discussion and agreement of outcomes of the workshop

12.00: Distribution of tasks for reporting and development of manuals with improved descriptions and illustrations.

14.00: End of workshop

Annex 3: Maturity scales currently in use

This annex presents an overview of the maturity coding keys currently in use. The original keys used in different countries are shown.

Stage	Description of appearence ovaries	Histology
1	Juvenile/Immature	
	No sex determination: juvenile below 15 cm, risk of mistaking gonads for bladder.	Oogonia / PN
	Sex determination: Juvenile-transparent ovaries.	PN
	Immature-translucent ovaries, coloration is pinkish to light orange, cast thin and clear. Blood vessels hardly discernable.	PN/CNR
2	Maturing: Firm, coloration ranges from reddish orange to creamy orange with granulated/oocytes clearly visible in issue. Blood vessels larger and diversified.	CA/T
3	Spawning: Distended, few to many hydrated eggs visible in tissue among vitelogenic oocytes or in lumen, occasionally running.	FM/HYD/POF
4	Spent: Slack with greyish cast, rich in blood vessels.	POF, perhaps atretia, PN, CNR
5	Resting/Skip of spawning*: No visible development-similar to Immature but simetimes with a greyish cast.	PN, CNR, perhaps atresia
6	Abnormal*: Hard parts (connective tissue), only one lope developed, intersex, or similar-fecundity at least partly reduced.	Variable

Stage	Description	Histology
I	Juvenile/Immature.	
	No sex determination: juvenile below 15 cm, gonads difficult to identify.	Germ cells/SG
	Sex determination: Juvenile-transparent testes.	Germ cells/SG
	Immature-testes with developing frills, coloration is reddish to white, vascularisation is limited.	SG/SC1
II	Maturing: Whitish to almost opaque reddish-white, blood vessels more prominent, empty transparent spermatoducts.	SC1/SC2/ST, spermatids/non-motile flagellate SZ
III	Spawning: Opaque creamy white colour to reddish late in stage, semen visible in spermatoduct, milt often flows at ligth pressure.	Aligned ripe SZ proximally and in sperm duct, cyst, no lobule walls.
IV	Spent: Contracted, empty and flabby lobules, colour deep pink to reddish-purple, bloodshot, potentially with greyish cast.	Migrating germ cells/SG, interlobular walls thickens, atretic spermatozoa
V	Resting/Skip of spawning*: No visible development, spermatoducts often with a greyish cast, similar to immature, early maturing.	Migrating germ cells/SG, resting cysts of SG and SC1.
VI	Abnormal*: Adipose tissue, only one lobe developed, intersex, or similar.	Variable

Ecosystem state indicators*

Figure A3.1. ICES standard maturity key (WKMSCWHS, 2007)

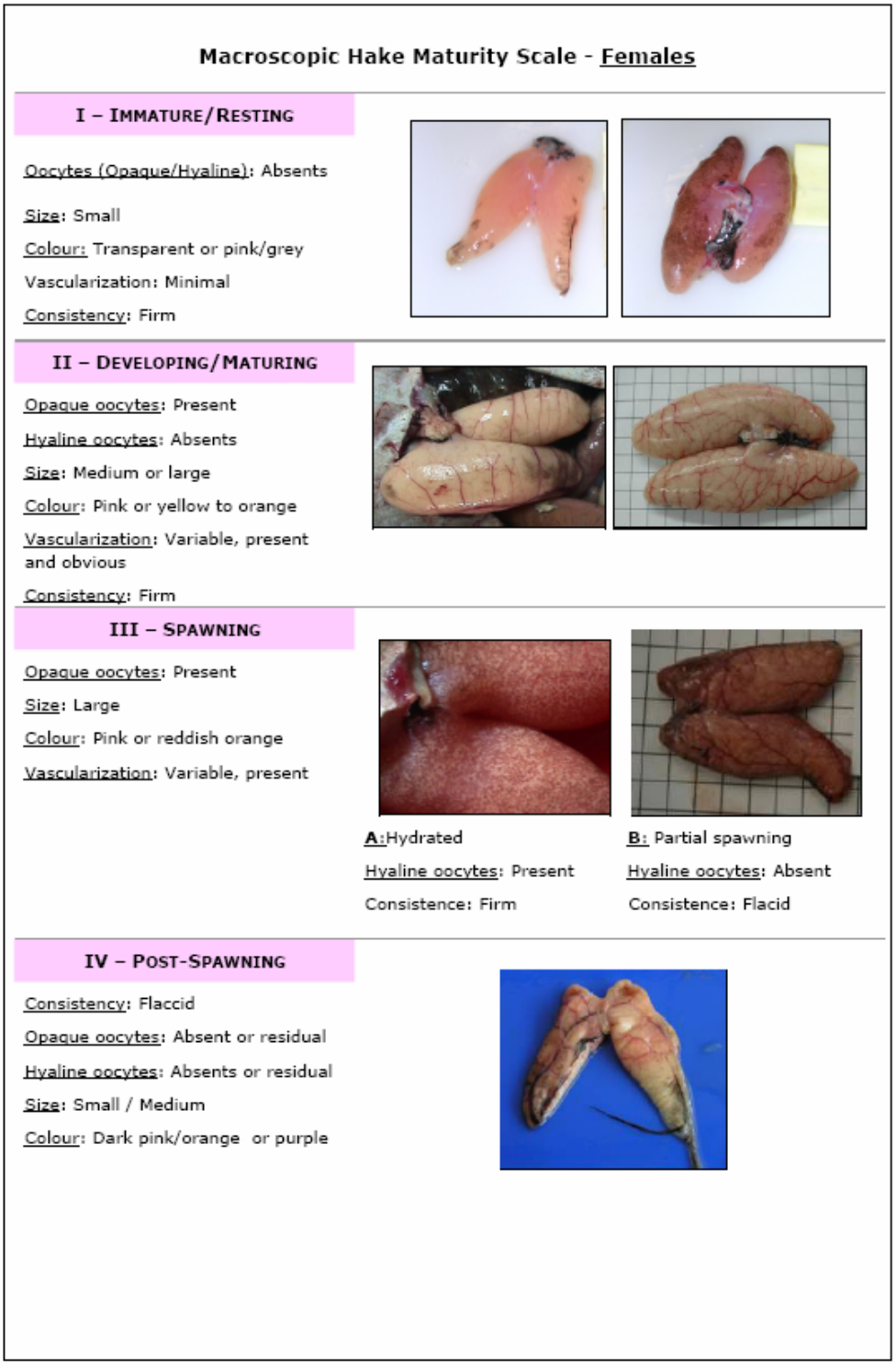


Figure A3.2. ICES standard maturity key for hake (WKMSHM, 2007)

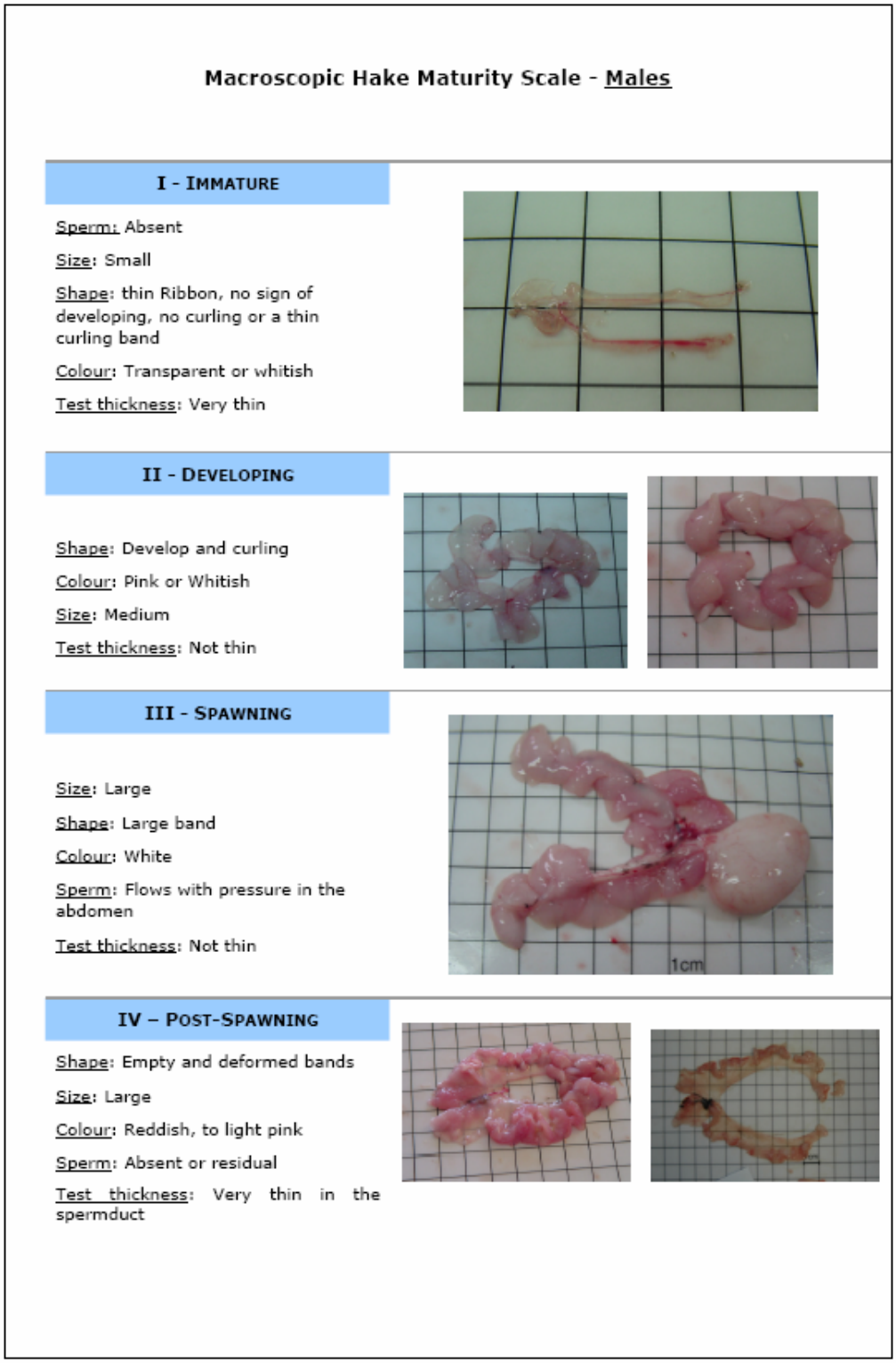


Figure A3.2(cont.). ICES standard maturity key for hake (WKMSHM, 2007)

SEX	GONAD ASPECT	MATURATION STATE	STAGE	MEDITS
I	Sex not distinguished by naked eye. Gonads very small and translucid, almost transparent. Sex undetermined.	UNDETERMINED	0	0
F	Small pinkish and translucent ovary shorter than 1/3 of the body cavity. Eggs not visible by naked eye.	IMMATURE=VIRGIN	1	1
M	Thin and withish testis shorter than 1/3 of the body cavity.			
F	Small pinkish/reddish ovary shorter than ½ of e body cavity. Eggs not visible by naked eye.	VIRGIN-DEVELOPING*	2a	2
M	Thin withish testis shorter than 1/2 of the body cavity.			
F	Pinkish-reddish/ reddish-orange and translucent ovary long about ½ of the body cavity. Blood vessels visible. Eggs not visible by naked eye.	RECOVERING*	2b	
M	Withish/pinkish testis, more or less symmetrical, long about ½ of the body cavity			
F	Ovary pinkish-yellow in colour with granular appearance, long about 2/3 of the body cavity. Eggs are visible by naked eye trough the ovaric tunica, which is not yet translucent. Under light pressure eggs are not expelled.	MATURING	2c	
M	Withish to creamy testis long about 2/3 of the body cavity. Under light pressure sperm is not expelled.			
F	Ovary orange-pink in colour, with conspicious superficial blood vessels, long from 2/3 to full length of the body cavity. Large transparent, ripe eggs are cleary visible and could be expelled under light pressure. In more advanced conditions, eggs escape freely.	MATURE/SPAWNER	3	3
M	Whitish-creamy soft testis long from 2/3 to full length of the body cavity. Under light pressure, sperm could be expelled. In more advanced conditions, sperm escapes freely.			
F	Reddish ovary shrunked to about 1/2 length of the body cavity. Flaccid ovaric walls; ovary may contain remanants of disintegrating opaque and/or translucent eggs.	SPENT	4a	4
M	Bloodshot and flabby testis shrunken to about 1/2 length of the body cavity			
F	Pinkish and translucent ovary long about 1/3 of the body cavity. Eggs not visible by naked eye.	RESTING*	4b	
M	Whitish/pinkish testis, more or less simmetrical, long about 1/3 of the body cavity.			

**be careful, these stages can be easily confused*

Figure A3.3. Medits Standard maturity key for bony fish (Medits-handbook, 2012)

SWEDISH 9 STAGES MATURITY SCALE

MALES	SCALE 1- 9	FEMALES
Testes small almost glassy transparent **(immature)**	1	Yellow-red translucent ovaries **(immature)**
Testes bigger than 1-3 cm and varying in colours **(immature)**	2	Grey - red, non transparent ovaries with no visible oocytes **(immature)**
Testes with swelling grey lobules **(maturing)**	3	Yellow red ovaries with really small but visible oocytes **(maturing)**
Lobules fully grown but not purely white **(maturing)**	4	Ovaries fully grown and transparent oocytes are absent **(maturing)**
Lobules are partly white and swelling **(spawning)**	5	Ovaries with few transparent large oocytes **(spawning)**
Lobules are white and milky semen flows after a light pressure **(spawning)**	6	More than a half of the oocytes are transparent and flow after a light pressure **(spawning)**
Flabby lobules; some rest of milky semen **(spent)**	7	Shrunk ovaries, often blood stains and some oocytes are still present **(spent)**
Lobules are contracted and empty **(resting)**	8	Ovaries are contracted and totally empty **(resting)**
Abnormal	9	**Abnormal**

Figure A3.4. Maturity key for all species (Sweden) (Modified from Maier, 1908)

Baltic cod maturity **Female scale**

STAGE	MACROSCOPIC CHARACTERS TO DETERMINE GONADAL MATURITY OF FEMALES
I	Juvenile Ovaries emerge as tiny, paired organs close to bladder; glassy transparent to orange-reddish translucent in larger specimens. L_T rarely above 30 cm; *GSI* < 1.
II	Preparation Ovaries small, but easily distinguishable posterior in body cavity; soft with even surface (flattens on a solid sheet); blurred translucent, reddish-orange. L_T: 25-60cm; *GSI* < 1.5.
III	**Ripening 1: Oocyte recruitment** Ovaries still small and restricted to posterior body cavity; firmer than II and roe shaped (keep form on a solid sheet), surface uneven; opaque orange-red to dark orange with greyish cast in large females. Tiny opaque oocytes emerge towards end of stage. L_T rarely below 30 cm; *GSI*: 1-7.5.
IV	Ripening 2: Late vitellogenesis Ovaries enlarged to mid body cavity; plump and firm with prominent blood vessels; opaque, orange to creamy yellow. Oocytes clearly visible and densely packed. *GSI*: 3-14.
V	Spawning 1: Initiation of spawning Ovaries extending into anterior body cavity; distended and soft; opaque, orange to creamy yellow. Single glassy, hydrating oocytes among abundant opaque, vitellogenic oocytes (as in IV, but round and larger). Viscous fluid or hydrated eggs in lumen may occur. *GSI*: 12-25.
VI	**Spawning 2: Main spawning period** Ovaries fill most of body cavity; very distended and soft; appear granulated orange- to reddish-grey from mixture of opaque and glassy oocytes. Lumen containing viscous fluid in excess or hydrated eggs. *GSI*: 15-60.
VII	Spawning 3: Cessation of spawning Ovaries shrunk to posterior body cavity; flabby with prominent blood vessels; unclear reddish- grey. Hydrated oocytes present; opaque oocytes few or absent. Lumen with excess fluid and frequently hydrated eggs. *GSI*: 3-8.
VIII	**Regeneration 1: Spent** Ovaries contracted; slack with greyish cast; rich in blood vessels; dim translucent reddish-grey. Vitellogenic oocytes absent, but single hydrated eggs or atretic oocytes (opaque, irregular granules) may occur. *GSI* normally 2-3; with atresia up to 10.
IX	Regeneration 2: Resting and spawning omission Ovaries small as in II, but with signs of previous spawning; e.g. greyish cast and somewhat uneven walls; blurred translucent, reddish-grey, but more granulated and opaque than in II. *GSI*: 1-3.
X	Degeneration: Reduced fertility A: Ovaries with fibrous tissue formation; affected areas compact and hard, brownish-yellow opaque; non-affected parts with normal development. Observed in females from 65 cm. B: Other abnormalities.

Figure A3.5. Maturity key for cod (Maier 1908, revised by Tomkiewicz *et al.*, 2002) (Germany)

Baltic cod maturity **Male scale**

STAGE	MACROSCOPIC CHARACTERS TO DETERMINE GONADAL MATURITY OF MALES
I	**Juvenile** Testes emerge as a pair of thin strings along air bladder. Lobules tiny, glassy transparent to reddish translucent in larger specimens. L_T rarely above 30 cm; *GSI* < 0.1.
II	**Preparation** Testes small, but distinguishable along air bladder. Lobules small, blurred translucent and reddish. L_T: 20-50cm; *GSI*: 0.1-0.5.
III	**Ripening 1: Early spermatogenesis** Testes still small, close to air bladder. Lobules plump and soft, rich in blood vessels, completely or partially opaque, reddish. L_T rarely below 20 cm; *GSI*: 0.5-6.
IV	**Ripening 2: Late spermatogenesis** Testes enlarged and prominent dorsal in body cavity; Lobules plump and brittle; reddish-white. Empty, transparent spermaducts with prominent blood vessels; no sperm release. *GSI*: 1-18.
V	**Spawning 1: Initiation of spawning** Testes extending into ventral part of body cavity. Lobules distended and brittle, opaque creamy-white. Spermaducts filled with viscous semen and a viscous droplet may be released from vent. *GSI*: 3-22.
VI	**Spawning 2: Main spawning period** Testes large and prominent in body cavity (as in V). Lobules still plump, but soft; completely opaque, whitish. Spermaducts filled with fluid, milky semen that easily flows from vent. *GSI*: 3 to 25.
VII	**Spawning 3: Cessation of spawning** Testes shrunk to dorsal part of body cavity; soft and flabby. Lobules almost empty, opaque, reddish-white. Spermaducts still with fluid semen that easily flows from vent. *GSI*: 0.5 to 4.
VIII	**Regeneration 1: Spent** Testes contracted, close to air bladder; rich in blood vessels. Lobules empty, flabby, reddish potentially with a greyish cast. Spermaducts with signs of previous distension, often with visible remains of semen. *GSI* > 1.5.
IX	**Regeneration 2: Resting and spawning omission** Testes small (as in Stage II), but with signs of previous spawning; e.g. lobules slightly larger than in II; spermaducts often with greyish cast. *GSI* < 1.5.
X	**Degeneration: Reduced fertility** A: Testes with adipose tissue formation; affected parts undeveloped, hard, yellowish; non-affected parts with normal development. Observed in males from 50 cm. B: Other abnormalities.

Figure A3.5 (cont.). Maturity key for cod (Maier 1908, revised Tomkiewicz *et al.*, 2002) (Germany)

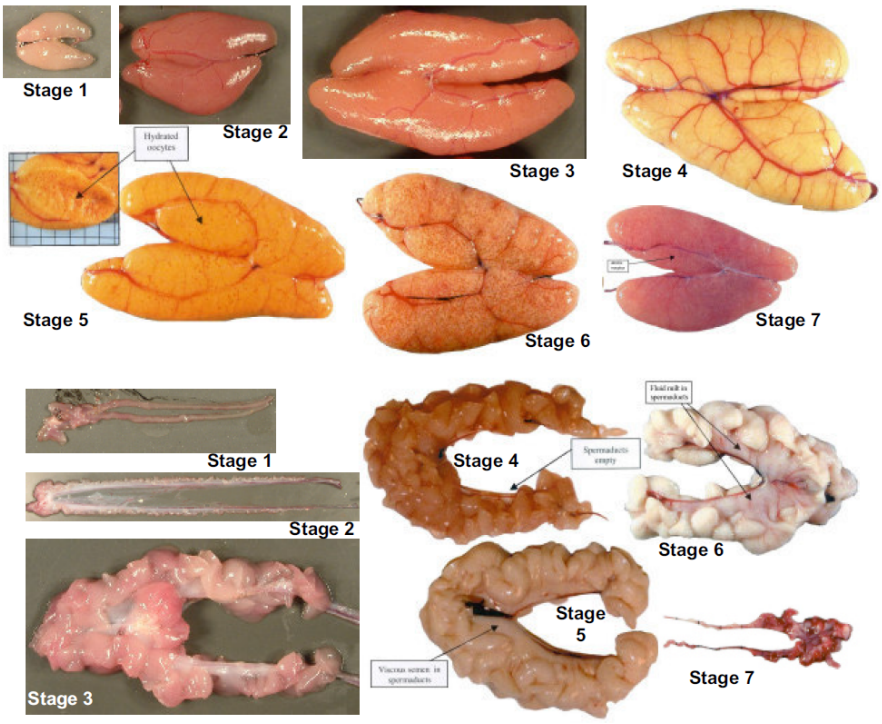
Stage	Description
Blank	**Undecided**/not checked
1	**Immature** Gonads are small. No visible eggs or milt.
2	**Maturing** Gonads are larger in volume. Eggs or milt are visible but not running.
3	**Spawning** Running gonads. Light pressure on the abdomen will release eggs or milt.
4	**Spent/Resting** Gonads small, loose and/or bloody. Regeneration starting, gonads somewhat larger and fuller than stage 1. No visible eggs or milt.
5	**Uncertain** Use only when difficult to distinguish stages 1 and 4.

Figure A3.6. Maturity key used in Norway (Mjanger *et al.*, 2011)

Gadoid Maturity Scale

(blue whiting, cod, haddock, hake, whiting, saithe)

Females	Males
1. Juvenile	
Translucent and very small, with thin walls.	Thin and translucent ribbons with tiny lobules
2. Developing virgin / Resting spent	
Blurred translucent, small.	Small lobules, blurred translucent.
3. Ripening 1	
Firmer than 2. Opaque. Yellow to orange colour visible when cut. Individual oocytes cannot be seen with the naked eye.	Lobules plump and soft, opaque. Rich in blood vessels.
4. Ripening 2	
Plump and frim. Colour yellow to orange, individual oocytes can be seen with the naked eye.	Lobules plump and brittle. No sperm in spermaducts.
5. Ripe	
Distended and soft. Hhydrated eggs present. Not yet running under moderate pressure.	Lobules distended and brittle. When testes are cut, some sperm is visible. Sperm in spermaducts. Not running under moderate pressure
6. Running	
Very distended and soft. Hydrated eggs can be extruded on slight pressure	Lobules plump but soft. Testes run on slight pressure
7. Recently spent	
Ovaries reduced in size and flaccid. Some residual eggs my occur.	Testes thin and flabby, some sperm may remain in spremaducts



Based on: (Tomkiewicz et al. 2002)

Figure A3.7. Maturity key used in Northern Ireland

Annex 4: Sampling protocol

Sampling protocol for the workshop on Sexual Maturity Staging of Cod, Whiting, Haddock, Saithe and Hake. (WKMSGAD follow-up of WKMSCWHS 2007 and WKMSHM 2007)

Sampling method (see table 1):

A sub sample of 5 individuals per 10 cm length group per sex (see Table 1) is taken randomly from the catch and stored on ice immediately. As it is likely that not all length groups are represented in one haul, the preferred sampling strategy is to commence the sampling by random selection of fish, but then as the length-groups are filled out, specific sampling for length groups not yet covered should be performed.

To cover the development of the gonads during the year, the sampling procedure should preferably be executed 3 times over the next 10 months for each species, covering the different seasons to include all stages for each species. Furthermore, the sampling should preferably be spread out on as many locations as possible; however, it is of higher priority to fill out as many length groups as possible.

Table1 Number of female and male individuals per length class.

Length group	Females	Males
<10 cm		
10–19 cm		
20–29 cm		
30–39 cm		
40–49 cm		
50–59 cm		
>60 cm		

Data collection (see table 2):

1) A random sample is taken from the haul/catch
2) From this a random fish is taken
3) If the fish is missing in the length group to which it belongs, move to point 4. If the length group to which the fish belongs is filled-up (i.e. you have already sampled 10 fish), you can throw away the fish and take a new fish
4) Give the fish an ID-number including station/area, date and fish number f.ex. ES 01-01032013/1 (Spain, 1 March 2013, fish number 1)

   5) To make the process easier, please prepare those labels in advance, before the sampling. This label has to be shown in the pictures (see pictures' examples below)

5) Total length (LT) of the fish is measured (with 1 decimal)
6) Total weight (WT) of the fish is noted (with 1 decimal)
7) Carefully cut the fish open, so that the gonads are not damaged (Photo a, see point 13)

8) The sex is noted
9) The maturity stage is noted for both males and females (according to scale normally used or possibly testing the WKMSCWHS 2007 and WKMSHM 2007 scales)
10) The weight of the entire gonad (W_{GO}) is noted (with 2 decimals)
11) Liver weight (W_L) of the fish is noted (with 2 decimal)
12) Note the gutted weight (W_{GT}) (with 1 decimal)
13) A series of photographs have to be taken during the process:
    a) Fish with the gonad inside (see example 1, remember to include ruler and identification number)
    b) Fish with the gonad lying next to it (see example 2)
    c) Close-up photo(s) of the gonad (see example 3)

For the preservation of the gonad tissue, take the entire gonad if it is small. If the gonad is big cut 3 transverse slices, approximately 2 cm wide, from the anterior, middle and posterior part respectively of one of the gonad lobes and preserve them as described below (points 14–15).Choose randomly whether preserving slices from the right or the left lobe.

Note from which lobe the slice is taken (right/left) and whether it is from the anterior, middle or posterior part.

1) Please wrap/roll the piece of gonad in gauze with an ID-tag (or put in a separate labelled jar).
2) Preserve the ovary in 4% buffered formaldehyde solution
3) Take out the otoliths
4) This procedure is repeated until 5 fish per length/maturity group are sampled

Note cruise name, station, date, latitude, longitude, the initials of the persons who collected the fish, and to which stock the fish belongs.

Histoformaldehyde:

4.0 g NaH_2PO_4-H_2O

7.5 g Na_2HPO_4-$2H_2O$

100 ml formaldehyde 37 %filling up to 1000 ml with distilled water

Contacts:

- Please send the applied maturity scale, a list over expected size of catch for each species, as well as pictures, data, total length–frequency distribution (if not all the fish is sampled for this purpose) per sample/cruise and **THE COLLECTED SAMPLES IF YOU CANNOT PROCESS THEM HISTOLOGICALLY** as soon as the samplings has been executed to:

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Cruise:_____ Station:_____ Date:_____ Species:_____

Stock:_____ Latitude:_____ Longitude:_____ Initials:_____

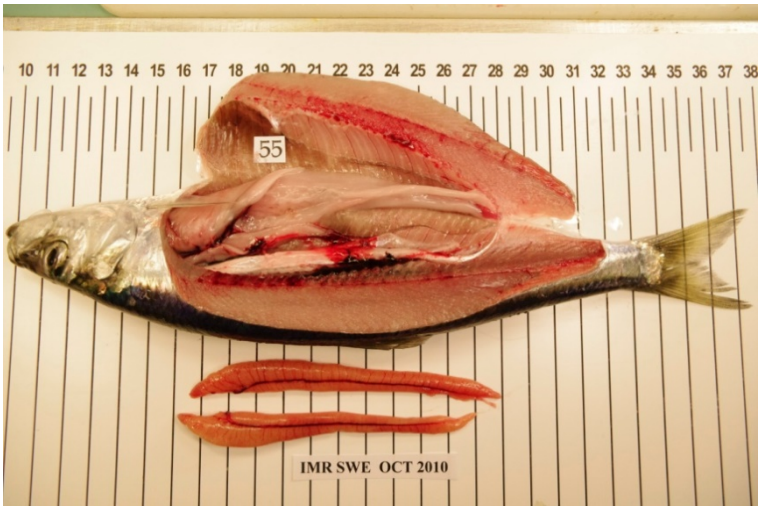
Table 2

Fish #	Total length (L_T/cm)	Total weight (W_T/g)	Sex	Maturity stage	Gutted weight (W_{GT}/g)	Gonad weight (W_{GO}/g)	Liver weight (W_L/g)	Comments

Photographing gonads

Example 1: Fish with the gonad inside

Example 2: Fish with the gonad lying next to it



Example 3:close-up of the gonad

Annex 5: Presentations

Gonad development and reproduction of European Hake in Western Central Mediterranean

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The present study presents a seven stages macroscopic maturity scale, histologically validated (Handbook MEDITS, 2012), for males and females of European hake (*Merluccius merluccius*) from Western Central Mediterranean (Southern Adriatic GSA18, Western Ionian Sea GSA19, Central-Southern Tyrrhenian Seas GSA 10, Sardinian Seas GSA 11, Ligurian and Tyrrhenian sea GSA 9, Northern Adriatic sea GSA 17). In particular the differences between the adult stages (recovering; maturing; mature/spawning; post-spawning; resting) and juveniles stages (virgin; virgin developing) are highlighted. This is extremely important for the maturity ogives estimations especially for species with a long spawning period, such as hake. Histological examination of ovarian sections shows an asynchronous organization, where oocytes of all stages are simultaneously present in the reproductively active ovaries. The oocyte size frequency distribution shows a polimodal pattern with a clear separation between advanced yolked oocytes and hydrated ones when hydration occurs. The monthly trends of the gonadosomatic index and maturity stages distribution highlight a long period of spawning with the presence of mature/spawning females through the whole year. A spawning peak is observed during winter in all subareas analysed (GSA 10, GSA 18, GSA 19 and GSA11). A further spawning peak during summer seems to occur in Tyrrhenian Sea (GSA9), Southern Adriatic (GSA18) and Western Ionian (GSA19) and spring-summer in Northern and Central Adriatic Sea (GSA 17). Monthly variations of male's maturity are also analysed in the investigated area (GSA 10, GSA 17, GSA18, GSA19 and GSA11).

Importance of histological information in the assessment of Southern Stock of European hake

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One of the main criteria for judging the status of an exploited fish population is the size of the spawning stock or spawning-stock biomass (SSB). Maturity data (mature/immature) are the basic information to estimate maturity ogives that allow the estimation of the SSB. Usually maturity ogives are estimated based on macroscopic observations of gonad. However, macroscopically, the virgin ovaries (immature) and

resting ovaries (mature) are similar. This distinction is only possible with histological maturity staging identification.

This work investigates differences in maturity (L_{50} and A_{50}) of Southern stocks of hake (ICES Div. VIIc and IXa) from two different geographic locations: Galicia (North of Spain) and Gulf of Cadiz (southern Atlantic coast). Maturity ogives based on macroscopic inspection and validated data by histology were compared. Moreover a correction factor based on histological samples was applied on macroscopic data. There are significant differences between females maturity ogives estimated with macroscopic and microscopic data. There is a tendency to overestimate L_{50} based on macroscopic data that leads to underestimation of SSB. The application of the correction factor based on histology minimizes significantly differences between macro and microscopic ogives and allows to estimate L_{50} more accurately.

Histological validation of macroscopic maturity staging on cod individuals from NAFO 3L

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The ovaries of cod (*Gadus morhua*) in different maturing stages were collected during 2012 this year in NAFO area 3L in August, when the population is in post-spawning-recovery period. The fresh ovaries have been classified on board using macroscopic criteria. Maturity stage was determined following the scale proposed in the Report of the Workshop on Sexual maturity Staging of Cod, Whiting, Haddock and Saithe (WKMSCWHS; ICES, 2007). A collection of pictures was made following the PGCCDBS 2010 recommendations (ICES, 2010). A lobe of the gonad was frozen and the other was conserved in formalin for posterior histological processing. This document presents the graphic comparison between the macro and microscopic classification of the 23 ovaries. Misclassifications occurred principally between those individuals that were finishing the recovering and starting the developing stage, because a big part of the individuals recorded macroscopically as 'recovering', presented cortical alveoli that mark the beginning of vitellogenesis.

Annex 6: Recommendations

Recommendation	Adressed to
Consider the optimal sampling time, specific for each species when using macroscopic assessing criteria, in order to minimize the uncertainties. Preferably cod, whiting, saithe and haddock should be collected in the prespawning period while hake should be collected during the peak of the spawing season.	All institutes
Histology or whole mount should always be used to validate local scales and produce correction factors on a length class basis for both sexes.	All institutes
Routine local training programs are highly recommended and an adequate level of knowledge and experience on the maturity of the species dealt with during a Workshop should always be ensured by national laboratories.	All institutes
Sex separated maturity ogives should always be preferred when data are available.	Assessment WGs
A standarized tool should be developed to evaluate the discrepancies among maturity stagers on these calibration exercises. This analysis should weight the differences between immature and mature and not only stages and consider the reproductive cycle is circular.	WGBIOP