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Report of the Workshop2 on Sexual Maturity Staging of sole, plaice, dab and flounder

9–13 January 2012

Oostende, Belgium



ICES

International Council for
the Exploration of the Sea

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Executive summary

WKMSSPDF met 9-13 January 2012 in Oostende, Belgium. 24 participants from 8 countries joined the meeting. The meeting aimed to validate the maturity stages for sole, plaice dab and flounder as proposed by WKMSSPDF in 2010.

Evaluation of maturity scales

The maturity scales as proposed by WKMSSPDF in 2010 have not been incorporated by all countries. Most labs, however, succeeded in translating the national (or local) scale into the newly proposed scales.

The translation of the BITS scale to the WKMSSPDF proposed scales proved difficult due to local regional use of the data, in specific the borderline between stage 2 and 3 (maturing to spawning). The group recommends that all the Baltic institutes keep their own national staging, and transfer it to the internationally DATRAS stages, from a certain date onwards. Old data should not be changed. There will be a clear break in the DATRAS time series with respect to the maturity. The BITS manual should describe this change well.

In general, it is important to realise that when countries move to the new maturity keys, a change in the number of spawning fish might occur as the definitions of the various stages might differ between the old national stages and the internationally agreed stage.

Changes in the maturity scale descriptions

As the descriptions of the stages were evaluated, some changes were made in the criteria, based on expertise and experiences.

It should be clear that the diagram is only relevant from two months prior to the spawning season until the end of spawning. After spawning a transition of the gonads takes place that is not described in the diagram. It is, however possible that in a survey in the spawning season specimens may be found that have spawned recently and are spent. The stage descriptions are modified for all species to incorporate this stage.

Female stage 5: Stage 5 as a resting stage cannot be staged macroscopically, and even when no development seems to be going on, histologically mostly development is visible. Normal gonad development is: stages 2-3-4-(outside spawning season)-and back to 2. Only when there is a problem with the condition of the fish during the spawning season stage 5 might occur. For this reason, stage 5 is only applicable directly prior to the spawning season. It was decided to remove 'Resting' from the diagrams.

Male stage 5: The general understanding is that male fish stage 5 looks too much like the other stages, so stage 5 is removed from the male staging diagram.

Distinction stage 2 and 3: It was decided that the presence of one hyaline egg will put fish in maturity stage 3, as spawning will happen within due time.

Staging exercises

Three staging exercises were carried out, one using fresh fish and two using pictures. In all exercises, for all species, the percentage agreement was higher than in 2010. As expected, the percentage agreement in the fresh staging was higher than the percent-

age agreement in the staging exercises from pictures since (a) touching is one of the components in maturity staging and (b) one hyaline egg is easier to identify in fresh samples than from pictures.

The general feeling was that it was easier to stage female fish than male fish. Analysis of the percentage agreement by sex over all species and calibration exercises support this feeling. There is also significantly higher agreement on the sexual maturity stage of fish in the spawning season (October-April) compared to outside the spawning season, proving that macroscopic maturity staging is a reliable method in the period from two months before the start of the spawning season until the end of spawning.

The macroscopic maturity stage was validated with the histological analysis after the calibration exercises. Thus, the results of the calibration exercises based on the modal stage were available and for fish with low agreement the staging was validated in plenary sessions using the microscopic smears or histological sections. The data reported in this report is based on the macroscopic maturity stage and not corrected in case the microscopic analysis proved the staging was incorrect.

WKMSSPDF recommends that in future workshops it should be decided if all stagings should be checked against the microscopic stage or the modal stage. If it is decided to continue using the modal stage it should then be decided to base the modal stage on all participants or only the modal of the expert stagers.

Next meeting

It was recommended that the meeting frequency should be once each 3-5 years and the group should not be expanded with more species (and so, more people). It was also recommended that the national institutes should be strongly encouraged to put effort into making pictures, and should find time and money to do so. Successful maturity staging workshops cannot be carried out without these pictures.

1 Opening of the meeting

WKMSSPDF met 9-13 January 2012 in Oostende, Belgium. 24 participants from 8 countries joined the meeting. The participant list is in Annex 1.

The meeting aimed to validate the maturity stages for sole, plaice dab and flounder as proposed by WKMSSPDF in 2010 (ICES, 2010).

The terms of reference for the meeting were:

- a) Report on the use of the common maturity scale proposed in 2010;
- b) Check the description of the characteristics of the stages of the 2010 scale;
- c) Calibrate staging of sole, plaice, dab and flounder using fresh fish, following the pattern of trial-discussion-retrial;
- d) Calibrate staging of sole, plaice, dab and flounder using photographs, following the pattern of trial-discussion-retrial;
- e) Validate macroscopic maturity determination with histological analysis.
- f) address the generic ToRs adopted for maturity staging workshops (see 'PGCCDBS Guidelines for Workshops on Maturity Staging')

ToR a and f were discussed in plenary, ToR b was dealt with in three subgroups (plaice, flounder, sole/dab), ToR c and d were individual exercises using WebGR as the stage recording tool. The objectives of WebGR can be found in Annex 5. ToR e was carried out in plenary, focussing on gonads where agreement was low.

The ToRs are discussed in separate chapters. Additionally, chapter 9 evaluates the use of WebGR during this workshop, and contains some recommendations for further development.

2 Adoption of the agenda

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

3 Use of the common maturity scale proposed in 2010 (ToR a)

The maturity scales as proposed by WKMSSPDF in 2010 have not been incorporated by all countries. Most labs, however, succeeded in translating the national (or local) scale into the newly proposed scales. Table 3.1. shows the current state regarding the implementation of the new stage descriptions, and the problems arising.

Table 3.1 Current state of maturity staging following WKMSSPDF 2010 (ICES, 2010) proposed scales.

COUNTRY	CURRENT SITUATION	PROBLEMS ARISING
Belgium	Incorporated the new stages, but maturity staging stopped as is was not defined in the national program	
Germany	vTI Rostock: haven't tried to use the new system, and still use the Maier scale as they used to. Transforming stages to old DATRAS scales. vTI Hamburg: tried to start to use 6-point scale next to 4 point scale.	Translation to DATRAS leads to some problems, mainly related to stage V (Maier) into new stage maturing or spawning.
Latvia	Still use national staging system, more detailed than new system. Transforming stages to old DATRAS scales.	No skipped spawners defined in the national scale. Translation to DATRAS leads to some problems, mainly related to the national stage 4 (late maturity) to new stage 2.
Lithuania	Still use national staging system, more detailed than new system. Transforming stages to old DATRAS scales.	No skipped spawners defined in the national scale. Translation to DATRAS leads to some problems, mainly related to the national stage 4 (late maturity) to new stage 3.
Netherlands	Changed from the 4-point scale to the proposed 6-point scale in January 2011	
Poland	Still use the Maier scale as they used to, and translate to the new scale when required. Transforming stages to old DATRAS scales.	Translation to DATRAS leads to some problems, mainly related to stage V (Maier) into new stage maturing or spawning.
Sweden	Different scales for IMR (plaice) and ICR (flounder) respectively. However, when proceeding the work of quality assurance at our departement a standardisation will hopefully take place. IMR still uses a 9-grade scale when staging plaice. When delivering data to DATRAS, national maturity data is translated to the WKMSSPDF scale ICR adopted the WKMSSPDF scale for flounder as national scale in November 2011. No delivery to DATRAS.	For flounder, the previously used scale (a 6-grade scale including 5 stages plus abnormal) has a different definition of the stage "spawning". Thus, it will not be possible to translate old data to the currently used WKMSSPDF scale (or any other 6-grade scale where any of the definitions are incomparable).
United Kingdom (England)	Still use own code, and translate to new stages for DATRAS. When PGCCDBS decides on final stages for sole, plaice, dab and flounder, CEFAS will shift to the new scale.	The current CEFAS staging includes a hyaline stage and the description of this does not translate to the new stage 3.

Rainer Oeberst presented the difficulties with respect to the translation of the BITS scale to the WKMSSPDF proposed scales, specifically related to the borderline between stage 2 and 3 (maturing to spawning). There are three options:

- 1) Use the current scale of the BITS
- 2) Start using new scale from a certain date (like IBTSWG and WGBEAM)
- 3) Re-upload all data for most countries, which is possible as most countries use more detailed national scales which can be translated into the new scales.

The third option is not possible without more manpower and a lot of time. The second option is not acceptable to WGBIFS as there will be a break in the timeseries for maturity staging.

The distinction between stage 2 and 3 is the presence of one or more hyaline eggs. If hyaline egg are visible the fish is in maturity stage 3, and so, formally it will contribute to the spawners. Sampling takes place in a specific timeframe. Especially in case of batch spawners, the presence of one hyaline egg means that the fish will be spawning.

The group recommends that all the Baltic institutes keep their own national staging, and transfer it to the internationally DATRAS stages, from a certain date onwards. Old data should not be changed. There will be a clear break in the DATRAS time series with respect to the maturity. The BITS manual should describe this change well. It is very important that all WKMSSPDF 2012 participants inform their national colleagues involved in WGBIFS about the current maturity stages and about the WKMSSPDF recommendation above.

However, it should be noted that the data in DATRAS should not be used for detailed maturity analysis and those wanting to carry out such work should contact the original institute for the original maturity information.

It is important to realise that when countries move to the new maturity keys, a change in the number of spawning fish might occur as the definitions of the various stages might differ between the old national stages and the internationally agreed stage.

4 Description of the characteristics of the stages (ToR b)

The descriptions of the maturity stages in this report should only be used in the period from 2 months before the spawning season until the end of spawning. As it is not possible to reliably stage the maturity of a fish macroscopically outside this period (see also section 7) the description of the different maturity stages focussed on the period 2 months before the spawning season until the end of spawning. Separate documents by species containing the maturity stage diagrams as well as reference pictures are available. The descriptions of the maturity stages as presented in ICES (2010) were discussed in three subgroups: one group dealing with flounder, one with plaice and one with sole and dab. The groups also decided on new reference pictures, as more pictures have become available over the last years.

Based on the discussions in the subgroups, WKMSSPDF decided the following:

- a) It should be clear that the diagram is only relevant from two months prior to the spawning season until the end of spawning. After spawning a transition of the gonads takes place that is not described in the diagram. It is, however possible that in a survey in the spawning season contains specimens that have spawned recently. The description is modified for all species to incorporate this stage.
- b) Female stage 5: plaice group discussed whether or not stage 5 should be seen as a regular stage. A normal gonad will go through the development stages 2-3-4-(outside spawning season)-and back to 2. Only when there is a problem in the fish condition during the spawning season stage 5 might occur. The question was raised if the characteristic 'resting' should be kept in the descriptions of the species? Currently, the diagrams show 2-3-4-5-2 development. Stage 5 as a resting stage cannot be staged macroscopically, and even when it looks like no development, there still might be development seen histologically. Stage 5 is only applicable directly prior to the spawning season. It was decided to remove 'Resting' from the diagrams. Put 'outside spawning season' in the diagram to show what should be done in that period.
- c) Male stage 5: All groups discussed the difficulty to describe male fish maturity stages accordingly to the 6-point scale. The general understanding is that male fish stage 5 looks too much like the other stages, so stage 5 will be removed from the male staging diagram.
- d) Females: add the clear definition of the presence of one hyaline egg in stage 3 as the distinctive characteristic compared to stage 2.
- e) Stage 6 will be added to the diagrams as otherwise people might think it is a 5-point scale. Abnormal gonads are relatively easy to identify so a simple description has been added to the keys.
- f) The terminology for all descriptions will be changed from the general term 'gonad' into 'ovary' or 'testis' for females and males respectively.
- g) Parasites in the gonad might be incorporated, but only if the gonad looks abnormal, and not if the gonad looks fine as fish might be able to spawn.
- h) Pictures: gonad in situ and outside the body, especially in stage 1 (line test).

- i) Females: add 'yolky eggs' in stage 2, as long as everybody understands what it means. This is very useful when identifying the ovary under the microscope.

It is important to realise that beginners as well as experts are going to use the descriptions, and so, they should be as clear and absolute as possible. It is however always recommended that people starting maturity staging of fish for the first time should always be guided by a more experienced person.

4.1 Sole

The subgroup checked the descriptions stage by stage and checked what should be changed. The changes are incorporated in the new diagrams (Figures 4.1.1, 4.1.2). The full report of the sole group can be found in Annex 6.

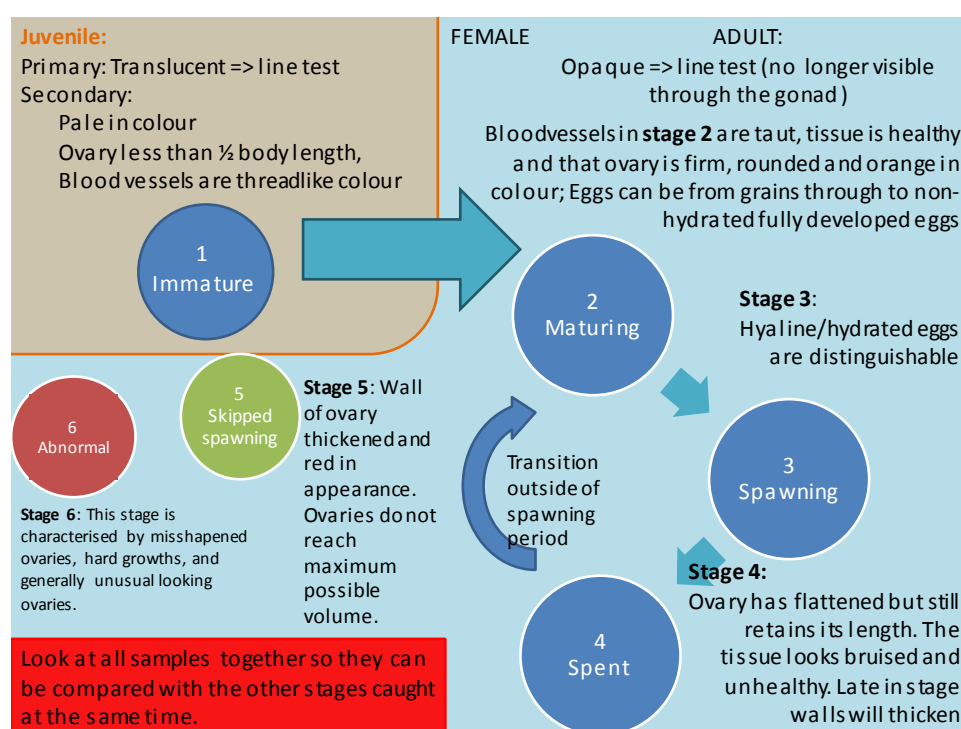


Figure 4.1.1 Maturity stages sole female

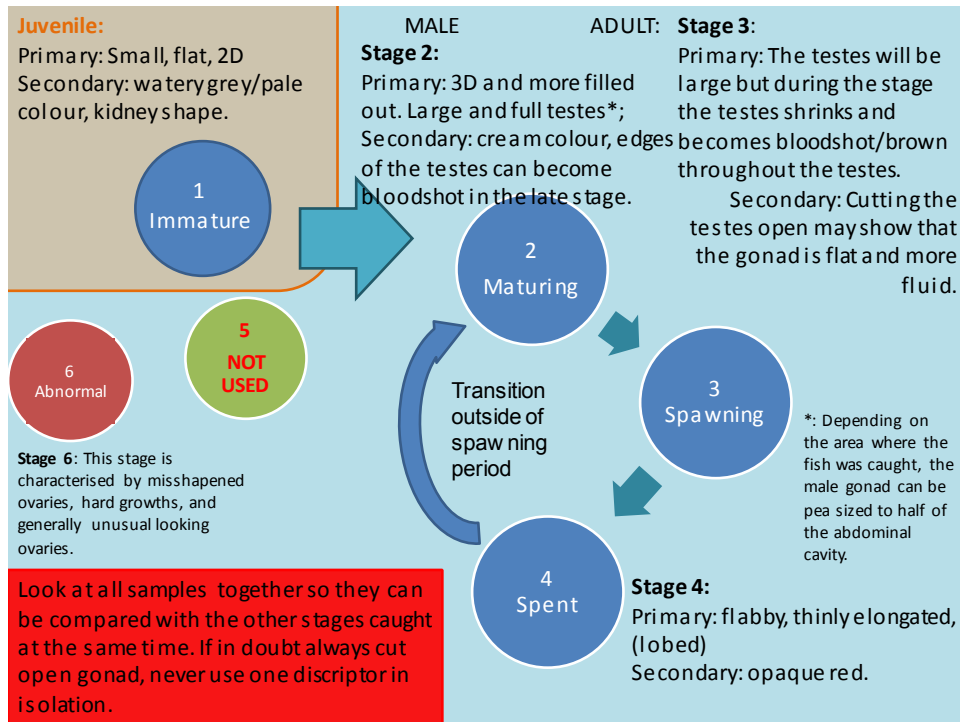


Figure 4.1.2 Maturity stages sole male

4.2 Plaice

The subgroup checked the descriptions stage by stage and checked what should be changed. The changes are incorporated in the new diagrams (Figures 4.2.1, 4.2.2). The full report of the plaice group can be found in Annex 6.

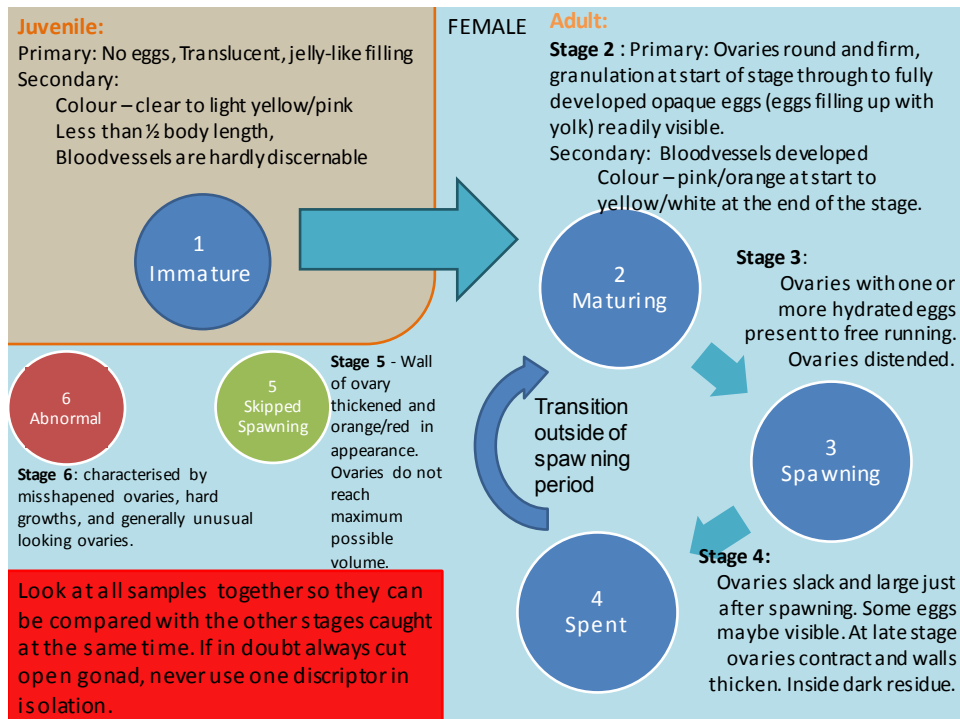


Figure 4.2.1 Maturity stages plaice female

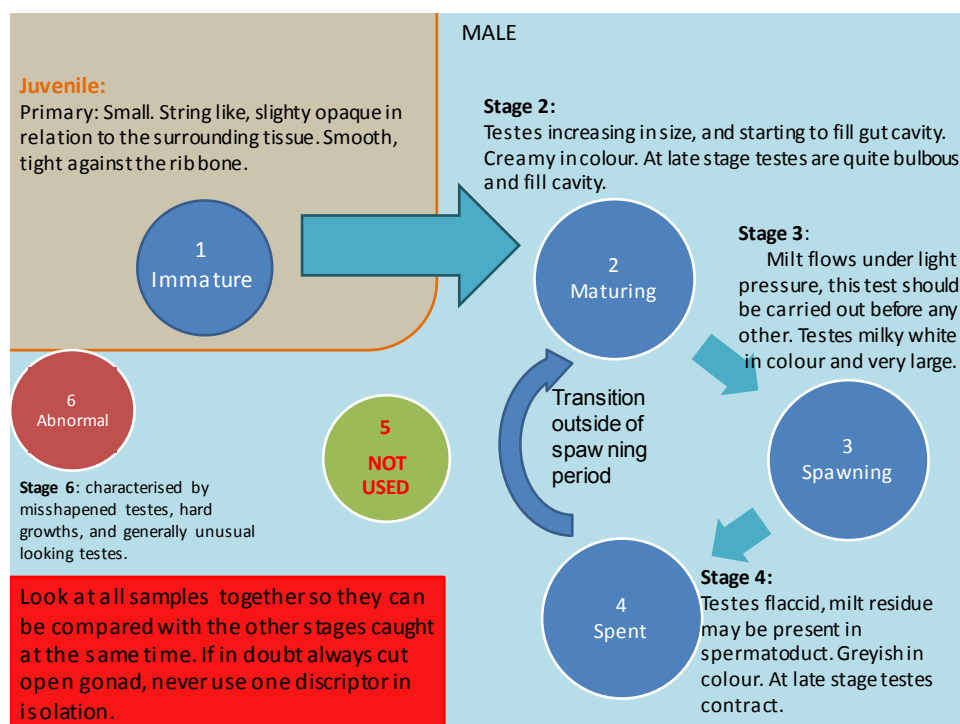


Figure 4.2.2 Maturity stages plaice male

4.3 Dab

The subgroup checked the descriptions stage by stage and checked what should be changed. The changes are incorporated in the new diagrams (Figures 4.3.1, 4.3.2). The full report of the dab group can be found in Annex 6.

The subgroup decided to separate the dab description from the plaice stages.

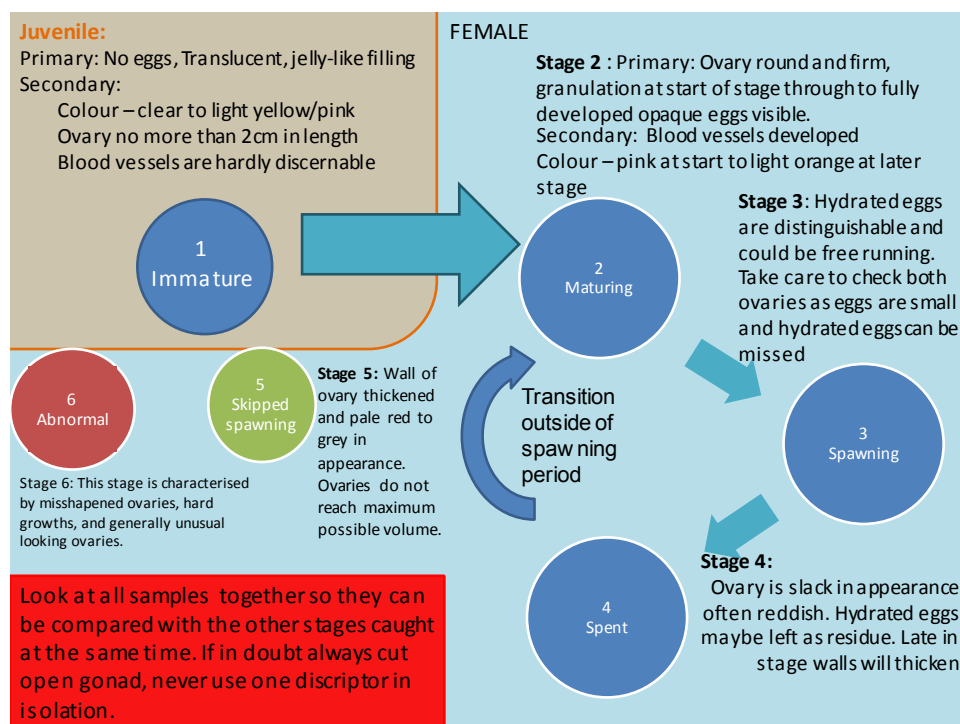


Figure 4.3.1 Maturity stages dab female

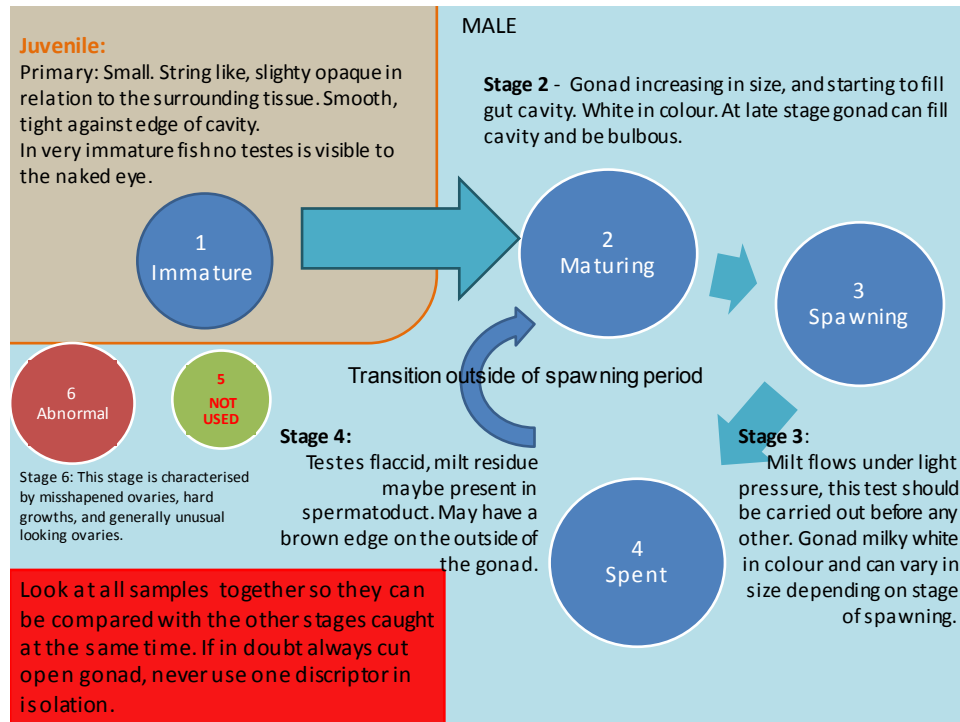


Figure 4.3.2 Maturity stages dab male

4.4 Flounder

The subgroup checked the descriptions stage by stage and checked what should be changed. The changes are incorporated in the new diagrams (Figures 4.4.1, 4.4.2). A summary of the discussion of the flounder group can be found in Annex 6.

The subgroup presented the draft diagram in plenary to get feedback on terminology.

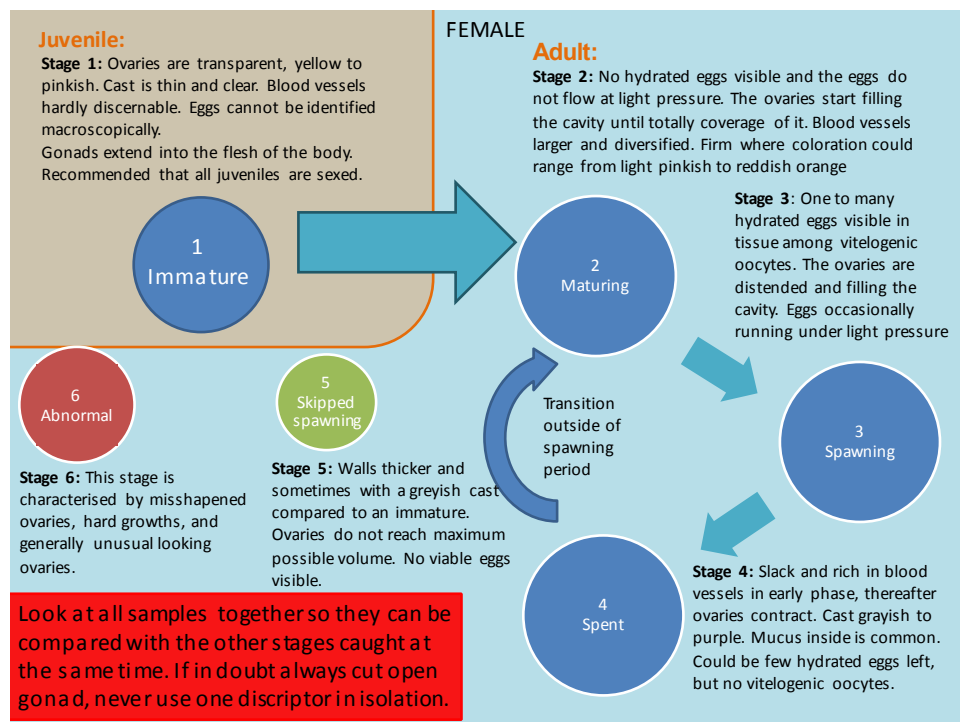


Figure 4.4.1 Maturity stages flounder female

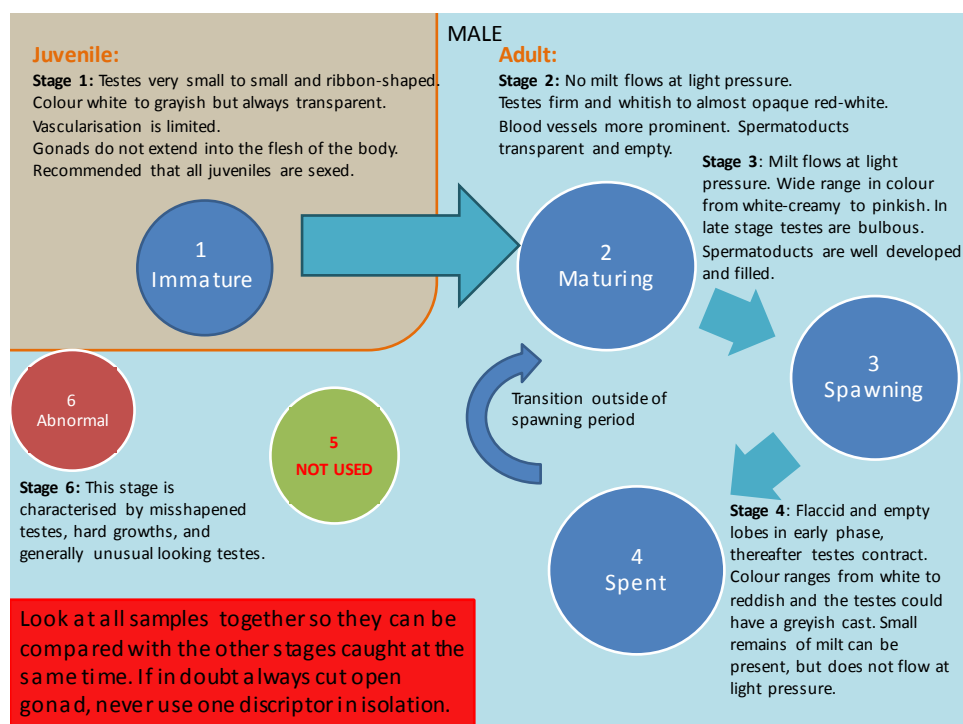


Figure 4.4.2 Maturity stages flounder male

5 Fresh fish calibration exercise (ToR c)

5.1 Fresh fish staging

The fresh fish staging was carried out on 25 fresh specimens per species for sole, plaice, dab and flounder. The fish were caught on the 5th of January 2012 and kept on ice until the fresh staging on the 9th of January. The fish were cut open on both sides and the gonads were left in the fish. All participants staged all species, independent of their expertise field. After staging, all the fresh fish were discussed and a maturity stage agreed upon. This created a fruitful exchange of views on fish stages. On some specimens the agreement on maturity stage during the discussion was low. Of these a swab of the gonad was taken and checked under the microscope and photographed to determine maturity stage. Based on this discussion, the criteria for maturity staging were refined in subgroups.

The general feeling is that staging female fish is easier than male fish, even when fresh.

Sex ratio and length frequencies per species is given in Annex 7. Since it was freshly caught fish not all maturity stages were present in the fresh samples.

Pictures were taken of all fish and after the staging, the pictures and relevant information of the fresh fish were uploaded to WebGR. All participants entered their original staging results from the fresh staging into WebGR.



Upper pictures: discussion after the fresh staging, to decide upon an agreed stage

Lower left: studying smears from fresh fish under the microscope

Lower right: staging from pictures

5.2 Statistics

In general, the agreement on the fresh specimens is higher than the agreement on pictures. Main reasons for that are (see also ICES (2010)):

- a) Touching the gonad is part of the staging;
- b) The possibility to look into more detail by cutting the gonad, is an advantage in comparison to staging from pictures;
- c) Fresh samples allow definitive staging especially for stage 3 hydrated eggs;
- d) In fresh samples, it is easier to quantify the transition to the next maturity stage compared to pictures;
- e) The ability to get an indication of the condition of the fish is higher in fresh samples;
- f) Photographs lack the depth of field

In case of uncertainty, putting a small amount of the content of a gonad under a microscope might clarify the maturity stage. It is however important to realise that during a survey, time to define the maturity stage is limited. It is not always feasible to study each part of the gonad using a microscope. However, if time allows, the group recommends using this method in case of disagreement or doubt on the maturity stage of a fish.

Over all, the percentage agreement per species in the fresh staging was higher than the percentage agreement in the 2010 WKMSSPDF (2010: min. 68%, max. 77%; 2012: min. 80%, max. 86%).

5.2.1 Sole

The sole in the fresh staging were all females and either stage 2 or 5 (Table 5.2.1a). Overall agreement on sole fresh staging was 82% (Table 5.2.1b). For the trainees it was difficult to identify the skipped spawning fish (Table 5.2.1b & c). Also an institute problem became evident. From one institute all experts did not recognise the stage 5 fish but identified these fish as stage 2 (Table 5.2.1b & c).

[illegible]

b) % agreement per stage																						
stage	Trainee	Intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Intermediate	Trainee	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	92%	92%	58%	96%	83%	100%	100%	92%	79%	100%	100%	100%	92%	88%	25%	79%	100%	88%	54%	88%	33%	83%
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	100%	100%	100%	100%	100%	0%	0%	100%	100%	100%	0%	0%	100%	100%	0%	100%	0%	100%	0%	0%	0%	57%
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	92.0%	92.0%	60.0%	96.0%	84.0%	96.0%	96.0%	92.0%	80.0%	100.0%	96.0%	96.0%	92.0%	88.0%	24.0%	80.0%	96.0%	88.0%	52.0%	84.0%	32.0%	82%

[illegible]

For sole 16 it was unclear whether this female was juvenile, stage 1, or maturing, stage 2. Under the microscope it was clear that oocytes were developing and the female was maturing (Figure 5.2.1.1).

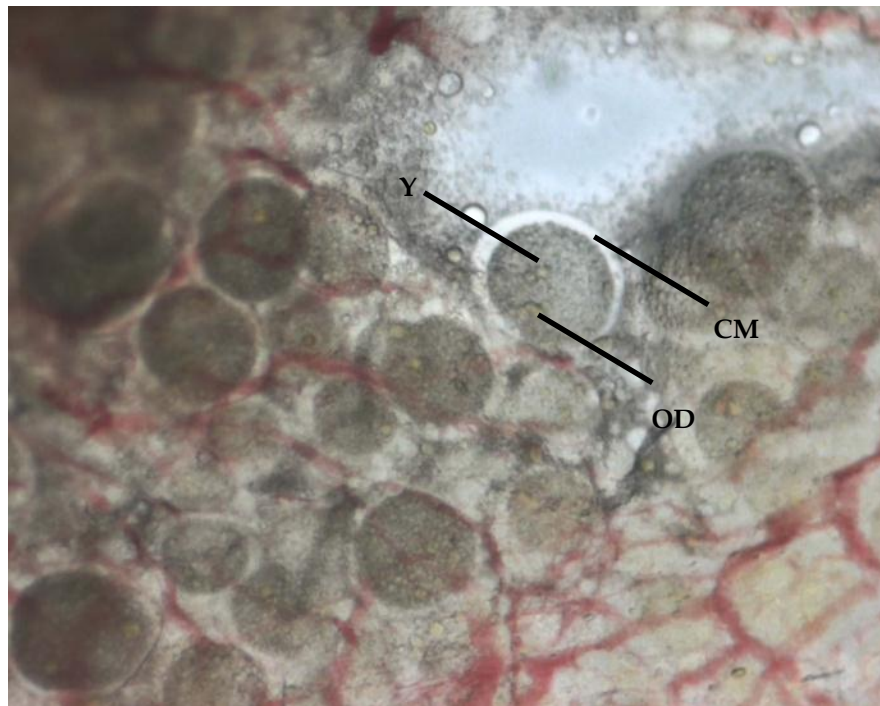


Figure 5.2.1.1. Developing oocytes in the vitellogenic stage in sole (CM: Cell membrane; Y: developing yolk (yolk vesicles and yolk granules); OD: oil droplets).

The maturity stage of sole 19 was unclear since the developing gonad looked strange. A smear of the gonad under the microscope revealed a lot of atretic oocytes (Figure 5.2.1.2). This female is resorbing eggs and will skip spawning the current spawning season, hence stage 5.

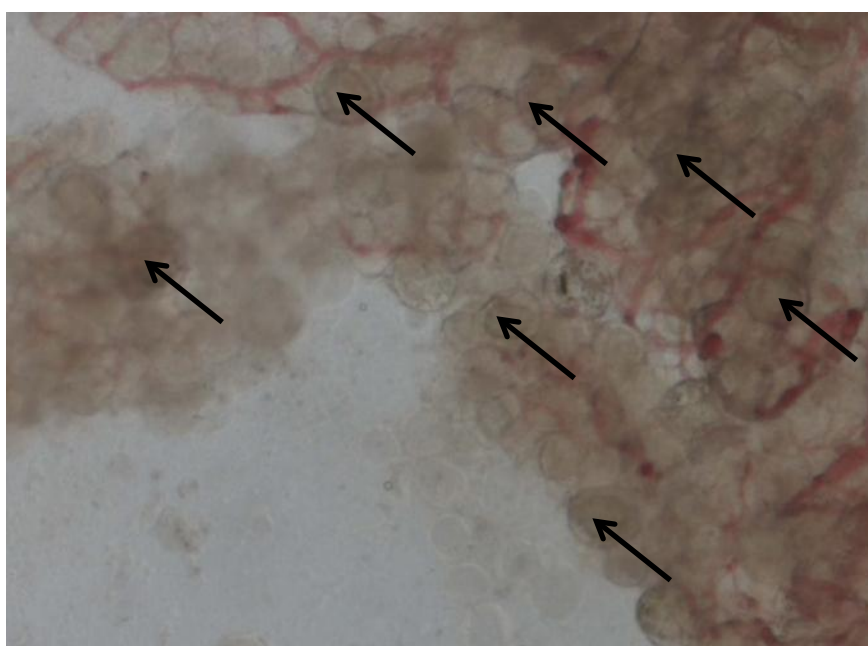


Figure 5.2.1.2 Atretic oocytes in skipped spawning (stage 5) sole.

5.2.2 Plaice

The fresh staging sample consisted of 21 females and 4 males. Maturity of both the males and females ranged from stage 2 to 4 (Table 5.2.2a). Overall agreement on plaice staging was 80% (Table 5.2.2b). The agreement by stage varied from 67% for stage 4 to 83% for stage 3. Generally, the lowest agreement occurred within the group of trainees, although some trainees had high agreements comparable to the expert stagers (Table 5.2.2b & c).

Table 5.2.2a. The number of stagings by modal stage for plaice.

[illegible]

Table 5.2.2b. Stage compositions by stage and reader for all stage readers for place. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																						
stage	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Expert	intermediate	Expert	Trainee	Expert	Trainee	Trainee	Expert	Trainee	intermediate	Trainee	intermediate	Trainee	intermediate	Trainee	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	71%	100%	100%	100%	100%	100%	86%	100%	14%	100%	86%	71%	86%	100%	100%	86%	86%	57%	14%	100%	86%	83%
3	75%	94%	94%	69%	94%	81%	100%	100%	88%	75%	88%	94%	56%	88%	50%	69%	88%	69%	94%	63%	69%	81%
4	50%	100%	100%	100%	100%	100%	100%	100%	100%	0%	50%	100%	0%	50%	100%	0%	100%	0%	0%	50%	100%	67%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	72.0%	96.0%	96.0%	80.0%	96.0%	88.0%	96.0%	100.0%	68.0%	75.0%	84.0%	88.0%	60.0%	88.0%	68.0%	68.0%	88.0%	60.0%	64.0%	72.0%	76.0%	80%

Table 5.2.2c. Bias in the comparison for plaice. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

5.2.3 Dab

All dab in the fresh staging were females and all were in either maturity stage 2 or 3 (Table 5.2.3a). Overall agreement in dab staging was 86%, with 64% agreement in stage 3 and 90% in stage 2 (Table 5.2.3b). Most experts had agreement above 96% (Table 5.2.3b & c). One expert had lower agreement but during the discussion it became clear that the problem was in the description of the maturity stage and whether one hydrated egg visible in the gonad means the fish is in maturity stage 3. In histological sections of females with macroscopically one hydrated egg is seen in the gonad, many more hydrated eggs are visible. Hence, it was agreed that one hydrated egg identified macroscopically means the fish is in maturity stage 3. In at least one institute this was interpreted differently up to now.

Table 5.2.3a. The number of stagings by modal stage for dab.

[illegible]

Table 5.2.3b. Stage compositions by stage and reader for all stage readers for dab. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																				
stage	Trainee	Intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Intermediate	Trainee	Expert	Expert	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	88%	94%	88%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	71%	82%	100%	94%	88%	90%
3	88%	75%	75%	25%	50%	75%	75%	50%	0%	75%	100%	100%	88%	75%	100%	88%	88%	0%	50%	64%
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	88.0%	88.0%	84.0%	76.0%	84.0%	92.0%	92.0%	84.0%	68.0%	92.0%	100.0%	100.0%	96.0%	92.0%	80.0%	84.0%	96.0%	64.0%	76.0%	86%

Table 5.2.3c. Bias in the comparison for dab. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

After discussion it remained unclear from the macroscopic staging whether dab 5 was a maturing (stage 2) or skipped spawning (stage 5). Under the microscope a smear of the gonad showed developing oocytes and no atresia (Figure 5.2.3.1).

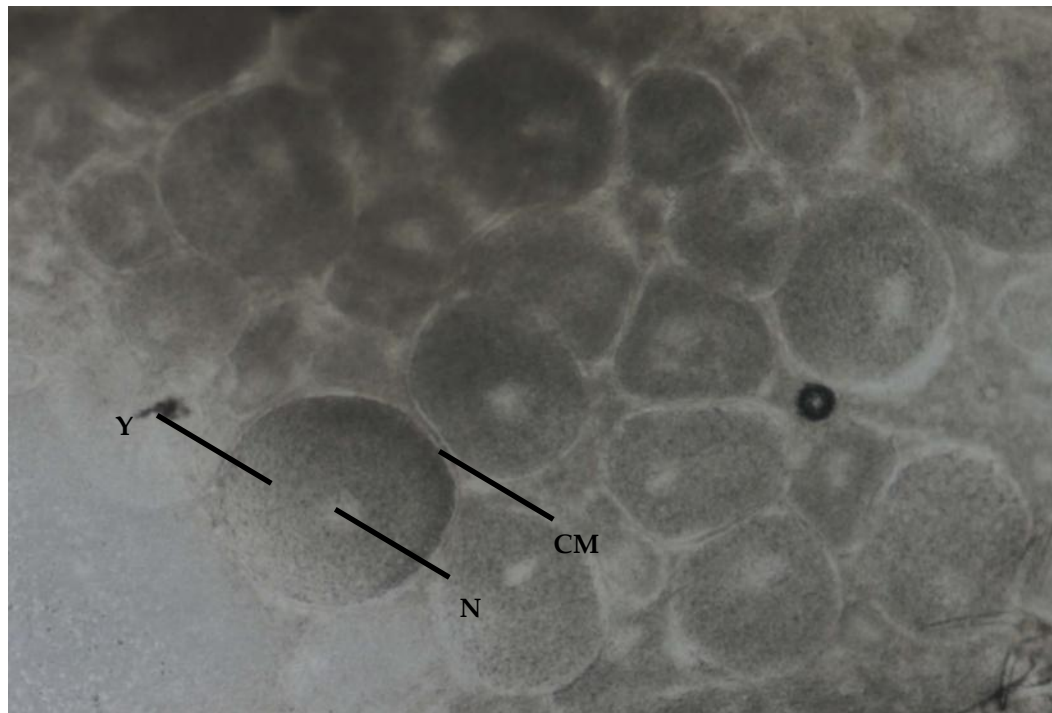


Figure 5.2.3.1 Developing oocytes in a maturing (stage 2) dab (CM: Cell membrane; Y: developing yolk (yolk vesicles and yolk granules); N: nucleus).

During the discussion on dab 13, 14 and 18 it became clear that it was difficult to decide if hydrated eggs were present in these gonads. Dab eggs are small and thus difficult to discern in a gonad. Under the microscope hydrated eggs were clearly visible (Figure 5.2.3.2). The text has been updated in the staging key to highlight the need for close examination of both the ovaries.

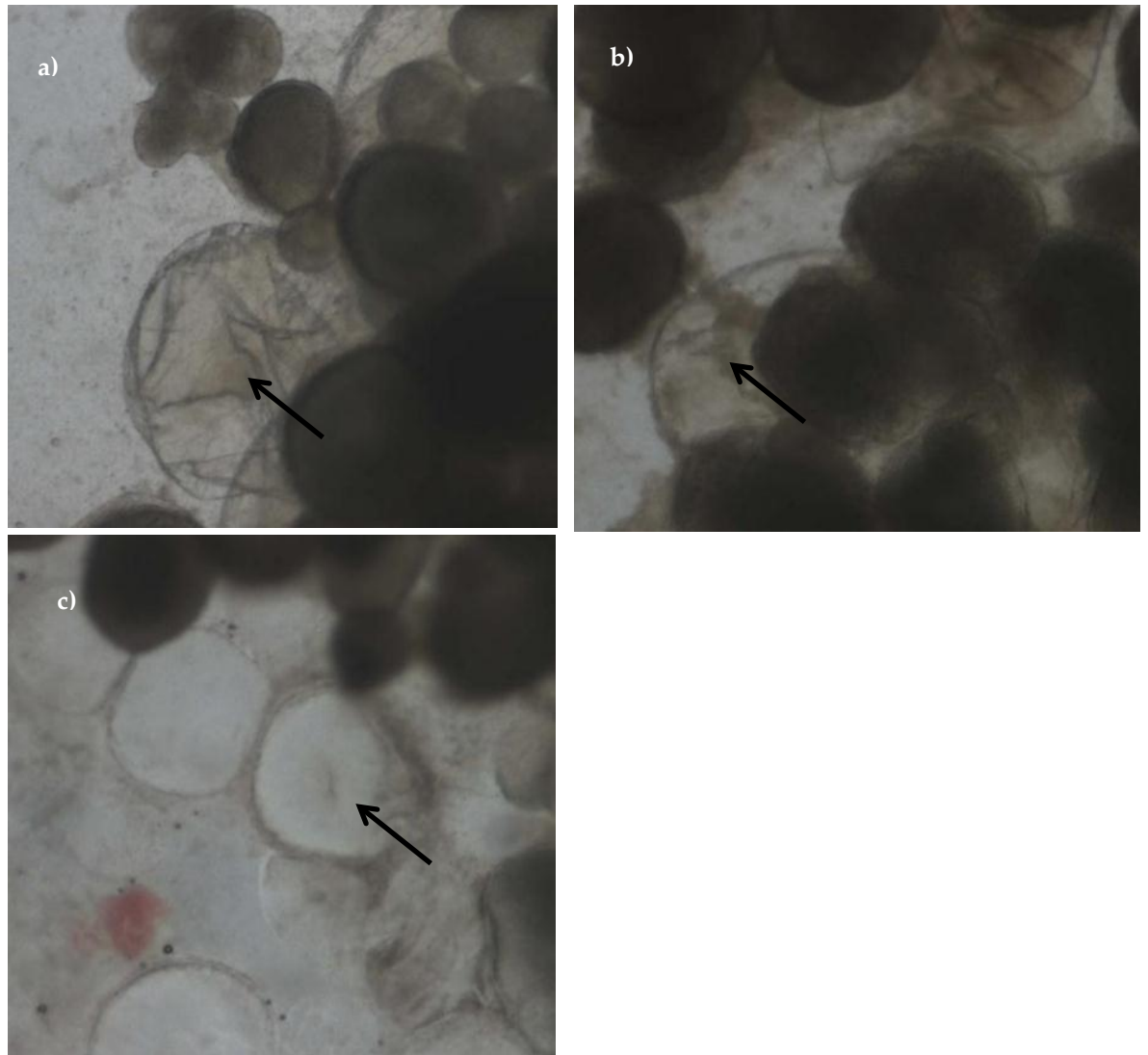


Figure 5.2.3.2. Hydrated oocytes in stage 3 a) dab 13, b) dab 14 and c) dab 18.

5.2.4 Flounder

Sex ratio in flounder in the fresh samples was 50:50. All flounder were in either maturity stage 2 or 3 (Table 5.2.4a). Overall agreement in maturity staging in flounder was 80% (Table 5.2.4b). As in dab the problem between stage 2 and 3 became apparent, especially for males. However in both experts and trainees individual agreement variation was the same (Table 5.2.4b & c).

Table 5.2.4a. The number of stagings by modal stage for flounder.

[illegible]

Table 5.2.4b. Stage compositions by stage and reader for all stage readers for flounder. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																					
stage	Trainee	Trainee	Trainee	Intermediate	Expert	Expert	Trainee	Expert	Expert	Intermediate	Expert	Expert	Intermediate	Expert	Trainee	Expert	Expert	Intermediate	Trainee	ALL	
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2	100%	85%	77%	100%	100%	100%	100%	100%	100%	100%	54%	54%	100%	100%	15%	100%	46%	100%	92%	81%	
3	100%	25%	67%	100%	75%	100%	67%	83%	8%	92%	83%	50%	92%	100%	42%	92%	92%	58%	67%	70%	
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-6	100.0%	56.0%	72.0%	100.0%	88.0%	100.0%	84.0%	92.0%	56.0%	96.0%	68.0%	52.0%	96.0%	100.0%	28.0%	96.0%	68.0%	80.0%	80.0%	80%	

Table 5.4.2c. Bias in the comparison for flounder. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

6 Picture calibration exercises (ToR d)

The second and third staging rounds were based on pictures. For all species, consensus on the maturity stages from the pictures (second round: min. 69%, max. 74%; third round: min. 69%, max. 83%) were lower compared to the fresh fish staging (min. 80%, max. 86%). However, agreement increased from the second to the third staging round. Also, agreement was higher compared to the results of the final picture staging of the 2010 workshop, when consensus varied from 58% to 67% (ICES, 2010).

The institutes followed the 2010 protocol for picture taking and pictures were of good quality. However, it remains difficult to assess maturity stages from pictures.

The general feeling was that it was easier to stage female fish than male fish. Analysis of the percentage agreement by sex over all species and calibration exercises support this (Figure 6.1, Table 6.1). There is also significantly higher agreement on the sexual maturity stage of fish in the spawning season (October-April) compared to outside the spawning season (Figure 6.1, Table 6.1). The macroscopic maturity staging is a reliable method in the period from two months before the start of the spawning season until the end of spawning.

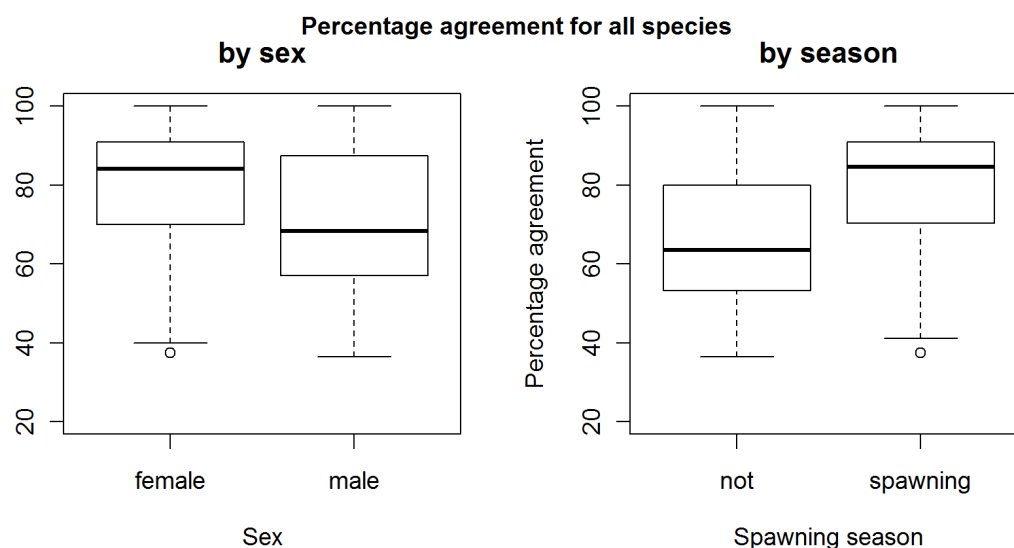


Figure 6.1 Percentage agreement by sex (left) and by season (right), for all species in all calibration exercises from WKMSSPDF 2012.

Table 6.1 Results independent 2-group T-test

	By sex	By season
n. observations	347	307
t	4.79	-5.84
df	192	129
p	<0.001	<0.001

6.1 First macroscopic picture staging

For the first staging exercise from pictures, 35 sole, 32 plaice, 26 dab and 63 flounder were used. All participants started with the species they are most familiar with, and carried on with other species if time allowed.

6.1.1 Sole

Maturity stages in sole varied from 1 to 3 and 5 (Table 6.1.1a), and agreement between participants was 73% (Table 6.1.1b). Again problems occurred deciding between stages 2 - 3 and 2 - 5 . However in some fish there was also a discussion between stages 4 and 5.

Table 6.1.1.a. The number of stagings by modal stage for sole

a) maturity stage composition																					
stage	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	TOTAL
1	2	-	2	1	-	-	-	-	-	-	1	-	-	-	-	-	1	1	1	-	9
2	25	3	23	27	-	-	-	-	14	-	22	-	7	17	-	-	22	24	1	-	185
3	4	-	3	4	-	-	-	-	2	-	6	-	-	17	-	-	6	5	3	-	50
4	-	3	-	-	-	-	-	-	-	-	6	-	-	-	-	-	5	6	1	-	21
5	5	5	8	4	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	46
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
1-6	36	11	36	36	0	0	0	0	16	0	35	0	9	36	0	0	34	36	6	0	291

Table 6.1.1.b. Stage compositions by stage and reader for all stage readers for sole. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																					
stage	Trainee	Intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	ALL
1	100%	-	100%	100%	-	-	-	-	0%	-	100%	-	-	0%	-	-	100%	100%	-	-	80%
2	100%	60%	91%	100%	-	-	-	-	52%	-	87%	-	100%	52%	-	-	91%	96%	0%	-	82%
3	67%	-	50%	67%	-	-	-	-	33%	-	83%	-	-	100%	-	-	83%	83%	-	-	71%
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	83%	67%	100%	67%	-	-	-	-	100%	-	0%	-	40%	17%	-	-	0%	0%	0%	-	46%
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	91.7%	63.6%	86.1%	88.9%					55.6%		74.3%		66.7%	52.8%			74.3%	77.8%	0.0%		73.1%

Table 6.1.1.c. Bias in the comparison for sole. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

6.1.2 Plaice

In plaice maturity stages varied from 1 to 5 (Table 6.1.2a). Agreement between participants was 68% (Table 6.1.2b). Participants had problems deciding between stages 2-3 or stages 2-4-5 (Table 6.1.2b & c).

Table 6.1.2a. The number of stagings by modal stage for plaice

a) maturity stage composition																						TOTAL
stage	intermediate	intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee		
1	3	-	2	3	3	-	3	-	3	-	2	-	3	2	3	-	3	3	2	-	35	
2	14	-	17	15	17	-	20	-	16	-	13	-	12	12	19	7	19	17	4	-	202	
3	2	-	5	11	7	-	6	-	6	-	12	-	11	12	5	5	8	8	18	-	116	
4	-	2	1	2	1	-	4	1	3	-	6	-	5	6	4	7	3	5	4	-	54	
5	9	8	8	2	5	-	-	1	-	-	-	-	2	-	2	4	-	-	4	-	50	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-6	28	10	33	33	33	0	33	2	28	0	33	0	33	32	33	23	33	33	32	0	452	

Table 6.1.2b Stage compositions by stage and reader for all stage readers for plaice. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																					
stage	intermediate	intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	ALL
1	100%	-	67%	100%	100%	-	100%	-	100%	-	67%	-	100%	100%	100%	-	100%	100%	67%	-	92%
2	65%	0%	71%	76%	88%	-	94%	0%	65%	-	76%	-	65%	59%	82%	38%	82%	76%	18%	-	66%
3	40%	-	56%	78%	78%	-	67%	-	33%	-	100%	-	89%	89%	33%	75%	67%	67%	88%	-	69%
4	0%	100%	50%	50%	50%	-	100%	100%	100%	-	100%	-	50%	50%	50%	100%	100%	100%	50%	-	71%
5	100%	0%	100%	50%	100%	-	0%	-	100%	-	0%	-	100%	0%	100%	0%	0%	0%	50%	-	48%
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	64.3%	10.0%	66.7%	75.8%	84.8%		81.8%	50.0%	63.6%		78.8%		75.8%	65.6%	69.7%	43.5%	75.8%	72.7%	43.8%		68.1%

Table 6.1.2c. Bias in the comparison for plaice. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

Maternity stages in dab varied from 2 to 5 (Table 6.1.3a). The agreement between all participants was 72% for dab (Table 6.1.3b). As in the other species, discussion was between stages 2-3 and 2-4-5 (Table 6.1.3b & c).

a) maturity stage composition																						
stage	intermediate	intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	TOTAL	
1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	2	
2	-	-	-	12	-	-	22	-	14	-	-	-	11	10	14	-	-	-	11	-	94	
3	-	-	-	11	-	-	4	-	3	-	3	-	7	12	5	-	-	3	9	-	57	
4	-	-	-	2	-	-	-	-	1	-	1	-	3	1	3	-	-	1	4	-	16	
5	-	-	-	1	-	-	-	-	-	-	-	-	5	2	4	-	-	-	1	-	21	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-6	0	0	0	26	0	0	26	0	18	0	4	0	26	26	26	0	0	4	26	0	182	

b) % agreement per stage																					
stage	Intermediate	Intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	Intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	85%	-	-	100%	-	62%	-	-	-	62%	77%	92%	-	-	-	77%	-	82%
3	-	-	-	100%	-	-	40%	-	30%	-	100%	-	50%	100%	50%	-	-	100%	90%	-	80%
4	-	-	-	100%	-	-	-	-	100%	-	-	-	100%	0%	100%	-	-	-	100%	-	81%
5	-	-	-	50%	-	-	-	-	100%	-	-	-	100%	50%	100%	-	-	-	50%	-	90%
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6				91.0%			90.8%		71.2%		75.0%		70.3%	79.6%	86.2%			75.0%	81.0%		81.2%

[illegible]

6.1.4 Flounder

For flounder maturity stages in the second staging varied from stage 1 to 5 (Table 6.1.4a). Agreement between participants was 74% (Table 6.1.4b). As in the other species, also in founder discussion was between stages 2-3 and 2-4-5 (Table 6.1.4b & c).

Table 6.1.4a The number of stagings by modal stage for flounder.

a) maturity stage composition																					TOTAL
stage	Trainee	Intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	
1	-	-	-	-	2	2	-	3	-	3	-	3	4	1	3	3	-	-	-	-	24
2	-	-	-	-	33	33	-	22	22	21	-	31	24	20	21	22	-	-	-	-	249
3	-	-	-	-	6	10	-	17	12	18	-	15	15	20	20	17	-	-	-	-	150
4	-	-	-	-	8	12	-	5	2	6	-	12	12	9	11	6	-	-	-	-	83
5	-	-	-	-	14	6	-	16	-	15	-	2	8	10	5	15	-	-	-	-	117
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	3
1-6	0	0	0	0	63	63	0	63	36	63	0	63	63	60	60	63	0	0	0	0	597

Table 6.1.4b Stage compositions by stage and reader for all stage readers for flounder. A weighted mean percent agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																						
stage	Trainee	Intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	ALL	
1	-	-	-	-	67%	67%	-	100%	0%	100%	-	100%	100%	33%	100%	100%	-	-	-	-	77%	
2	-	-	-	-	92%	88%	-	85%	54%	81%	-	88%	85%	68%	62%	85%	-	-	-	-	79%	
3	-	-	-	-	29%	53%	-	100%	53%	100%	-	65%	71%	71%	65%	100%	-	-	-	-	71%	
4	-	-	-	-	57%	100%	-	57%	14%	71%	-	86%	86%	83%	71%	57%	-	-	-	-	68%	
5	-	-	-	-	100%	30%	-	100%	90%	100%	-	20%	60%	70%	40%	90%	-	-	-	-	70%	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-6					71.4%	69.8%		88.9%	53.2%	88.9%		71.4%	77.8%	68.9%	61.9%	87.3%					74.0%	

Table 6.1.4c Bias in the comparison for flounder. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

6.2 Second macroscopic picture staging

For the second staging exercise from pictures, 20 sole, 25 plaice, 15 dab and 31 flounder were used. All participants started with the species they are most familiar with, and carried on with other species if time allowed.

6.2.1 Sole

Maturity stages in sole varied from 1 to 3 (Table 6.2.1a). Agreement in sole between participants increased to 79% in the third staging (Table 6.2.1b). Again problems occurred deciding between stages 2 - 3 and 2 - 5 (Table 6.2.1b & c).

Table 6.2.1a. The number of stagings by modal stage for sole.

a) maturity stage composition																						
stage	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	TOTAL	
1	1	-	1	1	-	-	-	-	-	-	1	-	-	-	-	-	1	1	-	-	6	
2	16	12	19	17	-	-	20	-	1	-	11	-	-	-	-	-	13	11	-	-	120	
3	2	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	2	4	-	-	13	
4	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	3	4	-	-	10	
5	-	-	-	2	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	11	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-6	19	12	20	20	0	0	20	0	10	0	20	0	0	0	0	0	19	20	0	0	160	

Table 6.2.1b. Stage compositions by stage and reader for all stage readers for sole. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																					
stage	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	ALL
1	100%	-	100%	100%	-	-	0%	-	-	-	100%	-	-	-	-	-	100%	100%	-	-	86%
2	94%	100%	100%	89%	-	-	100%	-	10%	-	61%	-	-	-	-	-	72%	61%	-	-	79%
3	100%	-	0%	0%	-	-	0%	-	-	-	100%	-	-	-	-	-	100%	100%	-	-	57%
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	94.7%	100.0%	95.0%	85.0%			90.0%		10.0%		65.0%						75.0%	65.0%			78.3%

Table 6.2.1c. Bias in the comparison for sole. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

c) bias per stage																					ALL
stage	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	
1	0.00	-	0.00	0.00	-	-	1.00	-	-	-	0.00	-	-	-	-	-	0.00	0.00	-	-	0.14
2	0.06	0.00	0.00	0.33	-	-	0.00	-	2.70	-	0.56	-	-	-	-	-	0.28	0.61	-	-	0.41
3	0.00	-	-1.00	-1.00	-	-	-1.00	-	-	-	0.00	-	-	-	-	-	0.00	0.00	-	-	-0.43
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

6.2.2 Plaice

In the third staging calibration, plaice maturity stages varied from 1 to 5 (Table 6.2.2a). Agreement between participants increased to 70% (Table 6.2.2b). Participants had problems deciding between stages 2-3 or stages 2-4-5 (Table 6.2.2b & c).

Table 6.2.2a. The number of stagings by modal stage for plaice.

a) maturity stage composition																					
stage	intermediate	intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	TOTAL
1	1	2	-	1	1	-	1	-	1	-	3	-	2	-	3	1	2	3	3	-	24
2	12	14	13	15	12	-	20	6	7	18	14	-	11	2	12	9	17	15	4	-	201
3	7	5	6	6	7	-	3	2	6	5	6	-	6	17	6	6	5	6	13	-	112
4	1	3	1	1	1	-	1	-	6	-	2	-	1	3	1	2	1	1	3	-	28
5	4	1	3	2	4	-	-	7	5	1	-	-	4	3	3	7	-	-	-	-	44
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
1-6	25	25	23	25	25	0	25	15	25	24	25	0	24	25	25	25	25	25	23	0	409

Table 6.2.2b Stage compositions by stage and reader for all stage readers for plaice. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																					
stage	intermediate	intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	ALL
1	100%	100%	-	100%	100%	-	100%	-	100%	-	100%	-	100%	0%	100%	100%	100%	100%	100%	-	93%
2	71%	79%	85%	86%	71%	-	100%	56%	50%	100%	86%	-	71%	14%	79%	57%	93%	86%	29%	-	72%
3	67%	67%	67%	83%	67%	-	50%	67%	67%	83%	83%	-	80%	100%	83%	67%	67%	83%	100%	-	75%
4	100%	0%	100%	100%	100%	-	100%	-	100%	0%	100%	-	0%	0%	100%	100%	100%	100%	100%	-	75%
5	100%	0%	100%	33%	100%	-	0%	100%	67%	33%	0%	-	33%	33%	67%	67%	0%	0%	0%	-	43%
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	76.0%	64.0%	82.6%	80.0%	76.0%		76.0%	66.7%	60.0%	83.3%	76.0%		66.7%	36.0%	80.0%	64.0%	76.0%	76.0%	45.8%		69.8%

Table 6.2.2c. Bias in the comparison for plaice. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

c) bias per stage																					ALL
stage	intermediate	intermediate	Expert	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	Trainee	
1	0.00	0.00	-	0.00	0.00	-	0.00	-	0.00	-	0.00	-	0.00	4.00	0.00	0.00	0.00	0.00	0.00	-	0.29
2	0.43	0.14	0.15	0.29	0.43	-	0.00	1.33	1.07	0.00	0.00	-	0.57	1.07	0.21	1.00	0.07	0.00	0.79	-	0.43
3	-0.33	0.00	-0.33	-0.17	-0.33	-	-0.50	-0.33	0.33	-0.17	-0.17	-	0.20	0.00	-0.17	0.17	-0.33	-0.17	0.00	-	-0.13
4	0.00	1.00	0.00	0.00	0.00	-	0.00	-	0.00	-2.00	0.00	-	1.00	-1.00	0.00	0.00	0.00	0.00	0.00	-	-0.06
5	0.00	-2.33	0.00	-2.00	0.00	-	-3.00	0.00	-0.33	-2.00	-2.67	-	-2.33	-0.67	-1.33	-0.33	-3.33	-3.33	-2.00	-	-1.51
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

6.2.3 Dab

Maturity stages in dab varied between 2, 3 and 5 (Table 6.2.3a). The agreement between all participants increased by 10% from the second to the third staging to 83% (Table 6.2.3b). As in the other species, discussion was again between stages 2-3 and 2-5 (Table 6.2.3b & c).

Table 6.2.3a The number of stagings by modal stage for dab.

a) maturity stage composition																				
stage	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	TOTAL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	7	-	-	10	-	8	-	-	-	-	4	-	-	-	-	8	37
3	-	-	-	6	-	-	5	-	4	-	-	-	-	9	-	-	-	-	7	31
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	2	-	-	-	-	3	-	-	-	-	2	-	-	-	-	-	7
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	0	0	0	15	0	0	15	0	15	0	0	0	0	15	0	0	0	0	15	75

Table 6.2.3b Stage compositions by stage and reader for all stage readers for dab. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																				
stage	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	intermediate	Trainee	Expert	Trainee	Trainee	Trainee	Trainee	Trainee	Expert	Expert	Trainee	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	100%	-	-	100%	-	83%	-	-	-	-	67%	-	-	-	-	100%	-
3	-	-	-	86%	-	-	71%	-	57%	-	-	-	-	100%	-	-	-	-	100%	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	100%	-	-	0%	-	100%	-	-	-	-	100%	-	-	-	-	0%	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6				93.3%			73.3%		73.3%					86.7%					86.7%	82.7%

Table 6.2.3c Bias in the comparison for dab. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage.

[illegible]

6.2.4 Flounder

For flounder maturity stages in the third staging varied from stage 1 to 3 and 5 to 6 (Table 6.2.4a). Agreement between participants had increased by 7% and was 81% in this round (Table 6.2.4b). As in the other species, discussion was between stages 2-3 and 2-5 (Table 6.2.4b & c).

Table 6.2.4a The number of stagings by modal stage for flounder.

a) maturity stage composition																				
stage	Trainee	Trainee	Trainee	intermediate	Expert	Expert	Trainee	Expert	Expert	intermediate	Expert	intermediate	Expert	Trainee	Expert	intermediate	Expert	Expert	Trainee	TOTAL
1	-	-	-	-	1	1	-	1	-	1	-	1	1	1	1	1	-	-	1	10
2	-	-	-	-	15	15	-	13	11	16	-	13	14	10	16	13	-	-	8	144
3	-	-	-	-	9	9	-	10	13	9	-	12	11	14	9	9	-	-	14	119
4	-	-	-	-	1	1	-	-	-	-	-	1	1	-	-	-	-	-	1	5
5	-	-	-	-	5	5	-	7	7	5	-	4	4	5	4	7	-	-	1	54
6	-	-	-	-	1	1	-	1	1	1	-	1	1	1	2	2	-	-	-	12
1-6	0	0	0	0	31	31	0	31	31	31	0	31	31	30	30	30	0	0	25	332

Table 6.2.4b Stage compositions by stage and reader for all stage readers for flounder. A weighted mean percentage agreement is given by stage reader and all stage readers combined.

b) % agreement per stage																				
stage	Trainee	Trainee	Trainee	intermediate	Expert	Expert	Trainee	Expert	Expert	intermediate	Expert	intermediate	Expert	Trainee	Expert	intermediate	Expert	Expert	Trainee	ALL
1	-	-	-	-	100%	100%	-	100%	0%	100%	-	100%	100%	100%	100%	100%	-	-	100%	-
2	-	-	-	-	86%	93%	-	93%	50%	93%	-	79%	93%	54%	100%	86%	-	-	30%	-
3	-	-	-	-	80%	90%	-	100%	70%	90%	-	90%	100%	90%	90%	80%	-	-	78%	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	67%	67%	-	100%	100%	67%	-	67%	67%	67%	67%	100%	-	-	20%	-
6	-	-	-	-	100%	100%	-	100%	100%	100%	-	100%	100%	100%	100%	100%	-	-	-	-
1-6					78.1%	84.4%		93.8%	62.5%	84.4%		78.1%	87.5%	67.7%	87.5%	84.4%			48.0%	78.5%

Table 6.2.4c Bias in the comparison for flounder. The bias is indicated by the percentage over- or under-estimation of each maturity stage, as estimated by each participant, in relation to the modal stage

c) bias per stage																				
stage	Trainee	Trainee	Trainee	intermediate	Expert	Expert	Trainee	Expert	Expert	intermediate	Expert	intermediate	Expert	Trainee	Expert	intermediate	Expert	Expert	Trainee	ALL
1	-	-	-	-	0.00	0.00	-	0.00	1.00	0.00	-	0.00	0.00	0.00	0.00	0.00	-	-	0.00	-
2	-	-	-	-	0.29	0.21	-	0.21	0.64	0.21	-	0.21	0.07	0.62	0.00	0.29	-	-	0.70	-
3	-	-	-	-	-0.20	-0.10	-	0.00	-0.30	-0.10	-	-0.10	0.00	-0.10	-0.10	0.20	-	-	-0.22	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-0.67	-0.67	-	0.00	0.00	-1.00	-	-0.67	-0.67	-1.00	-0.33	0.00	-	-	-2.00	-
6	-	-	-	-	0.00	0.00	-	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	-	-	-	-

7 Validation of macroscopic maturity with histological analysis (ToR e)

The macroscopic maturity stage was validated with the histological analysis after the calibration exercises. Thus the results of the calibration exercises based on the modal stage were available and for fish with low agreement the staging was validated in plenary sessions using the microscopic smears or histological sections. The data reported in this report is based on the macroscopic maturity stage and not corrected in case the microscopic analysis proved the staging was in correct.

WKMSSPDF recommends that in future workshops it should be decided if all stagings should be checked against the microscopic stage or the modal stage. If it is decided to continue using the modal stage it should than be decided to base the modal stage on all participants or only the modal of the expert stagings.

7.1 Histological development of gonads

7.1.1 Female

The maturation cycle of oocytes in a female starts by hormonal production, which in the species discussed in this report is triggered by an environmental change, such as daylight length. When a new oocyte maturation cycle starts, oocytes are recruited from the previtellogenic stage to the vitellogenic stage.

The first early maturation period of oocytes is the cortical alveoli stage. In this stage the cortical alveoli appear which will be used for further development of the cell membrane. After the cortical alveoli stage, yolk vesicles appear.

In the next stage, yolk granules appear which later form the yolk of the egg. Depending on the species, small oil globules are visible in the yolk vesicle and yolk granule stages, later fusing to form one or more bigger oil droplets in the final maturation.

In the final maturation stage, called the hydrated stage, oocytes take up water and so, extremely increase in size. This stage is quite short and thus spawning of the oocytes will occur in the near future after final maturation.

It should be noted that cortical alveoli appear almost immediately after spawning due to the hormonal production. In North Sea plaice cortical alveoli stage is in March-April, while the females spawn in December or January.

During the maturation of oocytes females might stop the maturation due to a low body condition. This can occur at any stage in the oocyte maturation period before final maturation. Macroscopically stage 5 'skip spawning' can show no development or some development.

Macroscopically the early maturation stages of the oocytes are difficult to assess and can only be reliably identified microscopically.

A good start for background information on gonad development and reproductive biology are these two references:

- McMillan, D. B. 2007. Fish histology: Female reproductive systems, Springer, Ontario, 598 pp.

- Jakobsen, T., Fogarty, M.J., Megrey, B.A., Moksness, E. 2009. Fish reproductive biology: Implications for assessment and management, Wiley-Blackwell, Oxford, 429 pp.

7.1.2 Male

In maturing males, spermatogonia start to divide and primary spermatocytes are formed. These develop first into secondary spermatocytes and later on to spermatids. At the final maturation the spermatozoa are developed which will be spawned. The maturation period in males is generally much quicker compared to females.

7.2 Smear method

A microscopic smear or swab of a female gonad is a quick and easy exercise to check the macroscopic maturity determination when in doubt. The gonad is cut length ways with a sharp scalpel and with the same scalpel you scrape some of the oocytes out of the gonad on a clear and clean glass plate. The smear should be viewed immediately, to prevent the oocytes from drying, under a dissecting microscope.

This smear method was used at WKMSSPDF 2012 after the fresh fish staging for those fish on which, after discussion, no agreement could be reached. Of these fish the discussion was either between stage 2 and 3 (hydrated eggs visible or not) or between stage 2 and 5 whether the development of gonad was normal or the fish was a skipped spawner. The smears easily show if hydrated oocytes or atretic (i.e. degenerated and subsequently resorbed) oocytes are present in the gonad. Results and pictures of the some of the discussed fish of the fresh staging can be found in section 5.

During the workshop fresh sole and plaice was available for preparing reference pictures for the different maturity stages. To these reference gonad pictures, also pictures of the smears were added (Annex 8).

7.3 Histological sections

The use of histological sections to validate the macroscopic maturity staging of gonads is the most precise method with the highest resolution, but also time consuming and more expensive than the smear method. Gonads need to be fixed for at least one week. After fixation the whole process of preparing blocks, cutting sections and examining the sections takes at least another full week. Hence, it was not possible to use this for the fresh fish staging.

For most of the pictures used in the calibration exercises histological sections were available to check the macroscopic maturity staging. The results of the picture calibration exercises again showed the problems between stages 2 and 3 and stages 2 and 5.

After each picture calibration exercise the fish with low agreement were discussed and validated using the histological sections (see also Annex 9). This revealed that it is difficult to macroscopically identify early stage 2 fish. Stage 2 fish showing cortical alveoli or vitellogenic oocytes up to the yolk vesicle-yolk granule stage in females or spermatocytes and spermatids in males were often misidentified as stage 5. Macroscopic maturity staging is a reliable method when used two months before the spawning season to assess maturity. Outside this period the macroscopic method can easily lead to misidentification and histological sections should be used to correctly identify the maturity stage. It is therefore recommended that maturity staging of fish only takes place in this period, unless it can be supported with histological sections.

8 Generic ToRs adopted for maturity staging workshops (ToR f)

8.1 Staging procedure

The procedure followed during WKMSSPDF 2012 was evaluated by the group and the following comments were made for future workshops:

- a) It was good that the workshop started with the fresh staging, to warm up. The discussion after the fresh staging was very fruitful, but it should be done in smaller groups.
- b) Although discussions about maturity stages are time consuming, they are very important as different countries are present in the workshop and so, enough time should be allowed for these discussions to take place.
- c) There is a wish to have more discussion about specific pictures. It is suggested to let people explain why they think it is a certain stage. The group (or subgroups per species) then can have a discussion on it about the reasons.
- d) To create more time for discussion, it might be worth to do the first calibration exercise before the meeting. This shows the potential discussion items before the workshop starts. A calibration exercise before the workshop will need time and effort from participants beforehand, also with respect to the upload of pictures.

8.2 Pictures

Staging from pictures is difficult, especially because there is no possibility to touch the gonad (distinction between stage 2 and 3, especially in males).

In general, the protocol for pictures worked fine, as the pictures were of higher quality than in WKMSSPDF in 2010.

When staging from pictures, it is necessary to standardise the way the pictures are taken. There have to be stringent procedures even down to equipment and/or settings used.

The choice of the period for taking pictures depends on the scope of the maturity staging workshop. Pictures from outside the spawning period might be used as example for discussion.

General marks for staging from pictures (see also Protocol data collection WKMSSPDF 2012 (Workshop on Maturity Staging of Sole, Plaice, Dab and Flounder) Version 2.0, April 2010):

- Pictures have to be taken on fresh fish.
- Add at least sampling time, area, unique sampling number, fish length and species in the picture. Ensure that maturity stage is not visible on the picture or in the filename.
- Take care that the samples should be clean/tidy, preferable without intestines.
- Take at least six pictures:
 - 1) Dorsal side: overview of the fish on a measuring board, with the gonads visible in the fish. The ability to look at the whole fish with the gonad intact is vital to get the ratio of gonad to body length.

- 2) Dorsal side: detail of picture 1, zoomed in on the gonads. Show the pressure characteristic on the picture to see if fish is running.
- 3) Ventral side: overview of the fish on a measuring board, with the gonads visible in the fish. The ability to look at the whole fish with the gonad intact is vital to get the ratio of gonad to body length.
- 4) Ventral side: detail of picture 3, zoomed in on the gonads. Show the pressure characteristic on the picture (or on an additional picture) to show if fish is running. As it is difficult to show the pressure test for male specimens, a homogenous background is needed as long as it is not white.
- 5) Picture of gonads outside the fish, placed on a measuring board, allowing to view the gonad in more detail, blood vessels etc.
- 6) Picture of longitudinally cut gonad.

In addition, a table including biological and sampling information should be available.

8.3 Maturity staging forum

There is an age readers forum active via an ICES groupnet page. The age readers use WebGR for the calibration exercises, and the forum for discussions. As everyone has access to the website, the information is available for everyone, also when the report is not ready yet. The forum is also accessible from the outside, so information can be shared with the wider audience.

The group recommends that a maturity-stagers forum is installed.

8.4 Meeting frequency

The group recommends that:

- a) The meeting frequency should be once each 3-5 years
- b) The group should not be expanded with more species (and so, more people)
- c) The national institutes should be strongly encouraged to put effort into making pictures, and should find time and money to do so. Successful maturity staging workshops cannot be carried out without these pictures.

9 Evaluation of the use of WebGR

All institutes that made pictures following the WKMSSPDF protocol, uploaded pictures to the WebGR server. Three calibration exercises were created for all fish (fresh, macroscopic 1 and macroscopic 2). It was decided that participants entered the stages on the first picture of a fish.

9.1 Uploading and data correction

Problems with uploading the pictures and fish information were generally solved easily. There is however a discrepancy between the manual and the oral instructions regarding the size of the pictures. The manual states that 5Mb is the maximum size of the picture, the new instruction is less than 0.5 Mb because otherwise the server will be stuck.

It is not possible to delete fish or fish related information from the database. This should be possible for data managers and workshop managers.

It is recommended that the WebGR manual is extended with at least the exact parameter descriptions for the upload, including mandatory fields, use of capitals, etc.. It is suggested that the manual is reviewed by users to improve it.

9.2 Use for maturity staging

A few issues occur while staging maturity using WebGR.

- 1) The resolution of the pictures is too low for maturity staging, as it should be possible to identify hyaline oocytes in a gonad. When picture resolution is low, the hyaline eggs are not always clearly visible, especially when there are only a few present. As the borderline between maturity stage 2 and 3 is the presence of hyaline eggs, it is very important that this can be seen. It is recommended to the WebGR team that it is possible to upload higher resolution pictures for maturity staging purposes.
- 2) For maturity purposes, multiple pictures per fish have to be studied in order to identify the maturity stage properly. However, the maturity stage is saved per picture, which is not preferred, as the maturity stage is related to a fish and the suite of pictures results to that decision. It is therefore recommended to the WebGR team that the possibilities for adding stage (and age) to a fish instead of to a picture are studied.
- 3) Consequently, use “fish” as a starting point, where all pictures from one fish are presented in a folder structure. Also, for workshop managers, it would be favourable if it was possible to select fish instead of pictures when preparing the calibration exercises.
- 4) Loading the pictures takes a lot of time, which seems to be a WebGR server problem. The system regularly threw people out of WebGR. It is suggested to have a ‘Resume button’.
- 5) Related to that, it is recommended to create an easily accessible standalone version of WebGR where people can work locally on their own computer after up- and/or downloading calibration exercises and/or results.
- 6) Especially when using more than one picture per fish it would be very useful for users to be able to select specific pictures in the application, even within the selection.

- 7) There is a strong wish for a user-defined appearance of the left screen in the annotation screen (as is possible in e.g. Windows Explorer).
- 8) Currently, statistics are available to everyone during the calibration exercise. This should only be possible for the workshop manager, and not for the readers. Readers however should be able to see their own statistics in order to check if they have staged all pictures in a calibration exercise.

9.3 Use for future maturity staging

Refreshing the pictures will take time and money from the institutes involved. It is important to maintain the system in order to use it well.

10 References

- Anon. 2010. Protocol data collection WKMSSPDF 2012 (Workshop on Maturity Staging of Sole, Plaice, Dab and Flounder) Version 2.0, April 2010.
- ICES. 2010. Report of the Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder (WKMSSPDF), 22-26 February 2010, IJmuiden, The Netherlands. ICES CM 2010/ACOM:50. 96 pp.
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Annex 2: Agenda

Monday, 9 January

13:00

General announcements, logistics, local arrangement, etc.

13:30

ToR c) Calibrate staging of sole, plaice, dab and flounder using fresh fish, following the pattern of trial-discussion-retrial;

Fresh fish staging

14:30

ToR b) Check the description of the characteristics of the stages of the 2010 scale; discussion fresh fish. Subgroups per species.

16:00

ToR a) Report on the use of the common maturity scale proposed in 2010;

Per institute, yes/no

Tuesday, 10 January

9:00 Start, connection computers etc.

ToR d) Calibrate staging of sole, plaice, dab and flounder using photographs, following the pattern of trial-discussion-retrial;

12:00 lunch

13:00

ToR b) Check the description of the characteristics of the stages of the 2010 scale; discussion pictures. Subgroups per species.

15:30

ToR e) Validate macroscopic maturity determination with histological analysis.

Plenary discussion about results, including histological pictures.

Wednesday, 11 January

10:00 Start, connection computers etc.

ToR d) Calibrate staging of sole, plaice, dab and flounder using photographs, following the pattern of trial-discussion-retrial;

12:00 lunch

13:00

ToR b) Check the description of the characteristics of the stages of the 2010 scale; discussion pictures. Subgroups per species.

15:30

ToR e) Validate macroscopic maturity determination with histological analysis.

Plenary discussion about results, including histological pictures.

Thursday, 12 January

9:00 Report writing in subgroups, finetuning descriptions per species, selection of pictures, etc.

11:00 Plenary major changes

12:00 lunch

13:00

ToR f) address the generic ToRs adopted for maturity staging workshops (see 'PGCCDBS Guidelines for Workshops on Maturity Staging')

Plenary about the organisation of MS workshops

15:30

Recommendations (or not)

Preparation of stand-alone version of flatfish maturity staging reference document (TB to be added after WKMSTB)

Finalise report writing

Friday, 13 January

9:00 Report checking

10:00 Stand-alone version, left-overs, etc.

Annex 3: WKMSSPDF terms of reference for the next meeting

WKMSSPDF 2012 assumes it is the responsibility of PGCCDBS to initiate a future workshop on maturity staging of sole, plaice, dab and flounder. Recommendations for the workshop can be found in Chapter 8 and Annex 4.

Annex 4: Recommendations

Recommendation	Adressed to
1. Macroscopic maturity staging is a reliable method when used from two months before the spawning season until the end of spawning to assess maturity. It is recommended that macroscopic maturity staging of fish only takes place in this period, unless it can be supported with histological sections.	National institutes through PGCCDBS
2. WKMSSPDF recommends that the Baltic institutes keep their national maturity staging scales, and transfer it to the internationally accepted maturity stage in DATRAS, from a certain date onwards. Old data should not be changed. There will be a clear break in the DATRAS timeseries with respect to the maturity. The BITS manual should describe this change well. (see section 3)	WGBIFS
3. The group recommends that a maturity-stagers forum is installed, following the lines of the age-readers forum facilitated by ICES	PGCCDBS
4. The group recommends that: <ul style="list-style-type: none"> a) The meeting frequency should be once each 3-5 years b) The group should not be expanded with more species (and so, more people) c) The national institutes should be strongly encouraged to put effort into making pictures, and should find time and money to do so. Successful maturity staging workshops cannot be carried out without these pictures. 	PGCCDBS
5. WKMSSPDF recommends that in future workshops it should be decided if all stagings should be checked against the microscopic stage or the modal stage. If it is decided to continue using the modal stage it should then be decided to base the modal stage on all participants or only the modal of the expert stagings. (see section 7)	Future WKMS through PGCCDBS
6. It is recommended that WebGR supports the upload of higher resolution pictures for maturity staging purposes. (see section 9.2)	WebGR coördinator (I. Quincoces, Azti)
7. It is recommended that for future development the comments of this groups are taken into account (see 9.2 for the full list)	WebGR coördinator (I. Quincoces, Azti)
8. It is recommended that the WebGR manual is extended with at least the exact parameter descriptions for the upload, including mandatory fields, use of capitals. It is suggested that the manual is checked by users to improve it.	WebGR coördinator (I. Quincoces, Azti)

Annex 5: WebGR

Objectives of WebGR (from <http://webgr.wiki.azti.es>)

The objective of this study is to develop a set of web services to support the organization and data analysis of calibration workshops, both for age and maturity information (WebGR). The most common exercises carried out during these workshops are counting otolith growth rings or classifying gonads, with subsequent analysis of the results in order to build age-length keys or maturity ogives and it must be possible to do this on line using WebGR services. WebGR must also implement procedures for training purposes, like browsing images, reading experts' annotations or simulating a calibration exercise. The services must be implemented in a coherent tool installable as a website.

The website should consist of a repository of images grouped or classified by workshop (species, date, area, etc) and accessible to all workshop participants. Each image must be annotated by several scientists. The annotations must include fields for the classification (age x or maturity stage y, etc), observations, scientist, etc. This information must be stored on a database so that the statistical analysis of the results can be automated as far as possible and made public as on line reports.

The software developed must be licensed by an Open Source license to promote transparency, technology transfer and peer review; and allow the scientific community to get involved in further developments, like linkage to statistical analysis engines, or any other specific features.

More information can be found at webgr.azti.es

Annex 6: Subgroup report on stage descriptions

Sole

The group discussed the descriptions and the photographs presented at the workshop in 2010. For all stages it was decided that a photograph of the gonad removed from the body cavity would be beneficial.

Female

Stage 1: The original description is good but a further photo of the gonad extracted from the body cavity would be useful (with line test evident).

Stage 2: The original text needed to be updated by adding “that gonad is firm and rounded” (as opposed to the skipped spawning of stage 5 that is slackened). If possible a better picture should be used

Stage 3: original text ok but if possible a better picture should be used

Stage 4: original text was okay but the word ‘big’ should be replaced with “gonad has flattened but still retains its length”. Once again a better picture is needed

Stage 5: The text should be replaced with ‘the gonad is recovering from spawning and is shrinking and shortened in length, gonad is slack in appearance often reddish’. A better picture should be used. Nb. After discussion with the rest of the group this text was changed a little.

Skipped spawning stage 5: The group decided that this is very difficult to identify unless you can then back it up by placing a sample of it under a microscope and this is not always possible. It was suggested that it is more important for a first time spawning fish to be identified as ‘skipped spawning’ to identify where it tried to spawn but failed.

Male

Stage 1: The use of ‘semi-skimmed milk’ to describe a colour was questioned and was replaced with ‘watery grey’. A better picture is needed

Stage 2: This text needed to be changed and is to be updated throughout the meeting. Also change ‘maximum’ to ‘large’ in the description.

Stage 3: Again the text needs changing but we should also add, ‘to distinguish between stage 2 and 3 is very difficult in the field but cutting the gonad open may show that the gonad is flatter and more fluid. Also the testes will be bloodshot throughout the testes’. A better photo is also required

Stage 4: No issues with the description or photo.

Stage 5: the sub-group expressed the opinion that it is not possible to distinguish between 5 and 2 for male sole

Plaice

General discussion: Exchange the word gonad for ovaries and testes respectively?

Terminology should preferably be consistent in all diagrams

The group decided to discuss the present text from 2010 stage by stage.

Female

Agreements 2010:

STAGE 1 Juvenile: We all agreed on the descriptions of Juvenile stage

STAGE 2: The group agreed on the present text but decided to incorporate the word yolk in the description as yolk is readily seen in the eggs in this stage. "Opaque eggs (filled with YOLK) visible".

STAGE 3:

The group decided to add to the text that from the moment one hyaline or hydrated egg is visible it is called stage 3.

STAGE 4: We had quite a discussion on how to describe stage 4. Unfortunately no pictures of this stage were available until the end of the workshop. A question that arose was whether blood vessels rupture or whether you can still see them. Also discussed the colouring and agreed on "dark residue".

STAGE 5: A stage hard to judge especially outside spawning season. We tend to use it before we can see growth in the gonad. Within the spawning season "Gonad contracted up to 50 % of spawning length, with walls looking darker then stage 2. Walls are too opaque (thick) to see through them OR Walls are not transparent. "Colouration from deep red to dark brown".

STAGE 6: Abnormal, by example stony gonads, both sexes in the same fish etc. How to deal with parasites? Figure needs to be added to the diagram. Ask the group! "Flo-kati"- abnormal protuberances inside the ovaries (Julia).

Pictures taken from smears:

The difference between the stages is quite well shown on page 10 in the 2010 ICES WKMSSPDF report, although these are herring eggs. The first picture is a stage 1 egg. Pictures 2, 3, 4 are eggs in stage two. Picture 5 shows a stage 3 egg. Herring microscopical pictures will be replaced by microscopical pictures of plaice.

Male

General: The word gonad is exchanged for TESTES (stage 2 en 3)

STAGE 1 Juvenile: We agree on the Juvenile description. The name (ANAL SPINE ref: WCGOP manual, chapter 8 p10: "Flatfish gonads are paired, are located posterior to the visceral cavity and extend just under the flesh on both sides of the fish. If the flatfish has an anal spine, the gonads will begin just behind this spine.") of the bone the gonad lies against could replace "bottom of cavity) STAGE 2: We agree on the description on stage 2.

STAGE 3: We agree on the description on stage 3

STAGE 4: We agree on the description on stage 4

STAGE 5: We agree on the description on stage 5 but with doubt on second thought. Can't really distinguish it from an early stage 2, is this really a valid stage?

STAGE 6: Abnormal – keep in mind that when the spermatids and spermatozoa are abnormal, no abnormality of the testes can be seen. (Lincoln, R. F. (1981), Sexual maturation in triploid male plaice (*Pleuronectes platessa*) and plaice x flounder (*Platichthys flesus*) hybrids. Journal of Fish Biology, 19: 415–426.

PICTURES – DISCUSSION

We think best pictures are taken from the coloured side of the plaice. Also a circle around the gonad can be added for clarity.

More pictures need to be put in for various stages.

Dab

The group discussed the descriptions and the photographs presented at the workshop in 2010. For all stages it was decided that a photograph of the gonad removed from the body cavity would be beneficial.

Female

Stage 1: the text should be changed to reflect that the gonad in the immature female dab would be no more than 2cm in length

Stage 2: a better photo is required. The text should be changed to 'round and firm' not 'rounder and firmer'. Also remove 'grey' as one of the distinguishing colours and change 'orange' to 'burnt orange'.

Stage 3: a better photo is required. The text should also say that 'when identifying you should take care to check both gonads and open gonad and check thoroughly due to the small size of the eggs'

Stage 4: Text should be modified to say that 'hydrated eggs maybe left as residue. Gonad is slack in appearance often reddish' rather than 'eggs maybe visible'

Stage 5: The text should add that 'the gonad is recovering from spawning and is shrinking and shortened in length'.

The difference between spent and recovering is timing. When sampling all year round it may be possible to distinguish between the two but looking at a snap shot it may be far more difficult. And then the difference between stage 5 and the start of stage 2 is not easily identified.

Male

Stage 1: a better photo is required

Stage 2: a better photo is required

Stage 3: The first line of the text should read 'apply pressure to the gonad area first and if running then stage 3'. A better photo is required

Stage 4: Text should be changed to 'greyish and might have a brown edge on the outside of the gonad, knobbly in appearance at late part of stage. A better photo is required

Stage 5: The subgroup commented that timing is the issue with this stage. Depending on when the original picture was taken it could very probably be stage 4. Therefore it may not be possible to distinguish this stage even by knowing the time of the year.

Flounder

To begin with, the WKMSSPDF 2010 descriptions for each of the maturity stages of flounder, male and female respectively, were discussed by splitting the descriptions of each stage into the following 4 categories:

- 1) Size of the gonad in relation to body cavity
- 2) Color of the gonad
- 3) Additional specific factors
- 4) Presence and characteristics of eggs or milt.

Key factors connected within each category were then pointed out and then for each stage the most important feature(s) was chosen and it was decided that these should be put at the beginning of each description in the report.

We tried to avoid using descriptions which were set in relation to any of the other maturity stages and for example, the previous descriptions relating to size differences between stages have been replaced by a more general text.

Furthermore, for maturity stage 2-4 in males, the part in the descriptions saying that there is a wide range of size was omitted.

Colour of the gonads was defined for most of the maturity stages. It was also pointed out when the gonads are transparent.

Additional factors specific for each stage (E.g. shape of the gonad, development of blood vessels or flexibility) have also been taken into account.

The absence or presence of reproductive products (eggs /milt) was noted, and if present the characteristics were described.

For spawning females, the most important conclusion was that even one hydrated egg present in the gonad classifies an individual as stage 3.

It was first stated in our sub-group discussion, that there might be difficult in macroscopic classification of gonads in between stage 4 (Spent) and stage 5 (Resting/Skipped spawning). Similar doubts might appear between stage 5 (Resting/Skipped spawning) and stage 2 (Maturing). Microscopically analysis (fresh smears) is recommended to solve this uncertainty.

However, when summing up the sub-group discussions in plenary, it was stated that WKMSSPDF 2012 will recommend that stage 5 only should be applied to skipped spawners and used only to identify female skipped spawners close to the spawning season (for more details see report section 4).

Last, the collection of the reference pictures for maturity stages of flounder in the report of WKMSSPDF 2010 has been enlarged. Photos of stages that were missing have been added and a few of the previous photos have been replaced by pictures that illustrate each maturity stage more precisely. We also decided to keep some of the previous photos. If helpful and photos were available, both the early and late phases of a maturity stage are illustrated.

Annex 7: Fish details per calibration exercise

Sex ratio by species

Staging exercise	Sex	Sole	Plaice	Dab	Flounder
(1) fresh	female	25	21	25	13
(1) fresh	male		4		12
(2) picture	female	27	20	19	33
(2) picture	male	9	13	7	30
(3) picture	female	14	21	11	18
(3) picture	male	4	3	4	14

Length frequency by species

Staging exercise	cm class	Dab	Flounder	Sole	Plaice
(1) fresh	20	1			
(1) fresh	21	1			
(1) fresh	22	2	1		
(1) fresh	23	3	3		
(1) fresh	24	2		1	
(1) fresh	25	6		1	
(1) fresh	26	1	3	2	
(1) fresh	27	3	1	3	2
(1) fresh	28	2		1	1
(1) fresh	29	3	1	3	1
(1) fresh	30		2	2	
(1) fresh	31		2	1	2
(1) fresh	32		3	2	1
(1) fresh	33	1	1		2
(1) fresh	34		3		2
(1) fresh	35		1	1	2
(1) fresh	36		1	2	2
(1) fresh	37		1	1	2
(1) fresh	38			2	1
(1) fresh	39		2	2	1
(1) fresh	40				1
(1) fresh	41				2
(1) fresh	42			1	1
(1) fresh	44				2
(2) picture	8				1
(2) picture	10				1
(2) picture	11				1
(2) picture	12		2		
(2) picture	15				1
(2) picture	17		1		
(2) picture	20	1		2	

(2) picture	21	1	1		
(2) picture	22	2	3		
(2) picture	23	2	1		
(2) picture	24	6	2		
(2) picture	25	3	5	3	
(2) picture	26	3	6	3	
(2) picture	27		6	4	1
(2) picture	28		3	3	4
(2) picture	29		4	2	
(2) picture	30	2	5		
(2) picture	31	3	6	1	1
(2) picture	32	1	6		3
(2) picture	33	2	3	1	1
(2) picture	34			1	3
(2) picture	35		2		3
(2) picture	36		1		2
(2) picture	37		1	1	1
(2) picture	38		1	3	1
(2) picture	39		2	1	2
(2) picture	40			2	1
(2) picture	41		2	3	
(2) picture	42			4	
(2) picture	43			1	2
(2) picture	44				2
(2) picture	45				1
(2) picture	47			1	
(2) picture	50				1
<hr/>					
(3) picture	17		1		
(3) picture	18				1
(3) picture	20	1	1		
(3) picture	21		2		
(3) picture	22		1		
(3) picture	23	1			
(3) picture	24	1		1	
(3) picture	25	2	3	1	
(3) picture	26	3	4	1	1
(3) picture	27		1		1
(3) picture	28		3	3	1
(3) picture	29		1	3	2
(3) picture	30	3	4		
(3) picture	31	2	3		
(3) picture	32	1	3		1
(3) picture	33	1	1		2
(3) picture	34		2	1	
(3) picture	35			1	1
(3) picture	36		1		4

(3) picture	37	2	1
(3) picture	38	2	1
(3) picture	40	1	
(3) picture	41	1	1
(3) picture	42	1	1
(3) picture	44	1	
(3) picture	45		2
(3) picture	47		2
(3) picture	49		1
(3) picture	52		1

Annex 8: Reference pictures fresh staging, macroscopic and micro- scopic

Dab

macroscopic

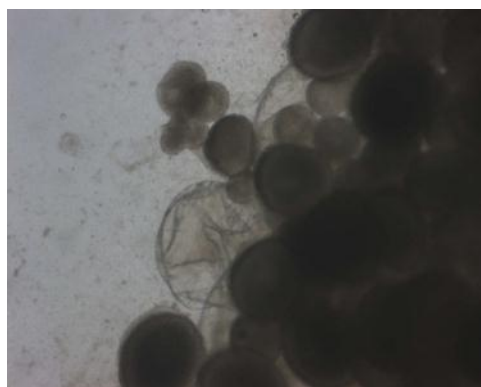
microscopic

agreed stage 2



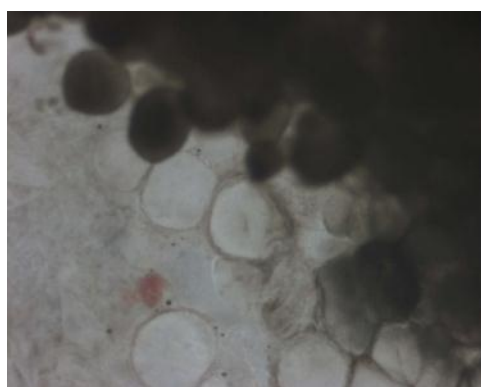
stage 2

agreed stage 3



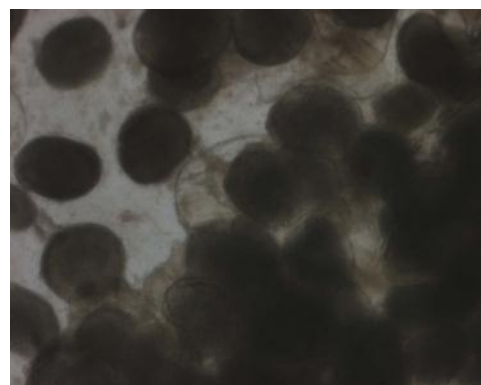
stage 3

agreed stage 3



stage 3

agreed stage 3



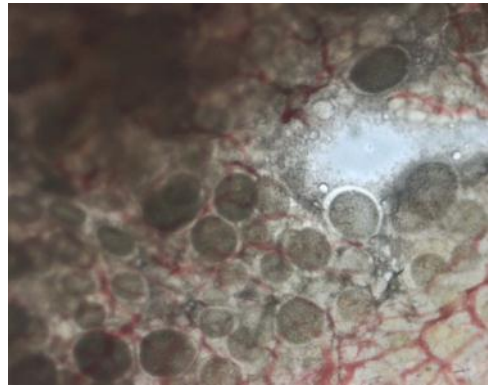
stage 3

Sole

agreed stage 2



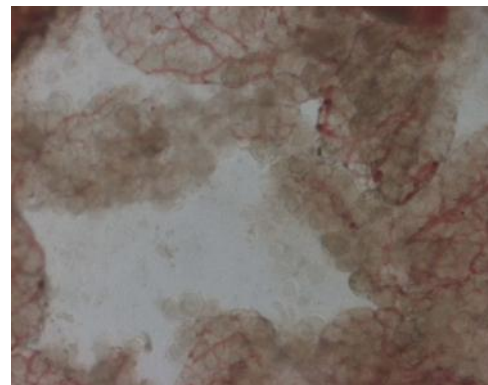
stage 2



agreed stage 5



stage 5



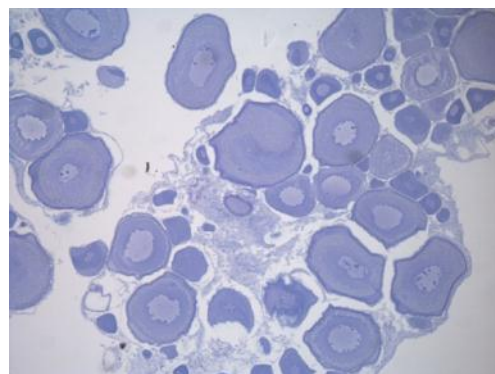
Annex 9: Reference pictures staging from pictures, macroscopic and histological

Second staging exercise (pictures)

macroscopic picture

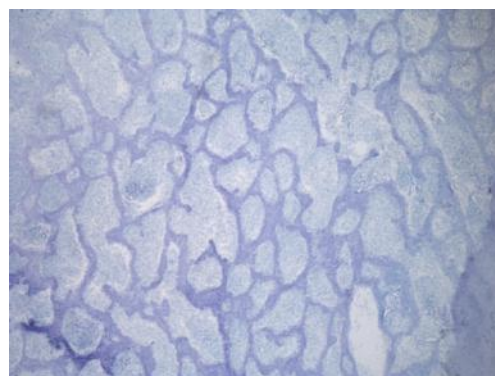
histological picture

modal stage 4



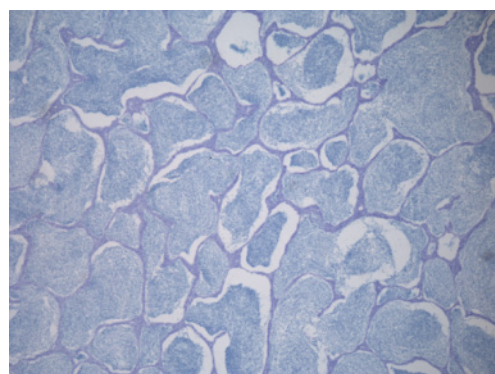
stage 2

modal stage 2



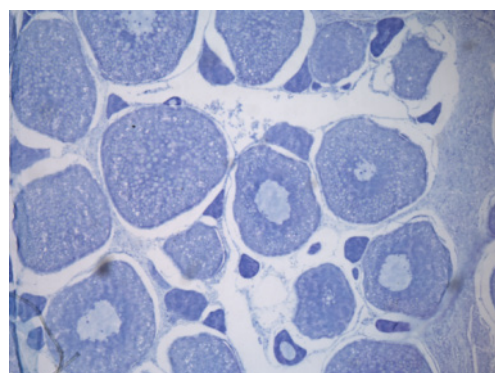
stage 2

modal stage 2



stage 2

modal stage 2



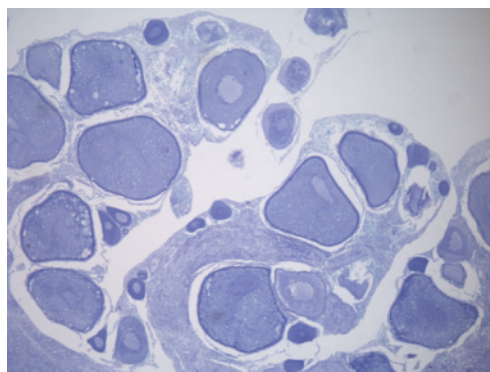
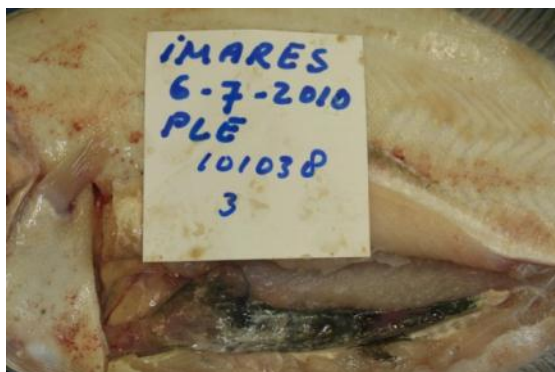
stage 2

Third staging exercise

macroscopic picture

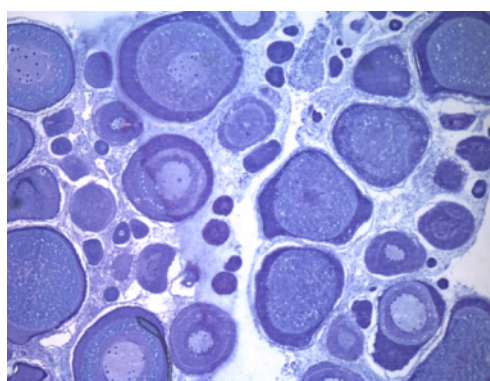
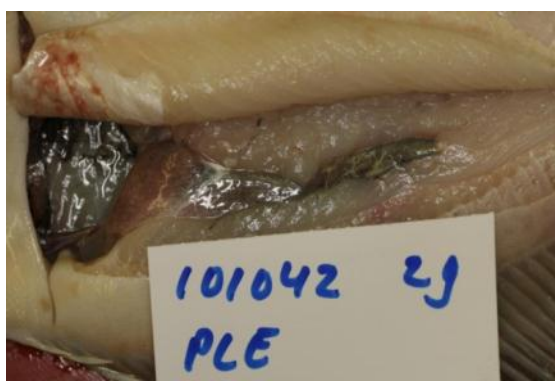
histological picture

modal stage 5



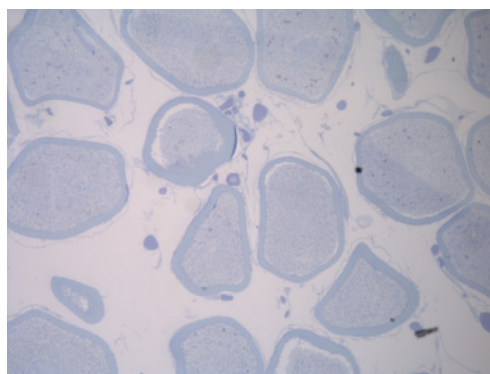
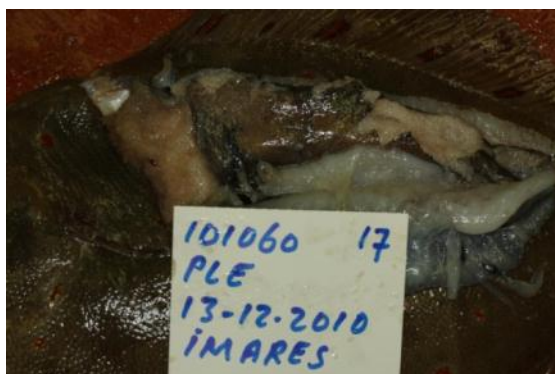
stage 2

modal stage 2



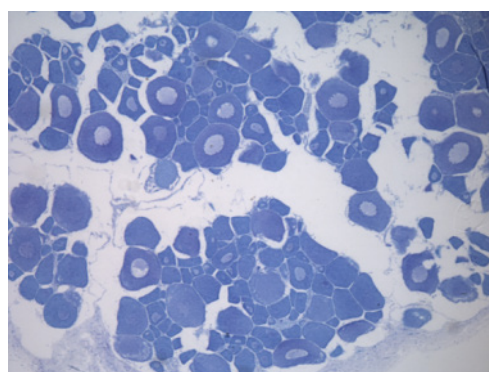
stage 2

modal stage 3



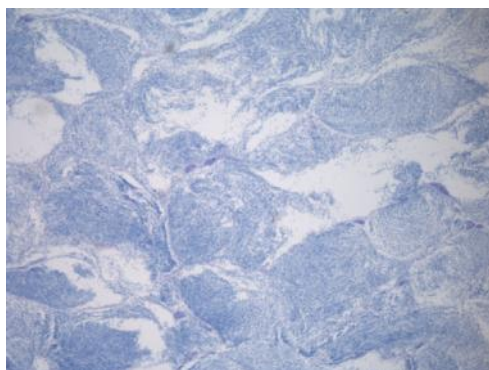
stage 2

modal stage 5



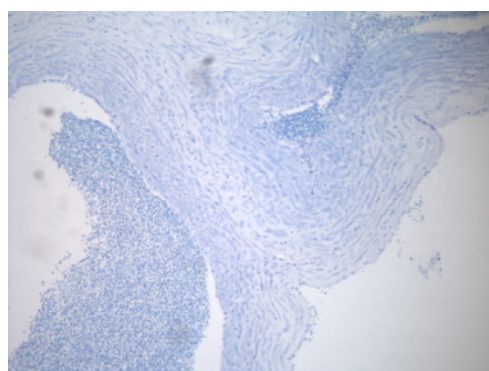
stage 4

modal stage 2



stage 3

modal stage 2



stage 2

Annex 10 – Working Documents (Separate)

1. Working Document 1: Protocol Data collection WKMSSPDF 2012 (separate)

2. Working Document 2: Reference Documents Maturity Stages of Dab, Flounder, Plaice and Sole (separate)
