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Report of the Workshop on Sexual Maturity Staging of Turbot and Brill (WKMSTB 2012)

5–9 March 2012

IJmuiden, Netherlands

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

WKMSTB met 5–8 March 2012 in IJmuiden, The Netherlands. Seven participants from three countries participated in the meeting. The meeting aimed to establish a common agreed maturity scale for turbot and brill and calibrate the maturity staging using the new proposed scale.

New proposed maturity scales

Currently, for turbot and brill maturity staging Poland uses the Maier scale and the UK (England) uses the CEFAS scale. The Netherlands has moved to the six point scale as proposed by WKMSSPDF.

WKMSTB proposes to adopt the six point scale as proposed by the previous ICES maturity staging workshops. The refined maturity staging scales from WKMSSPDF 2012 (ICES, 2012) were used as a basis for the descriptions of the maturity stages for turbot and brill.

During the calibration exercises and discussions it became apparent that the maturity stages for turbot and brill are very similar, so one common scale for both species is proposed.

It should be clear that the diagram with the maturity stage descriptions is only relevant from two months prior to the spawning season until the end of spawning. After spawning, a transition of the gonads, which is not described in the diagram, takes place. It is however possible that during a survey in the spawning season specimens are found that have spawned recently and are spent.

Female stage 5: 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.

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.

Staging exercises

Three staging exercises were carried out; one using fresh fish and two using pictures. The percentage agreement in the fresh staging was higher than the percentage 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. Percentage agreement in the fresh staging was 94% for both turbot and brill. Agreement in the second exercise from pictures was 79% for turbot and 73% for brill. In the last calibration exercise from pictures the agreement increased to 81% for both species.

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 does not support this feeling. There is, however, 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. After the exercises all fish and pictures were discussed in plenary and macroscopic staging was validated with microscopic smears or histological sections. The data reported in this report is based on the macroscopic maturity stage and is not corrected in case the microscopic analysis proved the staging was incorrect.

WKMSTB recommends that in future workshops, it should be decided whether 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 whether to base the modal stage on all participants or only the modal of the expert stagers.

Next meeting

It was recommended that it is not necessary to organise another workshop on turbot and brill in due time. Before organising another maturity staging workshop WKMSTB recommends to organise a WebGR calibration exercise. Based on the results of this exercise it should then be decided if it is necessary to organise a maturity staging workshop. It might also be worth considering combining turbot and brill in a joint workshop with other flatfish species.

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

The Workshop on maturity staging of turbot and brill (WKMSTB) met 5–8 March 2012 in IJmuiden, The Netherlands. Seven participants from three countries joined the meeting, of which one was by correspondence. The participant list is in Annex 1.

The terms of reference for the meeting were:

a) Agree on a common maturity scale for turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) across laboratories comprising a comparison of existing scales and standardization of maturity determination criteria;

b) Calibrate staging of turbot and brill using fresh fish, following the pattern of trial-discussion-retrial;

c) Calibrate staging of turbot and brill using photographs, following the pattern of trial-discussion-retrial;

d) Validate macroscopic maturity determination with histological analysis;

e) Establish correspondence between old and new scales to convert time-series;

f) Propose optimal sampling strategy to estimate accurate maturity ogives;

g) Address the generic ToRs adopted for maturity staging workshops (see 'PGCCDBS Guidelines for Workshops on Maturity Staging').

WKMSTB will report by 4 April 2012 for the attention of ACOM and PGCCDBS.

2 Adoption of the agenda

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

3 Agree on a common maturity scale for turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) across laboratories comprising a comparison of existing scales and standardization of maturity determination criteria (ToR a)

3.1 Agree on a common maturity scale for turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) across laboratories comprising a comparison of existing scales

The WKMAT 2007 (ICES, 2007) proposed a five point maturity scale. Afterwards, the WKMSCWHS 2007 (ICES, 2008) proposed to add an extra scale for skipped spawning and one for abnormal gonads. WKMSSPDF 2010 (ICES, 2012) proposed to adopt the six point scale as proposed by the gadoid workshop, which was refined during WKMSSPDF 2012 (ICES, 2012). As all participants also joined WKMSSPDF 2012, the benefits of a common scale were clear to everyone, and so, for turbot and brill the six point scale was proposed.

For the staging exercises during WKMSTB 2012, the proposed scale from WKMSSPDF 2012 was used. During the workshop species-specific topics were discussed, which are included in the stage descriptions per species (Section 3.2).

Table 3.1.1. Currently used systems of maturity staging of turbot and brill.

Country	Current situation
Netherlands	6-point scale as proposed by WKMSSPDF 2010 (ICES, 2010).
Poland	Use the Maier scale.
United Kingdom (England)	Use CEFAS maturity staging code, which can be translated to new stages for DATRAS.

3.2 Standardization of maturity determination criteria

Separate documents by species containing the maturity stage diagrams as well as reference pictures are available. The descriptions of the maturity stages of other flatfish species as presented during WKMSSPDF (ICES, 2012) were used as a starting point.

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.

When discussing the maturity stage descriptions all participants agreed that the development and maturity stage descriptions for both turbot and brill are similar. A report on the stage description discussion can be found in Annex 6.

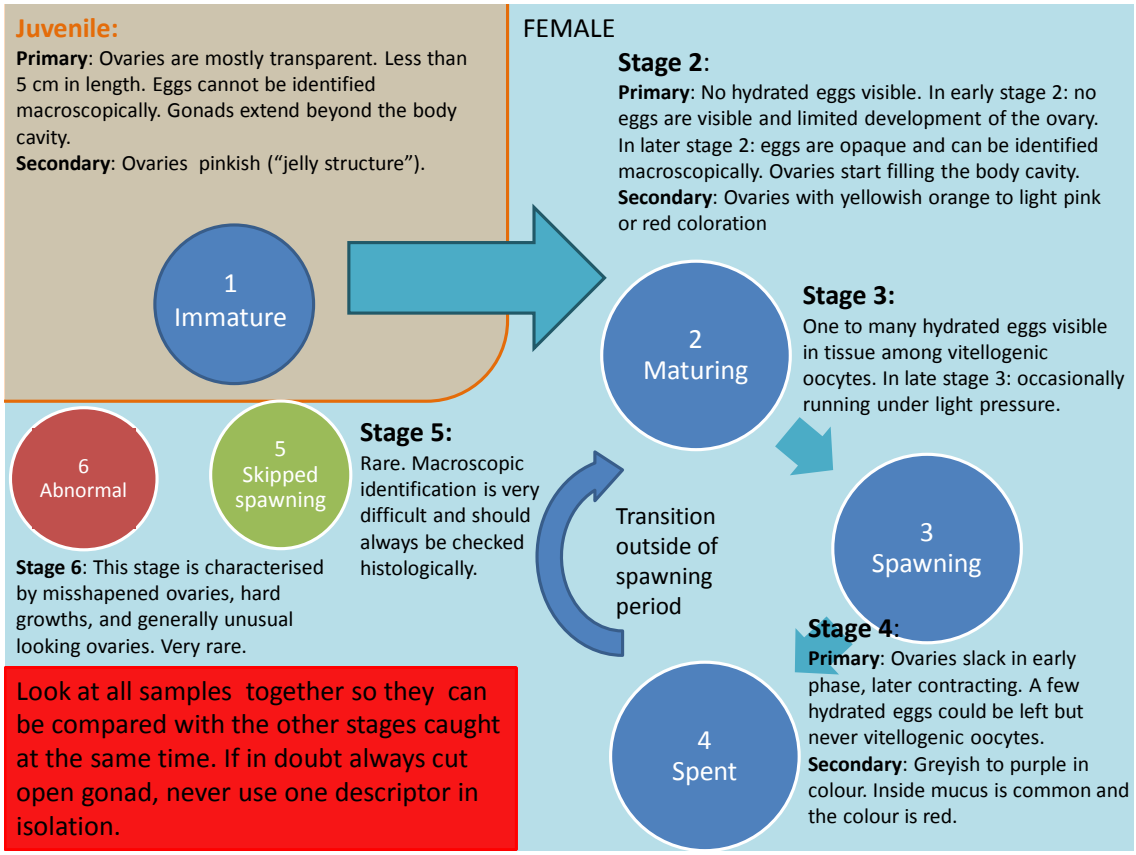

Figure 3.2.1. Maturity stages turbot and brill female.

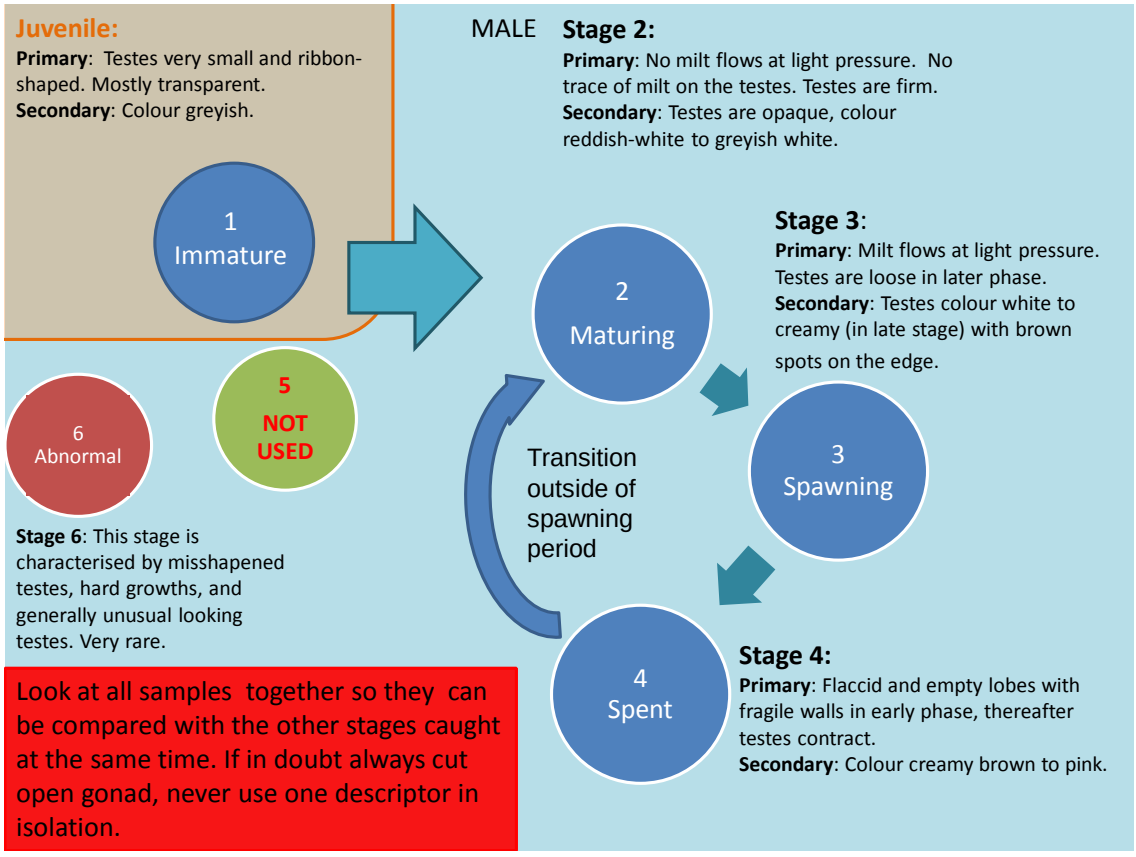

Figure 3.2.2. Maturity stages turbot and brill male.

4 Establish correspondence between old and new scales to convert time-series (ToR e)

The maturity scales as proposed by WKMSSPDF in 2010 (and refined by WKMSSPDF 2012) were used as a starting point. It was decided to also use the 6-point scale for turbot and brill.

Table 4.1 shows the current state regarding the use of maturity stages for turbot and brill, and the problems arising when changing to the 6-point scale.

Table 4.1. Currently used systems of maturity staging, and difficulties arising when changing to 6-point scale following WKMSSPDF 2010 (ICES, 2010) and WKMSSPDF 2012 (ICES, 2012).

Country	Current situation	Problems arising
Netherlands	6-point scale as proposed by WKMSSPDF 2010 (ICES, 2010).	
Poland	Use the Maier scale, which can be translated 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. It should be noted that Maier stage II converts to stage 2 in the new proposed scale.
United Kingdom (England)	Use CEFAS maturity staging code, which can be translated to new stages for DATRAS.	The current CEFAS staging includes a hyaline stage and the desription of this does not translate to the new stage 3.

For the Polish samples, the same problem as discussed in WKMSSPDF 2012 (ICES, 2012) arises, 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 time-series 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 WKMSTB 2012 participants inform their national colleagues involved in WGBIFS about the current maturity stages and about the WKMSTB 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.

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 turbot and brill. The fish were bought on the 2nd of March 2012 and kept on ice until the fresh staging on the 5th of March. 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.

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

Sex ratio and length frequencies per species are given in Annex 5. 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 (see Annex 4 and webgr.azti.es). All participants entered their original staging results from the fresh staging into WebGR.

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 (2012)):

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.

5.2.1 Turbot

Turbot in the fresh staging were both male and female. Males were all stage 2 while females were stage 1 and 2 (Table 5.2.1a). Overall agreement on turbot was 94% (Table 5.2.1b). Discussion occurred between stage 2 and 5 and stage 1 and 5 (Table 5.2.1b and c). This was probably due to the fact that turbot in the Baltic Sea are smaller compared to North Sea turbot, and thus Baltic turbot mature at smaller sizes.

Table 5.2.1a. The number of stagings by stage for turbot.

stage	Expert	Expert	Expert	Expert	
1	-	2	2	2	6
2	21	21	23	23	88
3	-	-	-	-	-
4	-	-	-	-	-
5	4	2	-	-	6
6	-	-	-	-	-
1–6	25	25	25	25	100

Table 5.2.1b. Stage compositions by stage and reader for all stage readers for turbot. A weighted mean percentage agreement is given by stage reader in relation to the agreed stage, and for all stage readers combined.

stage	Expert	Expert	Expert	Expert	ALL
1	0%	100%	100%	100%	60.0%
2	91%	91%	100%	100%	76.5%
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
1–6	84.0%	92.0%	100.0%	100.0%	94.0%

Table 5.2.1c. Bias in the comparison for turbot. 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.

stage	Expert	Expert	Expert	Expert	ALL
1	4.00	0.00	0.00	0.00	0.6
2	0.26	0.26	0.00	0.00	-0.29565
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-

For turbot 10 it was unclear whether this female was in stage 2 or 5. Under the microscope it was clear that the oocytes were small but clearly developing (Figure 5.2.1.1) and this female was stage 2 maturing.

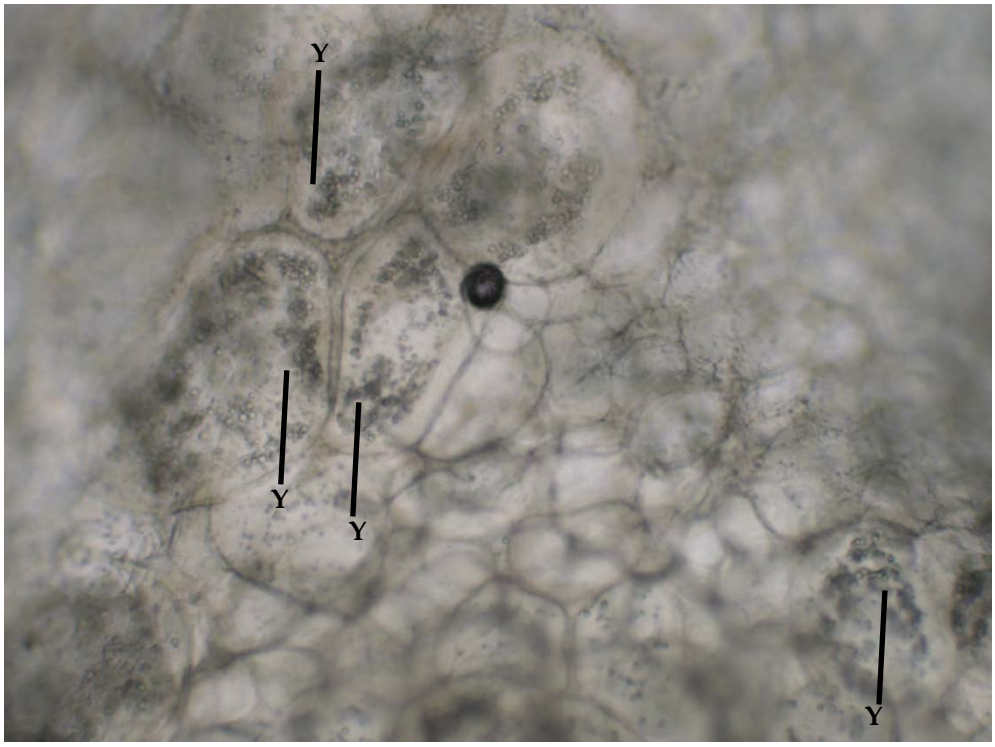

Figure 5.2.1.1. Developing oocytes in the vitellogenic stage in turbot 10 (Y: developing yolk).

For female turbot 15 the discussion was also between stage 2 and 5. The smear under the microscope showed developing oocytes and this female was in stage 2 (Figure 5.2.1.2).

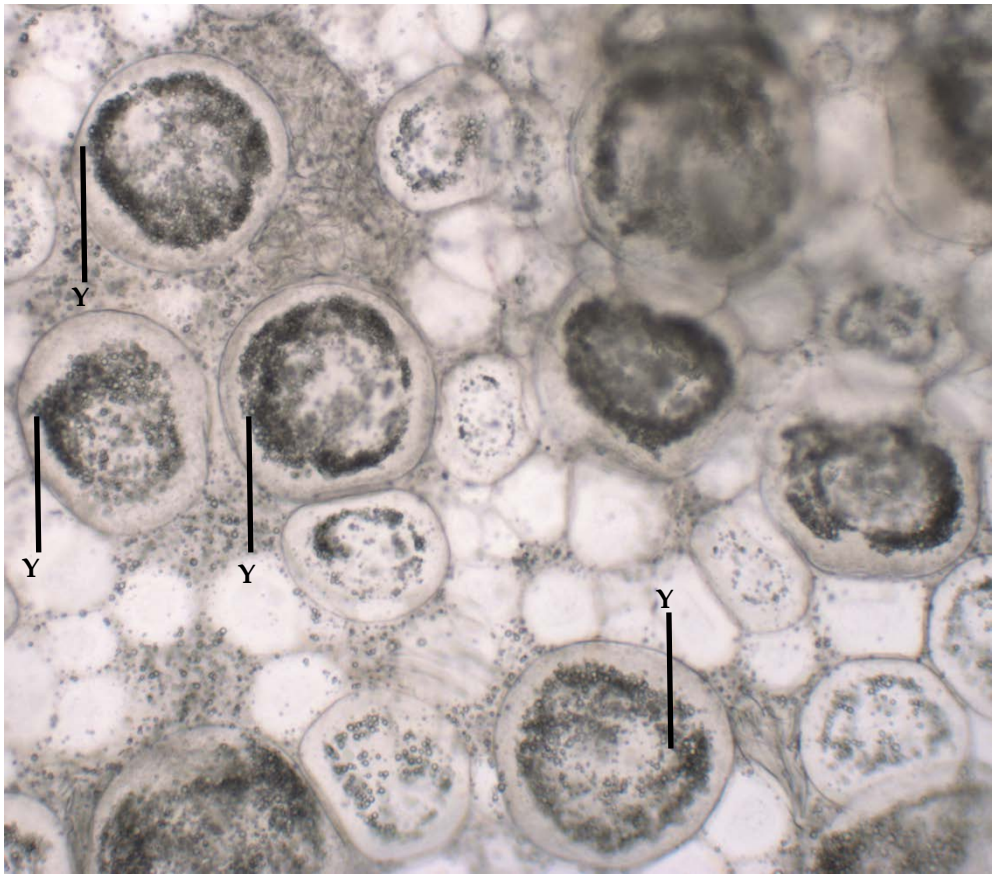

Figure 5.2.1.2. Developing oocytes in the vitellogenic stage in turbot 15 (Y: developing yolk).

The discussion on female turbot 22 was between stage 1 and 5. Under the microscope it was clear that all oocytes in the ovary were small and did not show any development (Figure 5.2.1.3). This female is juvenile, stage 1.

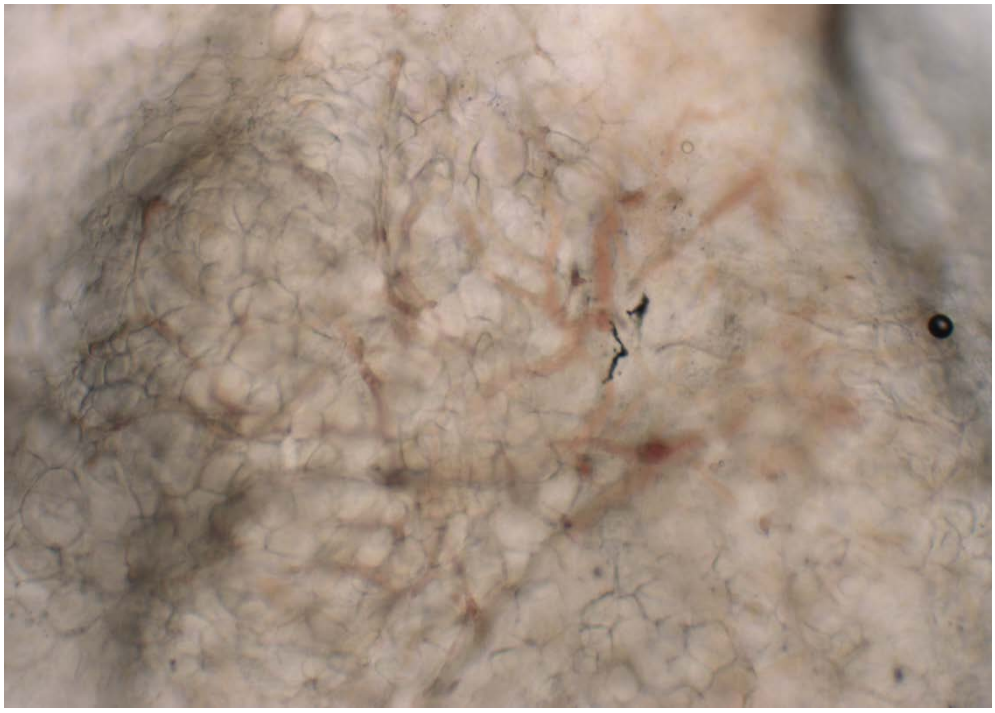

Figure 5.2.1.3. Undeveloped oocytes in female turbot 22.

In female turbot 23 the discussion focussed on stage 1 and 5. The smear showed small and undeveloped oocytes (Figure 5.2.1.4) and this female was a juvenile, stage 1.

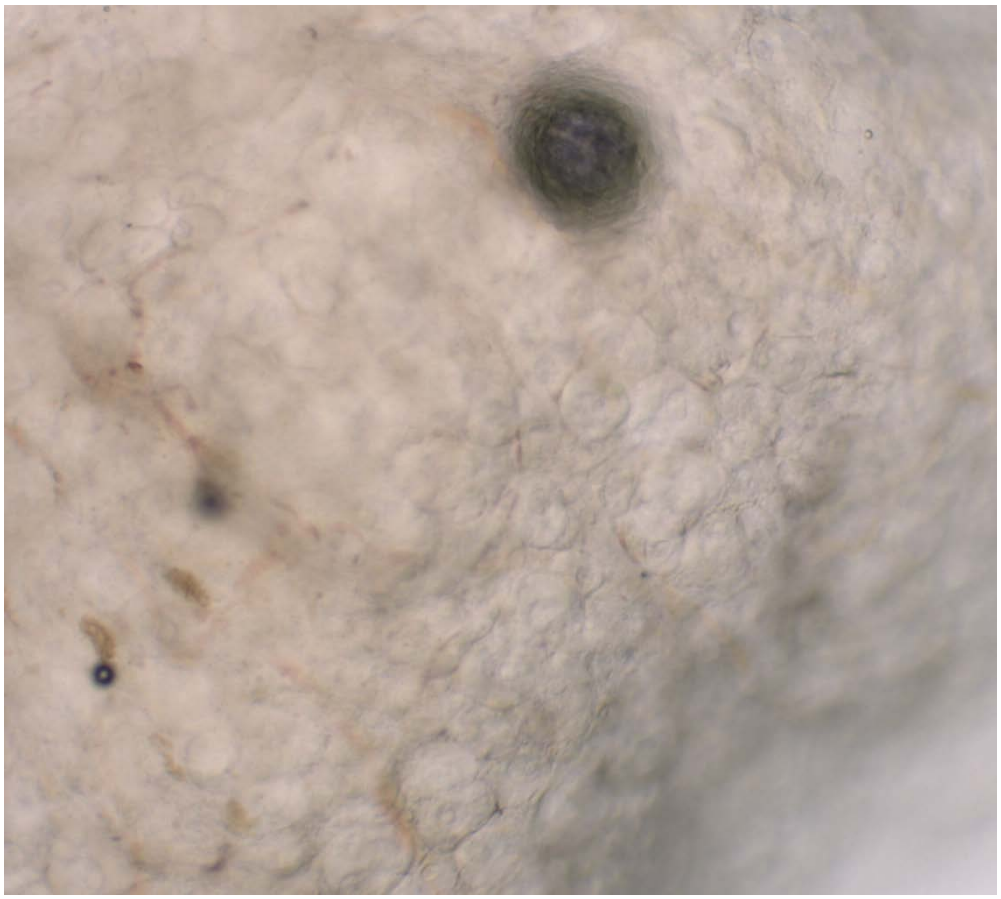

Figure 5.2.1.4. Small and undeveloped oocytes in female turbot 23.

5.2.2 Brill

Both female and male brill were available for the fresh staging exercise. Males were all stage 2 while females were stage in 2 and 3 (Table 5.2.2a). Overall agreement in brill was 94% (Table 5.2.2b). Discussion occurred between stage 2 and 3 and 2 and 5 (Table 5.2.2b and c). Brill is very rare in the Baltic. Like turbot, brill in the Baltic Sea are smaller compared to the North Sea, and thus Baltic brill mature at smaller sizes.

Table 5.2.2a. The number of stagings by stage for brill.

stage	Trainee	Trainee	Expert	Expert	TOTAL
1	-	-	-	-	-
2	20	20	22	22	84
3	3	2	3	3	11
4	-	-	-	-	-
5	2	3	-	-	5
6	-	-	-	-	-
1–6	25	25	25	25	100

Table 5.2.2b. Stage compositions by stage and reader for all stage readers for brill. A weighted mean percentage agreement is given by stage reader in relation to the agreed stage, and for all stage readers combined.

stage	Trainee	Trainee	Expert	Expert	ALL
1	-	-	-	-	-
2	91%	86%	100%	100%	75.5%
3	100%	67%	100%	100%	73.3%
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
1–6	92.0%	84.0%	100.0%	100.0%	94.0%

Table 5.2.2c. Bias in the comparison for brill. 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.

stage	Trainee	Trainee	Expert	Expert	ALL
1	-	-	-	-	-
2	0.27	0.41	0.00	0.00	-0.26364
3	0.00	-0.33	0.00	0.00	-0.66667
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-

For brill 6 the discussion was between stage 2 and 3. The coloration of this female gonad was somewhat different compared to the other stage 2 females and participants were unsure of hydrated oocytes were present. The smear under the microscope showed developing oocytes but no hydrated oocytes (Figure 5.2.2.1). Female brill 6 is in maturity stage 2.

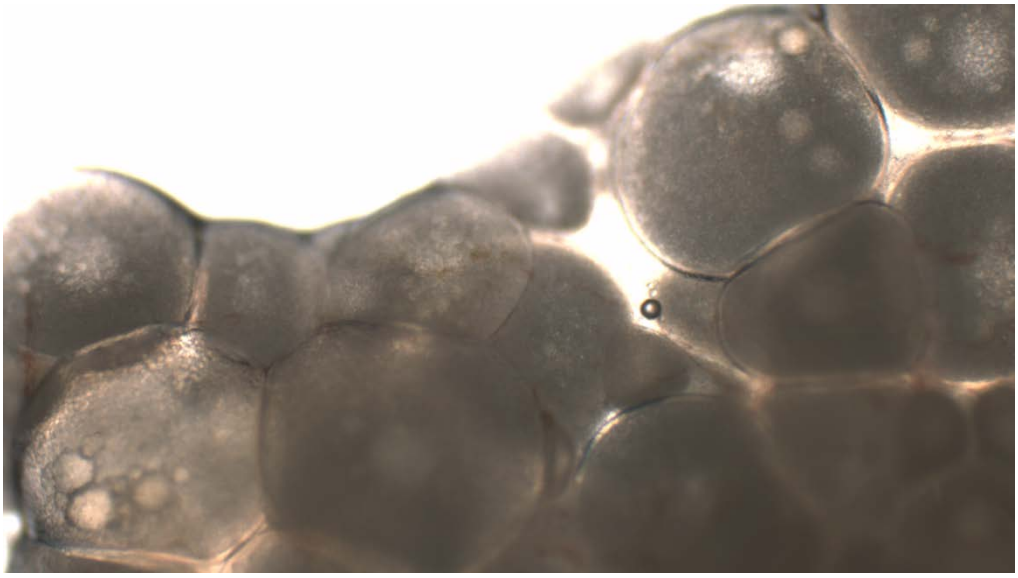

Figure 5.2.2.1. Developing oocytes in female brill 6.

It was unclear if female brill 20 maturing, stage 2 or stage 5. The smear showed that the oocytes were small but clearly developing (Figure 5.2.2.2). Brill 20 is maturing, stage 2.

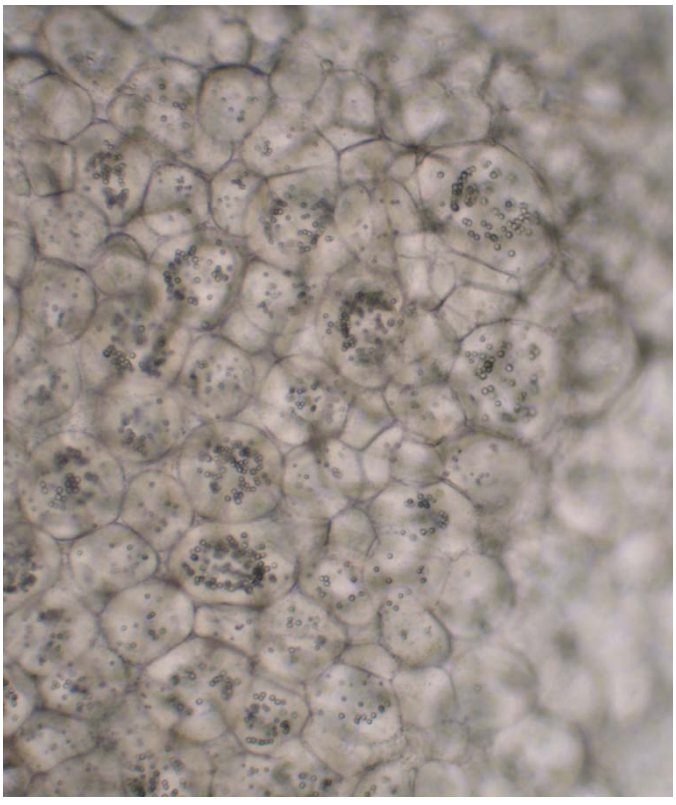

Figure 5.2.2.2. Developing oocytes in female brill 20.

For brill 21, a similar discussion as for brill 20 took place. It was unclear whether the female was in stage 2 or 5. Under the microscope the oocytes all appeared to be developing (Figure 5.2.2.3), meaning brill 21 is a maturing female, stage 2.

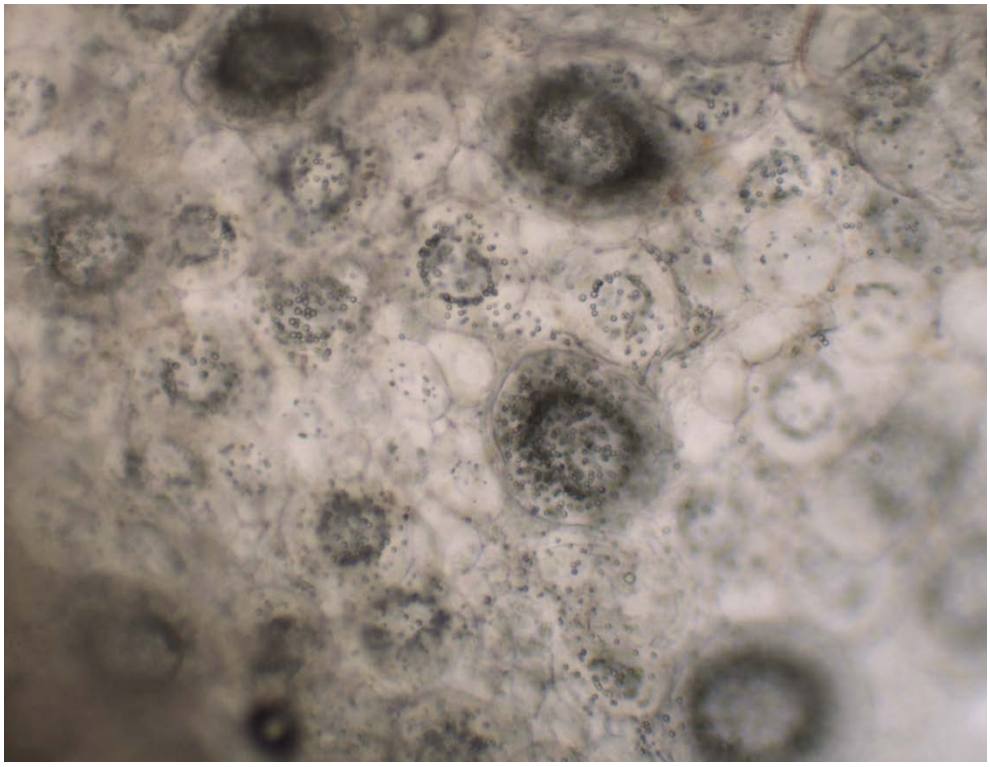

Figure 5.2.2.3. Developing oocytes in brill 21.

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. 68%, max. 87%; third round: min. 59%, max. 97%) were lower compared to the fresh fish staging (min. 84%, max. 100%). Overall agreement increased from the second to the third staging round.

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

The EU directive requires that sampling of turbot and brill is less intensive compared to the WKMSSPDF flatfish species (ICES, 2012), hence the pictures for the calibration exercises could not be taken throughout the year. Pictures from February, March, May, August and November were available for both species.

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 does not support this (Figure 6.1, Table 6.1). There is, however, significantly higher agreement on the sexual maturity stage of fish just prior to and in the spawning season (October–April) compared to outside the spawning season (Figure 6.1, Table 6.1). This supports the idea that macroscopic maturity staging is a reliable method only in the period from two months before the start of the spawning season until the end of spawning.

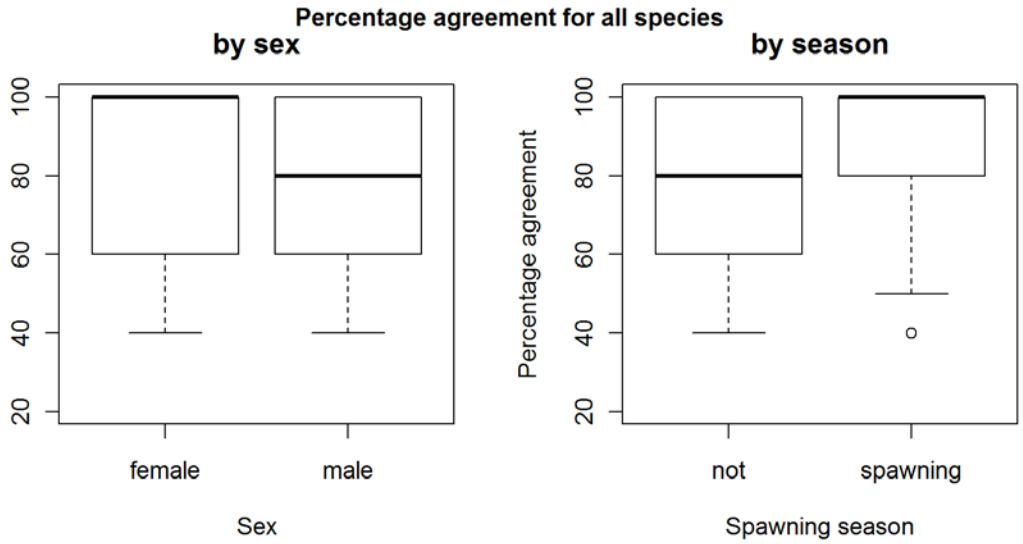

Figure 6.1. Percentage agreement by sex (left) and by season (right), for all species in all calibration exercises from WKMSTB 2012.

Table 6.1. Results independent 2-group T-test.

	BY SEX	BY SEASON
n. observations	182	171
T	0.36	-4.41
Df	133	143
p	0.72	<0.001

6.1 First macroscopic picture staging

For the first staging exercise from pictures, 35 turbot and 32 brill were used. All participants staged both species.

6.1.1 Turbot

Maturity stages in turbot varied from 1 to 4 (Table 6.1.1a), and agreement between participants was 79% (Table 6.1.1b). Like in the fresh staging problems occurred deciding between stages 2 and 4–5, but also between stages 2–3 (Table 6.1.1b and c).

After the staging, all pictures and the available histological pictures were projected and discussed. During the discussion the problem of translating the Maier scale stage 5 to the new proposed scale stage 2 or 4 became apparent. Participants normally using the Maier scale staged fish as 5, while the microscopic validation showed all these fish were stage 2 or stage 4. Some fish were staged 4 while these were already showing early development and were preparing for the next spawning season. Again, it appeared to be difficult to assess the early gonadal development macroscopically and so, macroscopic maturity staging can only be reliably carried out just prior to (approximately two months) and during spawning. The identification of hydrated eggs from pictures was difficult for all participants, resulting in problems distinguishing stages 2 and 3. The fish with 100% agreement on stage 3 showed microscopically hydrated eggs, while the fish were participants disagreed between stage 2 and 3 did not show hydrated eggs microscopically.

Table 6.1.1a. The number of stagings by stage for turbot.

stage	Expert	Expert	Expert	Expert	Expert	TOTAL
1	-	1	6	4	6	17
2	17	13	17	20	17	84
3	4	6	6	6	2	24
4	8	8	8	7	11	42
5	8	8	-	-	-	16
6	-	-	-	-	-	-
1–6	37	36	37	37	36	183

Table 6.1.1b. Stage compositions by stage and reader for all stage readers for turbot. A weighted mean percentage agreement is given by stage reader in relation to the modal stage, and for all stage readers combined.

stage	Expert	Expert	Expert	Expert	Expert	ALL
1	0%	33%	100%	100%	100.0%	68.4%
2	88%	76%	88%	94%	82.4%	85.9%
3	67%	100%	67%	83%	20.0%	69.0%
4	75%	88%	100%	88%	87.5%	87.5%
5	100%	100%	0%	0%	0.0%	40.0%
6	-	-	-	-	-	-
1–6	73.0%	80.6%	83.8%	86.5%	72.2%	79.2%

Table 6.1.1c. Bias in the comparison for turbot. 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.

stage	Expert	Expert	Expert	Expert	Expert	ALL
1	3.75	2.67	0.00	0.00	0	1.210526
2	0.29	0.65	0.12	0.06	0.294118	0.282353
3	-0.33	0.00	-0.33	-0.17	0	-0.17241
4	0.25	0.13	0.00	-0.25	-0.25	-0.025
5	0.00	0.00	-4.00	-3.00	-4	-2.2
6	-	-	-	-	-	-

6.1.2 Brill

Maturity stages in brill varied from 1 to 4 (Table 6.1.2a), and agreement between participants was 73% (Table 6.1.2b). Problems occurred deciding between stages 2–3 and 2–4, and also between stages 1 and 2–4 (Table 6.1.2b and c). Unlike in turbot, for brill less problems occurred with respect to the Maier scale translation.

Again, the identification of hydrated eggs from pictures was difficult for all participants, resulting in problems distinguishing stages 2 and 3. Additionally, the early gonadal development is often impossible to identify macroscopically, resulting in problems distinguishing between stage 1 and 2 or 2 and 4.

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

stage	Trainee	Trainee	Expert	Expert	Expert	TOTAL
1	6	1	4	4	3	18
2	19	17	7	8	15	66
3	5	6	8	15	7	41
4	2	7	12	5	5	31
5	-	1	-	-	1	2
6	-	-	-	-	-	-
1–6	32	32	31	32	31	158

Table 6.1.2b. Stage compositions by stage and reader for all stage readers for brill. A weighted mean percentage agreement is given by stage reader in relation to the modal stage, and for all stage readers combined.

stage	Trainee	Trainee	Expert	Expert	Expert	ALL
1	75%	25%	75%	75%	75.0%	65.0%
2	87%	87%	47%	53%	80.0%	70.7%
3	63%	75%	100%	100%	85.7%	84.2%
4	40%	80%	80%	80%	80.0%	72.0%
5	-	-	-	-	-	-
6	-	-	-	-	-	-
1–6	71.9%	75.0%	67.7%	71.9%	80.6%	73.4%

Table 6.1.2c. Bias in the comparison for brill. 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.

stage	Trainee	Trainee	Expert	Expert	Expert	ALL
1	0.25	2.00	0.75	0.75	0.25	0.8
2	-0.13	0.27	1.00	0.47	0.4	0.4
3	-0.38	-0.25	0.00	0.00	-0.14286	-0.15789
4	-1.40	-0.40	-0.60	-0.60	-0.4	-0.68
5	-	-	-	-	-	-
6	-	-	-	-	-	-

6.2 Second macroscopic picture staging

For the second staging exercise from pictures, 35 turbot and 32 brill were used. All participants staged both species.

6.2.1 Turbot

Maturity stages in turbot in the third calibration varied from 1 to 5, though not all participants identified the one stage 5 female (Table 6.2.1a). Agreement between participants increased to 81% (Table 6.2.1b). As in the fresh staging problems occurred deciding between stages 2 and 4, but also between stages 2–3 (Table 6.2.1b and c). The translation of the Maier stage 5 proved not to be a problem during this calibration exercise.

Participants agreed that in case of doubt between stage 4 and early stage 2gonads should always be cut open to check for developing vitellogenic oocytes in the ovary. During the discussion and validation with histological pictures it became apparent that for the female stage 5 every participant doubted and thought it was a strange gonad. However, no one staged it as 5.

Table 6.2.1a. The number of stagings by stage for turbot.

stage	Expert	Expert	Expert	Expert	Expert	TOTAL
1	3	3	1	2	1	10
2	26	24	23	23	15	111
3	3	2	6	3	7	21
4	2	5	2	6	10	25
5	-	-	1	-	1	2
6	-	-	-	-	-	-
1–6	34	34	33	34	34	169

Table 6.2.1b. Stage compositions by stage and reader for all stage readers for turbot. A weighted mean percentage agreement is given by stage reader in relation to the modal stage, and for all stage readers combined.

stage	Expert	Expert	Expert	Expert	Expert	ALL
1	100%	100%	33%	67%	33.3%	66.7%
2	100%	100%	86%	87%	56.5%	86.0%
3	100%	67%	100%	33%	33.3%	66.7%
4	40%	100%	40%	100%	100.0%	76.0%
5	-	-	-	-	-	-
6	-	-	-	-	-	-
1–6	91.2%	97.1%	75.8%	82.4%	58.8%	81.1%

Table 6.2.1c. Bias in the comparison for turbot. 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.

stage	Expert	Expert	Expert	Expert	Expert	ALL
1	0.00	0.00	0.67	0.33	2	0.6
2	0.00	0.00	0.23	0.17	0.652174	0.210526
3	0.00	-0.33	0.00	-0.67	-0.66667	-0.33333
4	-1.20	0.00	-1.00	0.00	0	-0.44
5	-	-	-	-	-	-
6	-	-	-	-	-	-

6.2.2 Brill

Maturity stages in brill varied from 1 to 5 and like in turbot participants did not identify the stage 5 (Table 6.2.2a) but thought it was a strange gonad. Agreement between participants increased in brill as well to 81% (Table 6.2.2b). Problems occurred deciding between stages 2–3 and 2–4 (Table 6.2.2b and c).

Table 6.2.2a. The number of stagings by stage for brill.

Stage	Expert	Expert	Expert	Expert	Expert	TOTAL
1	12	8	6	7	4	37
2	14	15	13	11	7	60
3	6	8	7	9	11	41
4	-	1	6	4	10	21
5	-	-	-	-	-	-
6	-	-	-	-	-	-
1–6	32	32	32	31	32	159

Table 6.2.2b. Stage compositions by stage and reader for all stage readers for brill. A weighted mean percentage agreement is given by stage reader in relation to the modal stage, and for all stage readers combined.

Stage	Expert	Expert	Expert	Expert	Expert	ALL
1	100%	78%	67%	78%	44.4%	73.3%
2	92%	100%	92%	82%	50.0%	83.1%
3	75%	100%	88%	100%	100.0%	92.5%
4	0%	33%	100%	100%	100.0%	66.7%
5	-	-	-	-	-	-
6	-	-	-	-	-	-
1–6	81.3%	87.5%	84.4%	87.1%	65.6%	81.1%

Table 6.2.2c. Bias in the comparison for brill. 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.

stage	Expert	Expert	Expert	Expert	Expert	ALL
1	0.00	0.22	0.78	0.22	1.444444	0.533333
2	-0.08	0.00	0.17	0.27	0.75	0.220339
3	-0.25	0.00	-0.13	0.00	0	-0.075
4	-2.67	-1.67	0.00	0.00	0	-0.86667
5	-	-	-	-	-	-
6	-	-	-	-	-	-

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.

WKMSTB 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.

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 larger 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. For example, 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 WKMSTB 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 reabsorbed) 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 turbot and brill was available for preparing reference pictures for the different maturity stages. To these reference gonad pictures, also pictures of the smears were added (Annex 7).

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 8). 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 4. There can be added that in some cases, where the macroscopically agreement (staging from pictures) was 100%, histological sections proved that everyone was wrong. For example brill NED2011_bll_131004_002_1.jpg and brill NED2011_bll_131007_035_1.jpg were staged as 3 (100% agreement) and it turned out to be very late 2. 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 Propose optimal sampling strategy to estimate accurate maturity ogives (ToR f)

As it is only possible to reliably stage the maturity of a fish macroscopically from two months before the spawning season until the end of spawning (see also Section 7, ICES (2010) and ICES (2012)), the descriptions of the maturity stages in this report should only be used within this period. If maturity staging outside this period is required, this should be based on histological information.

As a result, optimal sampling for maturity ogives is within the defined period. This may, however, result in inaccurate information for smaller fish, as this might depend on survey information. If maturity information of smaller fish cannot be obtained within the defined period, it is recommended to take histological samples.

9 Generic ToRs adopted for maturity staging workshops (ToR g)

9.1 Staging procedure, pictures and maturity stagers forum

As WKMSTB 2012 occurred shortly after WKMSSPDF 2012, and all participants of WKMSTB 2012 joined the WKMSSPDF 2012, there are no additions to the feedback given by the first maturity staging WK. The comments on staging procedure, pictures, and the maturity stagers forum are supported by WKMSTB and can be found in ICES (2012), Section 8.

Additionally, the following comments were made:

1) It would be good if the sex of the fish is added to the picture name.

9.2 Meeting frequency

The group concludes that:

a) There is no need for another workshop in due time.

b) It is recommended that before a next maturity staging workshop a calibration exercise using WebGR is conducted. Based on the results of this calibration exercise it should be decided if a new workshop is needed.

c) It should be checked beforehand if there is any country interested in a maturity staging workshop for these two species.

d) It might be worth to consider a joint workshop with other flatfish species.

e) 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.

10 Evaluation of the use of WebGR

The advantage of a web-based tool for maturity staging workshops was shown during WKMSTB, as one participant was not able to physically join the meeting, but was available to join the calibration exercises.

All institutes that made pictures following the WKMSTB 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.

WKMSTB 2012 agrees with WKMSSPDF 2012 (ICES, 2012, Section 9) with respect to the recommendations for future developments of WebGR.

Additionally, the following comments were made:

1) It would be good to invest in an easier format to delete selected images from a calibration exercise, e.g. by clicking boxes to select the pictures that have to be deleted, and then delete them at once.

2) It would be good if all pictures of one exercise could be downloaded to your own computer. It takes a long time to open the pictures. This occurred both during the WKMSSPDF 2012 in Oostende and during this workshop, so it is probably not an internet connection problem.

3) During the WKMSTB workshop a lot of times the WebGR program froze and the calibration exercise needed to be restarted. When an exercise is restarted it starts with the first picture of the exercise. It would be a big improvement if by restarting the exercise would automatically move to the last picture that was staged.

4) The image list currently shows tiles of the pictures. It would be good if it is possible for the user to change the view of the list of pictures to be able to easier scroll through the picture list.

11 References

Anon. 2010. Protocol data collection WKMSTB 2012 (Workshop on Maturity Staging of Turbot and Brill) Version 2.0, April 2010.

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

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

Annex 1: List of participants

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Annex 2: Agenda

Monday 5 March

13.00

Logistics

Introduction of people

Introduction to the workshop: terms of reference

Adoption of the agenda

13.30

Proposed maturity scale per species:

- Do the descriptions fit per species?

Adoption of new maturity scale

Fresh fish staging

Discussion on fresh fish stages→agreed stage

16.00 Discussion on maturity scales

Creating overview of currently used maturity scales

- Per species
- Per institute

17.30 Finish

Tuesday 6 March

9.00

Proposed maturity scale per species:

- Do the currently used scales fit into the proposed scale?
- Fine-tuning of the descriptions per species in subgroups

11.00 Staging from pictures

- Starting with familiar species from own country, staging in new scale
- Staging pictures 1

12.30 Lunch

14.30 Discussion

- Exchange of experiences using the new scale
- Comparison of results macroscopic staging
- Comparison macroscopic staging to histological slides

17.30 Finish

Wednesday 7 March

9.00

Proposed maturity scale per species:

- Fine-tuning of the descriptions per species in subgroups

11.00 Staging from pictures

- Starting with familiar species from own country, staging in new scale
- Staging pictures 2

12.30 Lunch

14.30 Discussion

- Comparison of results macroscopic staging, including discussion on specific pictures
- Comparison macroscopic staging to histological slides

17.30 Finish

Thursday 8 March

9.00

Proposed maturity scale per species:

- Finalising the descriptions per species, including diagram
- Report writing

12.30 Lunch

13.30 Recommendations, next meeting? Report checking

15.00 Finish

Annex 3: 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. It is recommended 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 (see section 8 and ICES (2012))	PGCCDBS
4. WKMSTB 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. (see section 7)	Future WKMS through PGCCDBS
5. It is recommended that for future development the comments of this groups are taken into account (see section 10 and ICES (2012) for the full list)	WebGR coördinator (Azti)
6. WKMSSPDF recommends that: There is no need for another workshop in due time It is recommended that before a next maturity staging workshop a calibration exercise using WebGR is conducted. Based on the results of this calibration exercise it should be decided if a new workshop is needed. It should be checked beforehand if there is any country interested in a maturity staging workshop for these two species It might be worth to consider a joint workshop with other flatfish species 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

Annex 4: 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 5: Fish details per calibration exercise

Sex ratio by species

Staging exercise	Sex	Turbot	Brill
(1) fresh	female	14	16
(1) fresh	male	11	9
(2) picture	female	23	21
(2) picture	male	13	11
(3) picture	female	17	23
(3) picture	male	15	9

Length-frequency by species

Staging exercise	cm class	Turbot	Brill
(1) fresh	25	1	
(1) fresh	26	1	
(1) fresh	27	1	
(1) fresh	28	2	
(1) fresh	30		2
(1) fresh	31	2	2
(1) fresh	32		1
(1) fresh	33		2
(1) fresh	34		1
(1) fresh	35	1	2
(1) fresh	36	2	
(1) fresh	37	1	
(1) fresh	38		1
(1) fresh	39		1
(1) fresh	40	1	
(1) fresh	41	3	2
(1) fresh	42	1	
(1) fresh	44	1	
(1) fresh	45	2	3
(1) fresh	48		1
(1) fresh	50	1	
(1) fresh	51	1	1
(1) fresh	52		2
(1) fresh	55		1
(1) fresh	56	2	
(1) fresh	58		1
(1) fresh	60	1	1
(1) fresh	62		1
(1) fresh	65	1	
(2) picture	24		1
(2) picture	25		2
(2) picture	26	1	

Staging exercise	cm class	Turbot	Brill
(2) picture	27	2	2
(2) picture	28	2	1
(2) picture	29	4	1
(2) picture	30	1	2
(2) picture	31	2	1
(2) picture	33	1	1
(2) picture	34	1	2
(2) picture	36	2	1
(2) picture	37	2	1
(2) picture	38	2	2
(2) picture	39	1	1
(2) picture	40	1	1
(2) picture	41	1	3
(2) picture	43		2
(2) picture	44	3	
(2) picture	46	2	
(2) picture	49	1	1
(2) picture	50		1
(2) picture	51		2
(2) picture	52		2
(2) picture	54		1
(2) picture	56	1	
(2) picture	57		1
(2) picture	58	1	
(2) picture	59	2	
(2) picture	63	1	
(2) picture	64	1	
(2) picture	70	1	
(3) picture	23		1
(3) picture	24	1	2
(3) picture	25	2	1
(3) picture	26	2	
(3) picture	27	1	1
(3) picture	28	1	3
(3) picture	29	4	1
(3) picture	31	1	1
(3) picture	32	1	4
(3) picture	33		1
(3) picture	35	1	
(3) picture	36		1
(3) picture	37	2	3
(3) picture	38	2	2
(3) picture	39	2	
(3) picture	40	3	1
(3) picture	43	1	2

Staging exercise	cm class	Turbot	Brill
(3) picture	46	1	
(3) picture	47	2	
(3) picture	48		2
(3) picture	50		1
(3) picture	53		1
(3) picture	54		1
(3) picture	56		1
(3) picture	57	1	1
(3) picture	59	2	
(3) picture	60		1
(3) picture	62	1	
(3) picture	66	1	

Annex 6: Discussion on maturity stage descriptions for turbot and brill

The gonadal development of turbot and brill is very similar. Hence, the description of the different maturity stages is identical for both species.

Females

- Juveniles: ovaries not always transparent, but sometimes semi-transparent. Ovaries are not yellow. Ovaries extend beyond the body cavity, but are short.
- Pressure tests for running eggs are not commonly used in stage 2, but running eggs under pressure only in stage 3.
- Length varies in stage 2.
- Stage 4: need to cut the ovary open to check on the inside of the ovary for a good identification of stage 4.
- Stage 5 and 6: very rare.
- Blood vessels not used for maturity staging.

Males

- Blood vessels not used for maturity staging.
- Spermatoducts difficult to see.
- In stage 3 the coloration is not the same over the whole testis.
- Stage 4: completely empty no milt residues ready.
- Stage 6 very rare.

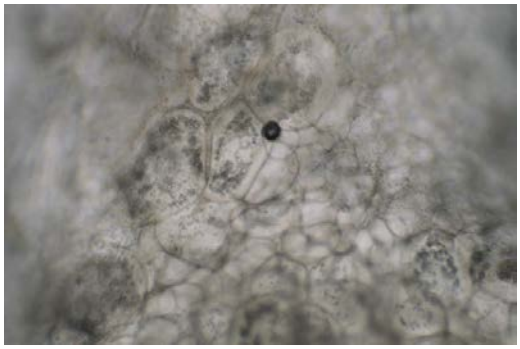
Annex 7: Reference pictures fresh staging, macroscopic and microscopic

Turbot

MACROSCOPIC

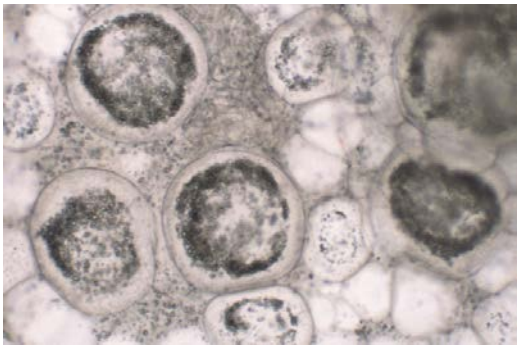
MICROSCOPIC

Agreed stage 2



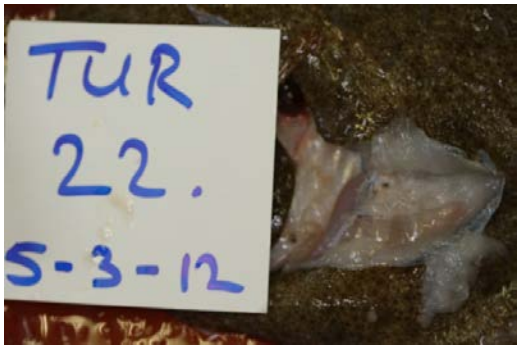
Stage 2

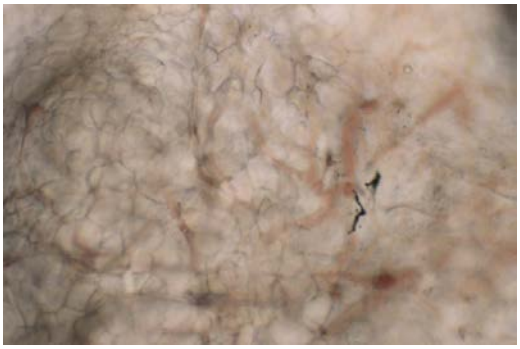
Agreed stage 2



Stage 2

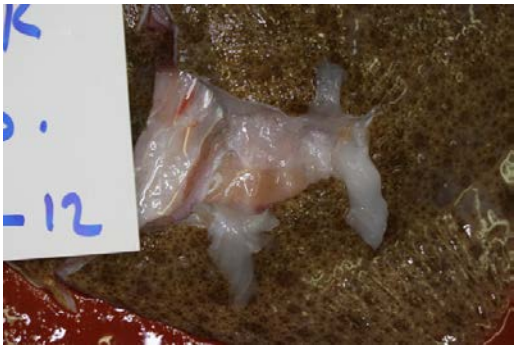
Agreed stage 1





Stage 1

Agreed stage 1



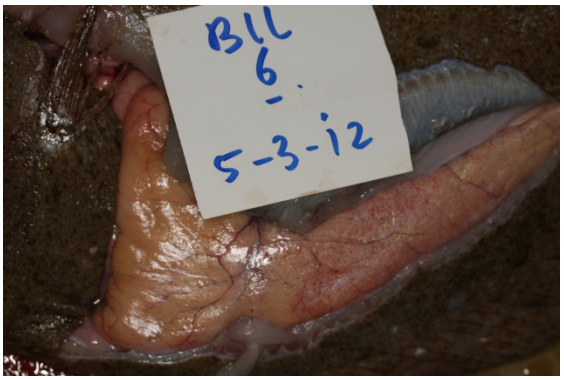
Stage 1

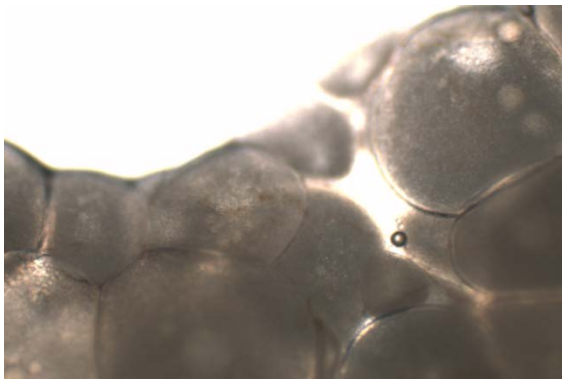
Brill

MACROSCOPIC

MICROSCOPIC

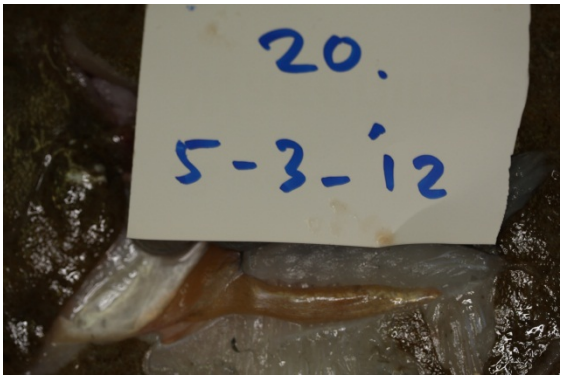
Agreed stage 2

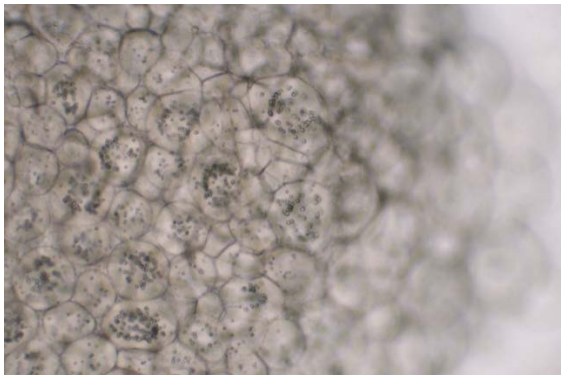




Stage 2

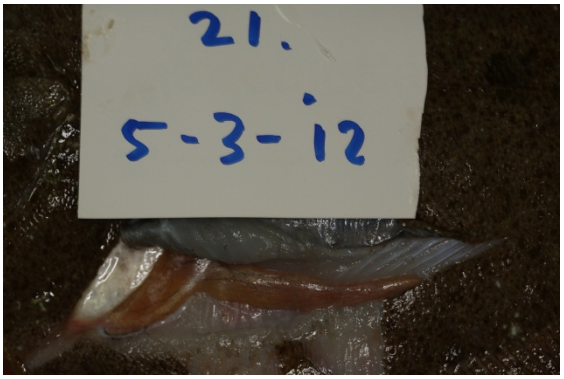
Agreed stage 2

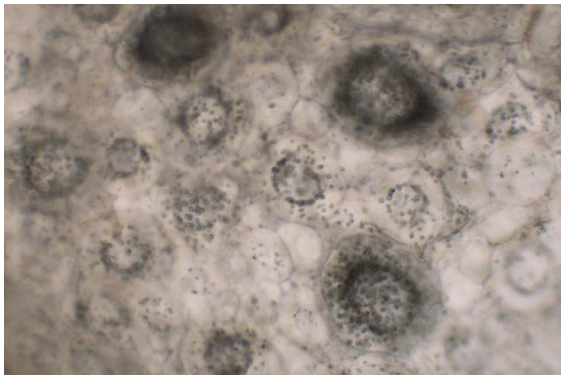




Stage 2

Agreed stage 2

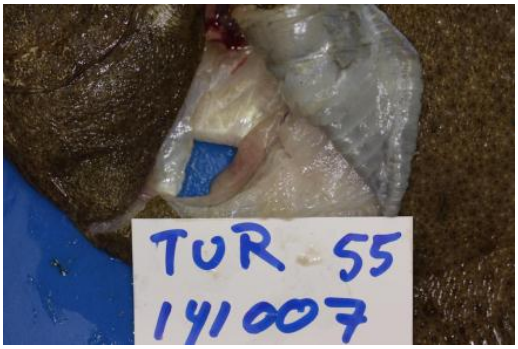
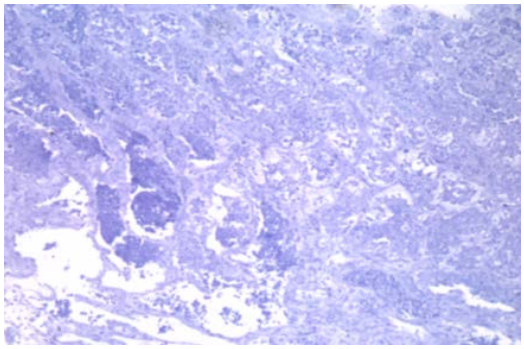
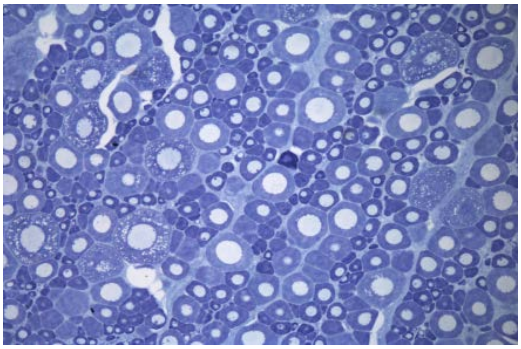
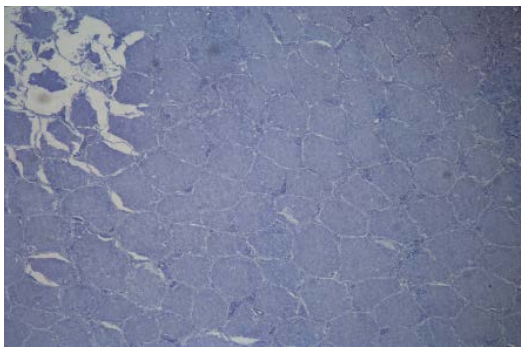
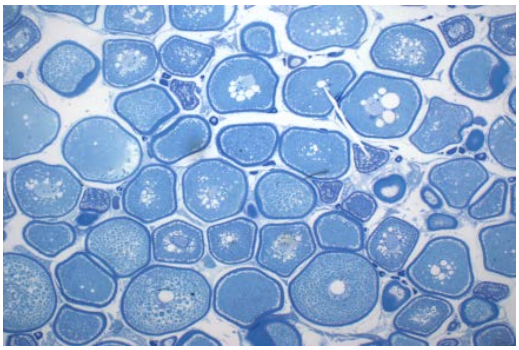




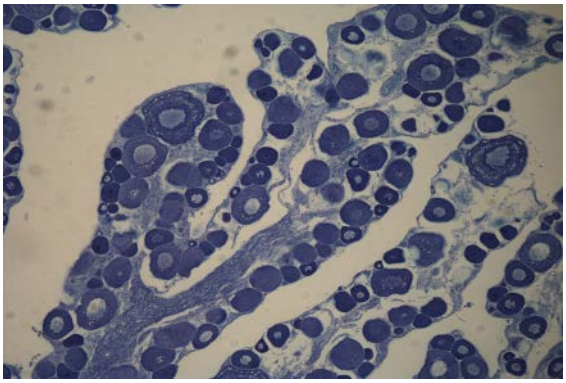
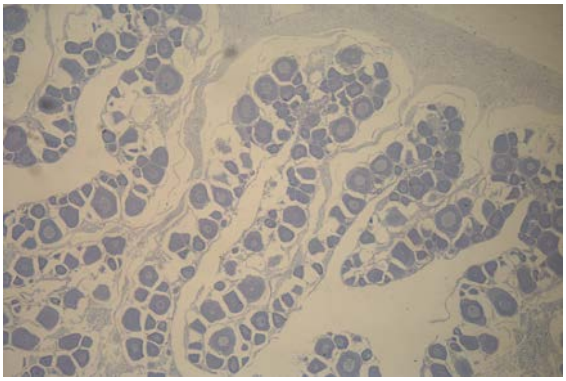
Stage 2

Annex 8: Reference pictures staging from pictures, macroscopic and histological

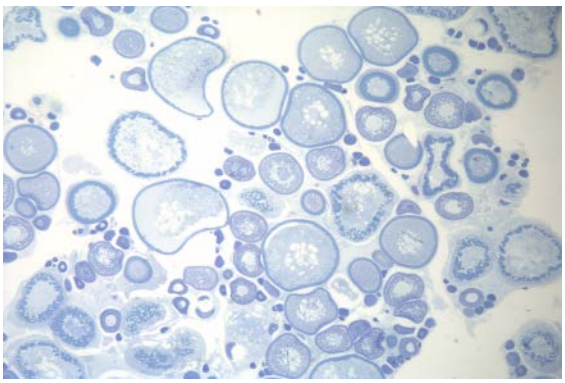
Turbot, second calibration exercise from pictures

	MACROSCOPIC	MICROSCOPIC	
Modal stage 3			Stage 2
Modal stage 5			Stage 2
Modal stage 5			Stage 1
Modal stage 3			Stage 2

Brill, second calibration exercise from pictures

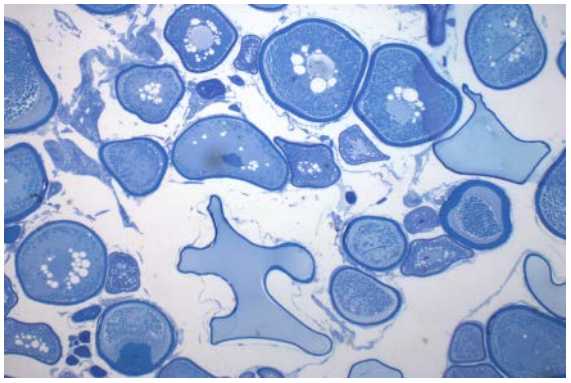
	MACROSCOPIC	MICROSCOPIC	
Modal stage 2			Stage 2
Modal stage 4			Stage 2
Modal stage 2			Stage 2

Modal stage 2



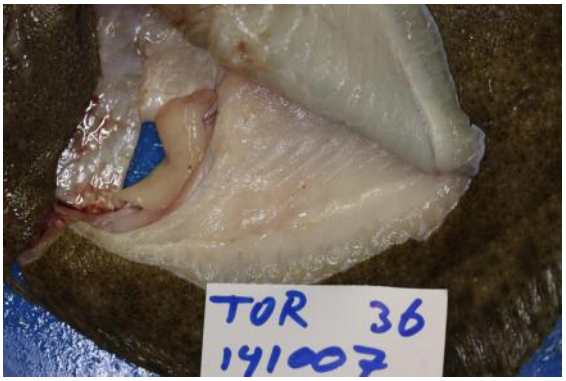
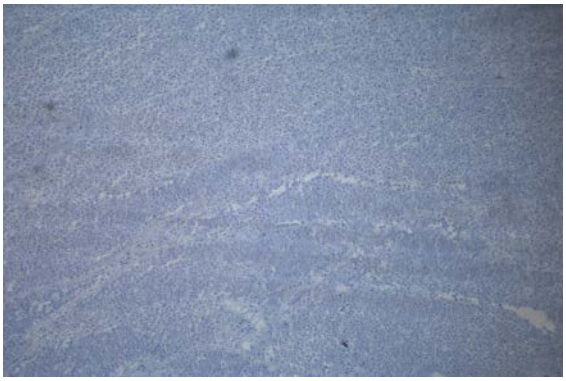
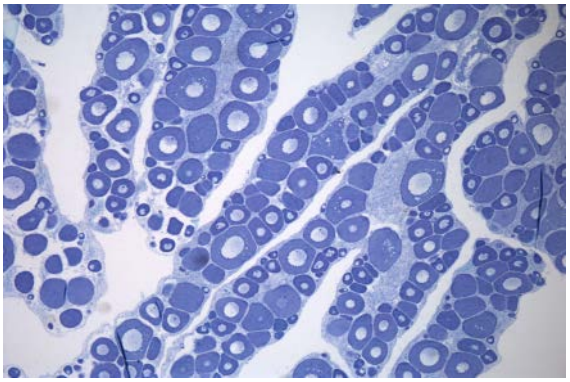
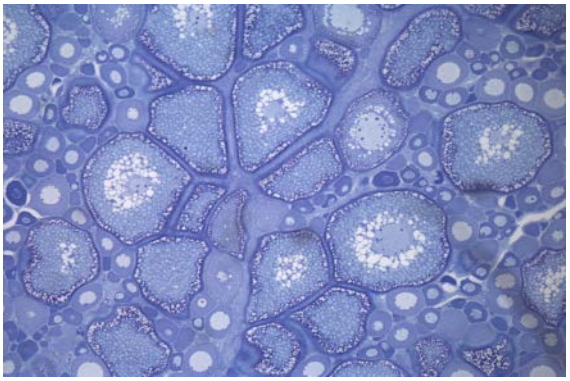
Stage 5

Modal stage 3

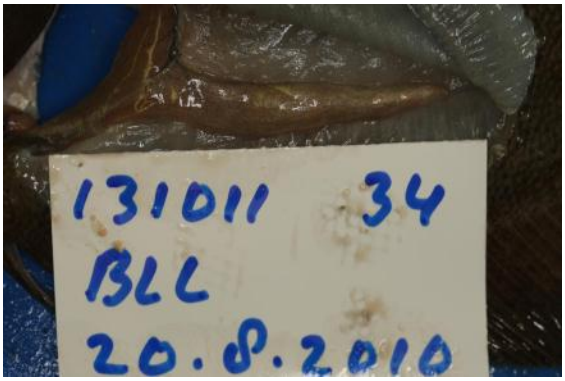
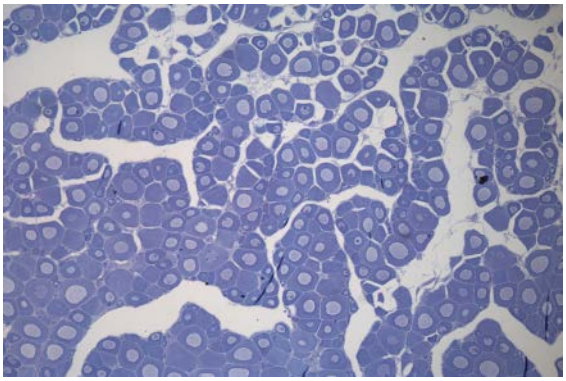
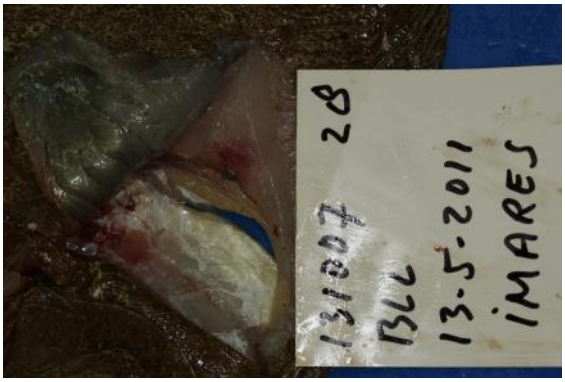
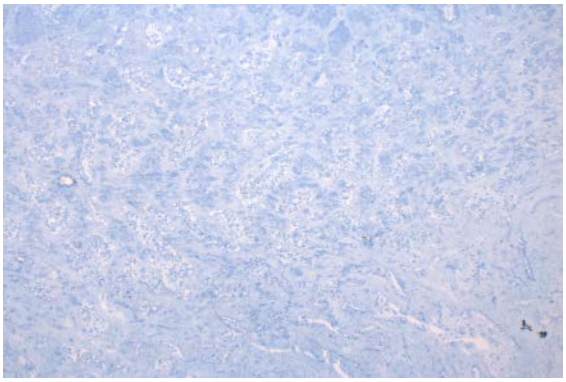


Stage 3

Turbot, third calibration exercise from pictures

	MACROSCOPIC	MICROSCOPIC	
Modal stage 3			Stage 2
Modal stage 4			Stage 2
Modal stage 2			Stage 5

Brill, third calibration exercise from pictures

	MACROSCOPIC	MICROSCOPIC	
Modal stage 1			Stage 1
Modal stage 1			Stage 1

Annex 9: Working documents (separate)

1) Working Document 1: Protocol Data collection WKMSTB 2010 (separate)

2) Working Document 2: Reference Documents Maturity Stages of Turbot and Brill (separate)