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Report of the Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder (WKMSSPDF)

22-26 February 2010

IJmuiden, Netherlands



ICES

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Contents

Executive summary	1
Introduction of common maturity scale	1
Classification criteria for maturity stages	1
Comparison of stagings	1
Comparison of macroscopic staging with histology	1
Optimal sampling time	1
Future workshops	2
1 Opening of the meeting	3
2 Adoption of the agenda	3
3 ToR a: Overview and comparison of currently used maturity scales	4
4 ToR d: Proposal of a common scale	5
5 ToR c: Standardisation of classification criteria and comparison of staging	6
5.1 Standardisation of classification criteria	6
5.1.1 Sole	6
5.1.2 Plaice	8
5.1.3 Dab	9
5.1.4 Flounder	10
5.2 Comparison of staging	12
5.2.1 First comparison, from pictures	12
5.2.2 Second comparison, fresh material	20
5.2.3 Third comparison, from pictures	30
6 ToR b: Validate macroscopic maturity determination with histological information	39
6.1 First comparison, macroscopic pictures	39
6.2 Second comparison, macroscopic pictures	39
7 ToR e: Identify optimal sampling time to estimate maturity ogives	41
7.1 Optimal sampling time	41
7.2 Understanding species biology	42
8 Guidelines for future workshops on maturity staging	42
8.1 Staging from fresh material vs. pictures	42
8.2 Guidelines for the pictures to be taken	42
8.3 Guidelines for the storage and use of pictures in a workshop	43
9 Next meeting	43

10	References	44
	Annex 1: List of participants.....	45
	Annex 2: Agenda WKMSSPDF IJmuiden (Netherlands), 22-26 February 2010	48
	Annex 3: WKMSSPDF terms of reference for the next meeting.....	50
	Annex 4: Recommendations	52
	Annex 5: Overview of currently used scales per country.....	53
	Annex 6: Length composition, sex ratio and sampling range of picture samples	62
	Annex 7: Reference pictures for maturity stages of flatfish	67
	Annex 8: Results of staging sessions	79

Executive summary

Introduction of common maturity scale

When assigning maturity stages to fish, it is important to have an understanding of the biology of the fish and its reproductive cycle in the sampling area. This helps to distinguish the transitions between the different stages.

WKMSSPDF 2010 proposed to adopt the 6 point scale as proposed by the gadoid workshop WKMSCWHS 2007 (ICES, 2008). From wide ranging discussions it has become evident that outside the spawning period it is not reliably possible to distinguish between what is described as a resting stage 5 and a re-maturing stage 2 (histological pictures have given credence to this). To this end it is recommended for flatfish, stage 5 should only be used during the proposed sampling period (see 'Optimal sampling time') and only to describe a skipped spawning fish.

It is recommended that institutes carry out in-house workshops on the reproductive biology of the fish and maturity staging, also as a follow-up of WKMSSPDF2010 to introduce the new common scale. An important aspect of the introduction of the new common scale is to take care that all institutes will be able to transpose their own scale into the common scale. This will give the flexibility for the institutes sometimes to keep their own scale but to internationalise their data in an easy way.

Classification criteria for maturity stages

For all species, classification criteria were defined in subgroups and presented in a similar way. Plaice and dab were discussed in the same subgroup since only a few experts on dab were present at the workshop. As far as possible, the subgroups worked on a reference picture collection per species per sex.

Comparison of stagings

The stagings were done by 19 readers, in three rounds (picture-fresh-picture). The first staging (from pictures) had low agreement (<60%) for all species. The fresh staging had a clearly higher agreement (>75%) for all species. In the third staging (from pictures) progress was made in percentage agreement compared to the first staging, but there was still a lower agreement on the stages than the staging from fresh material. This is not surprising since touching is one of the ways to identify maturity stages in fish.

Comparison of macroscopic staging with histology

The comparison between the macroscopic staging and histological slides shows that there is an early development stage in gonads seen in the histological samples which cannot easily be identified macroscopically. The general percentage of agreement between histological slides and macroscopic scales is very low. This might be caused by the number of samples outside the recommended sampling period.

Optimal sampling time

As it is difficult to identify the proper maturity stage when fish is not clearly developing, data collection for maturity ogives is recommended during the pre-spawning season. This implies that sampling for maturity staging for sole, plaice, dab and flounder should be done during late fourth quarter until the end of the first quarter.

For assessment purposes, only data from one month before the spawning season (to be identified based on the collected data) until the start of the spawning season should be used for the estimation of maturity ogives for sole, plaice, dab and flounder. Any macroscopic staging outside of this period can be misinterpreted and should not be used for maturity ogives.

However, collecting maturity information outside the defined period might be interesting for scientific purposes other than stock assessment. For these purposes, it is recommended that histological staging is done.

Future workshops

Judging from WKMSSPDF a workshop on maturity staging for other commercial flatfish species (turbot, brill, lemon sole, witch flounder) might be useful. However, the lemon sole staging during WKMSSPDF shows that having the expertise in staging one species of flatfish can be adequate to stage other species of flatfish.

To define whether a workshop is necessary, it is recommended to do a calibration exercise prior to organising a workshop based on pictures, for example using the WebGR tool (see <http://webgr.berlios.de/doku.php>).

Before setting up the next meeting, the number of pictures to stage during the workshop should be considered in order to meet the need for time to discuss individual cases as well as maintaining statistical accuracy. Based on the experiences of WKMSSPDF it is recommended that roughly 30 fish per species would be an adequate number to judge at each round during the workshop. This applies for fresh samples as well as pictures. It is recommended that the process of trial-discussion-retrial is based on fresh samples. This means that at least two staging sessions on fresh material have to be done during future workshops. As a consequence, for all species named in a workshop fresh material has to be available in the neighbourhood of the hosting institute.

A workshop on maturity staging should take place when the diversity in maturity stages is high and maturity stages are distinguishable.

WKMSSPDF 2010 developed a set of criteria for pictures to be made for a maturity staging workshop.

1 Opening of the meeting

The meeting started on 22 February 2010. In total, 21 participants from 14 institutes were represented from 12 countries (see Table 1.1). The list of participants can be found in Annex 1.

Table 1.1. Represented countries and institutes during WKMSSPDF 2010

COUNTRY	INSTITUTE
Belgium	ILVO
Denmark	DTU Aqua
France	IFREMER
Germany	vTI
Germany	vTI\OSF
Latvia	BIOR
Lithuania	FRL
Netherlands	IMARES
Poland	SFI
Portugal	IPIMAR
Sweden	ICR
Sweden	IMR
Turkey	EGE
UK-England	CEFAS

2 Adoption of the agenda

The adopted agenda is in Annex 2.

The terms of reference for the meeting were:

A Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder [WKMSSPDF] (Chairs: Ingeborg de Boois* and Cindy van Damme*, The Netherlands) will be established and will take place in IJmuiden, Netherlands, 22–26 February 2010 to:

- a) Compare applied maturity scales and main criteria followed by the scientists/technicians involved in the national sampling, to classify each maturity stage for males and females.
- b) Validate macroscopic maturity determination with histological analysis.
To be checked
- c) Standardise the criteria to classify each maturity stage.
- d) Propose a common scale, with common classification criteria, to be used by all laboratories.
- e) Identify the optimal sampling time to estimate maturity ogives.

WKMSSPDF will report by 8 March 2010 for the attention of PGCCDBS and ACOM.

3 ToR a: Overview and comparison of currently used maturity scales

WKMSSPDF made an inventory of maturity scales used by the different institutes. A complete overview of the scales used and the descriptions of the stages for male and female specimens are in Annex 5. A summary of the scales is in Table 3.1. Most countries use the similar scale for males and females, although in some cases (France, Turkey) the female scale is more extended. In those cases, however, the descriptions of the stages for both sexes are alike but some stages are left out of the scale for the male fish.

Table 3.1. Currently used scales for flatfish maturity staging

COUNTRY	INSTITUTE	MATURITY FEMALE	SCALE	MATURITY SCALE MALE
Belgium	ILVO	7 point scale		7 point scale
Denmark	DTU Aqua	6 point scale		6 point scale
France	IFREMER	8 point scale		4 point scale
Germany	vTI	4 point scale		4 point scale
Germany	vTI\OSF	8 point scale		8 point scale
Latvia	BIOR	5 point scale		5 point scale
Lithuania	FRL	6 point scale		6 point scale
Netherlands	IMARES	4 point scale		4 point scale
Poland	SFI	8 point scale		8 point scale
Portugal	IPIMAR	7 point scale		7 point scale
Sweden	IMR	9 point scale		9 point scale
Sweden	ICR	6 point scale		6 point scale
Turkey	EGE	5 point scale		4 point scale
UK-England	CEFAS	5 point scale		5 point scale

4 ToR d: Proposal of a common scale

The WKMAT 2007 (ICES, 2007) proposed a 5 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 proposed to adopt the 6 point scale as proposed by the gadoid workshop.

For the stagings during WKMSSPDF 2010 the proposed scale was used. During the workshop a number of general topics came up concerning the maturity staging in general and more specifically, the staging following the newly proposed scale. The main discussion items on this topic during WKMSSPDF 2010 were:

- Juvenile/immature: unlike the majority of roundfish, flatfish sexes can be identified at any length. Take care in using coloration as identification; only use it when coloration is obvious.
- The difference between resting and skipped spawning is a matter of timing. It has been suggested that skipped spawning is not often occurring in flatfish, however discussion during this workshop have cast doubts on this assumption, and the identification of skipped spawning is dependent on the spawning time. From wide ranging discussions it has become evident that outside the spawning period it is not reliably possible to distinguish between what is described as a resting stage 5 and a re-maturing stage 2 (histological pictures have given credence to this). To this end it is recommended for flatfish, stage 5 should only be used during the proposed sampling period (see 7.1) and only to describe a skipped spawning fish.
- The question was raised if it is possible to stage maturing male fish correctly without using histology information. Male fish might macroscopically show maturing characteristics even when they are still immature from a histological point of view. On the other hand, maturation of males can go very fast. Once gonads start to swell, only histology might be able to tell what percentage of the males is really maturing and still is immature.
- It is important to realise that one species might have different spawning strategies in different areas, which might for example lead to two spawning seasons in a year in one area and one spawning season in another area. So, when staging maturity of fish, it is important to know which area the fish comes from and to know its spawning strategy in that specific area.
- An important part of the introduction of a common scale is to take care that all institutes will be able to transpose their own scale into the common scale. This will give the flexibility for the institutes sometimes to keep their own scale but to internationalise their data in an easy way. All members of WKMSSPDF2010 felt it should easily be possible either to introduce the proposed scale in the institute, or at least make a key for the translation of the institute's scale to the common scale.
- The follow-up of the introduction of the common scale has to be done by the research institutes, by for example maturity staging workshops within the institute.

5 ToR c: Standardisation of classification criteria and comparison of staging

5.1 Standardisation of classification criteria

The proposed descriptions per species were developed in subgroups per species and discussed in plenary afterwards. Plaice and dab were discussed in the same group since there were only a few institutes involved in staging dab (IMARES, CEFAS). For flounder and plaice/dab after the plenary session the terminology was synchronised where relevant.

General dissection procedures for determination of the maturity stages of flatfish:

- UK England, Germany: cut dorsal side
- France, Sweden, Poland, Latvia, Lithuania: cut ventral side
- Turkey, Portugal, Netherlands, Belgium: cut both sides and weigh the gonads.

It is crucial not to use only one descriptor in isolation to describe any stage. Each stage should use as many of the descriptors as possible to ensure as robust an identification as possible. Always cut gonad open if in doubt about stage.

5.1.1 Sole

The development of sole gonads varies per area. In Turkey, it takes about 3 months for the females to mature, after 1 month the male is ready for spawning. In essence, males seem always ready for spawning and waiting for females to mature.

The minimum body size below which it is difficult to sex a sole is about 7 cm. Below this length a female will be recognised when something (her ovary) is visible. When nothing is visible, it is more likely for it to be a male, but is it? Sex determination is based on length (in relation to size of fish) and shape (female: elongated; male: kidney shaped).

The classification criteria for sole are in Figures 5.1.1.1 (female) and 5.1.1.2 (male). Pictures of agreed stages are in Annex 7.

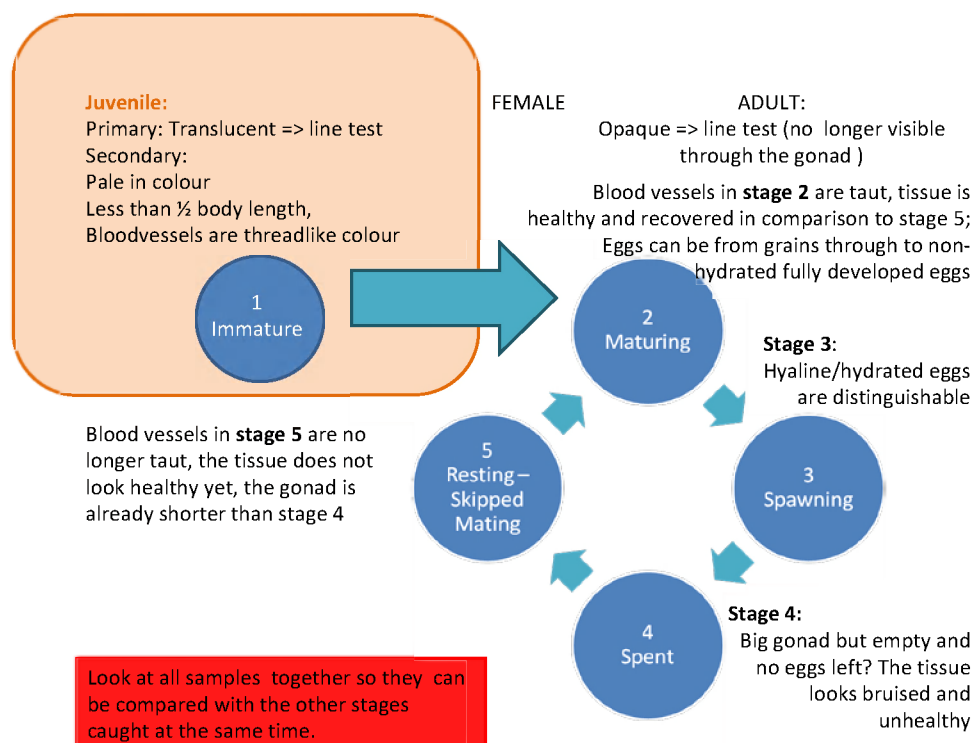


Figure 5.1.1.1. Classification criteria for maturity stages for female sole

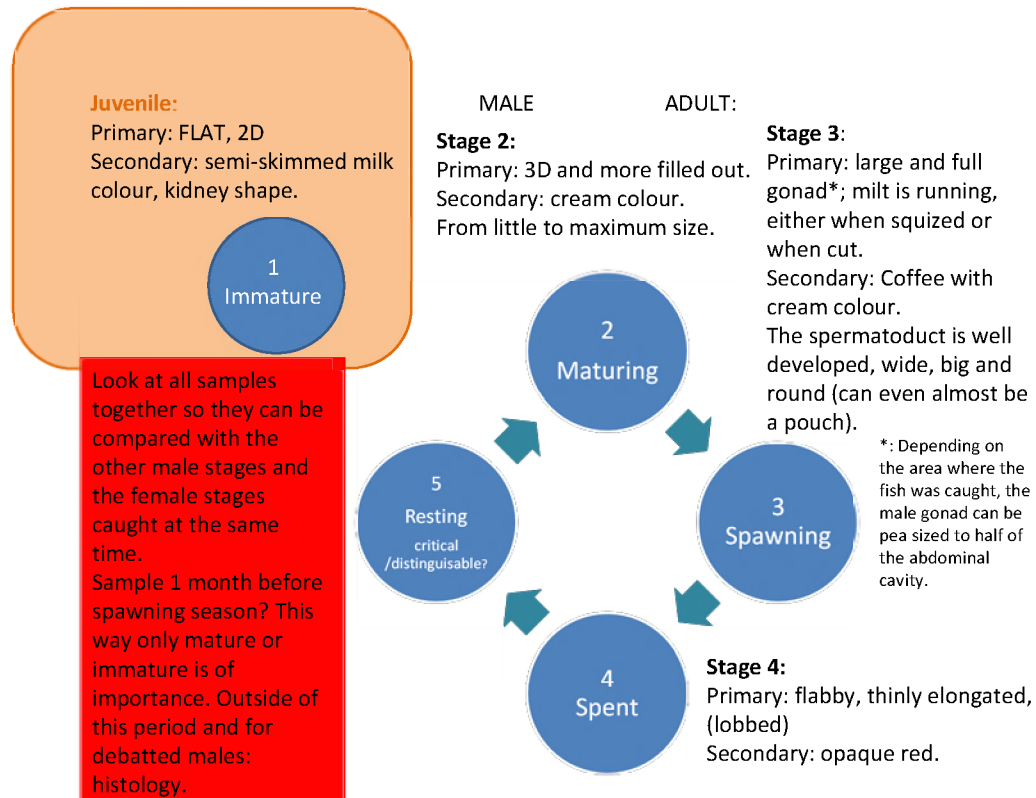


Figure 5.1.1.2. Classification criteria for maturity stages for male sole

5.1.2 Plaice

Since there were only a few dab experts present at the workshop and plaice and dab develop in the similar manner, the species were taken together in defining the classification criteria for the maturity stages. The classification criteria for plaice and dab are in Figures 5.1.2.1 (female) and 5.1.2.2 (male). Pictures of stages are in Annex 7.

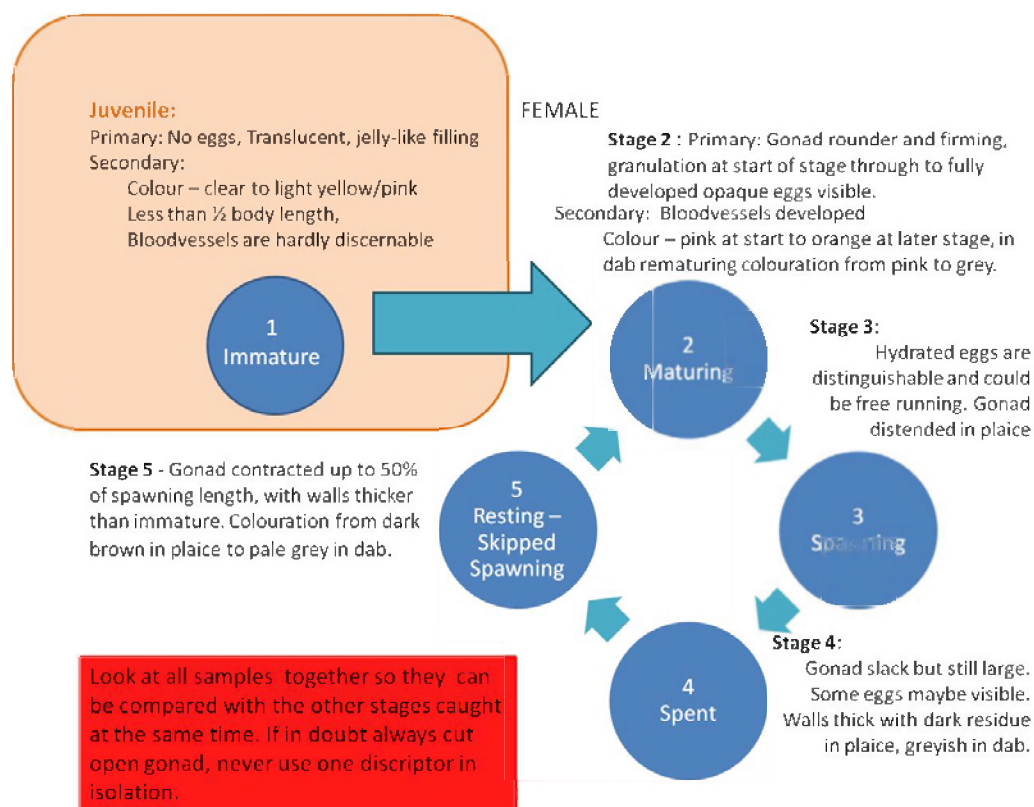


Figure 5.1.2.1. Classification criteria for maturity stages for female plaice and dab

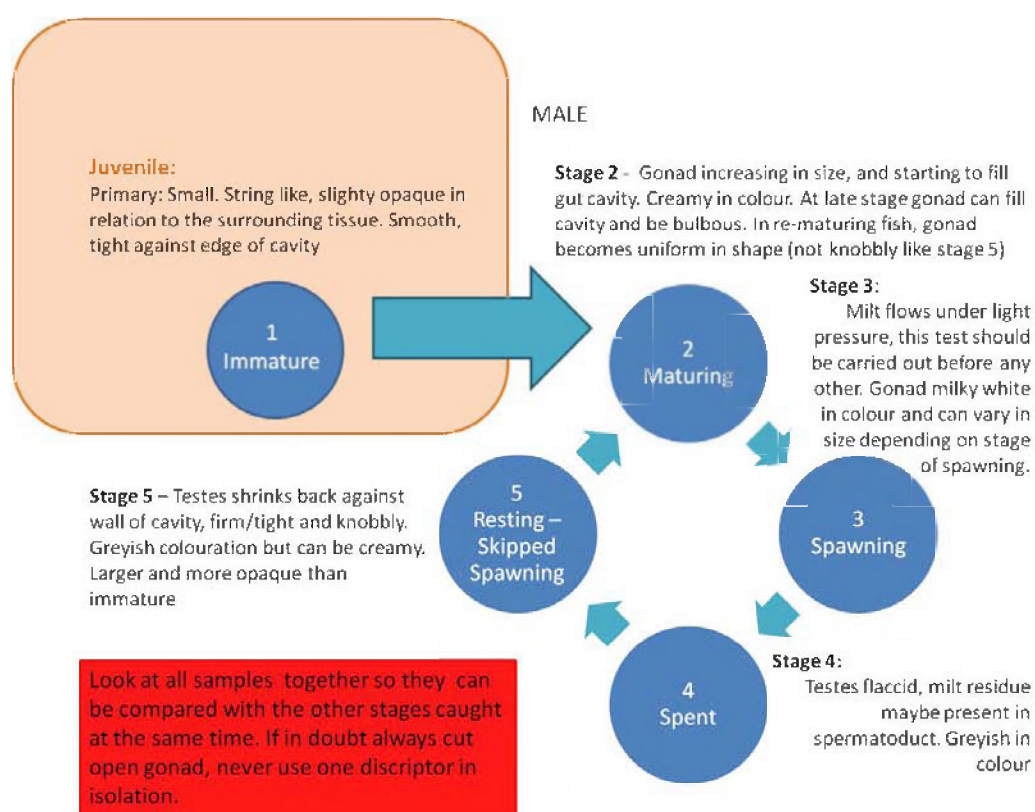


Figure 5.1.2.2. Classification criteria for maturity stages for male plaice and dab

5.1.3 Dab

The classification criteria for dab are in Figures 5.1.2.1 (female) and 5.1.2.2 (male). Pictures of stages are in Annex 7.

It should be noted that female dab gonads do not get proportionally as large as plaice and any comparisons of size when staging should take this into consideration. For example, the fully mature dab gonad is rarely longer than half the body length so when comparison between immature and mature gonad length is made, this should be taken into account.

Pay attention to the fact that in dab hyaline eggs are smaller than the eggs from other flatfish species. This makes it more difficult to see the hyaline eggs from the outside of an unopened gonad. If possible, it is best to open the gonad where bubbles or hyaline eggs are seen. In case dissection itself is still not sufficient, a small sample of the gonad should be checked under a microscope for presence of yolk. Figures 5.1.3.a-e show examples of the fresh gonad content for herring under a microscope.

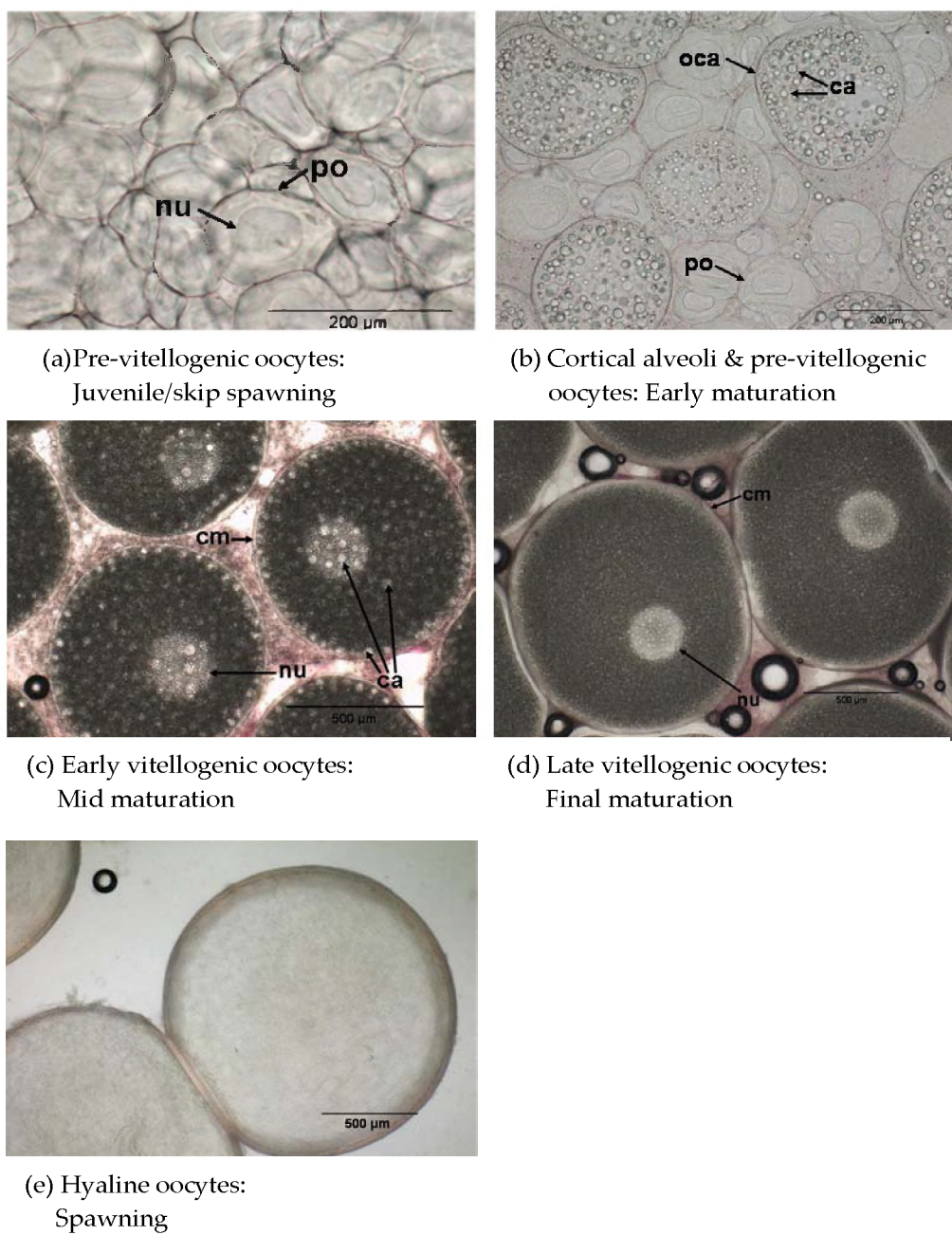


Figure 5.1.3.a-e Fresh samples of herring oocytes under a microscope.

5.1.4 Flounder

The classification criteria for flounder are in Figures 5.1.4.1 (female) and 5.1.4.2 (male). Pictures of stages are in Annex 7.

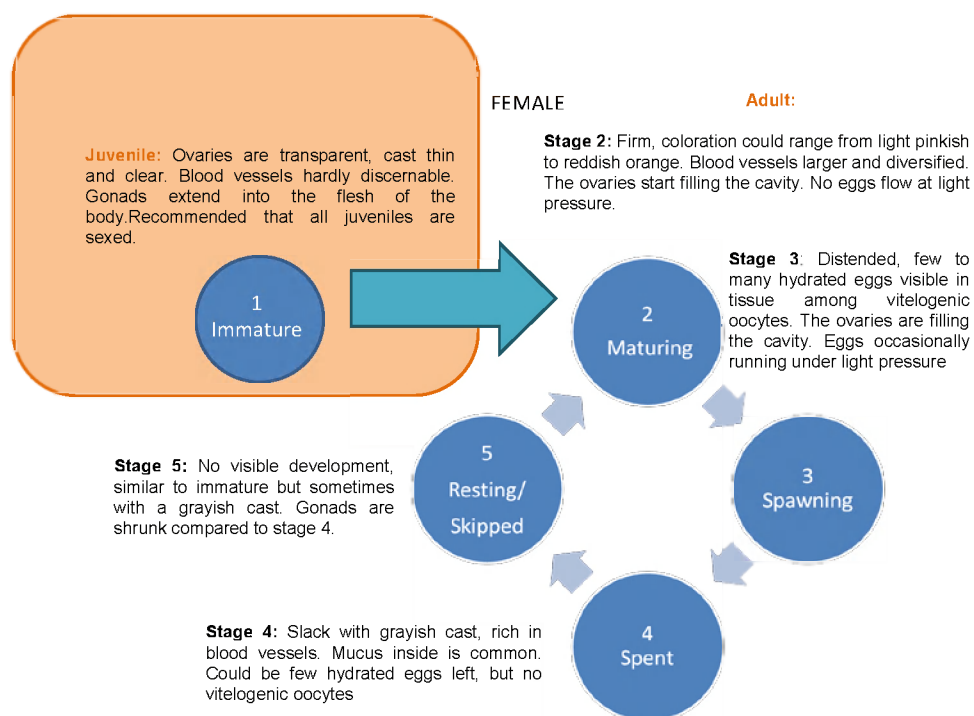


Figure 5.1.4.1 Classification criteria for maturity stages for female flounder

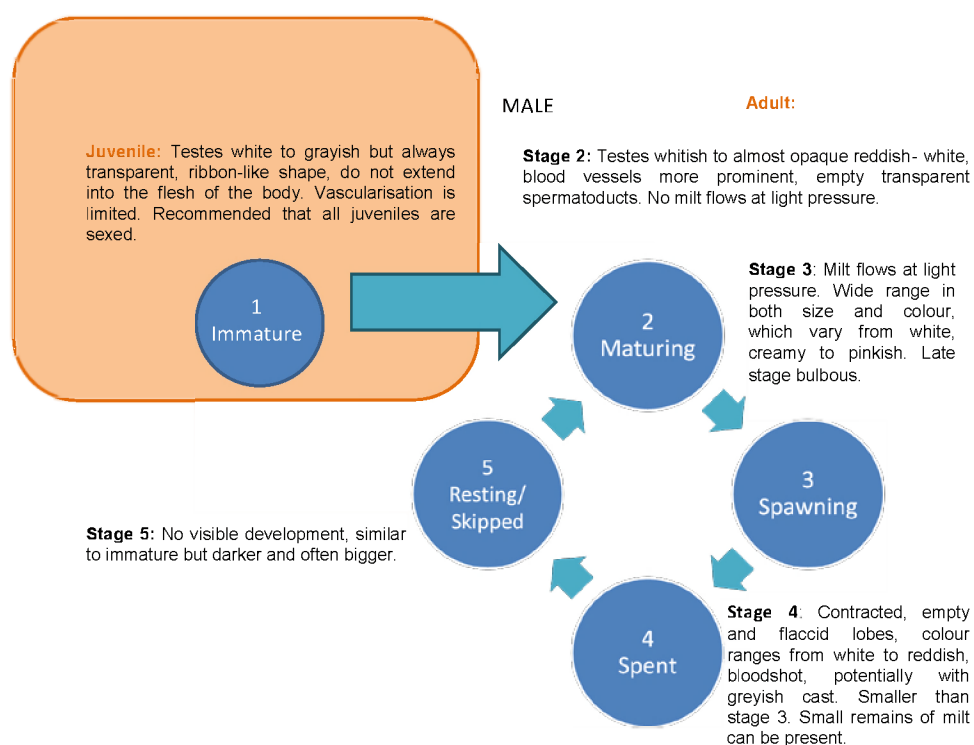


Figure 5.1.4.2 Classification criteria for maturity stages for male flounder

5.2 Comparison of staging

The comparison of the staging was done in three steps:

- a) staging of a subset of pictures for sole, plaice, dab and flounder and additionally lemon sole based on the proposed common scale
- b) staging of fresh sole, plaice, dab and flounder based on the proposed common scale
- c) staging of another subset of pictures for sole, plaice, dab and flounder and additionally lemon sole based on the proposed common scale

An overview of the length composition and sex ratio of the staging sets is given in Annex 6.

Results of the comparisons are in Tables 5.2.1.1.-5.2.1.5, 5.2.2.1-5.2.2.4, 5.2.3.1-5.2.3.5 and Annex 8. The readers had the same number throughout the comparisons.

5.2.1 First comparison, from pictures

The first staging round was based on pictures. Gonads were staged in the newly proposed maturity scale. Generally speaking, there was little consensus on the maturity stages of the samples. This applied for all species. The quality of the pictures was variable, which might have contributed to the low agreement level.

The general feeling was that it was easier to stage female fish than male fish. Table 5.2.1, showing the results for all readers by stage by sex, for all stages by sex and for all stages for all fish, does not give evidence for that.

Table 5.2.1. First (picture) staging by sex, % agreement by modal stage

	SOLE		PLAICE		DAB		FLOUNDER		LEMON SOLE	
	m	f	m	f	m	f	m	f	m	f
1	54.0	61.2	50.6	55.7	75.0	58.4	36.8	-	-	47.4
2	50.7	60.8	62.6	53.5	63.2	55.3	49.1	-	70.6	-
3	-	60.4	-	-	-	-	55.3	71.9	62.7	58.8
4	29.4	51.5	-	55.5	-	-	-	75.0	43.8	56.6
5	-	43.6	41.5	39.3	50.0	52.6	47.1	59.5	-	44.1
6	-	-	-	-	-	-	-	-	-	-
1-6	49.8	54.5	56.3	52.4	62.2	55.4	48.9	68.9	60.7	50.8
1-6	52.8		53.7		57.9		58.5		53.8	

5.2.1.1 Sole**Table 5.2.1.1.a The number of stagings by modal stage for sole**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	16	18	14	14	22	12	7	-	6	6	11	4	1	6	9	-	1	13
2	13	8	6	9	10	4	10	13	-	17	3	9	16	3	16	13	22	28	-
3	-	-	-	5	6	2	5	-	-	5	3	4	3	-	3	2	3	1	3
4	1	7	3	1	1	4	6	1	-	5	2	1	3	-	1	1	5	2	19
5	21	1	4	3	2	3	1	-	-	2	17	8	9	4	9	10	5	3	-
6	-	-	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	35	32	31	32	33	35	34	21	0	35	31	33	35	8	35	35	35	35	35

Table 5.2.1.1.b Stage compositions by stage and reader for all stage readers for sole. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	0	100	100	80	80	100	78	67	-	30	38	88	30	0	60	80	0	10	90	0
2	58	22	20	33	42	17	42	83	-	83	20	58	75	25	100	83	92	83	0	58
3	0	0	0	67	100	67	67	-	-	67	67	67	100	-	100	67	100	33	67	0
4	0	67	67	33	33	100	33	0	-	67	33	33	67	-	33	0	33	33	100	0
5	100	14	57	43	29	14	0	0	-	14	71	71	71	100	100	86	43	0	0	100
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	40.0	46.9	53.1	51.4	54.3	51.4	44.1	52.4		51.4	41.9	66.7	62.9	25.0	82.9	74.3	51.4	37.1	40.0	40.0
rank	15	12	6	8	5	8	13	7		8	14	3	4	18	1	2	8	17	15	15

Table 5.2.1.1.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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	3.10	0.00	0.00	0.60	0.60	0.00	0.67	0.56	-	1.20	2.50	0.50	1.30	2.00	0.70	0.20	1.30	1.20	0.30	0.88
2	1.17	-	-	-	-	-	0.17	-	-	-	1.60	-	0.33	1.25	0.00	0.17	0.17	0.50	1.25	0.24
3	-	-	-	1.00	0.00	0.67	0.67	-	-	-	-	-	0.00	-	0.00	0.33	0.00	-	0.33	-
4	1.00	-	-	-	-	0.00	-	-	-	-	-	0.00	0.33	-	-	1.00	-	-	0.00	-
5	0.00	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.00	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.2.1.2 Plaice

Table 5.2.1.2.a The number of stagings by modal stage for plaice

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	16	26	11	9	25	14	16	1	19	5	7	1	10	10	13	-	5	16
2	21	12	8	15	16	14	22	20	-	25	6	11	19	19	27	12	12	6	10
3	-	-	-	3	2	3	6	-	-	5	1	1	-	10	2	6	2	5	-
4	12	19	6	9	10	6	5	15	-	1	12	9	19	8	4	8	20	12	27
5	21	7	13	11	12	-	5	1	-	2	30	20	13	7	11	15	20	26	-
6	-	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	54	54	53	49	49	48	52	52	1	52	54	48	52	54	54	54	54	54	53

Table 5.2.1.2.b Stage compositions by stage and reader for all stage readers for plaice. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	0	88	88	73	60	80	50	60	-	87	31	58	7	44	63	63	0	31	75	0
2	87	60	57	71	71	57	47	73	-	73	40	57	79	80	80	47	73	27	57	87
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	67	87	40	40	47	18	27	53	-	0	73	64	73	33	27	53	73	53	93	67
5	75	50	13	50	50	0	13	0	0	14	100	88	38	38	13	63	50	75	0	75
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	53.7	74.1	54.7	59.6	57.7	45.8	36.5	53.8	0.0	48.1	55.6	64.6	50.0	50.0	50.0	55.6	48.1	42.6	64.2	53.7
rank	10	1	8	4	5	17	19	9	20	16	6	2	12	12	12	6	15	18	3	10

Table 5.2.1.2.c 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]

5.2.1.3 Dab

Table 5.2.1.3.a The number of stagings by modal stage for dab

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	7	9	4	3	10	9	5	5	6	-	1	7	5	3	6	6	6	6
2	2	8	6	6	6	4	9	9	9	7	1	2	2	5	9	6	6	8	1
3	-	-	-	1	1	4	1	-	-	1	-	-	-	-	-	-	1	-	-
4	-	-	-	-	-	1	-	5	5	2	-	-	-	-	-	-	-	-	12
5	17	4	4	5	5	-	-	-	-	2	16	12	10	9	7	7	5	4	-
6	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	1	1	-
1-6	19	19	19	16	15	19	19	19	19	18	17	15	19	19	19	19	18	18	19

Table 5.2.1.3.b Stage compositions by stage and reader for all stage readers for dab. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	0	100	86	60	50	71	14	57	57	83	0	33	86	71	43	86	86	86	86	63
2	20	100	80	60	60	60	20	80	80	60	20	40	40	60	60	100	80	100	20	60
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	100	57	57	71	71	0	0	0	0	29	100	100	100	71	29	86	57	57	0	52
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	42.1	84.2	73.7	64.7	62.5	42.1	10.5	42.1	42.1	55.6	47.1	66.7	78.9	68.4	42.1	89.5	73.7	78.9	36.8	57.9
rank	13	2	5	9	10	13	19	13	13	11	12	8	3	7	13	1	5	3	18	

Table 5.2.1.3.c 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	3.57	0.00	0.14	0.40	0.50	0.57	0.86	0.71	0.71	0.50	4.00	2.67	0.57	1.14	1.86	0.57	0.71	0.71	0.43	1.02
2	2.40	0.00	- 0.20	0.00	0.00	- 0.40	- 0.40	0.40	0.40	0.00	2.40	1.80	1.00	1.20	1.20	0.00	0.60	0.00	1.60	0.63
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	0.00	- 1.29	- 1.57	- 0.29	- 0.29	- 2.86	- 3.71	- 2.29	- 2.29	- 1.86	0.00	0.00	0.00	- 0.86	- 2.14	- 0.43	- 1.14	- 1.29	- 1.00	- 1.23
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.2.1.4 Flounder

Table 5.2.1.4.a The number of stagings by modal stage for flounder

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	1	3	-	1	7	1	2	2	3	-	-	-	-	-	-	-	-	3
2	4	6	2	3	3	1	4	6	6	5	-	2	2	3	3	2	4	3	3
3	3	-	2	5	5	3	6	2	2	4	5	5	4	5	4	4	3	4	6
4	2	2	2	4	3	1	-	3	3	-	2	2	3	2	2	6	3	3	1
5	5	4	4	1	-	-	2	1	1	1	6	3	4	3	5	2	4	4	-
6	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	14	13	13	13	12	12	13	14	14	13	13	12	13	13	14	14	14	14	13

5.2.1.5 Lemon sole

Since pictures were available for lemon sole, this species was taken into account in the comparison of the maturity staging. Although no specific descriptions for the maturity stages of lemon sole were available, the results were comparable to the other species. The group felt that this was because when one has experience of flatfish maturity staging, this can be transposed and used in staging other flatfish species, in this case lemon sole.

Table 5.2.1.5.a The number of stagings by modal stage for lemon sole

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	3	-	1	1	1	5	1	1	2	-	2	-	3	1	2	-	2	3
2	4	7	6	5	5	5	2	2	2	3	4	4	4	3	4	4	3	2	1
3	1	1	3	8	8	4	4	-	-	2	1	1	5	3	5	2	6	6	6
4	-	2	7	1	1	6	3	2	2	3	1	1	4	2	5	4	3	4	6
5	11	3	-	-	-	-	2	-	-	3	9	6	3	5	1	4	4	2	-
6	-	-	-	1	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-
1-6	16	16	16	15	15	16	16	5	5	13	15	14	16	16	16	16	16	16	16

Table 5.2.1.5.b Stage compositions by stage and reader for all stage readers for lemon sole. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	0	100	0	0	0	0	100	50	50	100	0	50	0	100	50	100	0	100	100	47
2	100	100	100	0	0	100	0	-	-	100	100	100	100	100	0	100	0	100	100	71
3	20	20	60	100	100	60	60	0	0	50	20	25	100	60	60	40	100	80	100	61
4	0	50	75	25	25	75	50	100	100	50	0	33	75	50	100	75	50	50	75	54
5	100	50	0	0	0	0	25	-	-	50	100	75	50	100	25	75	50	50	0	44
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	37.5	50.0	43.8	37.5	37.5	43.8	50.0	60.0	60.0	61.5	37.5	50.0	68.8	75.0	56.3	68.8	56.3	68.8	68.8	53.8
rank	16	11	14	16	16	14	11	7	7	6	16	11	2	1	9	2	9	2	2	

Table 5.2.1.5.c Bias in the comparison for lemon 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	4.00	0.00	1.00	1.00	1.00	1.00	0.00	0.50	0.50	0.00	4.00	2.00	1.00	0.00	0.50	0.00	1.00	0.00	0.00	0.92
2	0.00	0.00	0.00	1.00	1.00	0.00	2.00	-	-	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.35
3	-	-	-	0.00	0.00	-	-	-	-	0.00	-	-	0.00	-	-	-	0.00	-	0.00	-
	0.20	0.80	0.40			0.40	0.40	1.00	1.00		0.40	0.75		0.40	0.40	0.60		0.20		0.31
4	1.00	-	-	-	-	-	-	0.00	0.00	0.50	1.25	0.67	0.25	-	0.00	0.25	0.50	-	-	-
		0.25	0.50	1.75	1.75	0.75	0.50							0.50				0.50	0.25	0.16
5	0.00	-	-	-	-	-	-	-	-	-	0.00	-	-	0.00	-	-	-	-	-	-
		1.75	1.00	1.50	1.50	1.25	2.50			1.00		1.00	1.00		1.50	0.25	1.00	0.50	1.75	1.03
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.2.2 Second comparison, fresh material

The staging was done on 30 fresh specimens per species for sole, plaice, dab and flounder. The fish were cut open on both sides and the gonads were left in the fish. From all specimens pictures have been taken. After staging there was a discussion on some specimens where the general consensus was low. This created a lively and fruitful exchange of views on fish stages. Based on this discussion, the criteria for maturity staging were refined in subgroups.

No length distribution was made of the fresh samples, but since the fish was bought at the fish market, it was all commercially sized fish. Sex ratio per species is given in Annex 6.

In general, the agreement on the fresh specimen was higher than the agreement on the pictures. All participants agreed it was easier to stage fresh material compared to staging from pictures. Main reasons for that were:

- Touching the gonad is part of the staging.
- The possibility to look into more detail by cutting the gonad, is an advantage in comparison to staging from pictures.
- Fresh samples allow definitive staging especially for stage 3 hydrated eggs

- In fresh samples, it is easier to quantify the transition to the next maturity stage than on a picture
- The ability to get an overview of the condition of the fish is higher in fresh samples
- Photographs lack the depth of field

In case of disagreement, putting 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.

Table 5.2.2 shows a clear difference in the % agreement by modal stage for male and female fish. This is contradictory to the results in the first staging. This might be caused by either the time of the year the samples were taken or to the difference between staging fresh material and pictures.

In Annex 8 the figures for the results per reader per stage are shown.

Table 5.2.2. Second (fresh) staging by sex, % agreement by modal stage

	Sole		Plaice		Dab		Flounder	
	m	f	m	f	m	f	m	f
1	-	-	-	42.1	-	-	-	-
2	66.7	78.3	44.7	78.9	-	72.2	48.7	57.9
3	-	-	71.1	83.1	-	78.4	60.2	84.2
4	52.6	-	-	76.8	-	-	48.1	83.2
5	-	-	-	42.1	-	-	-	-
6	-	-	-	-	-	-	-	-
1-6	64.0	78.3	52.3	76.6		77.0	54.6	80.2
1-6	76.4		71.0		77.0		68.3	

5.2.2.1 Sole

Table 5.2.2.1.a The number of stagings by modal stage for sole

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	-	-	-	-	3	-	-	1	-	-	-	-	-	-	-	-	-	-
2	29	4	19	12	21	24	-	30	20	22	29	29	28	29	29	27	28	21	30
3	-	26	7	16	7	2	28	-	8	7	-	1	2	1	-	-	1	7	-
4	1	-	4	1	2	1	2	-	1	1	-	-	-	-	1	3	1	1	-
5	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	29	30

Table 5.2.2.1.b Stage compositions by stage and reader for all stage readers for sole. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	100	10	66	41	72	83	0	100	69	76	100	97	93	97	100	93	97	72	100	77
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	100	0	100	100	100	100	0	0	100	100	0	0	0	0	100	100	100	0	0	53
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	100.0	10.0	66.7	43.3	73.3	83.3	0.0	96.7	70.0	76.7	96.7	93.3	90.0	93.3	100.0	93.3	96.7	72.4	96.7	76.4
rank	1	18	16	17	13	11	19	3	15	12	3	7	10	7	1	7	3	14	3	

Table 5.2.2.1.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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.00	0.90	0.45	0.66	0.31	- 0.03	1.07	0.00	0.24	0.24	0.00	0.03	0.07	0.03	0.00	0.14	0.03	0.31	0.00	0.23
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	0.00	- 2.00	0.00	0.00	0.00	0.00	- 1.00	- 2.00	0.00	0.00	1.00	- 2.00	- 2.00	- 2.00	0.00	0.00	0.00	- 4.00	- 2.00	- 0.84
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

For sole, the discussion on male specimens that were difficult to stage focussed on fish staged 2/3/4/5 by different participants. When taking into account that females that were caught in the same area, at the same time are not ready for spawning, stages 3, 4 and 5 for male sole are not likely. Previous years have shown that it is too early for the spawning season in the catch area to have started.

5.2.2.2 Plaice

Table 5.2.2.2.a The number of stagings by modal stage for plaice

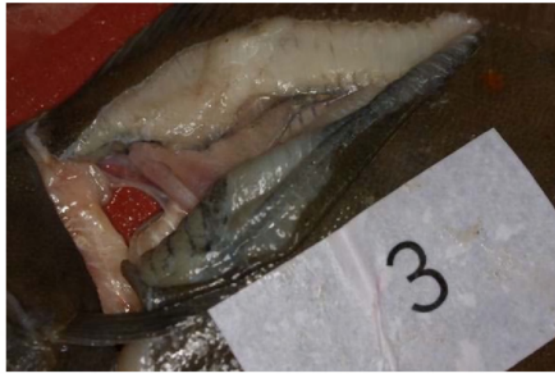
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	2	-	-	-	5	-	-	1	4	-	1	1	-	1	2	-	-	2
2	8	-	3	1	10	5	-	29	5	8	7	7	3	3	6	2	1	2	1
3	7	15	9	11	8	10	16	1	13	9	9	11	16	15	12	10	10	11	13
4	14	13	16	15	5	7	13	-	9	7	11	2	10	11	2	14	18	13	14
5	1	-	2	3	7	3	1	-	2	1	3	8	-	1	9	2	1	4	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	30	30	30	30	30	30	30	30	30	29	30	29	30	30	30	30	30	30	30

For plaice, it is important to realise that during the spawning period, once mature, male plaice are always ready to spawn, even before the females are. Therefore if the females are just ready for spawning, males can already be spent.

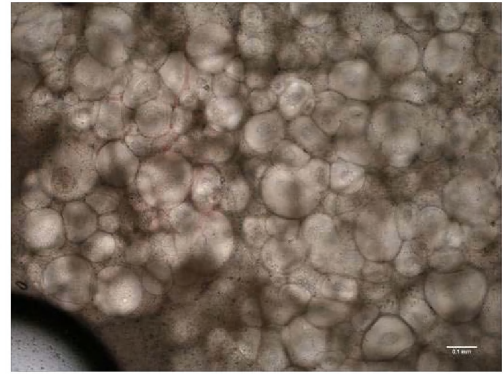
There were some discussions on specific fish, which resulted in fine-tuning of the staging criteria. For example:

- Fish 12: stage 4: gonad is loose, the fluid that is left in the spermatoduct/gonad is very runny/watery/has no male "tissue" left.
- Fish 16: stage 5 or 1; 5: strong, though wall of the gonad with grey cast, size of fish; 1: short, thin (not wide), transparent, floppy gonad that is not granular inside.
- Fish 22: stage 4: big, wide, floppy, flaccid gonad with no hyaline eggs left.
- Fish 3: stage 5, skipped spawning: the gonads' wall is too thick to be immature, the gonad already has a grey cast (should not be visible in immature), the inside of the gonad is too granular to have never spawned before. This is not stage 4 or 5 (recovering after this year spawning event) because the gonads are too small to have spawned this spawning season (that is still not over, just beginning)
- Fish 4: stage 3: the gonad still contains a large amount of hyaline and yolked eggs thus the eggs are still viable. In stage 4 some hyaline eggs might still be present but in stage 5 they should all be resorbed.

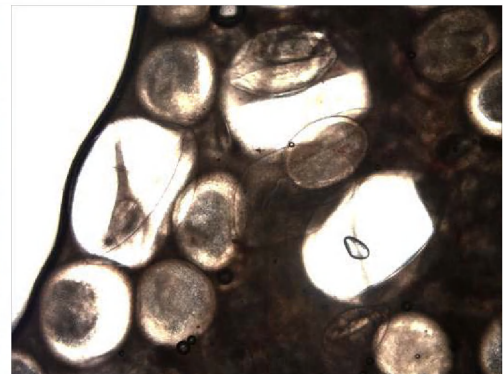
For a number of plaice with low agreement, microscopic pictures were made during the workshop (Figure 5.2.2.1) to ensure the maturity stage.



(a) Plaice 3 stage 5, developing eggs visible



(b) Plaice 6 stage 3, hyaline eggs visible



(c) Plaice 16 stage 5, developing eggs visible



Figure 5.2.2.1a-c Oocyte pictures and gonad pictures of three plaice staged during WKMSSPDF 2010

5.2.2.3 Dab**Table 5.2.2.3.a The number of stagings by modal stage for dab**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	8	-	5	8	10	8	-	14	23	12	5	13	2	8	29	15	12	5	3
3	21	30	24	21	20	22	30	5	7	18	25	17	28	22	1	15	18	25	27
4	1	-	1	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	30	30	30	29	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30

Table 5.2.2.3.b Stage compositions by stage and reader for all stage readers for dab. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	100	0	71	86	100	100	0	43	100	86	71	100	29	71	100	100	100	71	43	72
3	91	100	96	91	87	96	100	17	30	74	100	74	100	87	4	65	78	100	100	78
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	93.3	76.7	90.0	89.7	90.0	96.7	76.7	23.3	46.7	76.7	93.3	80.0	83.3	83.3	26.7	73.3	83.3	93.3	86.7	77.0
rank	2	13	5	7	5	1	13	19	17	13	2	12	9	9	18	16	9	2	8	

Table 5.2.2.3.c 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.00	1.00	0.29	0.14	0.00	0.00	1.00	1.00	0.00	0.14	0.29	0.00	0.71	0.29	0.00	0.00	0.00	0.29	0.57	0.30
3	0.00	0.00	0.04	-0.09	-0.13	-0.04	0.00	-0.13	-0.70	-0.26	0.00	-0.26	0.00	-0.13	-0.96	-0.35	-0.22	0.00	0.00	-0.17
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.2.2.4 Flounder

To identify spawning, firmly squeeze the gonads and maybe cut the gonad and scrape it while putting a small amount of pressure onto it.

Table 5.2.2.4.a The number of stagings by modal stage for flounder

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
2	14	-	1	1	9	9	-	19	9	17	11	6	3	2	10	4	7	1	7
3	3	16	10	12	8	3	14	-	7	10	8	11	14	14	5	14	5	14	12
4	13	14	19	10	10	18	15	11	9	1	11	5	12	14	8	11	18	15	11
5	-	-	-	7	3	-	1	-	4	1	-	4	1	-	7	1	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	30	30	30	30	30	30	30	30	29	30	30	26	30	30	30	30	30	30	30

Table 5.2.2.4.b 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	83	0	17	17	83	67	0	100	67	67	67	67	50	33	83	50	50	17	67	52
3	30	100	70	90	60	20	70	0	60	60	60	90	100	100	40	90	40	100	100	67
4	86	100	100	64	71	93	71	79	62	7	79	45	86	100	43	79	100	93	79	76
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	66.7	80.0	73.3	63.3	70.0	63.3	56.7	56.7	62.1	36.7	70.0	69.2	83.3	86.7	50.0	76.7	70.0	80.0	83.3	68.3
rank	12	4	7	13	8	13	16	16	15	19	8	11	2	1	18	6	8	4	2	

Table 5.2.2.4.c 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]

5.2.3 Third comparison, from pictures

The third staging round was based on pictures and carried out by 18 readers. Gonads were staged using the newly proposed maturity scale. Generally speaking, there was more agreement on the maturity stages of the samples than in the first round (pictures), but less than in the second (fresh). This applied for all species.

Apart from the general higher percentage of agreement, specially the agreement on stage 1 (immature) was higher compared to the first round. Agreement on this stage is very important for stock assessment purposes, since the maturity ogives are determined by the separation of immature and mature fish.

Table 5.2.3 shows the results for all readers by stage by sex, for all stages by sex and for all stages for all fish.

In Annex 8 the figures for the results per reader per stage are shown.

Table 5.2.3. Third (picture) staging by sex, % agreement by modal stage

	Sole		Plaice		Dab		Flounder		Lemon sole	
	m	f	m	f	m	f	m	f	m	f
1	79.2	86.1	64.6	55.7	83.9	100.0	-	-	87.0	78.3
2	65.6	62.6	62.4	67.8	52.9	54.2	66.8	86.3	57.8	57.8
3	-	-	-	61.3	50.0	-	67.6	-	100.0	100.0
4	40.0	53.3	-	42.3	-	-	-	50.0	50.0	50.0
5	40.0	48.1	40.0	57.2	46.4	54.9	49.0	61.7	40.0	40.0
6	-	-	-	-	-	-	-	-	-	-
1-6	65.4	64.1	62.3	61.3	57.5	60.4	64.6	72.4	62.3	61.0
1-6	64.3		61.7		58.7		67.4		58.5	

5.2.3.1 Sole**Table 5.2.3.1.a The number of stagings by modal stage for sole**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	21	9	7	6	5	18	13	6	-	13	9	13	9	-	7	9	6	9
2	2	16	9	12	16	20	26	17	-	27	19	19	19	-	16	22	26	2
3	3	-	-	1	1	3	2	3	-	3	-	1	1	-	1	-	-	-
4	1	7	-	6	4	3	1	2	-	1	7	3	1	1	2	-	3	3
5	17	11	6	-	-	-	1	-	-	-	8	6	13	-	17	10	7	12
6	-	-	-	-	-	-	1	-	-	-	1	1	-	-	-	-	-	-
1-6	44	43	22	25	26	44	43	28	0	44	43	42	43	1	43	41	42	26

Table 5.2.3.1.b Stage compositions by stage and reader for all stage readers for sole. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	89	100	83	86	57	100	89	100	-	100	67	89	89	0	88	67	44	100	83
2	8	60	62	83	92	58	73	82	-	92	54	64	65	-	58	78	80	8	63
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	0	50	-	100	50	50	0	100	-	0	50	50	0	-	100	0	50	100	47
5	86	57	100	0	0	0	14	0	-	0	57	57	83	-	100	57	50	60	47
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	36.4	67.4	72.7	72.0	65.4	56.8	63.6	78.6		75.0	56.8	67.4	69.8	0.0	72.1	68.3	66.7	50.0	64.3
rank	16	8	3	5	11	13	12	1		2	13	8	6	17	4	7	10	15	

Table 5.2.3.1.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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	0.44	0.00	0.17	0.14	0.57	0.00	0.56	0.00	-	0.00	0.33	0.11	0.11	3.00	0.13	0.67	1.56	0.00	0.33
2	0.81	1.08	0.54	0.25	0.15	- 0.08	0.00	0.18	-	- 0.08	0.81	0.36	0.77	-	1.19	0.30	0.20	2.08	0.51
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	- 1.00	- 1.00	-	0.00	- 1.00	- 1.00	- 2.50	0.00	-	- 1.00	- 1.00	- 1.00	1.00	-	0.00	1.00	- 1.00	0.00	- 0.57
5	- 0.57	- 0.43	0.00	- 1.50	- 2.25	- 3.00	- 2.57	- 3.00	-	- 2.86	- 0.71	- 1.14	- 0.50	-	0.00	- 1.43	- 1.50	- 0.80	- 1.37
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.2.3.2 Plaice

Table 5.2.3.2.a The number of stagings by modal stage for plaice

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	2	17	27	10	10	7	15	4	-	9	8	9	19	16	13	12	10	12	2
2	22	38	33	44	55	14	19	32	-	37	24	26	35	45	32	43	34	22	22
3	3	1	3	13	2	3	25	2	-	16	9	10	2	1	7	9	8	10	3
4	11	2	-	9	2	5	6	8	-	3	7	7	3	1	-	1	2	1	11
5	37	20	13	4	9	1	13	2	-	5	24	20	14	13	24	11	22	26	37
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	75	78	76	80	78	30	78	48	0	70	72	72	73	76	76	76	76	71	75

Table 5.2.3.2.b Stage compositions by stage and reader for all stage readers for plaice. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	6	82	88	47	47	40	53	43	-	78	57	64	71	82	59	71	47	75	60
2	40	78	72	60	89	61	22	94	-	67	51	51	74	98	43	76	74	54	65
3	100	50	50	0	0	100	100	50	-	100	50	50	50	50	0	100	100	100	61
4	100	50	0	0	0	50	100	-	-	50	0	0	100	50	0	0	50	50	42
5	83	83	33	25	18	0	25	0	-	33	92	83	73	75	17	64	83	100	56
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	42.7	78.2	67.1	48.8	66.7	50.0	33.3	70.8		62.9	58.3	58.3	72.6	88.2	40.8	71.1	69.7	67.6	61.7
rank	15	2	8	14	9	13	17	5		10	11	11	3	1	16	4	6	7	

Table 5.2.3.2.c 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]

5.2.3.3 Dab**Table 5.2.3.3.a The number of stagings by modal stage for dab**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	4	11	-	8	7	-	17	-	7	-	-	-	6	5	8	7	19	16
2	4	19	-	21	15	-	1	-	12	-	-	2	9	9	12	15	16	4
3	-	-	-	3	2	-	-	-	3	-	1	-	-	1	2	1	-	-
4	-	-	-	2	5	-	3	-	3	-	-	-	-	1	4	4	-	-
5	30	8	-	2	9	-	17	-	5	-	-	-	18	22	12	11	3	18
6	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	38	38	0	36	38	0	38	0	30	0	1	2	33	38	38	38	38	38

Table 5.2.3.3.b Stage compositions by stage and reader for all stage readers for dab. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	57	100	-	100	86	-	100	-	60	-	-	-	100	71	100	86	100	100	89
2	27	82	-	73	55	-	9	-	75	-	-	-	38	55	64	73	73	18	53
3	0	0	-	100	100	-	0	-	100	-	-	-	0	100	100	100	0	0	50
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	100	37	-	11	37	-	68	-	31	-	0	0	68	84	53	37	16	84	52
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	68.4	60.5		47.4	52.6		55.3		50.0		0.0	0.0	63.6	73.7	65.8	57.9	47.4	65.8	58.7
rank	2	6		11	9		8		10		13	13	5	1	3	7	11	3	

Table 5.2.3.3.c 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	1.71	0.00	-	0.00	0.57	-	0.00	-	0.40	-	-	-	0.00	0.71	0.00	0.57	0.00	0.00	0.34
2	2.18	0.18	-	0.36	0.64	-	1.09	-	0.00	-	-	-	1.88	1.36	0.82	0.82	-	-	0.74
3	-	-	-	0.00	0.00	-	1.00	-	0.00	-	-	-	-	0.00	0.00	0.00	-	-	-
	1.00	1.00											1.00				1.00	1.00	0.33
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2.05		2.21	1.58		1.26		1.81		2.00	3.00	1.00	0.37	1.16	1.53	3.00	0.58	1.39
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.2.3.4 Flounder

Table 5.2.3.4.a The number of stagings by modal stage for flounder

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-	-	2	-	2	9	1	3	4	-	-	-	1	-	-	-	2	-
2	22	27	21	14	18	13	14	22	22	-	19	20	21	19	14	23	22	22
3	2	1	6	10	4	7	5	2	1	-	10	10	1	2	5	2	3	2
4	2	1	3	5	5	7	9	7	8	-	2	-	4	1	1	5	-	1
5	10	8	4	7	7	-	6	2	2	-	5	6	9	4	16	5	9	12
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
1-6	36	37	36	36	36	36	35	36	37	0	36	36	36	26	36	35	36	37

Table 5.2.3.4.b 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	88	88	80	52	72	52	46	84	81	-	68	72	76	80	48	80	84	73	72
3	100	0	50	100	100	100	0	100	50	-	100	100	50	50	100	50	50	50	68
4	100	50	50	50	100	100	0	100	100	-	0	0	50	50	0	100	0	0	50
5	100	86	43	86	86	0	71	14	14	-	43	57	29	50	86	14	71	86	55
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	91.7	81.1	69.4	61.1	77.8	47.2	45.7	72.2	67.6		61.1	66.7	63.9	73.1	55.6	66.7	75.0	70.3	67.4
rank	1	2	8	13	3	16	17	6	9		13	10	12	5	15	10	4	7	

Table 5.2.3.4.c 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]

5.2.3.5 Lemon sole**Table 5.2.3.5.a The number of stagings by modal stage for lemon sole**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	3	3	-	1	3	8	8	-	-	-	4	3	3	1	3	2	1	-
2	9	11	-	10	9	2	7	-	-	-	7	10	3	-	3	10	-	-
3	3	1	-	8	7	6	3	-	-	-	4	4	-	-	4	4	-	-
4	-	-	-	2	1	5	1	-	-	-	-	1	-	-	1	4	-	-
5	6	6	-	-	-	-	2	-	-	-	6	2	-	-	10	1	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	21	21	0	21	20	21	21	0	0	0	21	20	6	1	21	21	1	0

Table 5.2.3.5.b Stage compositions by stage and reader for all stage readers for lemon sole. A weighted mean percent agreement is given by stage reader and all stage readers combined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALL
1	75	75	-	25	50	100	100	-	-	-	100	100	100	100	75	50	100	-	77
2	67	75	-	50	42	17	50	-	-	-	58	75	100	-	25	67	-	-	54
3	50	50	-	50	50	100	50	-	-	-	100	100	-	-	50	100	-	-	70
4	0	0	-	100	0	100	0	-	-	-	0	100	-	-	100	100	-	-	50
5	100	50	-	0	0	0	0	-	-	-	100	50	-	-	50	50	-	-	40
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-6	66.7	66.7		42.9	38.1	42.9	52.4				71.4	80.0	100.0	100.0	42.9	66.7	100.0		58.5
rank	6	6		10	13	10	9				5	4	1	1	10	6	1		

Table 5.2.3.5.c Bias in the comparison for lemon 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 ToR b: Validate macroscopic maturity determination with histological information

The comparison between the macroscopic staging and histological slides shows that there is an early development stage in gonads seen in the histological samples which cannot easily be seen macroscopically. The general percentage of agreement between histological slides and macroscopic scales is very low. This might be caused by the high number of samples outside the recommended sampling period. Since histological samples were low in number, no analysis is done on the agreement inside and outside the recommended sampling time (see 7.1).

6.1 First comparison, macroscopic pictures

Table 6.1 shows the % agreement for the different stages based on the validated histological slides for the first staging (based on pictures). For dab, only one histological slide was present so this species is not taken into account in this comparison. For sole, plaice and flounder the % agreement was definitely lower than the agreement based on the modal stage.

Table 6.1. % agreement per maturity stage for macroscopically staged samples compared to the histological slides

	SOLE	PLAICE	FLOUNDER
1	12.1	52.7	-
2	28.8	37.1	25.0
3	-	19.3	55.3
4	-	19.5	37.8
5	-	26.3	-
6	-	-	-
1-6	23.2	32.3	41.4
Number of samples	6	26	9

WKMSSPDF looked at some of the macroscopic and histological pictures for species with a very low agreement on modal stage. From this, the question was raised to what extent macroscopic staging is reliable (see 7.1).

6.2 Second comparison, macroscopic pictures

Table 6.2. shows the % agreement for the different stages based on the validated histological slides for the first staging (based on pictures). For dab, only three histological slides were present so this species is not taken into account in this comparison. For sole, plaice and flounder the % agreement was definitely lower than the agreement based on the modal stage. However, it was higher than the % agreement as shown in Table 6.1.

Table 6.2. % agreement per maturity stage for macroscopically staged samples compared to the histological slides

	SOLE	PLAICE	FLOUNDER
1	-	64.7	-
2	-	66.8	88.2
3	-	35.8	29.4
4	-	10.8	-
5	28.9	40.6	42.9
6	-	-	-
1-6	28.9	46.9	41.9
Number of samples	6	31	9

7 ToR e: Identify optimal sampling time to estimate maturity ogives

7.1 Optimal sampling time

In maturity ogives, only a distinction in mature and immature is made. So, the distinction between stage 1 on one hand and 2-5 on the other, is the most important to be made from an assessment point of view.

Figure 7.1 shows the increase of absolute bias in maturity stage per month for cod (Vitale *et al.*, 2006). The bias decreases clearly just prior to the spawning season. From experience, this pattern applies for other fish species, too. As it is difficult to identify the proper maturity stage when fish is not clearly developing, data collection for maturity ogives is recommended during the pre-spawning season. This implies that sampling for maturity staging for sole, plaice, dab and flounder should be done during late fourth quarter until the end of the first quarter.

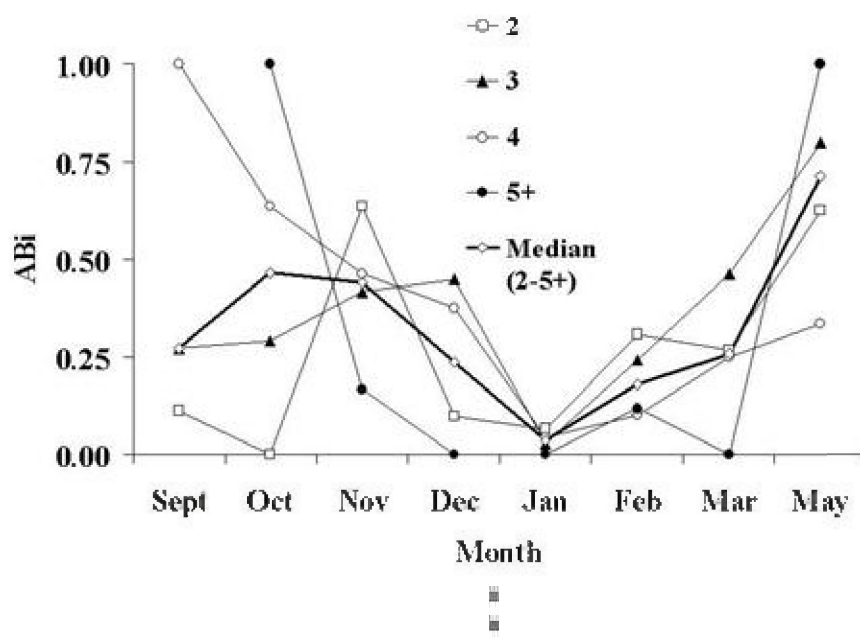


Figure 7.1. Monthly trend of the absolute bias (ABi) within age classes for cod (Vitale *et al.*, 2006). The absolute bias was resulting from the comparison between histological analysis and visual inspection of gonads for each age class on a monthly basis.

For assessment purposes, only data from one month before the spawning season (to be identified based on the collected data) until the start of the spawning season should be used for the estimation of maturity ogives for sole, plaice, dab and flounder. Any macroscopic staging outside of this period can be misinterpreted and should not be used for maturity ogives.

For sole, the best sampling period is dependent on the geographical area. The spawning season in Turkey's waters is from November till March. Sole along the Portuguese coasts spawn from January till March (April?). In the Bay of Biscay sole spawns the second half of February and March and when summer temperatures are above normal some sole in the Bay of Biscay have a second spawning in September. In the English Channel and southern North Sea spawning occurs from half March till the end of May. In the Irish Sea spawning has been observed from November till February.

For plaice, dab and flounder, there was a discussion on what time of year macroscopic maturity staging can be carried out usefully. First quarter is best for these species but the end of fourth quarter can be used, too.

However, collecting maturity information outside the defined period might be interesting for scientific purposes other than stock assessment. For these purposes, it is recommended that histological staging is done.

7.2 Understanding species biology

When staging fish, it is important to have an understanding of the biology of the fish and its reproductive cycle in the sampling area. This helps to distinguish the transitions between the stages.

It is recommended that institutes carry out in-house workshops on the reproductive biology of the fish and maturity staging.

8 Guidelines for future workshops on maturity staging

8.1 Staging from fresh material vs. pictures

The WKMSSPDF clearly showed that staging from pictures is more difficult than staging from fresh material (see 5.2.2).

Generic comments were that some of the stage descriptions were only suitable for fresh samples and the characteristics were not visible on the pictures. There is a need for clear descriptions on pictures to be taken (see 8.2).

It is recommended that the process of trial-discussion-retrial is based on fresh samples. This means that at least two staging sessions on fresh material have to be done during future workshops. As a consequence, for all species named in a workshop fresh material has to be available in the neighbourhood of the hosting institute.

This means that the best time of year to do a workshop on maturity staging is when the diversity in maturity stages is high (see 7.1).

It is recommended to use images as a tool for calibration prior to a workshop or to identify the need for future workshops.

Also, pictures will be useful as reference material during a workshop (see 8.2).

8.2 Guidelines for the pictures to be taken

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

- General marks for staging from pictures:
- Pictures have to be taken on fresh fish.
- Add at least sampling time, area, unique sampling number, fish length and species in the picture.
- Take care that the samples should be clean/tidy, preferable without intestines.
- Take at least six pictures:

- 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 to see if fish is running.
 - 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.
- For the best results is there a certain time when the photos are not that useful or not required (see 7.1). However, getting as many different stages is useful as an educational tool.

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

8.3 Guidelines for the storage and use of pictures in a workshop

When using pictures for a comparative workshop, the ICES groupnet is not suitable to use. The pictures take up a lot of space and the system gets very slow when many people try to use it simultaneously. When organising a maturity workshop where stagings from pictures will be done, a server prepared for this purpose has to be used. The WebGR tool (<http://webgr.berlios.de/doku.php>) might be the right application to support maturity staging workshops.

9 Next meeting

WKMSSPDF discussed the need for a next maturity workshop on the same species (sole, plaice, dab and flounder). Since all countries intend to accept the proposed common scale, the group felt the need for a calibration workshop in January 2012. Proposed location for this meeting is Oostende, Belgium.

Judging from WKMSSPDF a workshop on maturity staging for other commercial flatfish species (turbot, brill, lemon sole, witch flounder) might be useful. However, the lemon sole staging during WKMSSPDF shows that having the expertise in staging one species of flatfish can be adequate to stage other species of flatfish.

To define whether a workshop is necessary, it is recommended to do a calibration exercise prior to organising a workshop, for example using the WebGR tool (see <http://webgr.berlios.de/doku.php>).

Before setting up the next meeting, the number of pictures to stage during the workshop should be considered in order to meet the need for time to discuss individual cases as well as maintaining statistical accuracy. Based on the experiences of WKMSSPDF it is recommended that roughly 30 fish per species would be an adequate number to judge at each round during the workshop.

10 References

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Annex 2: Agenda WKMSSPDF IJmuiden (Netherlands), 22-26 February 2010

Monday 22 February

AM

Logistics

Introduction of people

Introduction to the workshop: terms of reference

Adoption of the agenda

Appointment of rapporteurs

Creating overview of currently used maturity scales

- Per species
- Per institute

PM

Proposed maturity scale per species:

- Do the descriptions fit per species?

Do the currently used scales fit into the proposed scale?

- Fine-tuning of the descriptions per species in subgroups

Adoption of new maturity scale

Tuesday 23 February

AM

Staging from pictures

- Starting with familiar species from own country, staging in new scale
- Staging pre-defined selection of pictures – set1

PM

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

Wednesday 24 February

AM

- Staging from fresh samples

PM

- Comparison of results fresh staging

Thursday 25 February

AM

- Staging pre-defined selection of pictures – set2

PM

- Comparison of results macroscopic staging
- Comparison macroscopic staging to histological slides

Friday 26 February

AM

Evaluation of the new maturity scales:

- Did we miss things in the description?
- Did we include all recommendations made during the WKMSSPDF?

Recommendations

Finalise report – text check

Annex 3: WKMSSPDF terms of reference for the next meeting

The Workshop on sexual maturity staging of sole, plaice, dab and flounder [WKMSSPDF] (Chairs: Ingeborg de Boois and Cindy van Damme, The Netherlands) will meet in Oostende, Belgium, 9-13 January 2012 to:

- a) Report on the use of the 2010 proposed common scale;
- 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.

WKMSSPDF will report by XXXXX-2012 for the attention of ACOM and PGCCDBS.

Supporting Information

PRIORITY:	The maturity stage is an important biological parameter to be used in the calculation of maturity ogives (and therefore of Spawning Stock Biomass), for the definition of the spawning season of a species, for the monitoring of long-term changes in the spawning cycle, and for many other research needs regarding the biology of fish.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	<p>During the 2010 workshop a common maturity scale with objective common criteria was proposed for sole, plaice, dab and flounder. Laboratories involved in collection maturity data agreed to use the common scale for reporting.</p> <p>This workshop has the objective to report on the use of the 2010 proposed scale and to calibrate maturity staging between the different laboratories involved in staging.</p> <p>The expectation of TOR a) has the goal of measuring the usefulness of the new 2010 maturity scales.</p> <p>TOR b) to validate the criteria and descriptions to classify maturity stages of the new 2010 scales.</p> <p>TOR c and d) calibrate maturity staging between the different laboratories.</p> <p>TOR e) validate with histological analysis the macroscopic maturity stage, mainly the resting stages that are incorrectly classified as immature.</p> <p>It is recommended that the Workshop be organised in January 2012. Participating institutes will be able to test the new scale and collect samples during 2010 and 2011.</p>
RESOURCE REQUIREMENTS:	<p>Before the Workshop the chairs will setup a sampling plan for collecting samples for to be used during workshop. The sampling will be carried out during 2010-11.</p> <p>For all species, the sampling parameters are: total length; gonad visual inspection - maturity stage by the new common maturity scale; total weight; gonad weight; liver weight; gutted weight; gonad photo; age; histological maturity stage; microscopic preparation photo.</p> <p>This workshop will be based on the analysis of both digital photos of gonads and fresh gonads. Therefore facilities suitable to examine fresh biological material must be available during the workshop. It would be necessary to have a web server for storage and easy access to the photos collected by the participants before the workshop.</p>
PARTICIPANTS:	In view of its relevance to the DCR, the Workshop is expected to attract wide interest from ICES Member States that participate in biological sampling of sole, plaice, dab and flounder.

SECRETARIAT FACILITIES:	
FINANCIAL:	<p>To obtain all biological data before the Workshop, funding is needed for buying fresh ungutted fish, to estimate age and to process gonads histology.</p> <p>To ensure wide attendance of relevant experts, additional funding will be required, preferably through the EU, e.g. by making attendance to the Workshop eligible under the DCR.</p>
LINKAGES TO ADVISORY COMMITTEES:	ACOM
LINKAGES TO OTHER COMMITTEES OR GROUPS:	<p>This workshop is proposed by PGCCDBS. Outcomes from this Workshop will be of interest to all Working and Study Groups related to sole, plaice, dab and flounder, namely WGNSSK, WGBFAS, WGSSDS and WGNSDS, as well as to survey groups like the IBTSWG and WGBEAM.</p>
LINKAGES TO OTHER ORGANIZATIONS:	There is a direct link with the EU DCR.

Annex 4: Recommendations

RECOMMENDATION	FOR FOLLOW UP BY:
1. As it is difficult to identify the proper maturity stage when fish is not clearly develop-ing, data collection for maturity ogives is recommended during the pre-spawning season. This implies that sampling for macroscopic maturity staging for sole, plaice, dab and flounder should be done during late fourth quarter until the end of the first quarter.	Research institutes
2. For assessment purposes, only data from one month before the spawning season (to be identified based on the collected data) until the start of the spawning season should be used for the estimation of maturity ogives for sole, plaice, dab and floun-der. Any macroscopic staging outside of this period can be misinterpreted and should not be used for maturity ogives.	WGNSSK and other assessment groups using maturity data of sole, plaice, dab and flounder.
3. Collecting maturity information outside the defined period might be interesting for scientific purposes other than stock assessment. For these purposes, it is recommended that histological staging is done.	Research institutes
4. It is recommended that institutes carry out in-house workshops on the reproductive biology of the fish and maturity staging.	Research institutes
5. It is recommended that the process of trial-discussion-retrial is based on fresh samples. This means that at least two staging sessions on fresh material have to be done during future workshops.	Maturity workshop organisers/PGCCDBS to add in the guidelines for maturity workshops
6. It is recommended to use images as a tool for calibration prior to a workshop or to identify the need for future workshops.	Maturity workshop organisers/PGCCDBS to add in the guidelines for maturity workshops
7. Based on the experiences of WKMSSPDF it is recommended to set the maximum fish to stage in one session to 120. This applies for fresh samples as well as pictures.	Maturity workshop organisers
8. If time allows during a survey, it is recommended to put the content of a gonad under a microscope in case of disagreement or doubt on the maturity stage of a fish.	Research institutes

Annex 5: Overview of currently used scales per country

Table VI.1 Female descriptions

COUNTRY	INSTITUTE	SPECIES	SCALE NAME	STAGES	STAGES_TEXT	DESCRIPTION
DEN	DTU Aqua	all	IBTS scale (gadoids)	1	Juvenile/immature	No sex determination: risk of mistaking gonads for bladder, Sex determination: Transparent, later pinkish/light orange, cast thin and clear, blood vessels hardly discernible.
DEN	DTU Aqua	all	IBTS scale (gadoids)	2	maturing	Firm, colouration ranges from reddish orange to creamy orange with granulated/oocytes clearly visible in issue. Blood vessels larger and diversified.
DEN	DTU Aqua	all	IBTS scale (gadoids)	3	spawning	Distended, few to many hydrated eggs visible in tissue among vitelogenic oocytes or in lumen, occasionally running.
DEN	DTU Aqua	all	IBTS scale (gadoids)	4	spent	Slack with greyish cast, rich in blood vessels.
DEN	DTU Aqua	all	IBTS scale (gadoids)	5	resting/skipped spawning* (ecosystem state indicators*)	No visible development - similar to Immature but sometimes with greyish cast.
DEN	DTU Aqua	all	IBTS scale (gadoids)	6	abnormal* (ecosystem state indicators*)	Hard parts (connective tissue), only one lobe developed, intersex, or similar - fecundity at least partly reduced.
NED	IMARES	all	4 point scale	2	immature	No signs of development
NED	IMARES	all	4 point scale	4	mature	Developing or resting after spent and recovering
NED	IMARES	all	4 point scale	6	spawning	Eggs run with slight pressure, translucent eggs in ovary or cavity
NED	IMARES	all	4 point scale	8	spent/resting	Ovaries empty, a few eggs remain. Solid, no signs of development.
UK-ENG	CEFAS	all	5 point scale	I	immature	Ovaries small. Ovary wall thin and easily broken. Internally yellowish-orange (although can be variable)
UK-ENG	CEFAS	all	5 point scale	M	maturing	Ovaries are extending down the side of the body of the fish. There are no signs of Hyaline eggs
UK-ENG	CEFAS	all	5 point scale	H	hyaline	Hyaline eggs present, may be just one or many but ovaries will not run, even under moderate pressure.
UK-ENG	CEFAS	all	5 point scale	R	running	Will extrude eggs under light pressure to advanced stage of extruding eggs freely with some eggs still in the gonad.
UK-	CEFAS	all	5 point scale	S	spent	Ovaries shrunken with few

ENG							residual eggs and slime. Ovaries becoming tighter, no sign of egg development.
SWE	IMR	DAB	no staging				
GER	vTI\OSF	FLE	8 point scale (cod)	1	juvenile		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	2	resting		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	3	maturing		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	4	maturing		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	5	any ripe eggs		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	6	spawning		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	7	partially spent		no description available
GER	vTI\OSF	FLE	8 point scale (cod)	8	spent		no description available
LAT	BIOR	FLE	5 point scale	2	immature		Ovaries small and firm; pale pink or reddish and somewhat transparent.
LAT	BIOR	FLE	5 point scale	3	ripening		Ovaries reddish and numerous blood vessels; opaque eggs are now visible to naked eye and give the ovaries a granular appearance.
LAT	BIOR	FLE	5 point scale	4	ripe		Has a few clear eggs at the earliest stage progressing to having mainly clear eggs but eggs do not extrude freely with slight pressure. Spawning has not started.
LAT	BIOR	FLE	5 point scale	5	spawning		As Stage IV but eggs running freely on slight pressure.
LAT	BIOR	FLE	5 point scale	6	spent		Ovaries soft; flabby and bloody; practically no eggs remain; purple in colour.
LIT	FRL	FLE	6 point scale	0	juvenile		unsexed
LIT	FRL	FLE	6 point scale	2	immature		gonads are firm, color greyish to pinkish
LIT	FRL	FLE	6 point scale	3	maturing		maturing. Eggs are visible in tissue. when you brush through tissue , the eggs remain inseparable
LIT	FRL	FLE	6 point scale	4	maturing		maturing. Eggs are clearly visible in tissue. when you brush through tissue , you get separable eggs
LIT	FRL	FLE	6 point scale	5	spawning		when slightly shake or push the fish, milt (male) flows and eggs are running (female)
LIT	FRL	FLE	6 point scale	6/2	spent/resting		spent/resting (egg bag empty, color greyish to redish)

POL	SFI	FLE	8 point scale	1	immature	
POL	SFI	FLE	8 point scale	2	resting	
POL	SFI	FLE	8 point scale	3	maturing	
POL	SFI	FLE	8 point scale	4	maturing	
POL	SFI	FLE	8 point scale	5	maturing	
POL	SFI	FLE	8 point scale	6	spawning	
POL	SFI	FLE	8 point scale	7	half spawned	
POL	SFI	FLE	8 point scale	8	spent	
SWE	ICR	FLE	6 point scale	1	juvenile	Ovaries small, stretched, whitish and translucent. No sign of development.
SWE	ICR	FLE	6 point scale	2	maturing	Development/growth started, started colouring, eggs developing and the ovaries start filling the body cavity, but no eggs expelled when putting a medium pressure on the body externally
SWE	ICR	FLE	6 point scale	3	spawning	Spawning or very near spawning. Gonads big, colour pink to yellow. The roe flows easily at medium pressure.
SWE	ICR	FLE	6 point scale	4	spent	Ovaries shrunk with very few eggs left and a lot of mucus.
SWE	ICR	FLE	6 point scale	5	resting	Ovaries firm, not translucent, no sign of growing.
SWE	ICR	FLE	6 point scale	9	abnormal	If possible, stage 1-5 and register sickness code "abnormal gonads". Examples abnormality hard parts (connective tissue), only one lobe developed, intersex.
SWE	IMR	FLE	no staging			
BEL	ILVO	PLE	7 point scale	1	immature	
BEL	ILVO	PLE	7 point scale	2	spent, recovering	
BEL	ILVO	PLE	7 point scale	3	half full	
BEL	ILVO	PLE	7 point scale	4	full	
BEL	ILVO	PLE	7 point scale	5	spawning	
BEL	ILVO	PLE	7 point scale	6	running	
BEL	ILVO	PLE	7 point scale	7	spent	
GER	vTI	PLE	4 point scale	1	juvenile	
GER	vTI	PLE	4 point scale	2	maturing	
GER	vTI	PLE	4 point scale	3	running	
GER	vTI	PLE	4 point scale	4	spent/resting	
GER	vTI\OSF	PLE	8 point scale (cod)	1	juvenile	no description available
GER	vTI\OSF	PLE	8 point scale (cod)	2	resting	no description available
GER	vTI\OSF	PLE	8 point scale (cod)	3	maturing	no description available
GER	vTI\OSF	PLE	8 point scale (cod)	4	maturing	no description available

GER	vTI\OSF	PLE	8 point scale (cod)	5	any ripe eggs	no description available
GER	vTI\OSF	PLE	8 point scale (cod)	6	spawning	no description available
GER	vTI\OSF	PLE	8 point scale (cod)	7	partially spent	no description available
GER	vTI\OSF	PLE	8 point scale (cod)	8	spent	no description available
POL	SFI	PLE	8 point scale	1	immature	
POL	SFI	PLE	8 point scale	2	resting	
POL	SFI	PLE	8 point scale	3	maturing	
POL	SFI	PLE	8 point scale	4	maturing	
POL	SFI	PLE	8 point scale	5	maturing	
POL	SFI	PLE	8 point scale	6	spawning	
POL	SFI	PLE	8 point scale	7	half spawned	
POL	SFI	PLE	8 point scale	8	spent	
SWE	IMR	PLE	9 point scale	1	juvenile	
SWE	IMR	PLE	9 point scale	2	juvenile	
SWE	IMR	PLE	9 point scale	3	maturing	
SWE	IMR	PLE	9 point scale	4	maturing	
SWE	IMR	PLE	9 point scale	5	maturing	
SWE	IMR	PLE	9 point scale	6	running	
SWE	IMR	PLE	9 point scale	7	spent	
SWE	IMR	PLE	9 point scale	8	resting	
SWE	IMR	PLE	9 point scale	9	abnormal	
BEL	ILVO	SOL	7 point scale	1	immature	
BEL	ILVO	SOL	7 point scale	2	spent, recovering	
BEL	ILVO	SOL	7 point scale	3	half full	
BEL	ILVO	SOL	7 point scale	4	full	
BEL	ILVO	SOL	7 point scale	5	hydrated eggs	
BEL	ILVO	SOL	7 point scale	6	running	
BEL	ILVO	SOL	7 point scale	7	spent	
FRA	IFREMER	SOL	8 point scale	0	not determined	
FRA	IFREMER	SOL	8 point scale	1	immature	
FRA	IFREMER	SOL	8 point scale	2	resting	
FRA	IFREMER	SOL	8 point scale	3	mature without egg	
FRA	IFREMER	SOL	8 point scale	4	maturing with eggs	
FRA	IFREMER	SOL	8 point scale	5	hyaline	
FRA	IFREMER	SOL	8 point scale	6	spawning	
FRA	IFREMER	SOL	8 point scale	7	afterlay	
GER	vTI	SOL	4 point scale	1	juvenile	
GER	vTI	SOL	4 point scale	2	maturing	
GER	vTI	SOL	4 point scale	3	running	
GER	vTI	SOL	4 point scale	4	spent/resting	
POR	IPIMAR	SOL	7 point scale	1	immature	
POR	IPIMAR	SOL	7 point scale	2	immature	

POR	IPIMAR	SOL	7 point scale	3	maturing
POR	IPIMAR	SOL	7 point scale	4	maturing
POR	IPIMAR	SOL	7 point scale	5	running
POR	IPIMAR	SOL	7 point scale	6	partially spent
POR	IPIMAR	SOL	7 point scale	7	spent
SWE	IMR	SOL	no staging		
TUR	EGE	SOL	5 point scale	1	immature
TUR	EGE	SOL	5 point scale	2	resting
TUR	EGE	SOL	5 point scale	3	mature
TUR	EGE	SOL	5 point scale	4	half spent
TUR	EGE	SOL	5 point scale	5	spent

Table VI.1 Male descriptions

COUNTRY	INSTITUTE	SPECIES	SCALE NAME	STAGES	STAGES_TEXT	DESCRIPTION
DEN	DTU Aqua	all	IBTS scale (gadoids)	1	Juvenile/immature	No sex determination: Gonads difficult to identify, Sex determinaiton: Transparent, later reddish to white, vascularisation is limited.
DEN	DTU Aqua	all	IBTS scale (gadoids)	2	maturing	Whitish to almost opaque reddish-white, blood vessels more prominent, empty transparent spermatoducts.
DEN	DTU Aqua	all	IBTS scale (gadoids)	3	spawning	Opaque creamy white colour to reddish late in stage, semen visible in spermatoduct, milt often flows at light pressure.
DEN	DTU Aqua	all	IBTS scale (gadoids)	4	spent	Contracted, empty and flabby lobules, colour deep pink to reddish-purple, bloodshot, potentially with greyish cast.
DEN	DTU Aqua	all	IBTS scale (gadoids)	5	resting/skipped spawning* (ecosystem state indicators*)	No visible development, spermatoducts often with greyish cast, similar to immature, early maturing.
DEN	DTU Aqua	all	IBTS scale (gadoids)	6	abnormal* (ecosystem state indicators*)	Adipose tissue, only one lobe developed, intersex, or similar.
NED	IMARES	all	4 point scale	2	immature	No signs of development
NED	IMARES	all	4 point scale	4	mature	Developing or resting after spent and recovering
NED	IMARES	all	4 point scale	6	spawning	Roe runs with slight pressure
NED	IMARES	all	4 point scale	8	spent/resting	Testes empty. Solid, no signs of development.
UK-ENG	CEFAS	all	5 point scale	I	immature	Gonads tight up against back of gut cavity and very small - not usually larger than 10mm

						x 2mm.
UK-ENG	CEFAS	all	5 point scale	M	maturing	Colour is progressing towards creamy white and the testes are filling more of the body cavity. Sperm cannot be extruded under moderate pressure. at lastest phase the gonad can become bulbus.
UK-ENG	CEFAS	all	5 point scale	H	hyaline	no used
UK-ENG	CEFAS	all	5 point scale	R	running	Sperm will extrude under light pressure to advanced stage of extruding sperm freely with some sperm still in the gonad. Sperm evident in the ducts.
UK-ENG	CEFAS	all	5 point scale	S	spent	Testes shrunk with little sperm in the gonads but often some in the gonoducts which can be extruded under light pressure. The gonad can shrink back to very small size but unlike immature stage the gonad will be thicker.
SWE	IMR	DAB	no staging			
GER	vTI\OSF	FLE	8 point scale (cod)	1	juvenile	
GER	vTI\OSF	FLE	8 point scale (cod)	2	resting	
GER	vTI\OSF	FLE	8 point scale (cod)	3	maturing	
GER	vTI\OSF	FLE	8 point scale (cod)	4	maturing	
GER	vTI\OSF	FLE	8 point scale (cod)	5	any ripe eggs	
GER	vTI\OSF	FLE	8 point scale (cod)	6	spawning	
GER	vTI\OSF	FLE	8 point scale (cod)	7	partially spent	
GER	vTI\OSF	FLE	8 point scale (cod)	8	spent	
LAT	BIOR	FLE	5 point scale	2	immature	Testes slender; appear as a clear
LAT	BIOR	FLE	5 point scale	3	ripening	Testes begin to turn white, no milt runs when pressure is applied.
LAT	BIOR	FLE	5 point scale	4	ripe	Testes distinctly wavy white and quite distended; a small amount of milt may be forced by pressure.
LAT	BIOR	FLE	5 point scale	5	spawning	Testes very white and fully extended; milt runs freely at the slightest pressure.
LAT	BIOR	FLE	5 point scale	6	spent	Testes shrunk and reddish;

						some blood left in organ.
LIT	FRL	FLE	6 point scale	0	juvenile	unsexed
LIT	FRL	FLE	6 point scale	2	immature	imature (never spawn before)
LIT	FRL	FLE	6 point scale	3	maturing	maturing. when you cut opaque, the edge of cut remains firm
LIT	FRL	FLE	6 point scale	4	maturing	maturing. when you cut opaque, the edge of cut is getting rounded, just like melting
LIT	FRL	FLE	6 point scale	5	spawning	spawning
LIT	FRL	FLE	6 point scale	6/2	spent/resting	spent/resting
POL	SFI	FLE	8 point scale	1	immature	ovaries small and firm, glassy to pale pink, virgin
POL	SFI	FLE	8 point scale	2	resting	ovaries small and firm, pale pink or reddish and somewhat transparent
POL	SFI	FLE	8 point scale	3	maturing	ovaries nontransparent, blood vessels present, opaque eggs visible to naked eye
POL	SFI	FLE	8 point scale	4	maturing	ovaries extended to a half of its size, nontransparent eggs filled with yolk visible
POL	SFI	FLE	8 point scale	5	maturing	ovaries fully elongated, eggs like stage 4, few fully developed transparent eggs
POL	SFI	FLE	8 point scale	6	spawning	eggs (most of them transparent) running freely on slight pressure, orange eggs still present
POL	SFI	FLE	8 point scale	7	half spawned	shortened ovaries flabby and bloody, fluids and eggs run after pressure
POL	SFI	FLE	8 point scale	8	spent	ovaries shortened and bloody, only few eggs visible, fluids inside
SWE	ICR	FLE	6 point scale	1	juvenile	Gonads very thin, translucent ribbons with non-vascularised blood vessel. No sign of development.
SWE	ICR	FLE	6 point scale	2	maturing	Development/growth started, colour creamy white and the gonads start filling the body cavity, but no milt expelled when putting a medium pressure to the body externally
SWE	ICR	FLE	6 point scale	3	spawning	Spawning or very near spawning. Gonads big, colour completely white. The milt flows easily at medium pressure.
SWE	ICR	FLE	6 point scale	4	spent	Gonads shrinkd with very little milt left. Often milt left

						in the gonadoduct.
SWE	ICR	FLE	6 point scale	5	resting	Gonads firm, not translucent, no sign of growing.
SWE	ICR	FLE	6 point scale	9	abnormal	If possible, stage 1-5 and register sickness code "abnormal gonads". Examples abnormality hard parts (connective tissue), only one lobe developed, intersex.
SWE	IMR	FLE	no staging			
BEL	ILVO	PLE	7 point scale	1	immature	
BEL	ILVO	PLE	7 point scale	2	spent, recovering	
BEL	ILVO	PLE	7 point scale	3	half full	
BEL	ILVO	PLE	7 point scale	4	full	
BEL	ILVO	PLE	7 point scale	5	spawning	
BEL	ILVO	PLE	7 point scale	6	running	
BEL	ILVO	PLE	7 point scale	7	spent	
GER	vTI	PLE	4 point scale	1	juvenile	
GER	vTI	PLE	4 point scale	2	maturing	
GER	vTI	PLE	4 point scale	3	running	
GER	vTI	PLE	4 point scale	4	spent/resting	
GER	vTI\OSF	PLE	8 point scale (cod)	1	juvenile	
GER	vTI\OSF	PLE	8 point scale (cod)	2	resting	
GER	vTI\OSF	PLE	8 point scale (cod)	3	maturing	
GER	vTI\OSF	PLE	8 point scale (cod)	4	maturing	
GER	vTI\OSF	PLE	8 point scale (cod)	5	any ripe eggs	
GER	vTI\OSF	PLE	8 point scale (cod)	6	spawning	
GER	vTI\OSF	PLE	8 point scale (cod)	7	partially spent	
GER	vTI\OSF	PLE	8 point scale (cod)	8	spent	
POL	SFI	PLE	8 point scale	1	immature	
POL	SFI	PLE	8 point scale	2	resting	
POL	SFI	PLE	8 point scale	3	maturing	
POL	SFI	PLE	8 point scale	4	maturing	
POL	SFI	PLE	8 point scale	5	maturing	
POL	SFI	PLE	8 point scale	6	spawning	
POL	SFI	PLE	8 point scale	7	half spawned	
POL	SFI	PLE	8 point scale	8	spent	
SWE	IMR	PLE	9 point scale	1	juvenile	
SWE	IMR	PLE	9 point scale	2	juvenile	
SWE	IMR	PLE	9 point scale	3	maturing	
SWE	IMR	PLE	9 point scale	4	maturing	

SWE	IMR	PLE	9 point scale	5	maturing
SWE	IMR	PLE	9 point scale	6	running
SWE	IMR	PLE	9 point scale	7	spent
SWE	IMR	PLE	9 point scale	8	resting
SWE	IMR	PLE	9 point scale	9	abnormal
BEL	ILVO	SOL	7 point scale	1	immature
BEL	ILVO	SOL	7 point scale	2	spent, recovering
BEL	ILVO	SOL	7 point scale	3	half full
BEL	ILVO	SOL	7 point scale	4	full
BEL	ILVO	SOL	7 point scale	5	hydrated eggs
BEL	ILVO	SOL	7 point scale	6	running
BEL	ILVO	SOL	7 point scale	7	spent
FRA	IFREMER	SOL	4 point scale	0	not determined
FRA	IFREMER	SOL	4 point scale	3	maturing
FRA	IFREMER	SOL	4 point scale	6	spawning
FRA	IFREMER	SOL	4 point scale	7	afterlay
GER	vTI	SOL	4 point scale	1	juvenile
GER	vTI	SOL	4 point scale	2	maturing
GER	vTI	SOL	4 point scale	3	running
GER	vTI	SOL	4 point scale	4	spent/resting
POR	IPIMAR	SOL	7 point scale	1	immature
POR	IPIMAR	SOL	7 point scale	2	immature
POR	IPIMAR	SOL	7 point scale	3	maturing
POR	IPIMAR	SOL	7 point scale	4	maturing
POR	IPIMAR	SOL	7 point scale	5	running
POR	IPIMAR	SOL	7 point scale	6	partially spent
POR	IPIMAR	SOL	7 point scale	7	spent
SWE	IMR	SOL	no staging		
TUR	EGE	SOL	4 point scale	1	immature
TUR	EGE	SOL	4 point scale	2	resting
TUR	EGE	SOL	4 point scale	3	mature
TUR	EGE	SOL	4 point scale	5	spent

Annex 6: Length composition, sex ratio and sampling range of picture samples

Table VI.1.1 Sex ratio for first picture set

	FEMALE	MALE	TOTAL
Sole	22	13	35
Plaice	34	20	54
Dab	11	8	19
Flounder	8	6	14
Lemon sole	12	4	16

Table VI.1.2 Length composition and sampling range for first picture set

SAMPLING MONTH	LENGTH CLASS (CM)	SOLE	PLAICE	DAB	FLOUNDER	LEMON SOLE
March	< 15					
	15-19				1	
	20-24					
	25-29		1		1	
	30-34		2		2	
	35-39				1	
	>= 40		1			
April	< 15					
	15-19					
	20-24				1	
	25-29	3			1	
	30-34	2			1	
	35-39					
	>= 40					
May	< 15					
	15-19					
	20-24					
	25-29	1				
	30-34	3				
	35-39					
	>= 40					
June	< 15					
	15-19					
	20-24					
	25-29	2				
	30-34					
	35-39					
	>= 40					

SAMPLING MONTH	LENGTH CLASS (CM)	SOLE	PLAICE	DAB	FLOUNDER	LEMON SOLE
July	< 15					
	15-19					
	20-24	2				
	25-29	1				
	30-34					
	35-39					
	>= 40					
August	< 15					
	15-19					
	20-24	2				
	25-29	1	4		2	
	30-34	1	1		2	
	35-39		1			
	>= 40	1	1		1	
September	< 15					
	15-19					
	20-24					
	25-29	2	1	1		
	30-34		1			
	35-39	1				
	>= 40		1			
November	< 15					
	15-19					
	20-24					
	25-29				1	
	30-34					
	35-39					
	>= 40					
December	< 15					
	15-19					
	20-24					
	25-29					
	30-34					
	35-39		1			
	>= 40		1			
Unknown	< 15			5		
	15-19	3	5	6		1
	20-24	4	9	4		7
	25-29	3	7	3		3
	30-34	2	4			5
	35-39	1	5			
	>= 40		8			
Total		35	54	19	14	16

Table VI.2.1 Sex ratio for the fresh samples

	FEMALE	MALE	TOTAL
Sole	26	4	30
Plaice	23	7	30
Dab	30	0	30
Flounder	16	14	30

Table VI.3.1 Sex ratio for first picture set

	FEMALE	MALE	UNKNOWN	TOTAL
Sole	28	15	4	47
Plaice	41	44	3	88
Dab	16	22		38
Flounder	14	22	1	37
Lemon sole	14	7		21

Table VI.3.2 Length composition and sampling range for second picture set

Note: For flounder, there were more pictures available in this set, but no length distribution was available for those pictures. However, the length of the fish was visible on the pictures since those were taken from fish on a measuring board. Also, some additional pictures for sole were available.

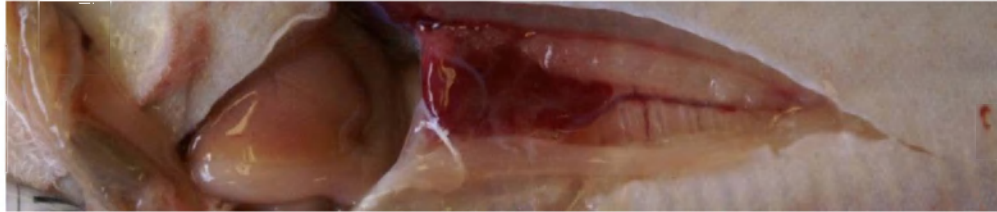
SAMPLING MONTH	LENGTH CLASS (CM)	SOLE	PLAICE	DAB	FLOUNDER	LEMON SOLE
March	< 15					
	15-19		1		1	
	20-24				1	
	25-29		2		2	
	30-34		2			
	35-39				1	
	>= 40		1			
April	< 15					
	15-19					
	20-24				2	
	25-29					
	30-34	1				
	35-39					
	>= 40					
May	< 15					
	15-19					
	20-24					
	25-29					
	30-34	1				
	35-39					
	>= 40					

SAMPLING MONTH	LENGTH CLASS (CM)	SOLE	PLAICE	DAB	FLOUNDER	LEMON SOLE
June	< 15 15-19 20-24 25-29 30-34 35-39 ≥ 40	2				
July	< 15 15-19 20-24 25-29 30-34 35-39 ≥ 40	1				
August	< 15 15-19 20-24 25-29 30-34 35-39 ≥ 40	1 2 1 1			2 2	
September	< 15 15-19 20-24 25-29 30-34 35-39 ≥ 40		1 1 5 2 1	1 1		
October	< 15 15-19 20-24 25-29 30-34 35-39 ≥ 40	3				
November	< 15 15-19 20-24 25-29 30-34 35-39 ≥ 40				1 2 1	

SAMPLING MONTH	LENGTH CLASS (CM)	SOLE	PLAICE	DAB	FLOUNDER	LEMON SOLE
December	< 15					
	15-19					
	20-24					
	25-29					
	30-34					
	35-39		2			
	>= 40		1			
Unknown	< 15		1	5		2
	15-19	4	7	7		2
	20-24	4	12	5		7
	25-29	4	7	3		3
	30-34	4	7	1		5
	35-39	2	8			2
	>= 40		9			
Total		42	71	24	16	21

Annex 7: Reference pictures for maturity stages of flatfish

Figure VII.1.1 Reference pictures for female sole

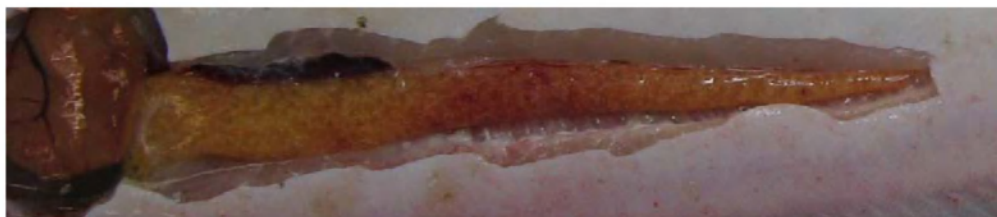


Stage 1: Primary: Translucent => line test

Secondary: Pale in colour, gonad less than $\frac{1}{2}$ body length, bloodvessels are threadlike
(original picture: UK2009_SOL_018d_Jul.jpg, fish length 19 cm)



Stage 2: Bloodvessels are taut, tissue is healthy and recovered in comparison to stage 5;
Eggs can be from grains through to non-hydrated fully developed eggs
(original picture: PT2009_SOL_04_Apr.jpg)



Stage 3: Hyaline/hydrated eggs are distinguishable
(original picture: PT2009_SOL_05_Apr.jpg)

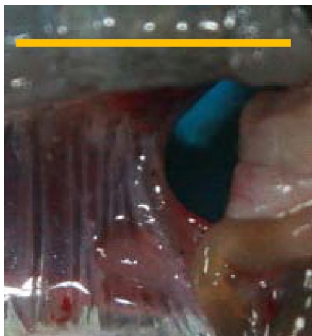


Stage 4: Big gonad but empty and no eggs left? The tissue looks bruised and unhealthy
(original picture: PT2009_SOL_13_Apr.jpg)



Stage 5: Bloodvessels are no longer taut, the tissue does not look healthy yet, the gonad is already shorter than stage 4
(original picture: PT2009_SOL_02_Jun.jpg)

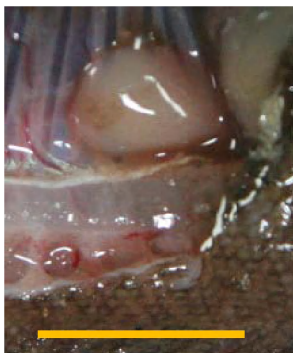
Figure VII.1.2 Reference pictures for male sole



Stage 1: Primary: FLAT, 2D

Secondary: semi-skimmed milk colour, kidney shape.

(original picture: UK2009_SOL_M 004 B_Sep.jpg)



Stage 2: Primary: 3D and more filled out. Secondary: cream colour, from little to maximum size.

(original picture: UK2009_SOL_M 006 B_Sep.jpg)

Stage 3: Primary: large and full gonad*; milt is running, either when squeezed or when cut. Secondary: Coffee with cream colour. The spermatoduct is well developed, wide, big and round (can even almost be a pouch).

(no picture available)



Stage 4: Primary: flabby, thinly elongated, (lobbed). Secondary: opaque red.

(original picture: NED2009_sol_111038045.jpg)

Stage 5: distinguishable?

(no picture available)

Figure VII.1.3a Reference pictures for female plaice–stage 1-3



Stage 1: Primary: No eggs, Translucent, jelly-like filling

Secondary: Colour – clear to light yellow/pink, gonad less than ½ body length, blood vessels are hardly discernable

(original picture: SWE2009_ple_1b.jpg)



Stage 2: Primary: Gonad rounder and firming, granulation at start of stage through to fully developed opaque eggs visible. Secondary: blood vessels developed

Colour – pink at start to orange at later stage.

(original picture: SWE2009_ple_19b.jpg)



Stage 3: Hydrated eggs are distinguishable and could be free running. Gonad distended.

(original picture: SWE_2009_ple_10b.jpg)

Figure VII.1.3b Reference pictures for female plaice –stage 4-5



Stage 4: Gonad slack but still large. Some eggs maybe visible. Walls thick with dark residue.

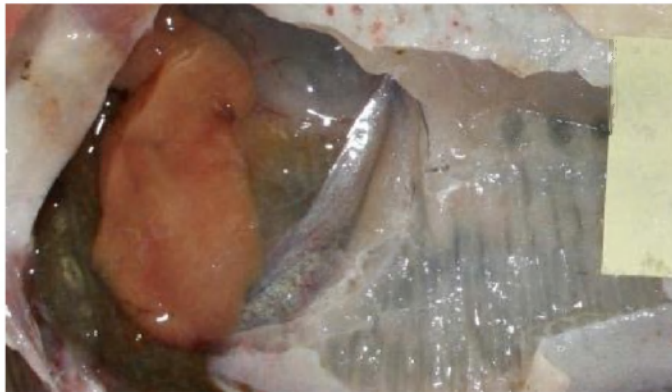
(original picture: UK2009_PLE_002c_jul_1.jpg)



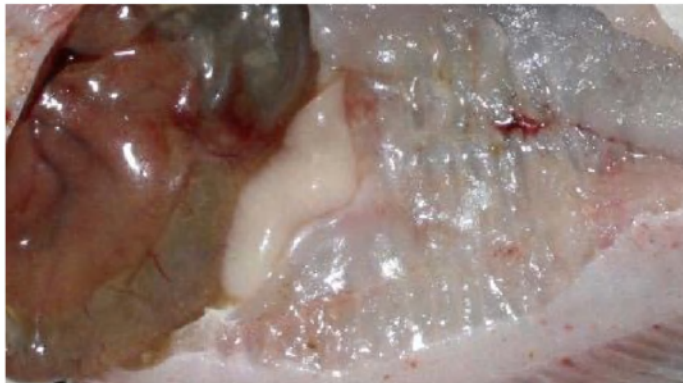
Stage 5: Gonad contracted up to 50% of spawning length, with walls thicker than immature. Colouration dark brown.

(original picture: UK2009_PLE_018b_jul_1.jpg)

Figure VII.1.4a Reference pictures for male plaice, stage 1-3.



Stage 1: Primary: Small. String like, slightly opaque in relation to the surrounding tissue. Smooth, tight against edge of cavity.
(original picture: SWE2009_ple_15a.jpg)



Stage 2: Gonad increasing in size, and starting to fill gut cavity. Creamy in colour. At late stage gonad can fill cavity and be bulbous. In re-maturing fish, gonad becomes uniform in shape (not knobbly like stage 5)
(original picture: SWE2009_ple_16a.jpg)

Stage 3: Milt flows under light pressure, this test should be carried out before any other. Gonad milky white in colour and very large.
(no picture available)

Figure VII.1.4b Reference pictures for male plaice, stage 4-5.

Stage 4: Testes flaccid, milt residue imaybe present in spermatoduct. Greyish in colour.
(no picture available)



Stage 5: Testes shrinks back against wall of cavity, firm/tight and knobbly. Greyish colouration but can be creamy. More opaque than immature.
(original picture: NED2009_ple_101042038a.jpg)

Figure VII.1.5 Reference pictures for female dab.



Stage 1: Primary: No eggs, Translucent, jelly-like filling

Secondary: Colour – clear to light yellow/pink, gonad less than $\frac{1}{2}$ body length, blood vessels are hardly discernable

(original picture: UK2009_DAB_031d_1.jpg)



Stage 2: Primary: Gonad rounder and firming, granulation at start of stage through to fully developed opaque eggs visible. Secondary: blood vessels developed

Colour – pink at start to orange at later stage, rematuring colouration from pink to grey.

(original picture: UK2009_DAB_018b_1.jpg)

Stage 3: Hydrated eggs are distinguishable and could be free running. Gonad distended. (no picture available)

Stage 4: Gonad slack but still large. Some eggs maybe visible. Walls thick with greyish residue.

(no picture available)



Stage 5: Gonad contracted up to 50% of spawning length, with walls thicker than immature. Colouration pale grey.

(original picture: UK2009_DAB_020b_1.jpg)

Figure VII.1.6 Reference pictures for male dab.



Stage 1: Primary: Small. String like, slightly opaque in relation to the surrounding tissue. Smooth, tight against edge of cavity.
(original picture: UK2009_DAB_034a_1.jpg)



Stage 2: Gonad increasing in size, and starting to fill gut cavity. Creamy in colour. At late stage gonad can fill cavity and be bulbous. In re-maturing fish, gonad becomes uniform in shape (not knobbly like stage 5)
(original picture: NED2009_dab_121012037a.jpg)

Stage 3: Milt flows under light pressure, this test should be carried out before any other. Gonad milky white in colour and very large.
(no picture available)

Stage 4: Testes flaccid, milt residue maybe present in spermatoduct. Greyish in colour.
(no picture available)



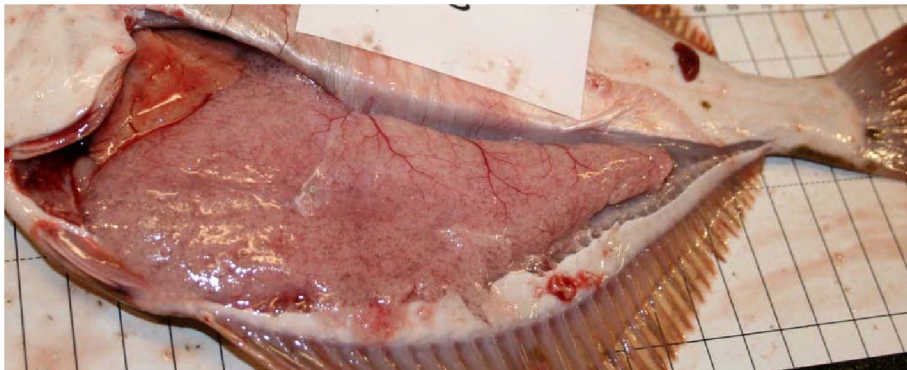
Stage 5: Testes shrinks back against wall of cavity, firm/tight and knobbly. Greyish colouration but can be creamy. More opaque than immature.
(original picture: UK2009_DAB_025b_1.jpg)

Figure VII.1.7a Reference pictures for female flounder, stage 1-3.

Stage 1: Ovaries are transparent, cast thin and clear. Blood vessels hardly discernable. Gonads extend into the flesh of the body. Recommended that all juveniles are sexed. (no picture available)



Stage 2: Firm, coloration could range from light pinkish to reddish orange. Blood vessels larger and diversified. The ovaries start filling the cavity. No eggs flow at light pressure. (original picture: LIT2009_FLO stage 2.jpg)



Stage 3: Distended, few to many hydrated eggs visible in tissue among vitelogenic oocytes. The ovaries are filling the cavity. Eggs occasionally running under light pressure. (original picture: DEN2009_flo_7a.jpg)

Figure VII.1.7b Reference pictures for female flounder, stage 4-5.



Stage 4: Slack with grayish cast, rich in blood vessels. Mucus inside is common. Could be few hydrated eggs left, but no vitellogenic oocytes.
(original picture: DEN2009_flo_10a.jpg)

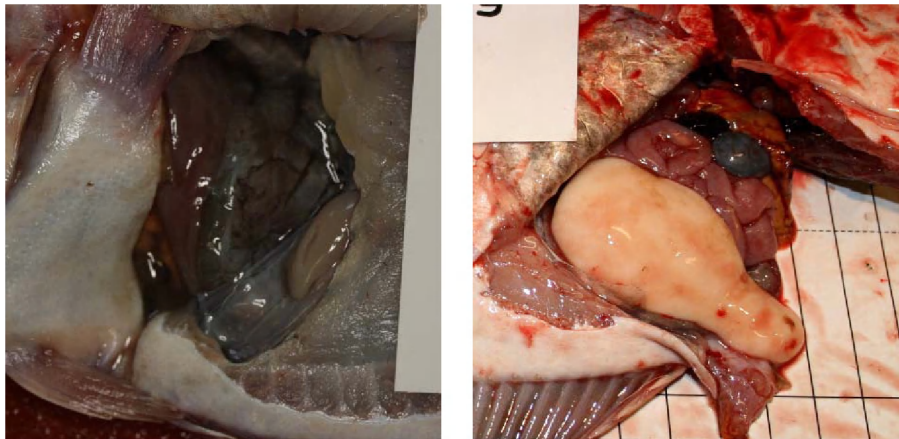


Stage 5: No visible development, similar to immature but sometimes with a grayish cast. Gonads are shrunk compared to stage 4.
(original picture: NED2009_flo_151015025a.jpg)

Figure VII.2.7a Reference pictures for male flounder, stage 1-3.

Stage 1: Testes white to grayish but always transparent, ribbon-like shape, do not extend into the flesh of the body. Vascularisation is limited. Recommended that all juveniles are sexed.

(no picture available)



Stage 2: Testes whitish to almost opaque reddish-white, blood vessels more prominent, empty transparent spermatoducts. No milt flows at light pressure.

(original pictures: NED2009_flo_151015003b.jpg (left), DEN2009_flo_5a.jpg (right))



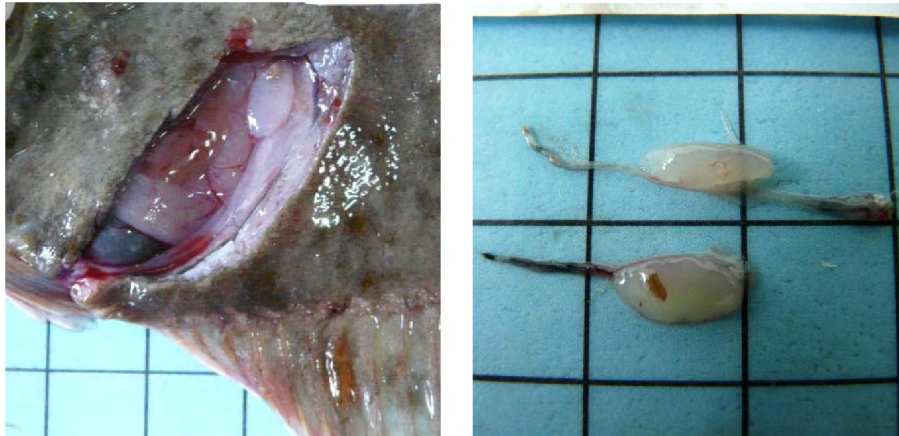
Stage 3: Milt flows at light pressure. Wide range in both size and colour, which vary from white, creamy to pinkish. Late stage bulbous.

(original picture: POL2009_fle_2b.jpg)

Figure VII.2.7b Reference pictures for male flounder, stage 4-5.

Stage 4: Contracted, empty and flaccid lobes, colour ranges from white to reddish, bloodshot, potentially with greyish cast. Smaller than stage 3. Small remains of milt can be present.

(no picture available)



Stage 5: No visible development, similar to immature but darker and often bigger.

(original pictures: POL2008_fle_5a.jpg (left), POL2008_fle_5b.jpg (right))

Annex 8: Results of staging sessions

Figure VIII.1.1 Results by reader for first staging SOLE (pictures)

In the maturity stage bias plots below the mean maturity stage recorded +/- 2stdev of each stage reader and all stage readers combined are plotted against the MODAL maturity stage. The estimated mean maturity stage corresponds to MODAL maturity stage, if the estimated mean maturity stage is on the 1:1 equilibrium line (solid line). Bias is the maturity stage difference between estimated mean maturity stage and MODAL maturity stage.

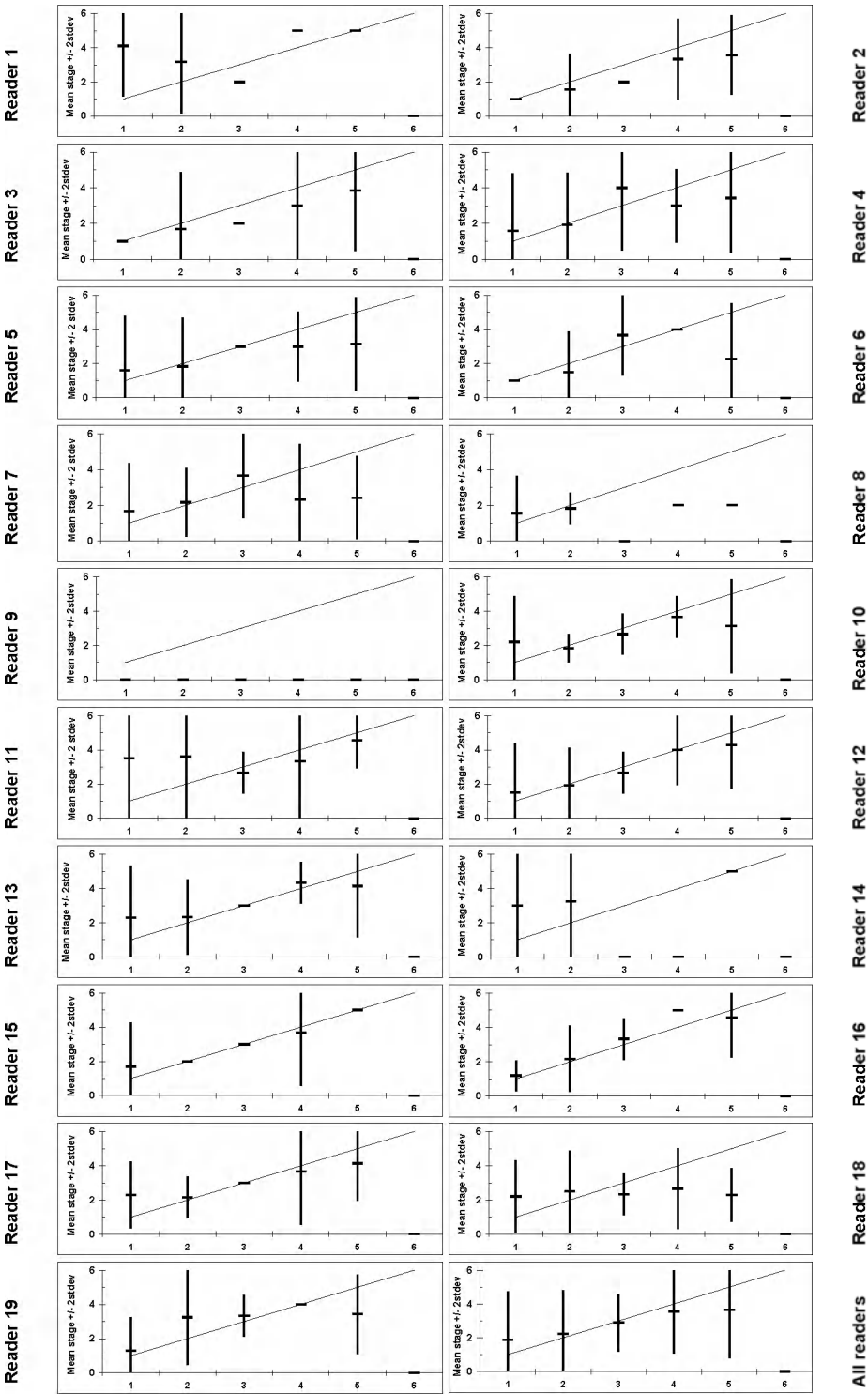


Figure VIII.1.2 Results by reader for first staging PLAICE (pictures)

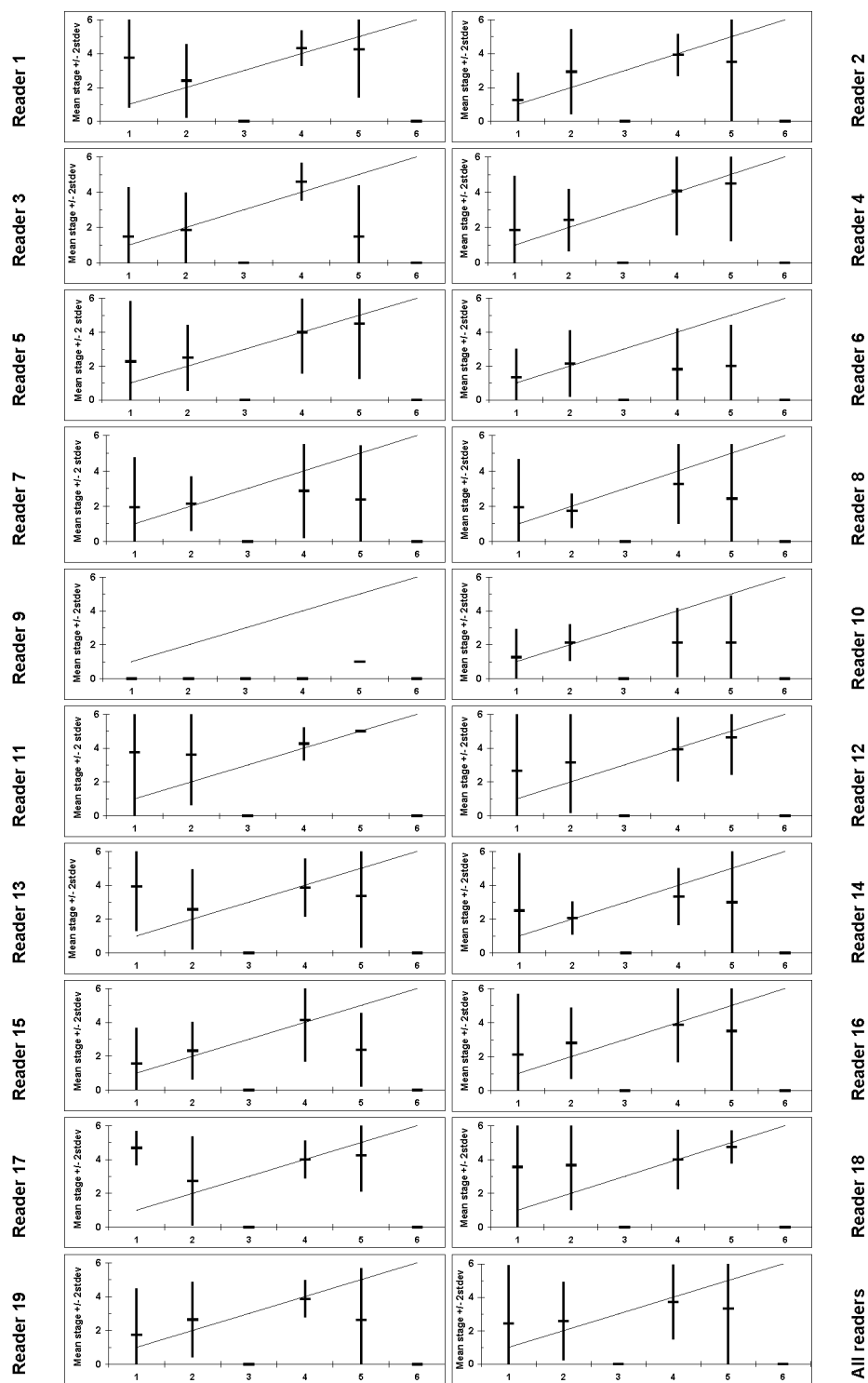


Figure VIII.1.3 Results by reader for first staging DAB (pictures)

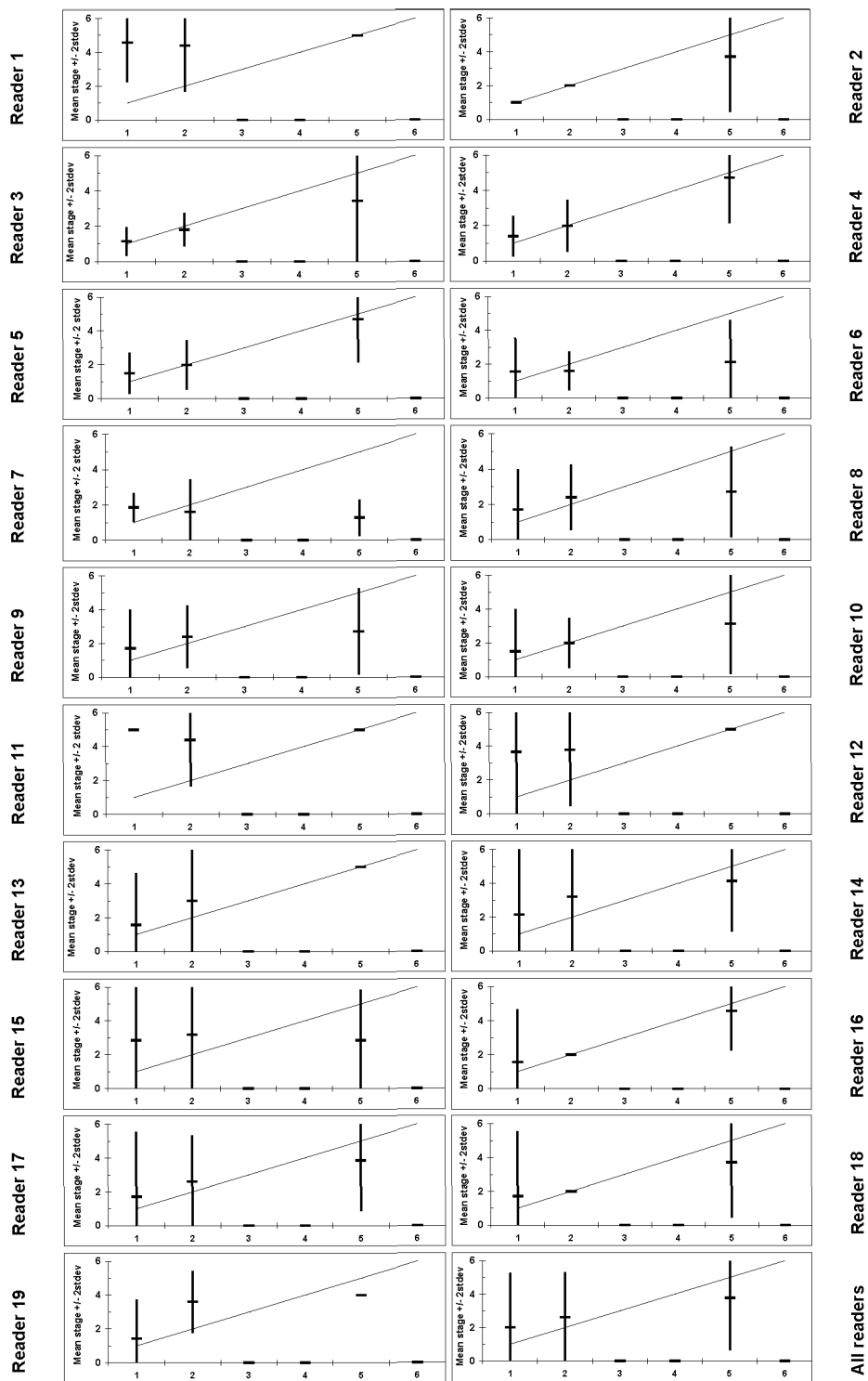


Figure VIII.1.4 Results by reader for first staging FLOUNDER (pictures)

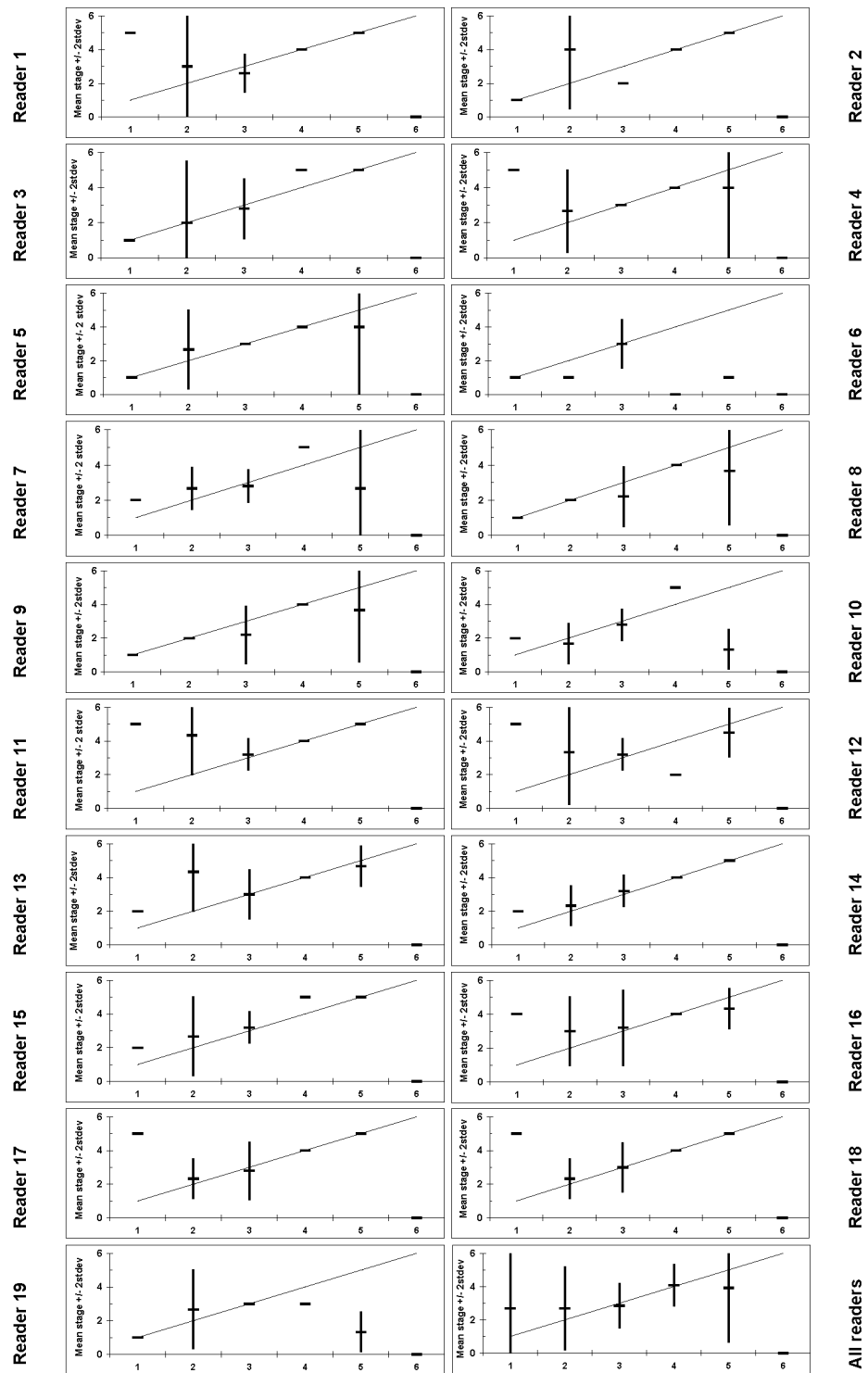


Figure VIII.1.5 Results by reader for first staging LEMON SOLE (pictures)

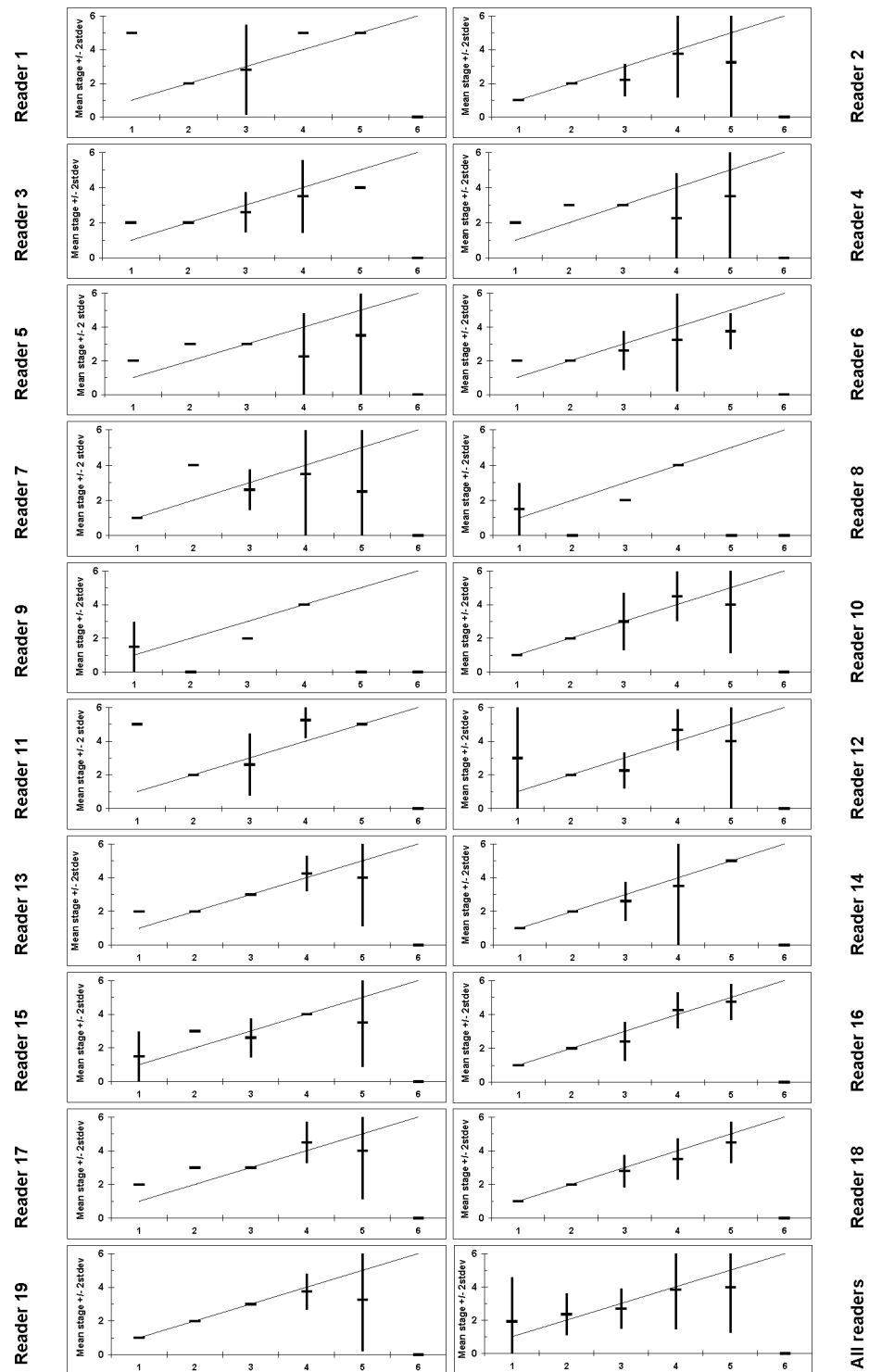


Figure VIII.2.1 Results by reader for second staging SOLE (fresh)

In the maturity stage bias plots below the mean maturity stage recorded ± 2 stdev of each stage reader and all stage readers combined are plotted against the MODAL maturity stage. The estimated mean maturity stage corresponds to MODAL maturity stage, if the estimated mean maturity stage is on the 1:1 equilibrium line (solid line). Bias is the maturity stage difference between estimated mean maturity stage and MODAL maturity stage.

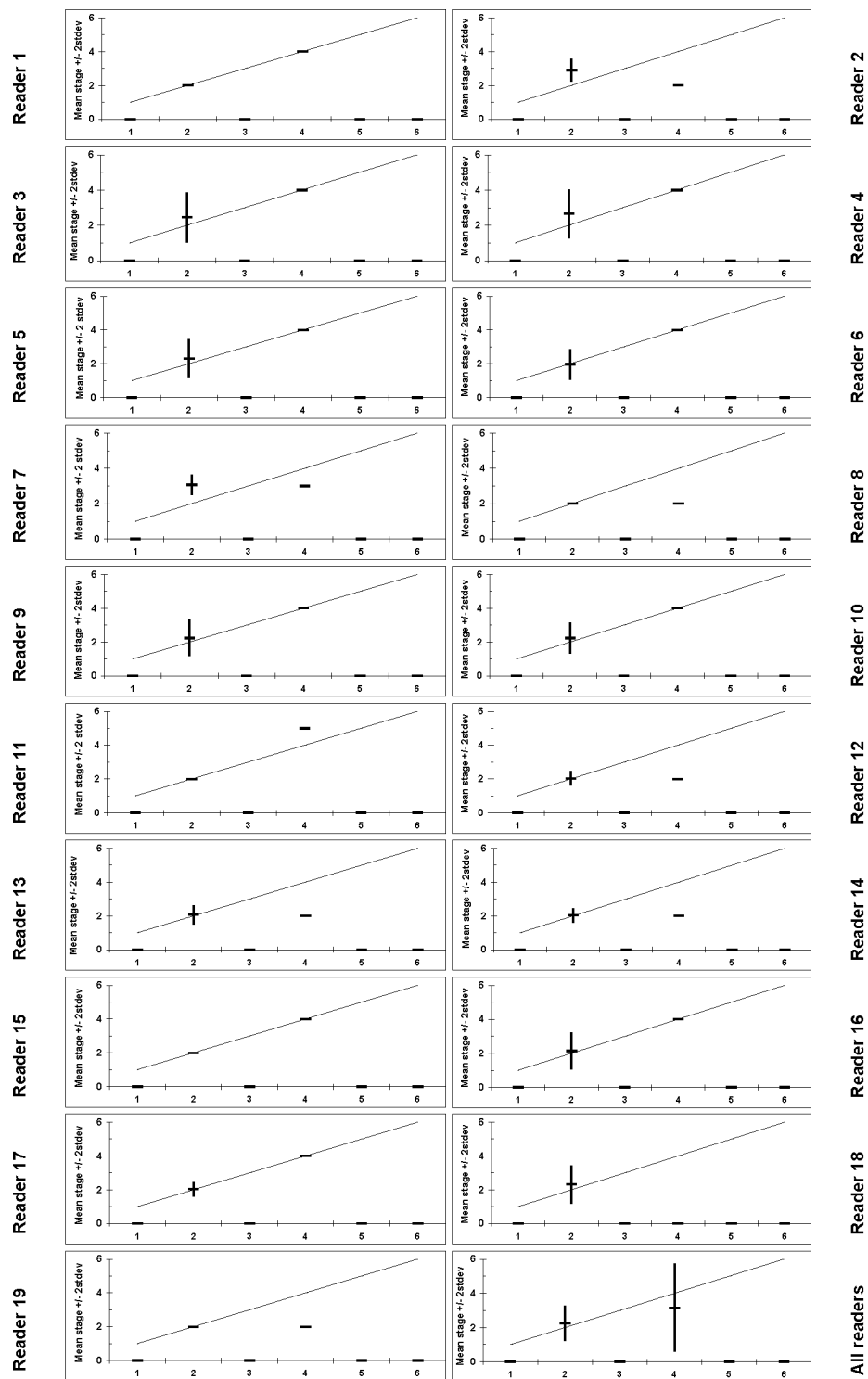


Figure VIII.2.2 Results by reader for second staging PLAICE (fresh)

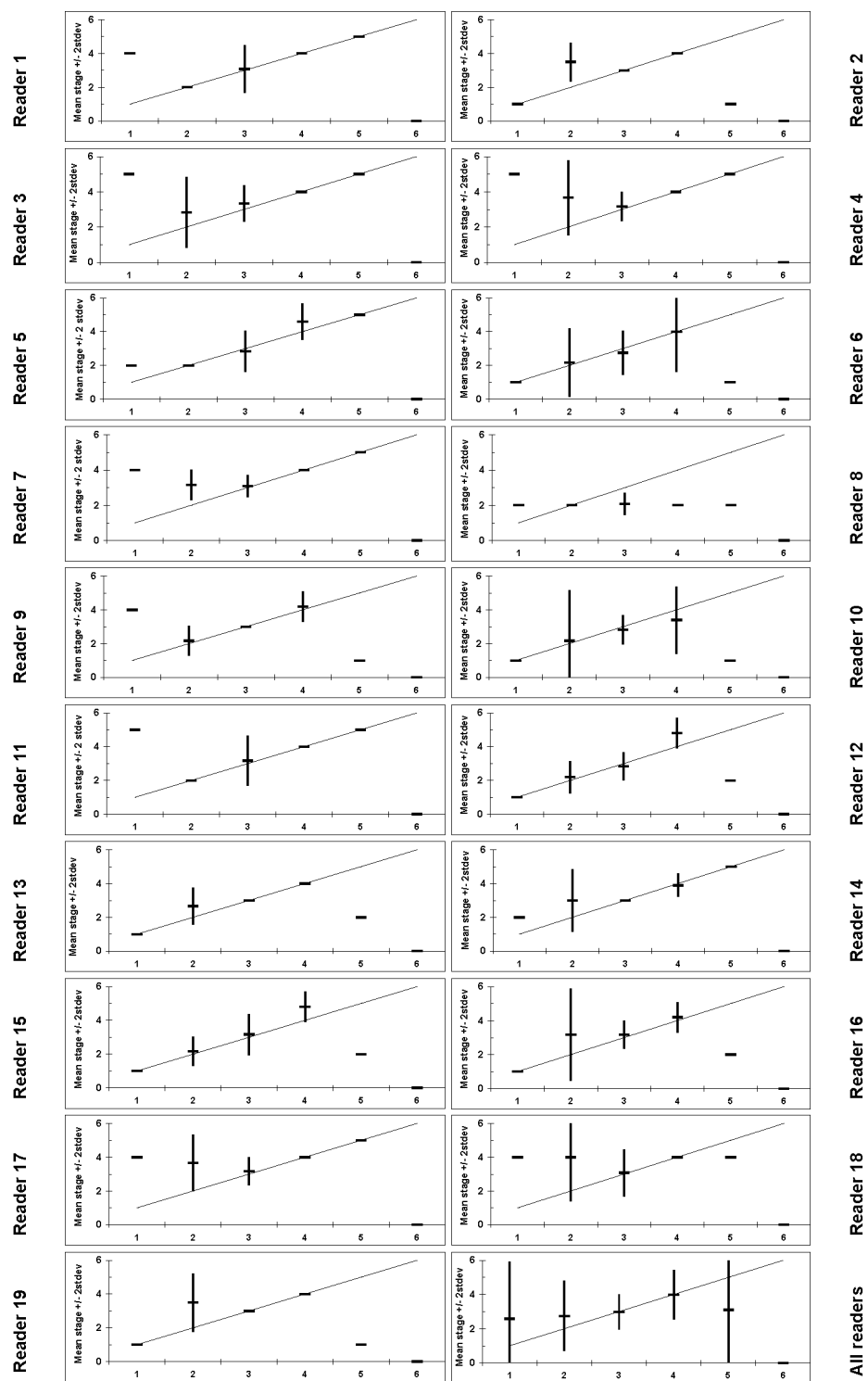


Figure VIII.2.3 Results by reader for second staging DAB (fresh)

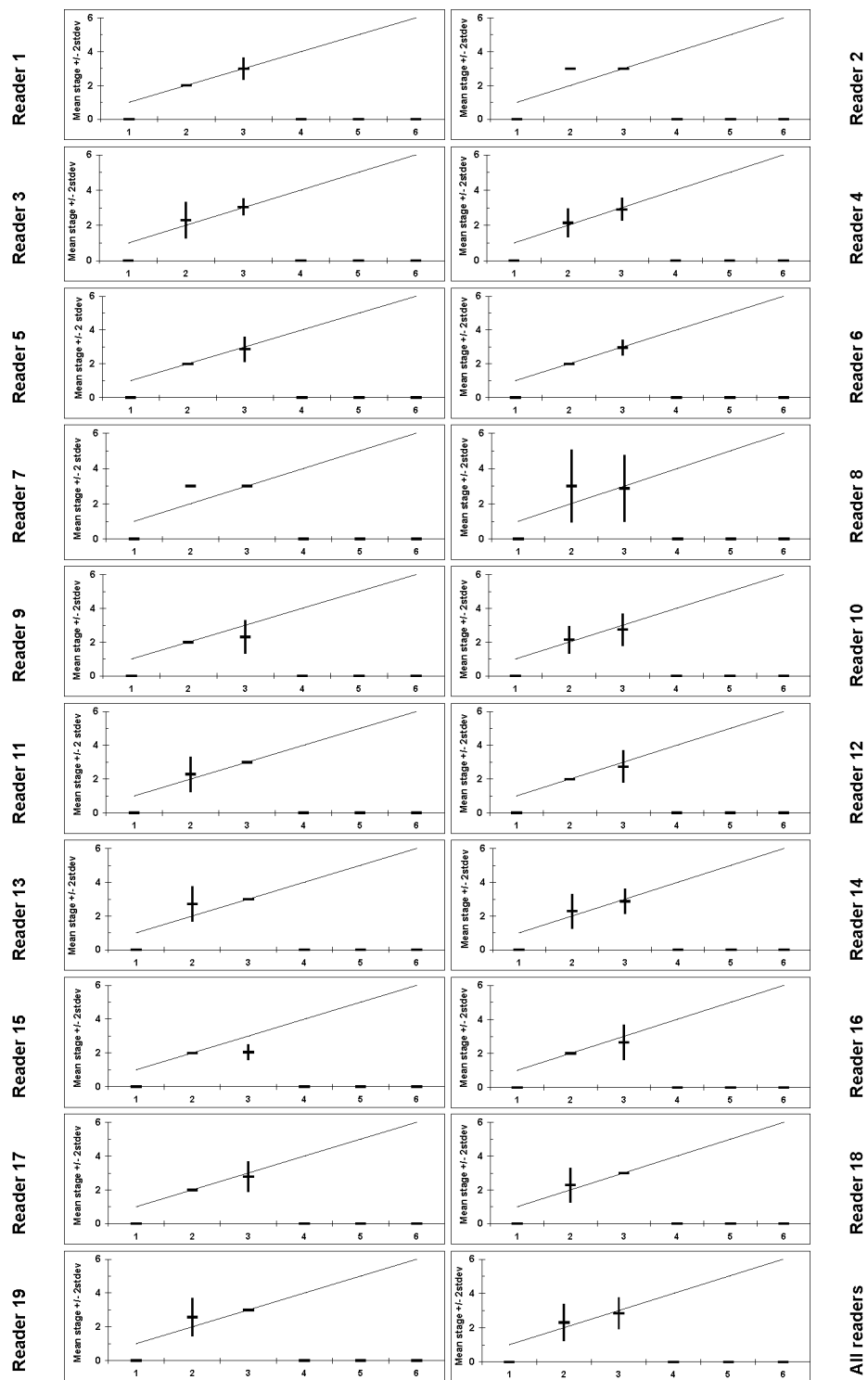


Figure VIII.2.4 Results by reader for second staging FLOUNDER (fresh)

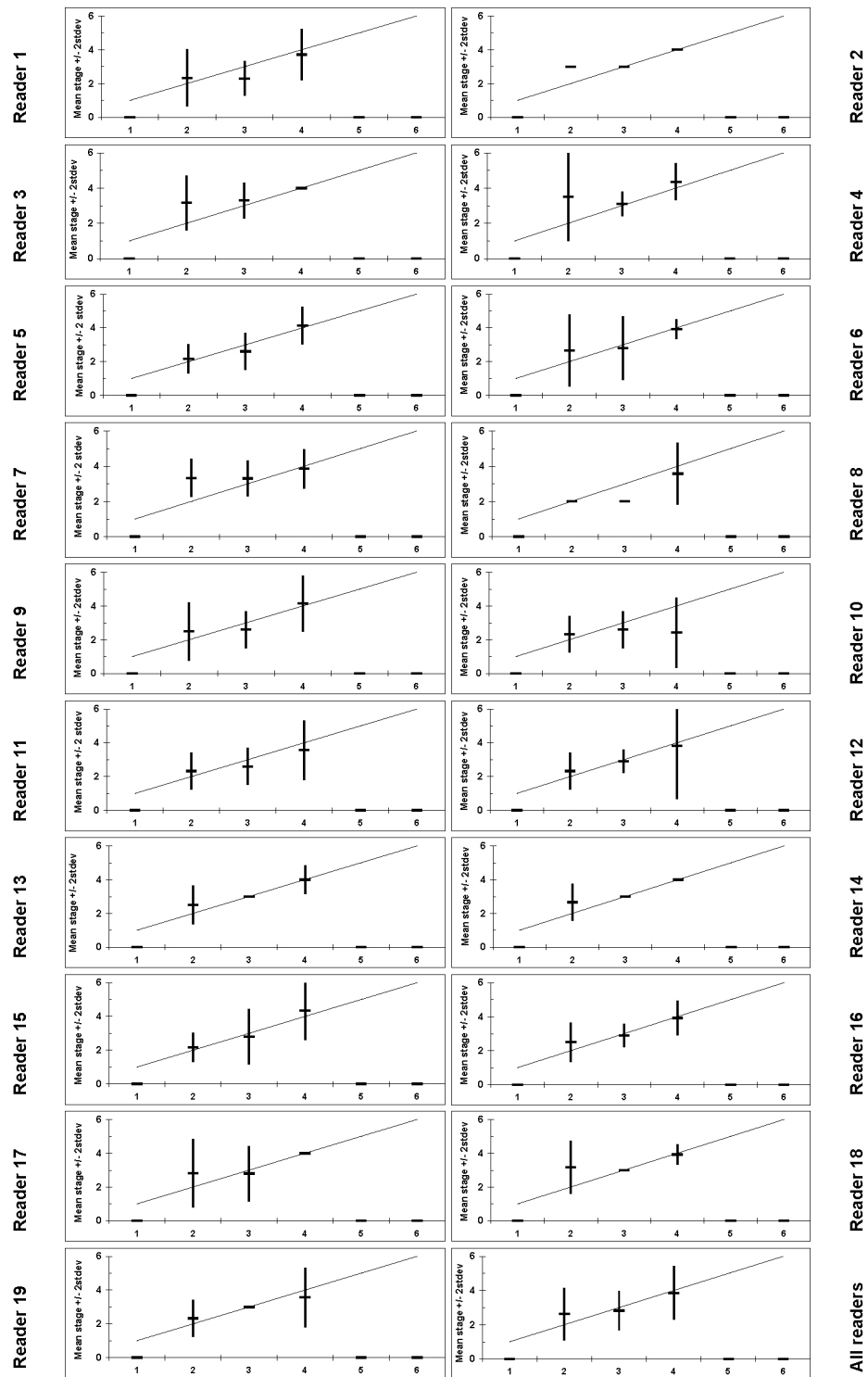


Figure VIII.3.1 Results by reader for third staging SOLE (pictures)

In the maturity stage bias plots below the mean maturity stage recorded ± 2 stdev of each stage reader and all stage readers combined are plotted against the MODAL maturity stage. The estimated mean maturity stage corresponds to MODAL maturity stage, if the estimated mean maturity stage is on the 1:1 equilibrium line (solid line). Bias is the maturity stage difference between estimated mean maturity stage and MODAL maturity stage.

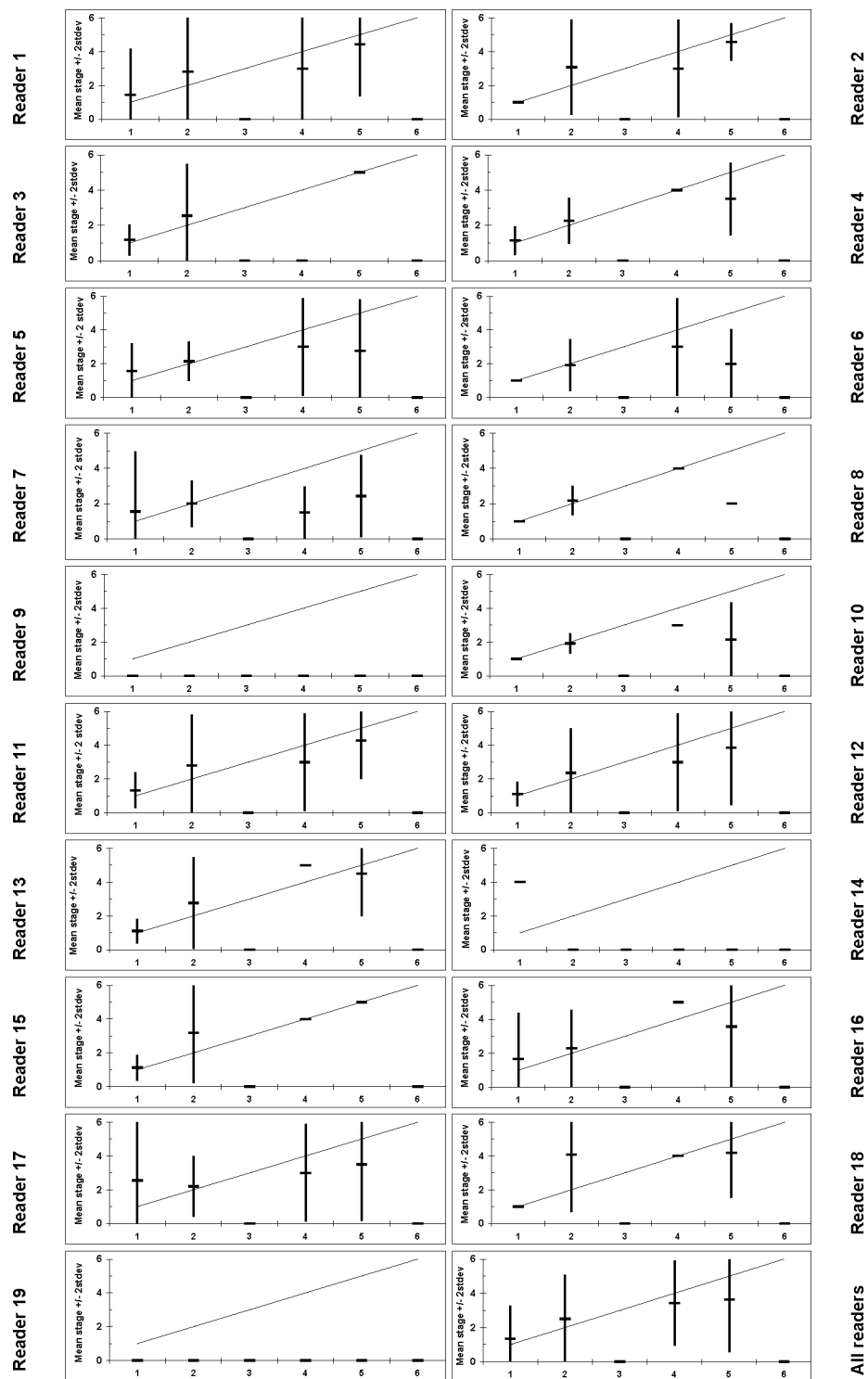


Figure VIII.3.2 Results by reader for third staging PLAICE (pictures)

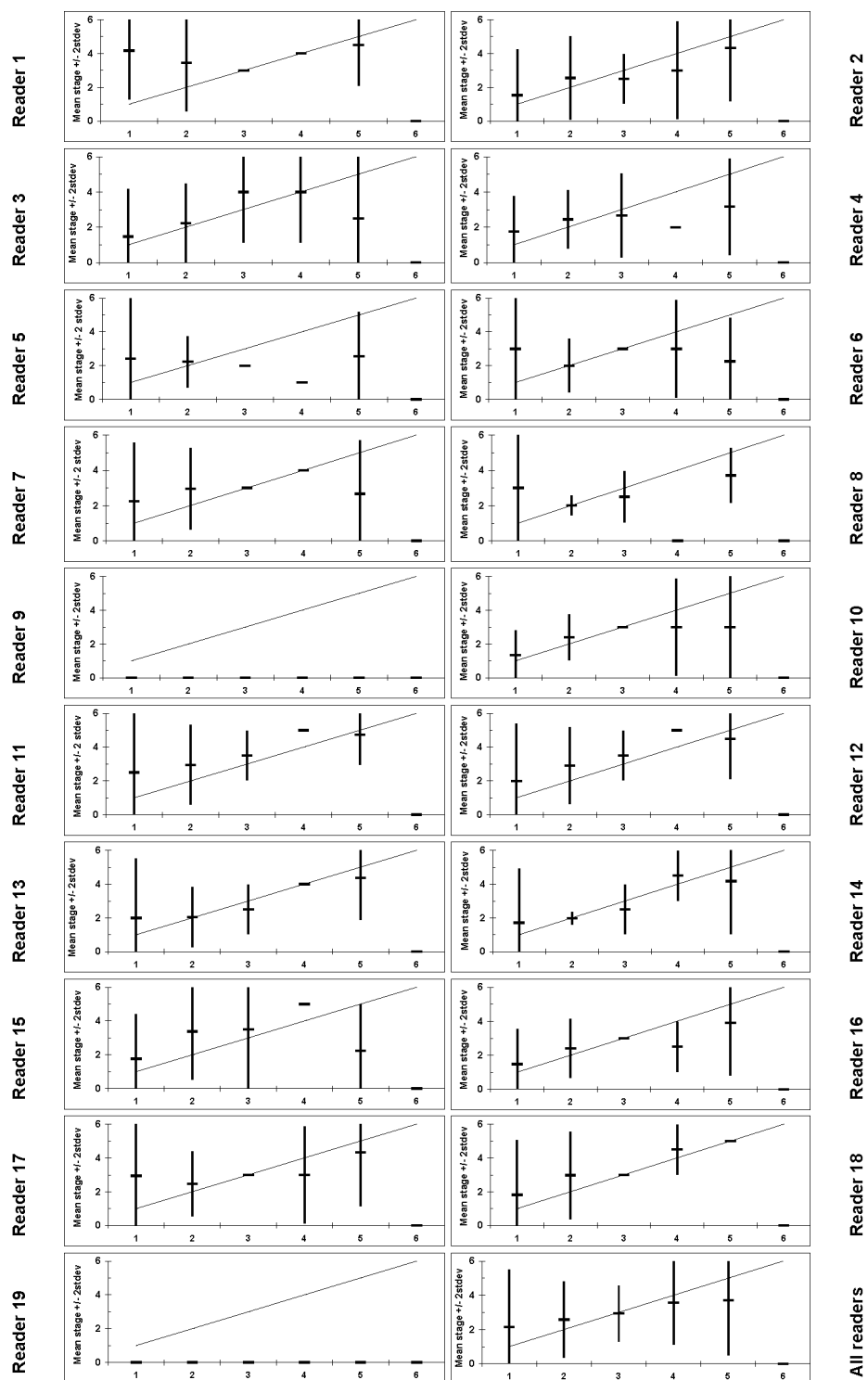


Figure VIII.3.3 Results by reader for third staging DAB (pictures)

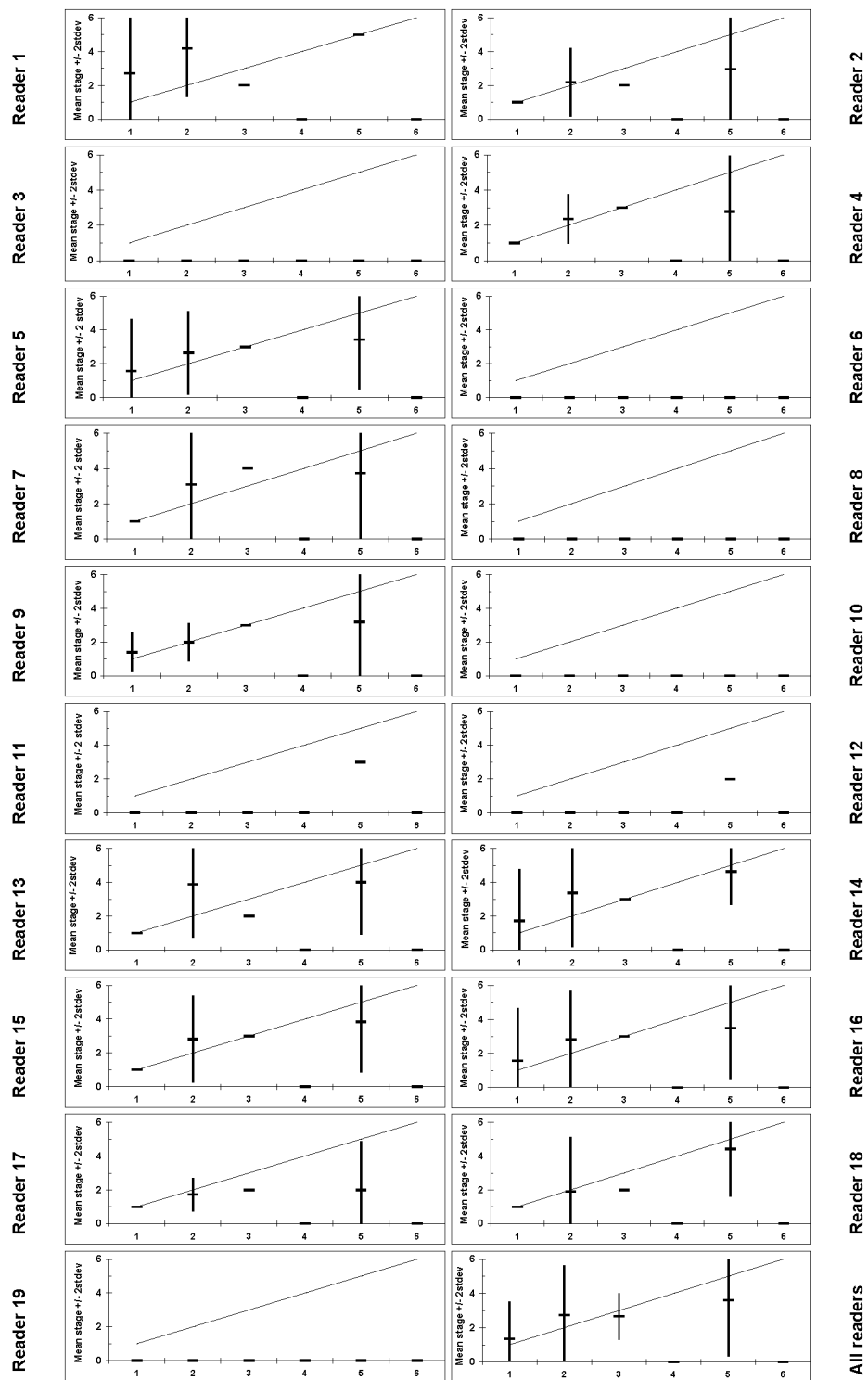


Figure VIII.3.4 Results by reader for third staging FLOUNDER (pictures)

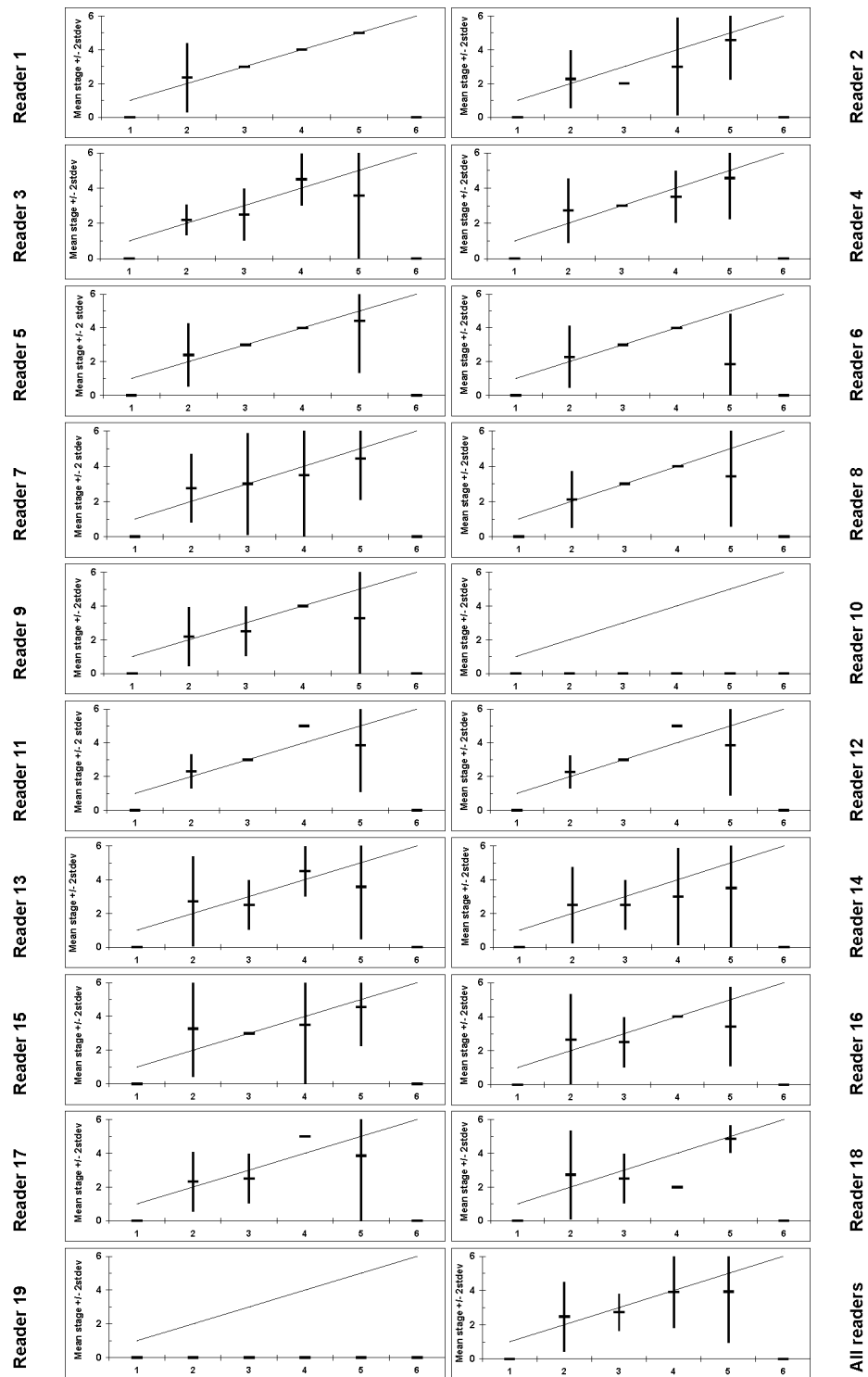


Figure VIII.3.5 Results by reader for third staging LEMON SOLE (pictures)

