WORKSHOP ON ADULT EGG PRODUCTION METHODS PARAMETERS ESTIMATION IN MACKEREL AND HORSE MACKEREL (WKAEPM)

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Contents

i Executive summary

The Workshop on Adult Egg Production Methods Parameters estimation in Mackerel and Horse Mackerel (WKAEPM) looked at the imprecision between institutes when processing survey samples. A number of protocol improvements were proposed, and these will be in place prior to the adult parameter analysis in 2022.

A calibration exercise was carried out prior to the workshop using standard mackerel and horse mackerel egg survey (MEGS) protocols. A second exercise was conducted during the workshop using a newly developed SmartDots module. Subgroups dealt with issues regarding annual egg production method (AEPM) and daily egg production method (DEPM) and added their recommendations to the manuals.

Descriptions for spent and massive atresia terms used during the screening process were redefined by adding further text and higher quality images into the ICES Survey Protocol Manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel (SISP- 5). Differences were noted in post ovulatory follicle (POF) and early alpha atresia identification, showing the difficulty associated with this work. In fecundity samples, there was high variance when identifying small oocytes close to the 185μm size threshold. It was recommended to have all fecundity samples analysed by two readers, and new criteria for the measurement of small oocytes were agreed. In atresia analysis, high variance was observed in both point and profile counting. High-resolution images are essential for this work, thus in 2022 slide scanner pictures will be taken for atresia analysis and will be sent around to all labs. There is still poor consensus on POF staging. Misclassifications between recent POFs and artefacts have now been clarified. Recent POF stages are used in spawning fraction estimation thus it is vital to be clear about them. A reference catalogue of images of early alpha atresia and POFs will be compiled and stored in SmartDots.

The desired number of gonad samples to be collected during the egg surveys, including North Sea samples, was defined. WGMEGS has requested that additional mackerel and horse mackerel female gonad samples would be collected by the Blue whiting survey, the Irish WESPAS survey and the Dutch Pelagic Fisherman's Association, PFA. An updated version of the ICES Fecundity and Atresia database will be ready for testing at the beginning of 2022 and the survey protocol manual will be updated in 2024.

ii Expert group information

Expert group name	Workshop on Adult Egg Production Methods Parameters estimation in Mackerel and Horse Mackerel (WKAEPM)
Expert group cycle	Annual
Year cycle started	2021
Reporting year in cycle	1/1
Chair(s)	Maria Korta, Spain
Meeting venue(s) and dates	22-26 November 2021, Online, (27 participants)

1 ToR A

Inter-calibrate the estimation of adult parameters in egg production methods (Annual and Daily Egg Production Methods), in particular, screening (histological maturity assignment), (batch) fecundity and atresia estimation, and POF staging; ICES Science plan 3.1, 3.3, 5.1

The goal of ToR-a is to carry out a calibration exchange on screening, fecundity, atresia and postovulatory follicles (POF) staging for mackerel and horse mackerel among the institutes participating in the next adult parameter survey analysis. The aim is to find out how much imprecision there is between institutes when processing the samples, and to propose measures for improvement. All the parameters above will be calculated in the 2022 survey to provide an SSB index for mackerel using the Egg Production Methods. Therefore, knowing the precision related to estimate is essential.

The calibration exercise was designed and sufficient time to carry out the analysis was given prior to the workshop in order to have the results ready for presenting at the workshop. The samples and templates as well as the freeware resources were shared among participants beforehand. Guidelines and additional useful information were provided for a successful calibration exercise (Annex 1).

1.1 Pre-survey calibration exercise

1.1.1 Set up

Four different analyses were set up in the calibration exercise, screening, fecundity, atresia and POF staging. The same material and as well as the same settings and templates were used during the calibration as in the survey.

- **Screening:** 19 digital slides images (*.npdi) were prepared from which the following markers were to be noted: oocyte stage, hydration state, presence-absence eggs, presence/absence POF and presence/absence early alpha atresia. It was also necessary to note whether the ovary was spent or if there were signs of massive atresia. Finally, it should also be noted if the sample should be discarded. For this exercise, histological screening images were analysed using NPDi.viewer (Annex 1).

- **Fecundity:** 10 fecundity samples, i.e., each sample consisting of 4 to 8 jpg pictures. Each sample had its own ObjectJ project to processes with (*.ojj). Oocytes above 185 µm should be manually counted if they were not automatically counted when processing the image. ImageJ along with the Object plugin was used for this analysis following the manual for whole mount analysis in ImageJ (SISP-5 Manual, Annex 7).

- **Atresia:** Profile counting was carried out on 7 atresia samples, i.e., each sample consisting of 3 to 8 jpg pictures. This was performed using the usual ObjectJ project 8 (*.ojj).

Point counting was carried out on other 7 atresia samples, i.e., each consisting of 1 jpg picture covering a large area of histological slide. In this case, a new ObjectJ project (*.ojj) used. It was used for the first time within the group to compare atretic markers positions among participants. ImageJ along with the Object plugin was used for this analysis following the manual for atresia analysis in ImageJ (SISP-5 Manual, Annex 7) and new manual for atresia analysis (Annex 2).

- **POF staging:** 25 digital slides images (*.npdi) were provided to stage the postovulatory follicle based on the 7 stages degeneration key (SISP- 5 Manual). For this exercise, histological images were analysed using NPDi.viewer (Annex 1).

Statistical analysis

Average percent error (APE) and the coefficient of variation (CV*)* precision indices were used to statistically analyse the results. Considering the precision as how close individual measurements on a given structure are to each other, i.e., reproducibility, thus APE and CV were considered proper indices of precision (imprecision) than the percent agreement (PA) used in previous calibration exercises, as they can either be scaled to percentage or used as proportion. Besides, CV is recognized as having a greater meaning and it is easier to interpret (Vitale et al., 2019).

1.1.2 Results and discussion

All tables and graphs with the results can be found in the Annex 3.

- **Screening:** in general, higher discrepancies were seen in samples which were identified as spent or having massive atresia, particularly when accounting for "oocyte_stage" and "POF_presence/absence" and "Early alpha atresia" variables.

  In such type of samples, there was a minor confusion in terms of considering the most developed stage of oocytes among the atretic oocytes as well. The oocyte stage that should be assigned is the most advanced healthy oocyte stage. In the case of "POF_presence/absence, some readers had difficulty distinguishing POFs from old atresia in ovaries which were spent or showed massive atresia. Other readers considered early alpha atresia a key characteristic in assigning massive atresia. However, it was not clear among all readers whether having massive atresia implies being spent, i.e., a massive atretic fish may further develop oocytes. Both latest issues, POF and Early alpha atresia in such type of samples have no straightforward solution. In the end, both spent and massive atresia samples will not be selected for fecundity and batch fecundity analysis.

  The definitions of both spent and massive atresia concepts required further detail and the SISP-5 Manual was updated accordingly:

  Spent
  A spent ovary is characterised by the absence of healthy vitellogenic oocytes at the end of the spawning period for that individual. POFs are present and atretic vitellogenic oocytes and residual eggs may be visible. This should not be confused with an ovary that

has just spawned but is not at the end of its spawning season. In this case the ovary has POFs and atresia, but there is a healthy generation of vitellogenic oocytes.

Massive atresia

Some massive atresia samples may have the most advanced batch of oocytes atretic but may also contain some healthy, less developed vitellogenic oocytes. Then not the 90% of the vitellogenic atretic as defined in the manual. These samples would impact in atresia intensity estimation. Massive atresia is considered when vitellogenic oocytes are at any of the steps in the atretic degeneration, not only in early alpha atresia. Otherwise, samples could be used for potential fecundity calculation.

Finally, Egg stage absence/presence was quite precisely performed by participants. They were slight discrepancies in Hydration state, i.e., some readers considering migratory oocyte stage rather than early hydration state. This would not impact in the batch fecundity results though as only hydration state 2 and 3 are used. This definition was improved in the manual for easier interpretation.

Early hydration state

Yolk granules start to fuse and hydrate and the nucleus still visible at the animal pole (end of migration). The size of the oocyte increases significantly.

Fecundity: Most Institutes got the same number of oocytes when automatically counted, however MSS scored a lower number of oocytes than others. When accounting for manually counted oocytes, they were some institutes that scored slightly higher than others and thus over the overall average score. When looking at results at readers level, however the readers consistently scoring higher that the average score did not belong to the same institute. Similarly, there were readers from different institutions scoring below the average score.

The F11 fecundity sample, which had the highest discrepancy in results, was selected to see why the scoring varied that way among readers. The sample showed a high percentage of "questionable" oocytes to count. These oocytes could be broken or half-hidden under more developed oocytes. The discrepancy most probably relied on the different interpretation on what to do with these oocytes, i.e., there was not accurate criteria for dealing with these oocytes. This showed that criteria should be reviewed among all:

Countable oocytes

Countable oocytes are any that fill the circle in ImageJ, even if the oocyte is "questionable" due to irregular structure or damaged. The "dark edge" around the oocyte should be inside the floating circle to be counted (Figure 1.1). If there is high percentage of "questionable" oocytes accounting for 30% in the sample, the guideline is to discard it and process the second sample that is taken also during the survey.

It is considered a good practice to press "Recalculate" in ImageJ before reporting the results, as it seems that it does not automatically refresh the counting and measurements, which may also have been a source of variance in results.

Finally, it was agreed to double check fecundity samples with a second reader to find uncounted oocytes. Quality check of fecundity samples is set up in the routine fecundity sampling processing and thus, the SISP-5 manual was updated accordingly.

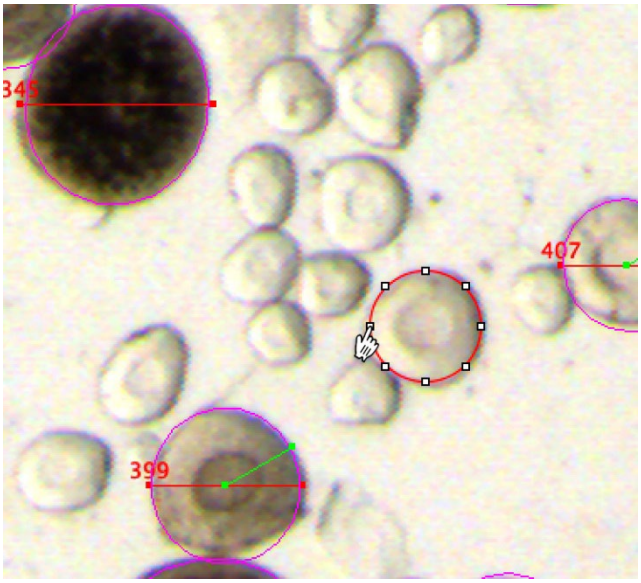


Figure 1.1. Optical effect of "dark edge" around the oocyte should be inside the floating circle to be counted.

- **Atresia:** profile counting, and point counting were carried out on different images due to the new ObjectJ project, set up for the first time during this workshop to compare the positioning of counted points among readers. Ideally both profile and counting results are treated together, i.e., a profile should correspond to an area fraction in the same image. In this exercise, they were treated separately. However, testing the new point counting ObjectJ project was considered essential to corroborate or refute the hypothesis that even if readers get the same number of points, they may not have the same position over the histological slide and thus may not be the same oocytes, which would uncover the lack of standardisation of atresia reading.

  Results of point counting from ATR10 and ATR13 atresia samples, i.e., atresia samples with highest discrepancies, showed that many times, the mismatch among readers relied on "borderline" atretic oocytes. The term of borderline refers to those atretic oocytes that are either at the beginning of early atresia or between early and late atresia. Many times, artifacts have been taken as breaks as well.

  When looking at the profile counting exercise, some images did not have a sufficiently high resolution, which complicated to get harmonised results. This analysis requires high resolution images which were not available for the exercise. Thus, in many cases, the break of the chorion was not clear enough to identify the oocytes within the early atresia category. This was emphasised in the youngest vitellogenic oocyte stages which, when they become atretic it is not always possible to identify both layers of zone radiata, making it difficult to stage early alpha stage.

Thus, the SISP-5 manual was updated by extending the description of atresia and adding new explanatory and high-resolution images showing the zona radiata appearance through oocyte development.

POF staging: some samples showed a wide range of stages which indicated that there is still low consensus on the POF staging. Although the discrepancy was improved when the POF stages were grouped into daily cohorts, it did not really solve the problem. In many cases POFs assigned as recent POFs were follicles detached from the oocytes during the sample manipulation and processing. In other samples assigned as later POFs with high discrepancies among readers, i.e., readings did not show a mode, the assigned stages were not contiguous stages but rather were quite far apart in stages rank, these samples were looked at in more detail in plenary.

The discussion on POF staging revealed the need for higher resolution images in the SISP-5 manual as well as a further effort on improving the description of POFs histomorphological characteristics and distribution on slides and how to approach staging from expert readers for a common interpretation.

Recent POFs

As a clue, there is frequently an oocyte next to it that has lost its follicle. In POFs the granulosa and theca are often not clearly distinguishable.

2 ToR B

Harmonize the analysis and interpretation of results with those of previous surveys; ICES Science plan 3.1, 3.3, 5.1

This ToR-b intends to harmonize the analysis and interpretation of results obtained in the calibration exercise. It tries to find out as well where the factors that cause inaccuracy lie and establish measure to reduce their impact. ToR-b aims to find out whether there is an evolution on the imprecision of calibration exercise through time.

Besides, ToR-b tries get hold of other ways to perform the calibration exercises that are accessible, i.e., without the need to previously install specific software or share images, and friendly for participants. In this sense, newly developed SmartDots module was tested and evaluated.

Within ToR-b also it is agreed the guidelines to process the samples but also it is planned the collection of adult samples according to protocols revised in ToR-d.

2.1 Comparative conclusion on calibration exercise

Screening: spent and massive atresia terms were redefined by adding further helpful descriptions and higher quality images into the SISP- 5 manual. Disagreement in spent goes back to WKFATHOM2 (2018), where it was argued it was basically due to the different interpretation of the phase of the reproductive cycle; however, there were no problems with massive atresia that time. EGG stage was also not clearly enough identifiable, (WKFATHOM2, 2018), so the definition on early hydration stage was further improved during the present workshop. Participants had problems recognising the presence/absence of POFs in slides. As for POF and early alpha atresia identification some differences were detected in a limited set of samples during WGMEGS (2020), demonstrating that a solution may not reach easily.

- **Fecundity:** manual counting showed high variance probably due to differences in identifying small oocytes (WKFATHOM2, 2018). Small oocytes close to the size thresholds of 185, that cause most of the differences, can overlap some small oocytes. It was recommended, if possible, to have all fecundity samples analysed by two readers, and the criteria for "questionable" oocytes was agreed in order to improve the agreement in fecundity results.

- **Atresia:** high variance in both point and profile counting was also observed during WKFATHOM2 (2018); with the differences up to 100 oocytes/g. Slide scanner pictures will be taken for atresia analysis and will be sent around to all labs to ensure uniform and high-resolution images. A reference catalogue of images of early alpha atresia will be generated in SmartDots rather than in the manual that has a limited space.

- **POF Staging**: There is still poor consensus on the POF staging. The overall agreement in previous calibration exercise was below 50% (WKFATHOM2, 2018) and differences among readers in many cases were considerable (WGMEGS, 2021). However, misclassification of recent POFs with artifacts has been clarified; these recent stages are used on

the spawning fraction estimation thus it is vital to be clear about them. A reference catalogue of images of POFs will be generated in SmartDots as well.

2.2 SmartDots Fecundity and atresia module

WGMEGS and ICES collaborated during 2020 to develop an online module for fecundity and atresia calibration exchanges. A beta version was ready to be tested during the workshop. It consisted of 4 different exercises, i.e., screening, fecundity, POF staging and atresia, which simulate the main work that is being carried out on the survey samples.

Screening and POF staging relied on digital scanned slides. Pre-processed images were used in fecundity exercise. Large images with Weibel grid stamped on top were available for atresia exercise.

2.2.1 Set up event 386

A new event was created in SmartDots for WKAEPM which was automatically assigned the number of 386. 15 digital scanned slides were uploaded for both screening and POF staging, while 3 images were available for fecundity and atresia exercises.

In the screening images, oocyte stage, hydration stage, presence/absence of eggs, POFs and early alpha atresia had to be marked on top of the image. Participants were also asked to note if the sample was spent or showed massive atresia. The markings done were ratified in the questionnaire at the end of the image analysis.

In POF staging, the POF structure had to be marked on top of the image with the corresponding stage.

Oocytes which were not marked during the pre-processing of the images, and those above 185 microns, had to be marked on fecundity images. Oocytes incorrectly marked during the pre-processing to the images had to be fixed.

In atresia exercise, point counting had to be done, i.e., the ends of the Weibel grid falling over early alpha atresia oocytes had to be marked, along with the profile counting, i.e., the corresponding oocyte developmental stage labelling.

All participants were granted access to the event. Before starting, instructions on how to navigate through the event were given by the ICES developer Carlos Pinto. Guidelines on image analysis were almost like pre-survey calibration exercise with slight modifications to suit the event.

2.2.2 Results and discussion

All tables and graphs with the results can be found in the Annex 4.

- **Screening:** 3 digital slides were analysed and surprisingly there was less agreement that in the pre-survey calibration exercise when accounting for oocyte stage. This was mainly due to participants marking all the stages present in the image and not the most

advanced one as usual. Hydration stage and egg stage were identified correctly. However, when looking at presence/absence of POF and early alpha stage participants faced the same difficulties when using this tool; similar issues occurred when assigning spent and massive atresia.

- **Fecundity:** 3 images were analysed and the results for those participants who had no technical problems there was broad consensus (see Flat_F19_A.jpeg). The discussion held on the pre-survey calibration exercise was noted here. It was possible to compare marked images in plenary, i.e., the tool allows you to compare markings on pairs of images. In general, the reason for scoring under and over the average oocyte count were related to the tool constrains, i.e., tags from one annotation frequently covered the oocyte next to it making it very difficult, or impossible, to measure. It was also difficult to delete previous annotations.

- **Atresia:** only one big image was analysed by all participants due to time constraints. Apart from 3 incorrectly scored results, i.e., profile counting was missing, or point counting was extremely low, profile and points counting ranged from 8 to 14 and 63 to 75 respectively. Two groups were distinguishable, i.e., one with an average ratio between profile and points counting of 0.12 and the other with 0.17. Looking at pairs of tagged images, the same problem arose as in pre-survey calibration, not the same cells were tagged by participants mainly due to borderline atretic cells.

- **POF Staging**: in 2 of the 3 digital scanned images, the annotations belonging to the same daily cohort showed a mode, and only, one or two annotations fell on another POF daily cohort. In the third image although participants agreed on the POF daily cohort, stages were different, confirming what has already been mentioned in the pre-survey calibration results.

This exercise above tested the fecundity and atresia module itself and participants suggested several improvements, at the request of the developer; the main and most common ones among participants are listed below:

Access

- It takes a long time to refresh the image: the resolution in these images is too large for efficient zooming and navigation through the image. Highly unnecessary time-consuming.

Images

- There is no possibility to change color/brightness/contrast on the images, which is especially useful when analysing atresia.

Sample information

- Sample information and image icon below are too close to each other in the layout.

Input fields

- No comment field, it would be good to add it especially for explaining why one would discard a sample or have a tag to write comments in areas of interest in the images.
- Histological questionnaire is "No" by default. It would be helpful that it takes the marks being done on the image.

Measurement tools

- In fecundity analysis the labels are big and show unnecessary information. The labels also get in the way of oocytes being measured.
- It is necessary to start exactly in the middle of the oocyte to measure correctly, otherwise the measurement has to be deleted and then the webpage has to be refreshed.
  Maybe it is easier to start a circle from the side of an oocyte. This would also be very useful for broken oocytes.
- When deleting a measured oocyte, the annotation is not deleted completely, even after hitting refresh.
- In the fecundity exercise the tools should have explanatory labels.

User friendliness

- In the fecundity exercise the tools are confusing and unintuitive.
- Hard to get it on the correct point.
- Error correction is inefficient.

2.3 Adult sampling

In 2022 mackerel gonad samples will be collected for both the Annual (AEPM) and Daily Egg Production method (DEPM) in the southern and western spawning areas (Tables 2.3.1 to 2.3.3). For horse mackerel and mackerel in the North Sea only DEPM gonad samples will be collected (Tables 2.3.4 and 2.3.5).

For the AEPM females in maturity stages 3 to 6 (Walsh scale) are requested to be sampled, while for the DEPM females in maturity stages 2 to 6 (Walsh scale) need to be sampled. For the sampling and data collection see the manuals (SISP-5 and SISP-6).

The ideal situation is that the gonad samples are spread over different hauls which are spread over the sampling area as much as possible. The tables below show the desired temporal and spatial distribution of the samples per survey period and institute sampling. For the DEPM it is requested to carry out a fishing haul each transect for the collection of gonad samples. If it is not possible to fish or get the number of females in a certain haul, please add extra females in a consecutive haul containing high numbers of females.

In previous surveys it was not possible for various reasons to collect the desired number of gonad samples during the egg surveys. WGMEGS therefor contacted other ICES expert survey groups and commercial fishing vessels to help with the collection of gonad samples. These extra samples are included in the tables below. Through WGIPS the blue whiting survey are requested to collect mackerel female gonad samples to the west of Ireland and Scotland in period 3 (Table 2.3.3). The Pelagic Freezer Trawler Association (PFA) will be requested to collect mackerel samples in periods 2, 3 and 4 (Tables 2.3.2 and 2.3.3). And in periods 6 and 7, the PFA and Ireland, (WESPAS survey), will be requested to collect horse mackerel gonad samples (Table 2.3.4).

Table 2.3.1. Desired temporal and spatial distribution of mackerel gonad sampling in the southern area in 2022.

Fecundity sampling (numbers of fish) — **Southern Area (Cantabrian and Biscay)** — **Southern Area (Cadiz to Galicia)**

MACKEREL

Week	Date	Period*	Lon ° 11W	10	9	8	7	6	5	4	3	2	1	Lat ° 36N	37	38	39	40	41	42	Total
3	9/jan/22	1												10							10
4	16/jan/22	2														10					10
5	23/jan/22	2															10				10
6	30/jan/22	2																			0
7	6/feb/22	2																	10		10
8	13/feb/22	2																			0
9	20/feb/22	2																			100
10	27/feb/22	2																			0
11	6/mrt/22	3																			0
12	13/mrt/22	3																			0
13	20/mrt/22	3																			0
14	27/mrt/22	3																			0
15	3/apr/22	3																			0
16	10/apr/22	4																			0
17	17/apr/22	4																			0
18	24/apr/22	4																			0
19	1/mei/22	4																			0
20	8/mei/22	5																			0
21	15/mei/22	5																			0
22	22/mei/22	5																			0
23	29/mei/22	6																			0
24	5/jun/22	6																			0
25	12/jun/22	6																			0
26	19/jun/22	6																			0
27	26/jun/22	6																			0
28	3/jul/22	7																			0
29	10/jul/22	7																			0
30	17/jul/22	7																			0
31	24/jul/22	7																			0
																					140

100 (prespawning (purseine/trawl))

See Table for the DEPM sampling

		per period				
	1	2	3	4	total
IEO	100				100
AZTI					0
IPMA	40				40
Total:	140	0	0	0	140

Table 2.3.2. Desired temporal and spatial distribution of mackerel Annual Egg Production gonad sampling in the western area in 2022. Note: in period 3 and 4 gonad sampling for the AEPM will be collected together with the DEPM samples (see table 2.3.3).

Fecundity sampling — **Western Area**

MACKEREL

Week	Date	Period*	42N	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	PFA commercial trawlers	Total
3	9/jan/22	1																								0
4	16/jan/22	2																								0
5	23/jan/22	2																								0
6	30/jan/22	2																								0
7	6/feb/22	2																								200
8	13/feb/22	2																								0
9	20/feb/22	2																								20
10	27/feb/22	2																								20
11	6/mrt/22	3																								0
12	13/mrt/22	3																								0
13	20/mrt/22	3																								0
14	27/mrt/22	3																								0
15	3/apr/22	3																								0
16	10/apr/22	4																								0
17	17/apr/22	4																								0
18	24/apr/22	4																								0
19	1/mei/22	4																								0
20	8/mei/22	5																								55
21	15/mei/22	5																								55
22	22/mei/22	5																								55
23	29/mei/22	6																								0
24	5/jun/22	6																								40
25	12/jun/22	6																								45
26	19/jun/22	6																								40
27	26/jun/22	6																								0
28	3/jul/22	7																								30
29	10/jul/22	7																								40
30	17/jul/22	7																								20
31	24/jul/22	7																								0
																										620

100 (prespawning (purseine/trawl))

50 (prespawning (purseine/trawl))

50

See table 2.3.3 for the DEPM sampling

		per period							
	1	2	3	4	5	6	7	Total
AZTI			270		45			315
TI			450	630				1080
MI		100	450			50		600
SCO		90		360	45		90	585
WMR					45	45		90
IEO			360	540				900
FAR					30			30
NOR						30		30
WGIPS			200					200
PFA		50	100	100				250
Total:	0	240	1830	1630	165	125	90	4080

Table 2.3.3. Desired temporal and spatial distribution of mackerel Daily Egg Production gonad sampling in the western area in 2022.

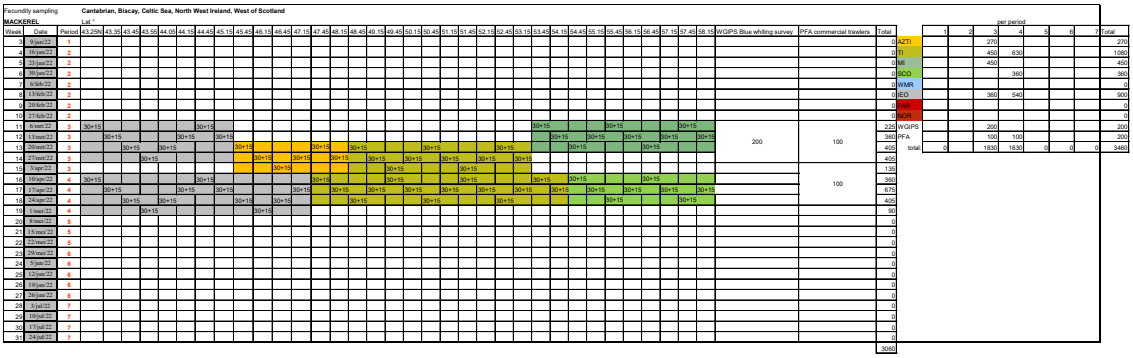


Table 2.3.4. Desired temporal and spatial distribution of horse mackerel Daily Egg Production gonad sampling in the western area in 2022.

Fecundity sampling — **Biscay, Celtic Sea, North West Ireland, West of Scotland**

HORSE MACKEREL — Lat °

Week	Date	Period	46.45N	47.15N	47.45	48.15	48.45	49.15	49.45	50.15	50.45	51.15	51.45	52.15	52.45	53.15	53.45	54.15	WESPAS	PFA commercial trawlers	Total
3	9/jan/22	1																			0
4	16/jan/22	2																			0
5	23/jan/22	2																			0
6	30/jan/22	2																			0
7	6/feb/22	2																			0
8	13/feb/22	2																			0
9	20/feb/22	2																			0
10	27/feb/22	2																			0
11	6/mrt/22	3																			0
12	13/mrt/22	3																			0
13	20/mrt/22	3																			0
14	27/mrt/22	3																			0
15	3/apr/22	3																			0
16	10/apr/22	4																			0
17	17/apr/22	4																			0
18	24/apr/22	4																			0
19	1/mei/22	4																			0
20	8/mei/22	5																			0
21	15/mei/22	5																			0
22	22/mei/22	5																			0
23	29/mei/22	6																			0
24	5/jun/22	6	30+15					30+15	30+15					30+15	30+15	60+30			100	100	270
25	12/jun/22	6		30+15			30+15			30+15			30+15								225
26	19/jun/22	6			30+15	30+15					30+15	30+15						30+15			225
27	26/jun/22	6																			0
28	3/jul/22	7	60+30												60+30						225
29	10/jul/22	7			60+30				60+30				60+30				60+30		100	100	225
30	17/jul/22	7					60+30				60+30										270
31	24/jul/22	7																			0
																					1440

	per period							
	1	2	3	4	5	6	7	Total
AZTI			270					270
TI			450	630				1080
MI			450					450
SCO				360				360
WMR								0
IEO			360	540				900
CAR								0
NOR								0
WGIPS			200					200
PFA			100	100				200
total:	0	0	1830	1630	0	0	0	3460

Table 2.3.5. Desired temporal and spatial distribution of mackerel Daily Egg Production gonad sampling in the North Sea in 2022.

Fecundity sampling — North Sea

MACKEREL — Lat °

Week	Date	Period	53.15	53.45	54.15	54.45	55.15	55.45	56.15	56.45	57.15	57.45	58.15	58.45	59.15	59.45	60.15	60.45	61.15	61.45	Total
3	9/jan/22	1																			0
4	16/jan/22	2																			0
5	23/jan/22	2																			0
6	30/jan/22	2																			0
7	6/feb/22	2																			0
8	13/feb/22	2																			0
9	20/feb/22	2																			0
10	27/feb/22	2																			0
11	6/mrt/22	3																			0
12	13/mrt/22	3																			0
13	20/mrt/22	3																			0
14	27/mrt/22	3																			0
15	3/apr/22	3																			0
16	10/apr/22	4																			0
17	17/apr/22	4																			0
18	24/apr/22	4																			0
19	1/mei/22	4																			0
20	8/mei/22	5																			0
21	15/mei/22	5																			0
22	22/mei/22	5																			0
23	29/mei/22	6																			0
24	5/jun/22	6																			0
25	12/jun/22	6	30+15									30+15	30+15				30+15				180
26	19/jun/22	6		30+15						30+15	30+15			30+15				30+15			225
27	26/jun/22	6			30+15			30+15	30+15						30+15				30+15		225
28	3/jul/22	7				30+15	30+15									30+15				30+15	180
29	10/jul/22	7																			0
30	17/jul/22	7																			0
31	24/jul/22	7																			0
																					810

	per period							
	1	2	3	4	5	6	7	Total
DEN						180		180
ENG						450		450
NOR						180		180
total:	0	0	0	0	0	810	0	810

Samples will be labelled the same way as for the 2019 survey. For example, label B003c_J refers to a horse mackerel sample collected by the Marine Institute in Ireland (B), female sampled number (3), it is a 100 µl pipette sample (c) and this sample will need to be sent to Wageningen Marine Research, The Netherlands for analyses (J). Table 2.3.6 provides an overview of the institute's codes for sample collection and analyses (see also SISP-5 manual).

Table 2.3.6, Coding for the collecting and analysing institutes for the mackerel and horse mackerel gonad samples during the survey in 2022.

Code	Country	Species
A	Ireland	mac
B	Ireland	hom
C	Scotland	mac
D	Scotland	hom
E	Norway	mac
F	Norway	hom
G	Germany	mac
H	Germany	hom
I	Netherlands	mac
J	Netherlands	hom
K	AZTI	mac
L	AZTI	hom
M	IEO	mac
N	IEO	hom
O	Portugal	mac
P	Portugal	hom
Q	Faroer	mac
R	Faroer	hom
S	Eng	NS mac
T	Denmark	NS mac
U	WGIPS Ireland	mac
V	WGIPS Netherlands	mac
W	WESPAS	hom
X	PFA	mac
Y	PFA	hom

Sample labels for mackerel will be printed and sent to the survey participants by Merete Fonn and Anders Thorsen, Institute for Marine Research, Norway. Sample labels for horse mackerel will be printed and sent to the survey participants by Cindy van Damme and Ewout Blom, Wageningen Marine Research, The Netherlands.

2.4 Updates ICES Fecundity and Atresia database

WGALES has been asked to comment on the beta version of the fecundity and Atresia database. There will be a more updated version of the database at the beginning of 2022. This should be tested in February 2022 and ready to start populating from second quarter of 2022.

3 ToR C

Review current, previously utilized and new developed methods and calculations for realised fecundity estimation as well as batch fecundity and spawning fraction estimation, and document changes in procedures and their consequences in a protocol to be stored on the WGMEGS GitHub; ICES Science plan 3.1, 3.3, 5.1

The aim of the ToR-c is to review the methods and the calculations currently in used in the estimation of adult parameters in each egg production method. It pretends to document all of them to guarantee reproducibility and store them in a way that makes it available to everyone such as ICES GitHub (https://GitHub.com/ices-eg/ wg_WGMEGS).

Two subgroups each dealing with an egg production method were created to comply with the objective of this ToR. All the issues discussed and agreed modification were communicated to ToR-d in case they were changes that need to include in the manuals.

3.1 AEPM subgroup

- **WGMEGS GitHub.**

Within the WGMEGS repository on the ICES GitHub some new folders were created to host the scripts to be used when estimating adult parameters for AEPM and DEPM implementation, i.e., survey samples processing and calibration exercise scripts.

Collin Miller from ICES was contacted to request permission to these repositories for more people within the group.

- **Series of Biodata**

Biodata for Atlantic mackerel from all years is in the SharePoint now. There were some slight changes in the naming of the variable from year to year; it was agreed to properly describe the data there is in templates for all years and include the label of the sample as well. In a second step, variables names should match with the fields ICESvocabulary (https://vocab.ices.dk/). It was agreed to it ready for the report in autumn for the survey 2022.

It was also discussed the need to collect the series of biodata for horse mackerel.

- **Oocyte recruitment threshold**

Results from dos Santos Schmidt et al. (2021) suggest that previtellogenic oocytes recruit to vitellogenic oocytes at a size of around 230 microns. This is larger than the 185 microns currently considered by WGMEGS for Atlantic mackerel potential fecundity estimation. It was therefore agreed that during the 2022 MEGS survey oocytes above 185 microns would be measured, as well as counted. The idea is to identify the batch of oocytes that pivot between both thresholds to know the magnitude of the overestimation.

It was agreed as well to double check the fecundity images analysis to avoid missing oocytes. This procedure was included accordingly in the manual SISP-5 and modified whole mount template.

3.2 DEPM subgroup

- **Getting enough samples to batch fecundity**

As the number of valid samples for batch fecundity remains low, during the workshop a change in the choice of samples valid for batch fecundity was decided. In the 2019 survey only ovaries that were in hydration stage 2 were considered valid for batch fecundity. Hydration 3 samples were discarded to ensure that spawning had not started.

During the 2021 North Sea mackerel egg survey there were very few samples in hydration stage 2, so samples in hydration stage 3 were used, resulting in a considerable increase in the number of valid samples for batch fecundity.

It was therefore decided to use samples in hydration stages 2 and 3 in the 2022 survey. Appropriate changes will be made to the screening filters. To make sure that spawning has not started, whole mount samples will be checked for the absence of POFs in those ovaries that show a batch.

- **Batch fecundity calculations**

In mackerel, the existence of a group of separate oocytes forming the batch must be visually checked. In order to visualise the distribution of oocyte diameters, different size groupings were tested. The most suitable for the range of diameters were 25 microns and 50 microns groups. It was decided to use the 50 microns size because the separation of the batch was well visualised and the 25 microns size produced separations at different diameter sizes, which did not correspond to the spawning batch. During the workshop, a working document was presented that supports the 50-micron groups in identifying the batch. The work compares the results of batch fecundity performed in the WGMEGS (with 50 microns separation) and batch fecundity performed by the traditional gravimetric method and concludes that there are no differences.

- **Notes on the data before submission to the group**

The weight of the ovary must be taken fresh. Laboratories taking the weight after fixation of the ovary shall submit the data after conversion to fresh weight. The conversion factor shall be specified in observations.

It was agreed that all participants had to check outliers and quality of the data before submission. It is advisable to make a size-weight graphs to avoid outliers; batch fecundity data from image analysis should be submitted in a single excel file and it should be checked that there are no blank lines between samples. For this purpose, it is advisable to make a dynamic table and check that all the samples that have been worked on appear.

- **Fishing time**

To record the fishing time (GMT) was considered of significance in order to clarify any diel periodicity of the spawning activity of the Atlantic mackerel. The fishing haul template was modified accordingly to include the fishing time (SISP-5).

- **Small Pelagic Symposium**

Small Pelagic Symposium will be held in Lisbon 7-11 November 2022 where there will be a workshop on egg production methods, co-chaired by a member of WEGMEGS, Cristina Nunes. It was considered an opportunity to contact people from other areas that can help WGMEGS dealing with new ideas to apply DEPM.

The link to the symposium website is following one:
https://meetings.pices.int/meetings/international/2022/pelagic/scope.

4 ToR D

Review available documentation on adult parameters estimation, both textual and figures, to redefine the standard protocols and update the survey manual; ICES Science plan 3.1, 3.3, 5.1

The ToR-d targets to review the protocols and survey manuals as well as templates available to incorporate where appropriate the improvements resulting from the discussions in the preceding ToRs.

- **Overview**

The SISP-5 manual on fecundity for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel has been deeply reorganized in favour of a more comprehensive structure, i.e., repeated sections were deleted and referenced, and further explanations were added in vague sections. This was considered a progress work in view of the future transformation of the manual into Times Series.

Discussions held in both AEPM and DEPM subgroups came to several agreed decision for changes to be made in the manual. The main modifications are more detailed in ToR-c section of the present report and listed below. They are also collected in the Annex 1 of the revised SISP-5 manual.

- New ovary sampling and analysis diagram.
- Samples containing the oocytes stage 5 hydration state 2 and 3 will be analysed for batch fecundity.
- New screening template accordingly.
- Count and measure all oocytes above 185 μm in fecundity samples analysis.
- Double check of fecundity samples analysis.
- New whole mount template accordingly.
- Large counting frame in atresia analysis.

- **North Sea Survey**

The North Sea mackerel egg survey will be carried out in June 2022, at the same time as the western spawning area survey. Sampling differences were discussed in the manual. The North Sea survey will collect only batch fecundity mackerel samples as only DEPM is applied in this area. All samples collected in Western and North Sea areas will be processed by those laboratories involved in this task, so the laboratory carrying out the North Sea survey will also process samples coming from western spawning area. For that reason, additional sample codes have been specified in the manual SISP-5.

5 References

ICES. 2021. ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS: outputs from 2020 meeting) ICES Scientific Reports. 3:11. 88pp. https://doi.org/10.17895/ices.pub.7899

ICES. 2019. Manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel. Series of ICES Survey Protocols SISP 5. 89 pp. http://doi.org/10.17895/ices.pub.5139.

ICES. 2019. Manual for mackerel and horse mackerel egg surveys, sampling at sea. Series

of ICES Survey Protocols SISP 6. 82 pp. http://doi.org/10.17895/ices.pub.5140

ICES. 2018. Report of the Workshop on egg staging, fecundity, and atresia in horse mackerel and mackerel (WKFATHOM2). 8-12 October and 19-23 November. Bremerhaven, Germany and IJmuiden, Netherlands. ICES CM 2018/EOSG:22. 74pp.

Vitale, F., Worsøe Clausen, L., and Ní Chonchúir, G. (Eds.) 2019. Handbook of fish age estimation protocols and validation methods. ICES Cooperative Research Report No. 346. 180 pp. http://doi.org/10.17895/ices.pub.5221

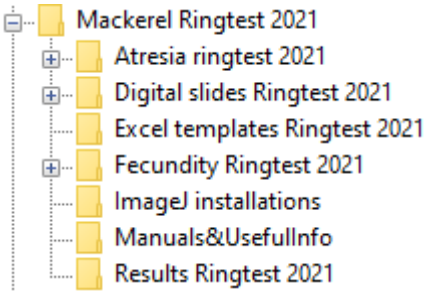
Annex 1: Guidelines for calibration exercises

Workshop on Adult Egg Production Methods Parameters estimation in Mackerel and Horse Mackerel (WKAPEM)

San Sebastian, 22-26 November 2021

CALIBRATION EXERCISES PREVIUOS TO WK

All the material we need for the calibrations is already within the folder "Mackerel Ringtest 2021" in the ftp.imr.no.



For those that did not access into the FTP previously, this is the information you need to type in the corresponding fields in FileZilla (client access to the ftp).

ftp.imr.no
usename: ########
PASSword: ######
Port: ##

- MANUALS AND ADDITIIONAL INFORMATION

In the "Manuals&UsefulInfo" folder we will find several documents which will assist us in the exercises.

SISP 5 - WGMEGS Manual for AEPM and DEPM.pdf
WGMEGS_2020.pdf
WKFATHOM report 2018.pdf

- EXERCISES

There are four calibration exercises we should perform before the workshop: Screening, POF staging, Fecundity and Atresia.

Screening: 27 ovary images.
- Check section 5.1 of SISP5 and 4.4.1 section of WKFATHOM (2018) to proceed.

POF staging: 25 ovary images.
- Check 8.7.2 section of SISP 5 to proceed.

Fecundity: 10 samples.
- Check 6.1 section of SISP 5 and section 5.3.4. and Annex 3 of WKFATHOM (2018) to proceed.

Atresia: numbers 1-7 samples for profile counting/numbers 10-16 for point counting

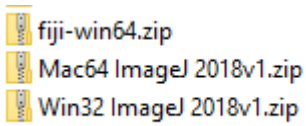
- Check section 7.2 of SISP 5 and section 5.3.3. of WKFATHOM (2018) to proceed.
- Beware: there is a new ojj project "Weibel_9.6". This is for point counting only. This will allow to compare markings collectively. "Cell-Count-Weibel-8.ojj" project is for profile counting only this wk. During August Anders will send us detailed instructions for the use of "Weibel 9.6".

- SOFTWARE

The first two exercises are based on images from scanned slides. We will need a viewer software, which we can easily download from the following link (it is free):

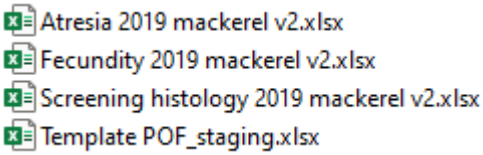
https://www.hamamatsu.com/eu/en/product/type/U12388-01/index.html

For the other two, we will need ImageJ to be installed. If not, you will find the executable in the folder named accordingly.



- TEMPLATES

In the "Excel templates Ringtest 2021" folder we will find several templates, one for each type of exercise.



- RESULTS

Results should be pasted into respective template. Name the results file with the name of exercise and your institute name, for example:

"MAC2021RINGTEST_atresia_IEO.xlsx", "MAC2021RINGTEST_fecundity_MI.xlsx", etc.

Place the results files within the "Results" folder. Each participating institute should get 4 different files at the end. If more than one person is performing the exercise within the same institute, please gather all the different results in a one file.

- DEADLINE

The deadline for sending the results is mid-October. Then we can start when it suits us best and adjust it according to our schedules. Nevertheless, we can make an online meeting in September to discuss doubts, etc.

Annex 2: Manual for atresia Weibel project version 9.5

These samples use only one large image for each sample and also use a new Weibel grid project file with a different user interface. The reason that we want to use this different setup for this part of the ringtest is that we then can compare afterwards all the different results directly on top of each other in ImageJ.

How to do the analysis

When you first open the project file you should link the image as usual (e. g. push the Link all button). After that you ad the Weibel grid (ObjectJ/Initialize Grid [F3].

Next step is to open the "Panel" (ObjectJ/Show Panel [P]. The panel shows the different counting categories and their numeric shortcut.

When you want to mark a grid point you hold the cursor above the grid point and then press the the numeric shortcut for that category on your keyboard.

For marking large areas of grid points (like sometimes for negative grid). You can use the swatter (ObjectJ/Swatter All/None [A]; paint the area with the swatter and then hit the shortcut on the keyboard for that category.

There are other functionality of this project file also. If you need them look in the manual that are attached.

Note that for this exercise we do not do the profile counting.

Project file name

To compare the results afterwards it is important that we all give the project file a name that identifies the person in imageJ (If you want you can use a secret alias name).

The name of the project files should contain 3 underscores and contain the the ID character and the observer name as follows:

ID: single character between 2nd and 3rd underscore

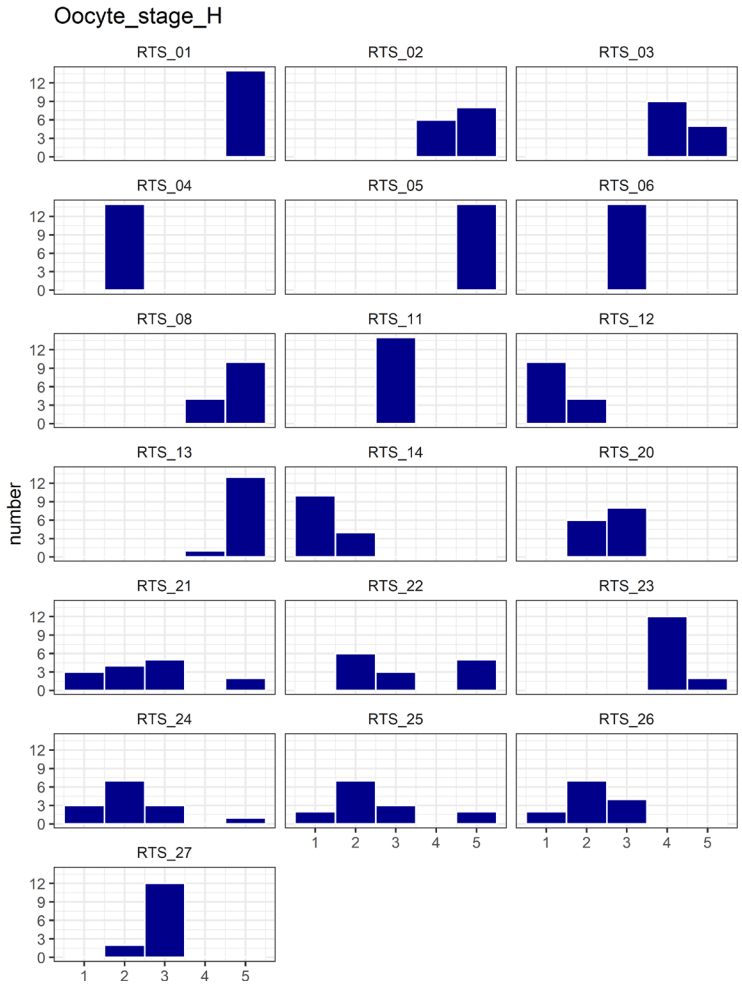
Name: part between last underscore and .ojj extension

Example: Weibel_9.6_a_Arie.ojj ID = 'a', name = 'Arie'

The Netherlands: a-f
IEO: g-k
AZTI: l-p
Scotland: q-t
IMR: v-z

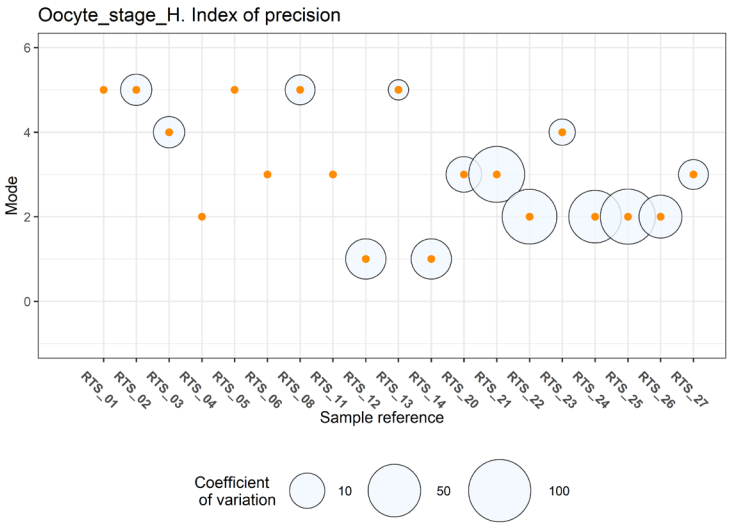
Annex 3: Results calibration exercise (ToR A)

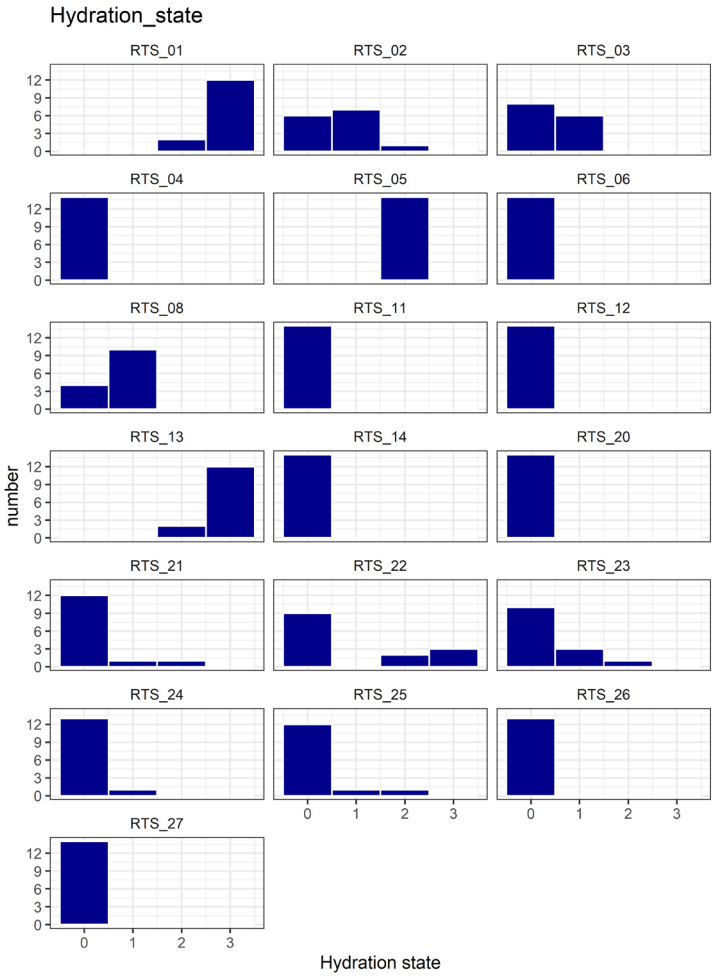
- **Screening**



Results of screening: Oocyte stage

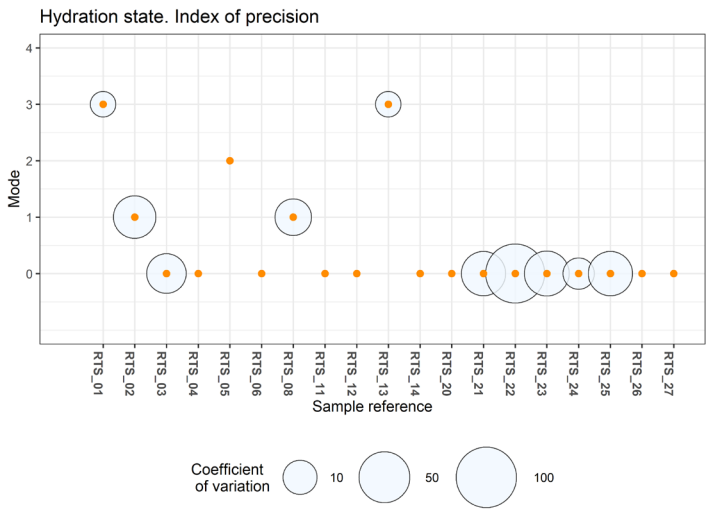
Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	5.00	5	14	0	0
RTS_02	4.57	5	14	11	6
RTS_03	4.36	4	14	11	6
RTS_04	2.00	2	14	0	0
RTS_05	5.00	5	14	0	0
RTS_06	3.00	3	14	0	0
RTS_08	4.71	5	14	9	5
RTS_11	3.00	3	14	0	0
RTS_12	1.29	1	14	32	17
RTS_13	4.93	5	14	3	1
RTS_14	1.29	1	14	32	17
RTS_20	2.57	3	14	19	10
RTS_21	2.57	3	14	39	64
RTS_22	3.29	2	14	37	58
RTS_23	4.14	4	14	6	3
RTS_24	2.21	2	14	33	50
RTS_25	2.50	2	14	37	60
RTS_26	2.15	2	13	24	22
RTS_27	2.86	3	14	8	5

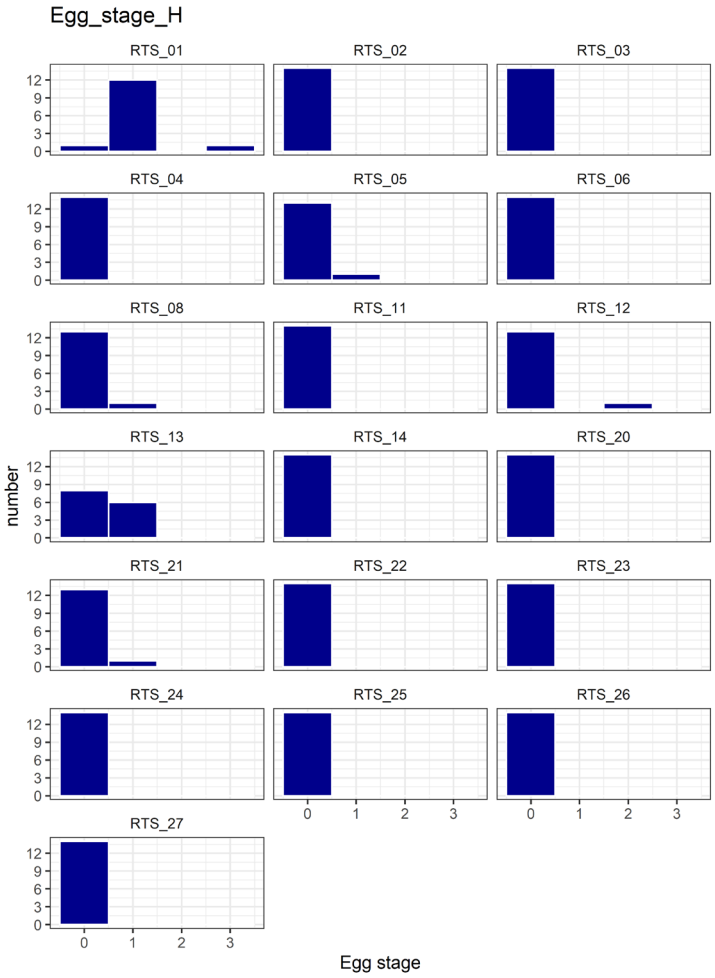




Results of Hidaratation state

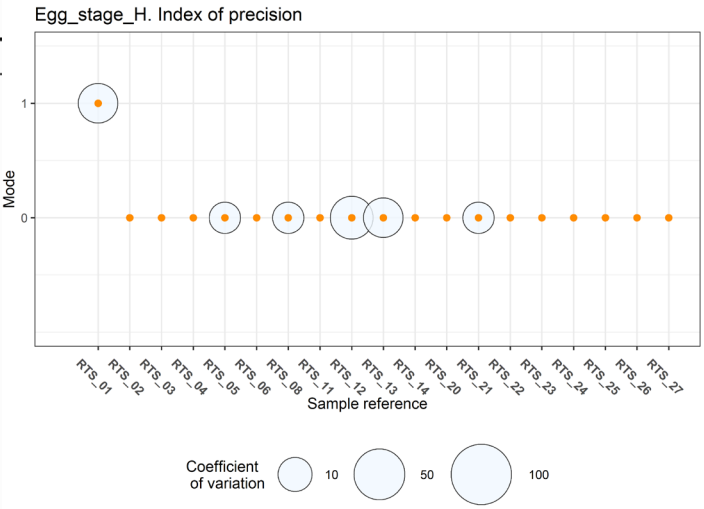
Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	2.86	3	14	6	3
RTS_02	0.64	1	14	34	24
RTS_03	0.43	0	14	34	18
RTS_04	0.00	0	14	0	0
RTS_05	2.00	2	14	0	0
RTS_06	0.00	0	14	0	0
RTS_08	0.71	1	14	24	13
RTS_11	0.00	0	14	0	0
RTS_12	0.00	0	14	0	0
RTS_13	2.86	3	14	6	3
RTS_14	0.00	0	14	0	0
RTS_20	0.00	0	14	0	0
RTS_21	0.21	0	14	30	28
RTS_22	0.93	0	14	62	91
RTS_23	0.36	0	14	38	29
RTS_24	0.07	0	14	12	7
RTS_25	0.21	0	14	30	28
RTS_26	0.00	0	13	0	0
RTS_27	0.00	0	14	0	0

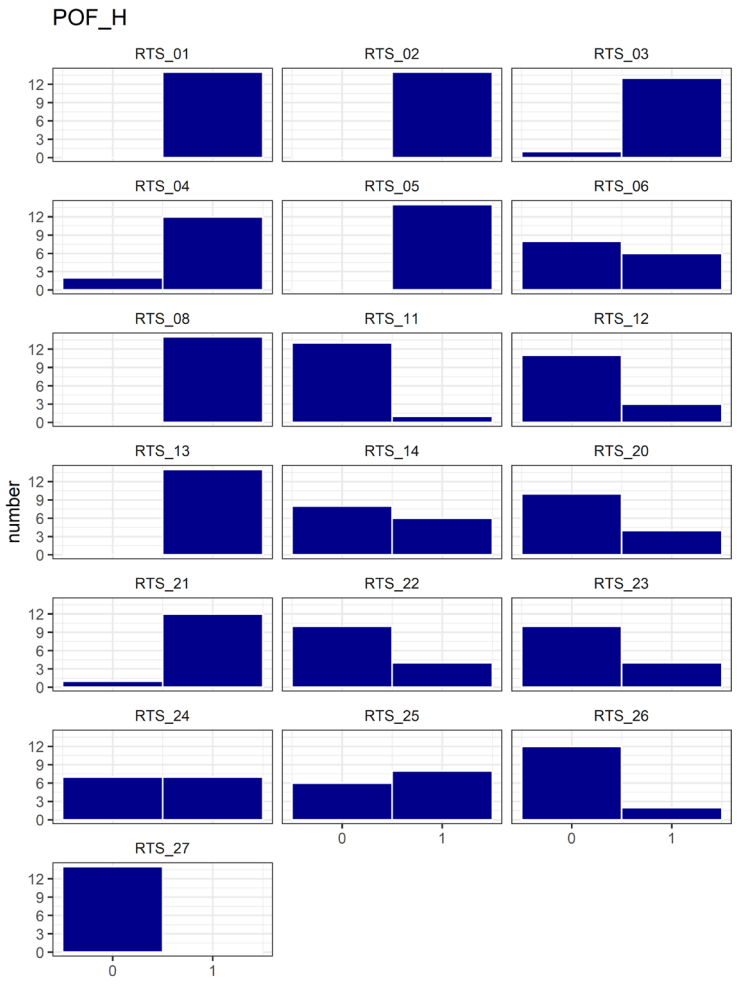




Results of screening: Eeg stage

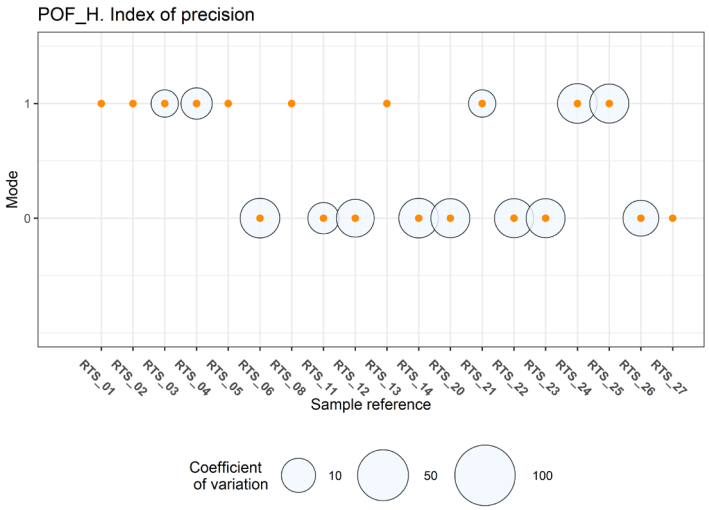
Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	1.07	1	14	13	18
RTS_02	0.00	0	14	0	0
RTS_03	0.00	0	14	0	0
RTS_04	0.00	0	14	0	0
RTS_05	0.07	0	14	12	7
RTS_06	0.00	0	14	0	0
RTS_08	0.07	0	14	12	7
RTS_11	0.00	0	14	0	0
RTS_12	0.14	0	14	23	25
RTS_13	0.43	0	14	34	18
RTS_14	0.00	0	14	0	0
RTS_20	0.00	0	14	0	0
RTS_21	0.07	0	14	12	7
RTS_22	0.00	0	14	0	0
RTS_23	0.00	0	14	0	0
RTS_24	0.00	0	14	0	0
RTS_25	0.00	0	14	0	0
RTS_26	0.00	0	14	0	0
RTS_27	0.00	0	14	0	0

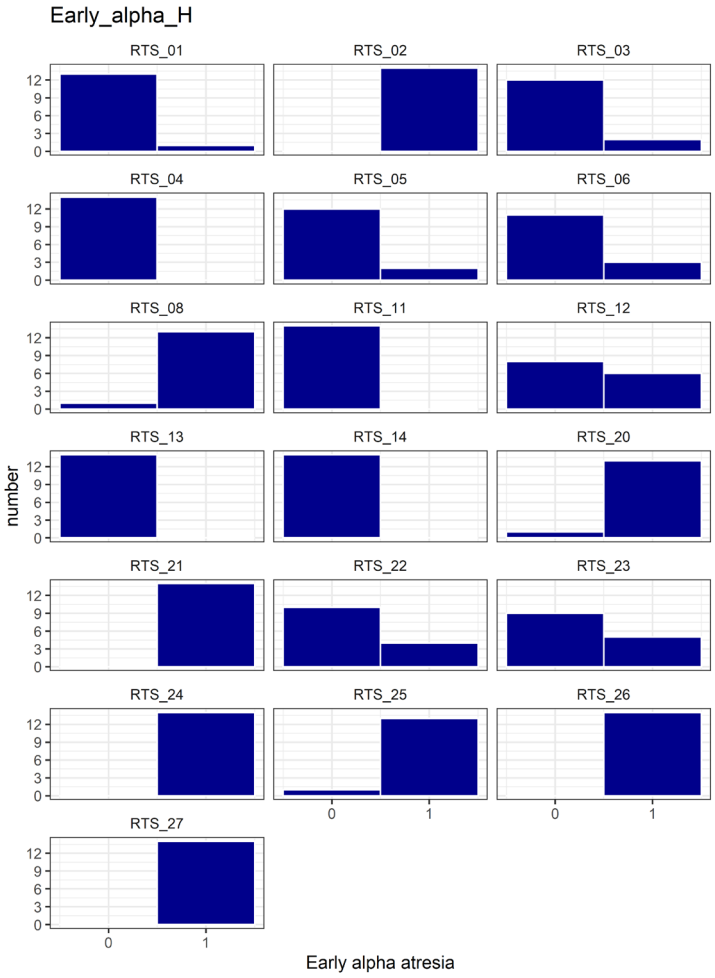




Results os screening: POF

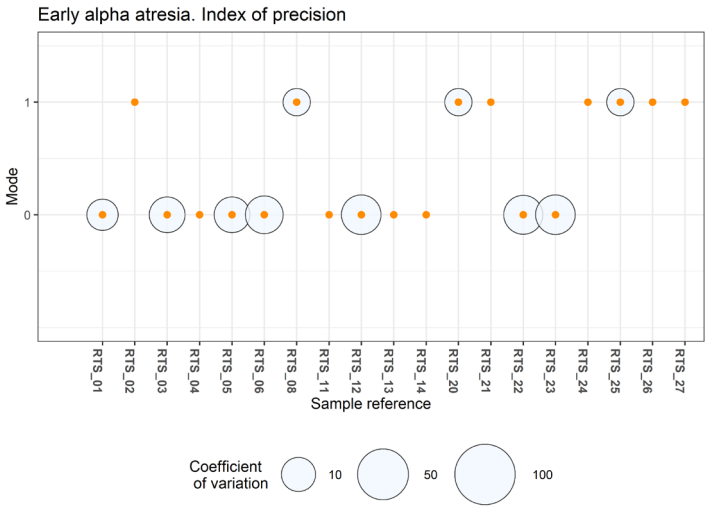
Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	1.00	1	14	0	0
RTS_02	1.00	1	14	0	0
RTS_03	0.93	1	14	7	4
RTS_04	0.86	1	14	13	7
RTS_05	1.00	1	14	0	0
RTS_06	0.43	0	14	34	18
RTS_08	1.00	1	14	0	0
RTS_11	0.07	0	14	12	7
RTS_12	0.21	0	14	28	15
RTS_13	1.00	1	14	0	0
RTS_14	0.43	0	14	34	18
RTS_20	0.29	0	14	32	17
RTS_21	0.92	1	13	8	4
RTS_22	0.29	0	14	32	17
RTS_23	0.29	0	14	32	17
RTS_24	0.50	1	14	33	18
RTS_25	0.57	1	14	31	17
RTS_26	0.14	0	14	21	12
RTS_27	0.00	0	14	0	0

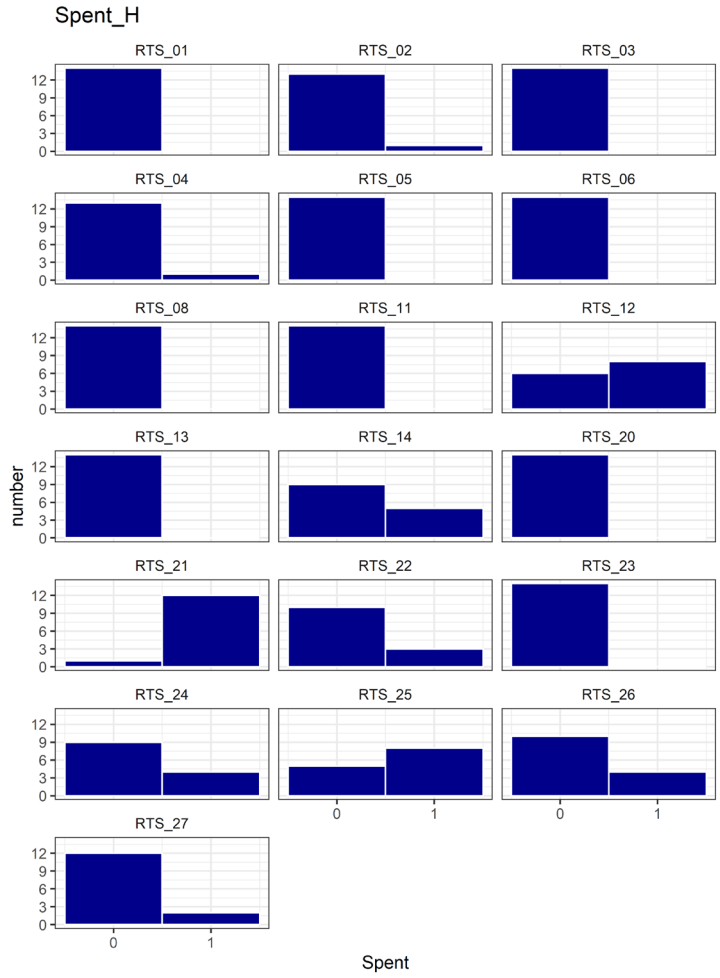




Results of screening: Early alpha atresia

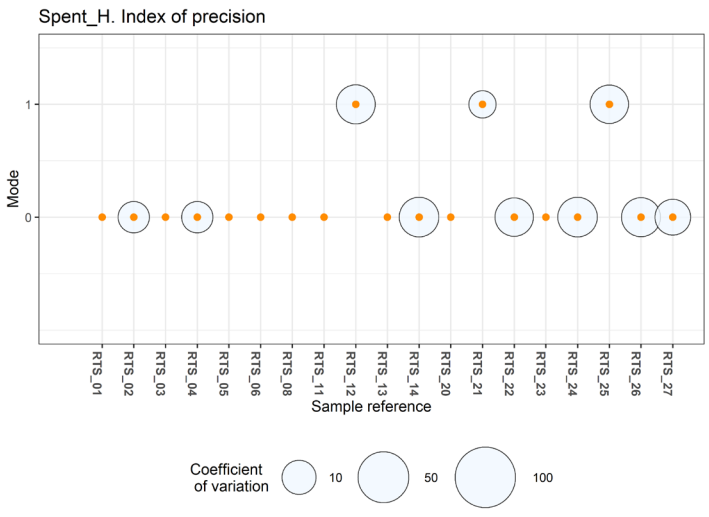
Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	0.07	0	14	12	7
RTS_02	1.00	1	14	0	0
RTS_03	0.14	0	14	21	12
RTS_04	0.00	0	14	0	0
RTS_05	0.14	0	14	21	12
RTS_06	0.21	0	14	28	15
RTS_08	0.93	1	14	7	4
RTS_11	0.00	0	14	0	0
RTS_12	0.43	0	14	34	18
RTS_13	0.00	0	14	0	0
RTS_14	0.00	0	14	0	0
RTS_20	0.93	1	14	7	4
RTS_21	1.00	1	14	0	0
RTS_22	0.29	0	14	32	17
RTS_23	0.36	0	14	34	18
RTS_24	1.00	1	14	0	0
RTS_25	0.93	1	14	7	4
RTS_26	1.00	1	14	0	0
RTS_27	1.00	1	14	0	0

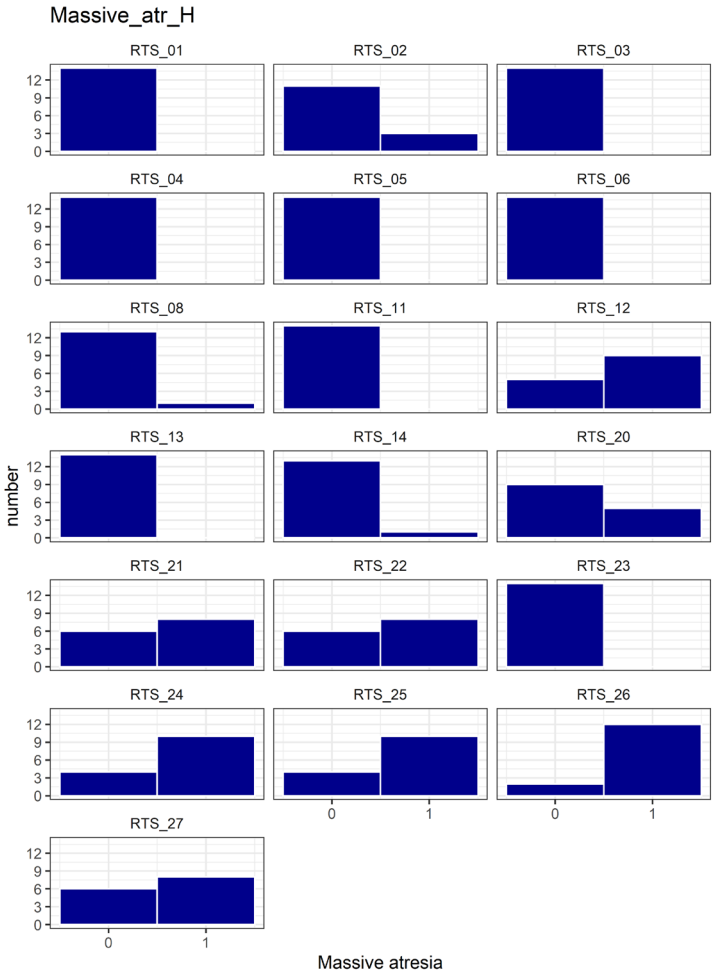




Results of screening: Spent

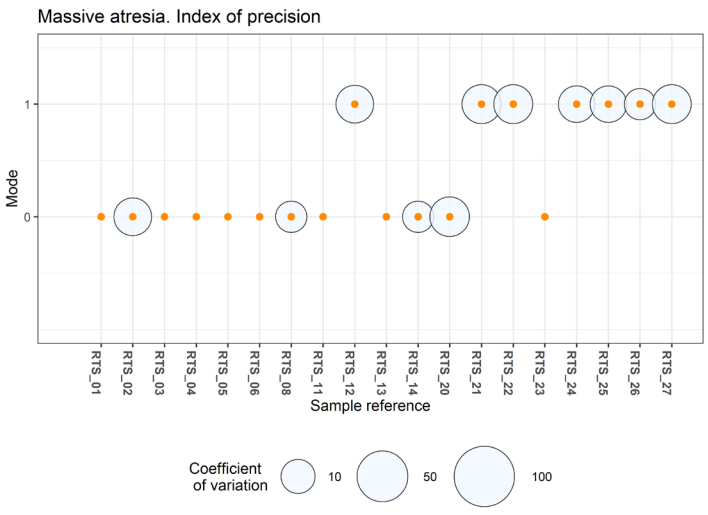
Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	0.00	0	14	0	0
RTS_02	0.07	0	14	12	7
RTS_03	0.00	0	14	0	0
RTS_04	0.07	0	14	12	7
RTS_05	0.00	0	14	0	0
RTS_06	0.00	0	14	0	0
RTS_08	0.00	0	14	0	0
RTS_11	0.00	0	14	0	0
RTS_12	0.57	1	14	31	17
RTS_13	0.00	0	14	0	0
RTS_14	0.36	0	14	34	18
RTS_20	0.00	0	14	0	0
RTS_21	0.92	1	13	8	4
RTS_22	0.23	0	13	29	16
RTS_23	0.00	0	14	0	0
RTS_24	0.31	0	13	33	18
RTS_25	0.62	1	13	29	16
RTS_26	0.29	0	14	32	17
RTS_27	0.14	0	14	21	12



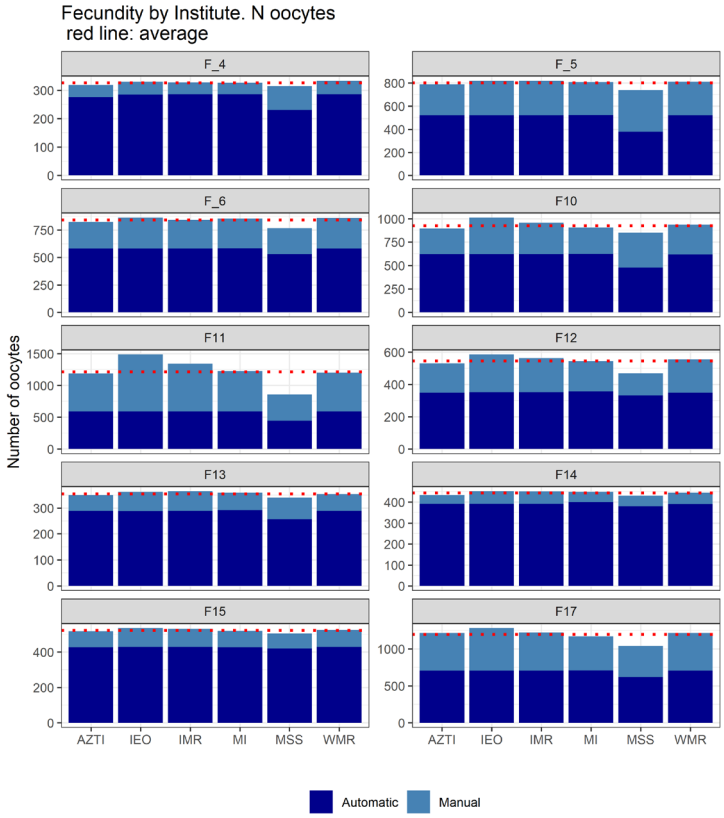


Results of screening: Massive atresia

Sample_ref	average	mode	total_reader	APE	CV_average
RTS_01	0.00	0	14	0	0
RTS_02	0.21	0	14	28	15
RTS_03	0.00	0	14	0	0
RTS_04	0.00	0	14	0	0
RTS_05	0.00	0	14	0	0
RTS_06	0.00	0	14	0	0
RTS_08	0.07	0	14	12	7
RTS_11	0.00	0	14	0	0
RTS_12	0.64	1	14	28	15
RTS_13	0.00	0	14	0	0
RTS_14	0.07	0	14	12	7
RTS_20	0.36	0	14	34	18
RTS_21	0.57	1	14	31	17
RTS_22	0.57	1	14	31	17
RTS_23	0.00	0	14	0	0
RTS_24	0.71	1	14	24	13
RTS_25	0.71	1	14	24	13
RTS_26	0.86	1	14	13	7
RTS_27	0.57	1	14	31	17

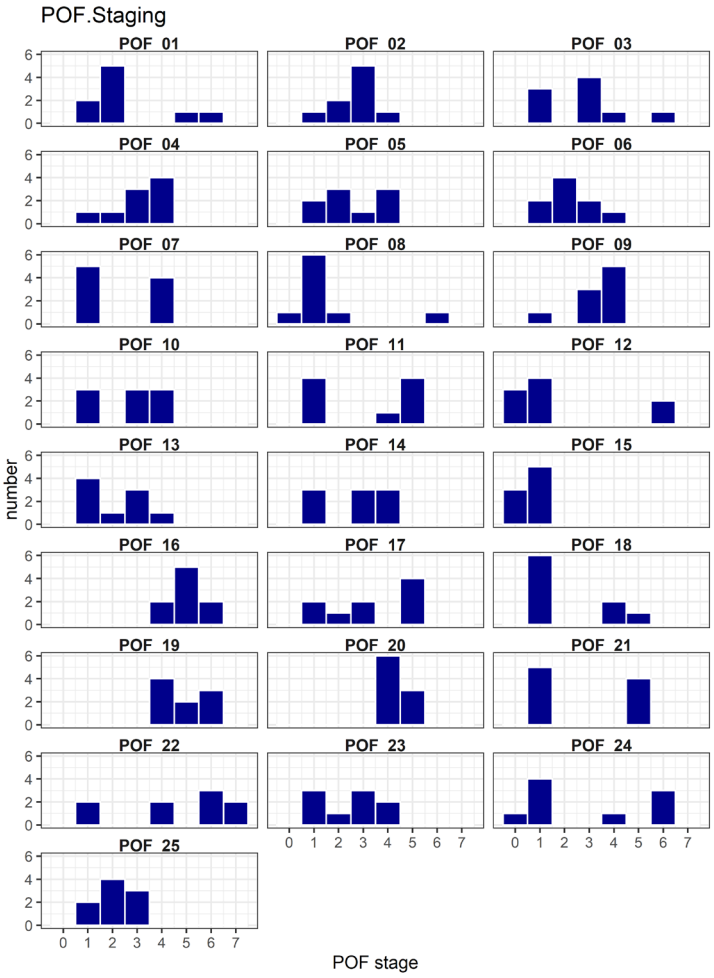


- **Fecundity**


Fecundity by Institute. N oocytes
red line: average
F_4
F_5
F_6
F10
F11
F12
F13
F14
F15
F17
Number of oocytes
AZTI
IEO
IMR
MI
MSS
WMR
Automatic
Manual

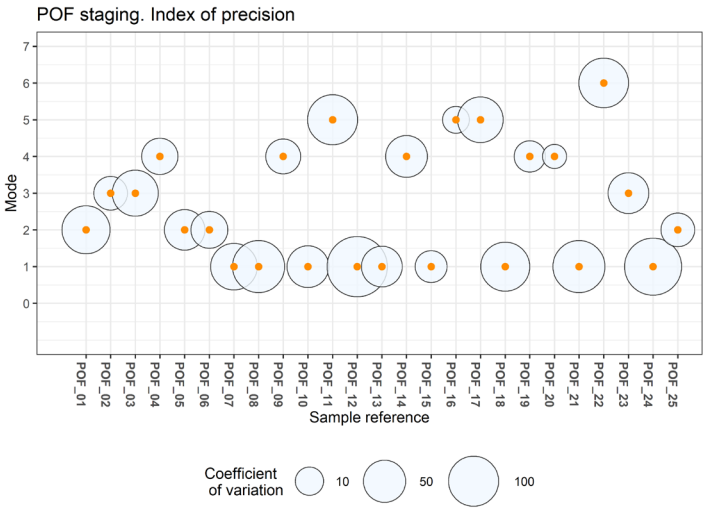

Fecundity by reader. N oocytes
red line: average
F_4
F_5
F_6
F10
F11
F12
F13
F14
F15
F17
Number of oocytes
AS
BOH
CP
CvD
DTY
EB
EKo
GNIC
GT
HH
HW
IM
IP
MK
PA
Reader
Automatic
Manual

- **POF staging**

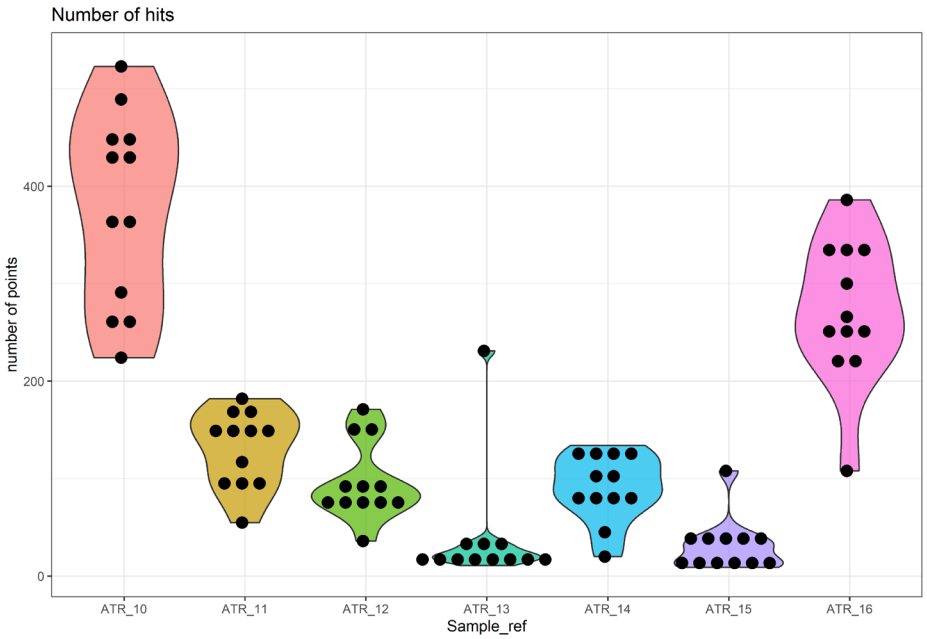


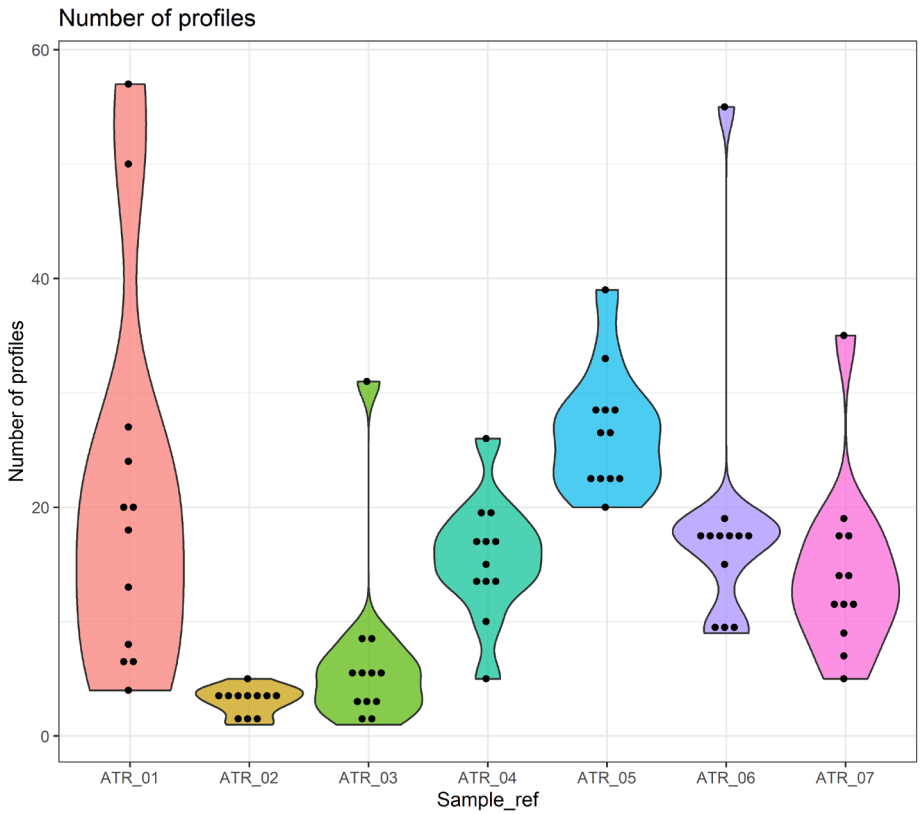
Results in POF staging

Sample_ref	average	mode	total_reader	APE	CV_average
POF_01	2.56	2	9	37	85
POF_02	2.67	3	9	18	20
POF_03	2.78	3	9	31	71
POF_04	3.11	4	9	19	27
POF_05	2.56	2	9	30	43
POF_06	2.22	2	9	23	29
POF_07	2.33	1	9	44	75
POF_08	1.56	1	9	43	118
POF_09	3.33	4	9	17	23
POF_10	2.67	1	9	30	48
POF_11	3.11	5	9	46	100
POF_12	1.78	1	9	68	214
POF_13	2.11	1	9	33	44
POF_14	2.67	4	9	30	48
POF_15	0.62	1	8	29	17
POF_16	5.00	5	9	7	8
POF_17	3.33	5	9	34	69
POF_18	2.11	1	9	48	92
POF_19	4.89	4	9	13	15
POF_20	4.33	4	9	8	5
POF_21	2.78	1	9	52	118
POF_22	4.67	6	9	34	97
POF_23	2.44	3	9	31	44
POF_24	2.89	1	9	60	170
POF_25	2.11	2	9	19	20

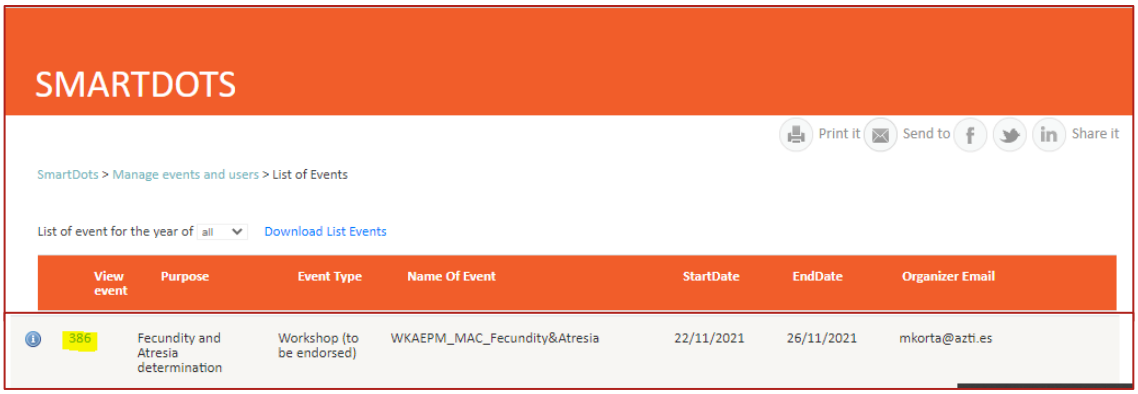


- **Atresia**

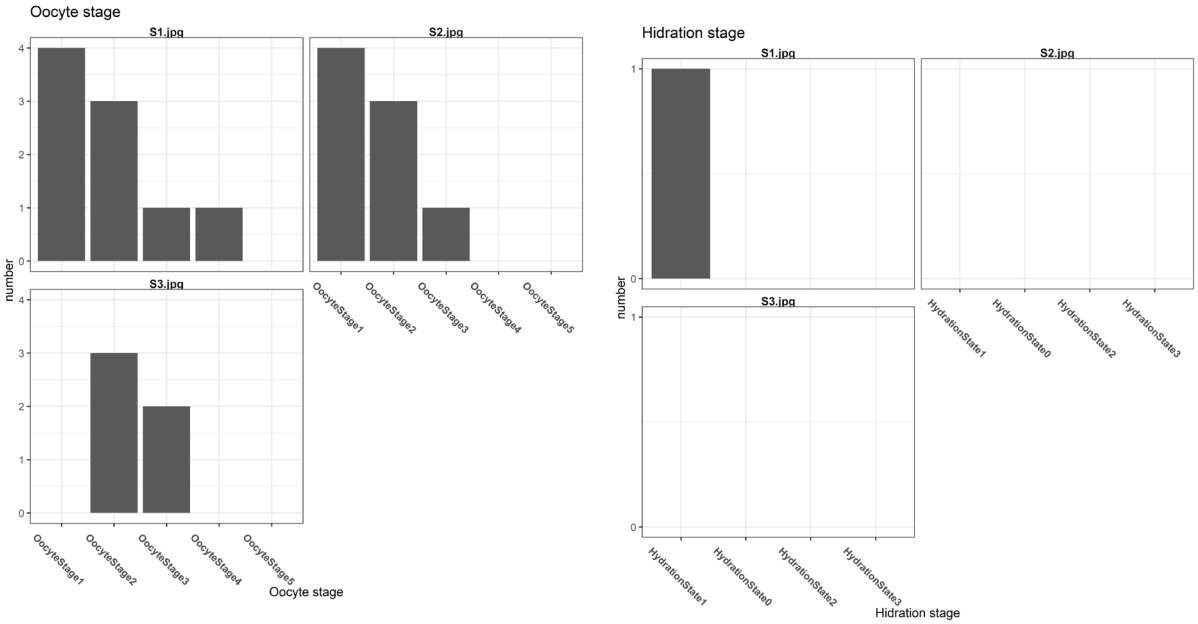

Number of hits
number of points
400
200
0
ATR_10
ATR_11
ATR_12
ATR_13
ATR_14
ATR_15
ATR_16
Sample_ref

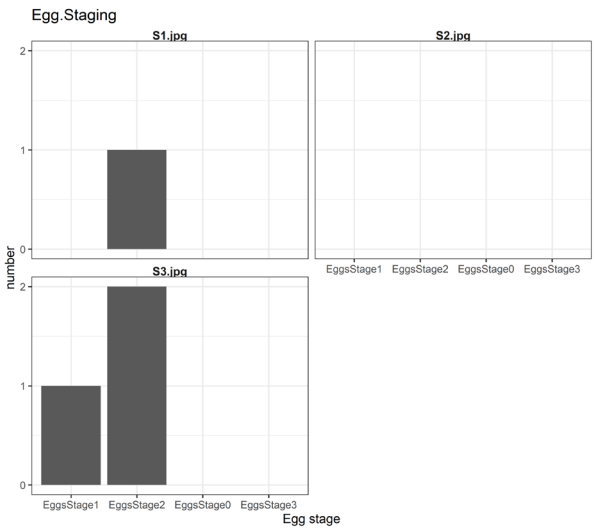

Number of profiles
Number of profiles
60
40
20
0
ATR_01
ATR_02
ATR_03
ATR_04
ATR_05
ATR_06
ATR_07
Sample_ref

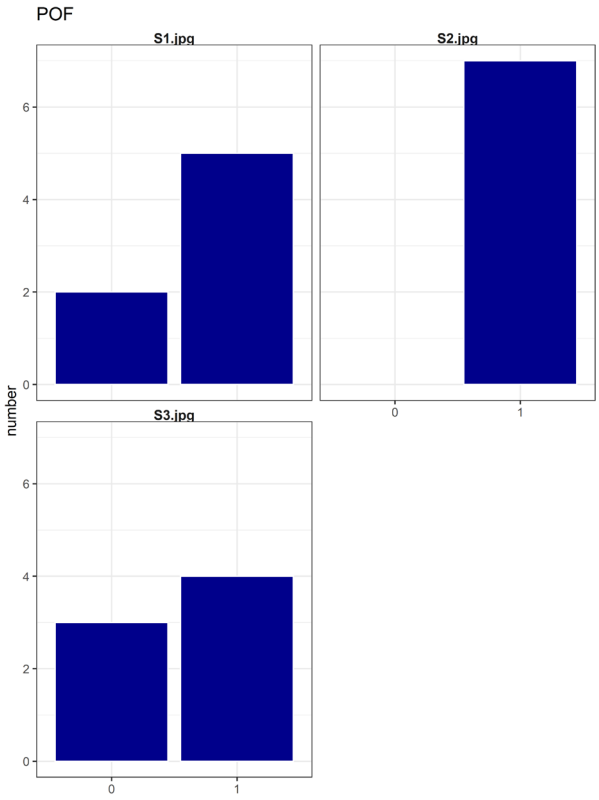
Annex 4: Results on SmartDots exercise (ToR B)

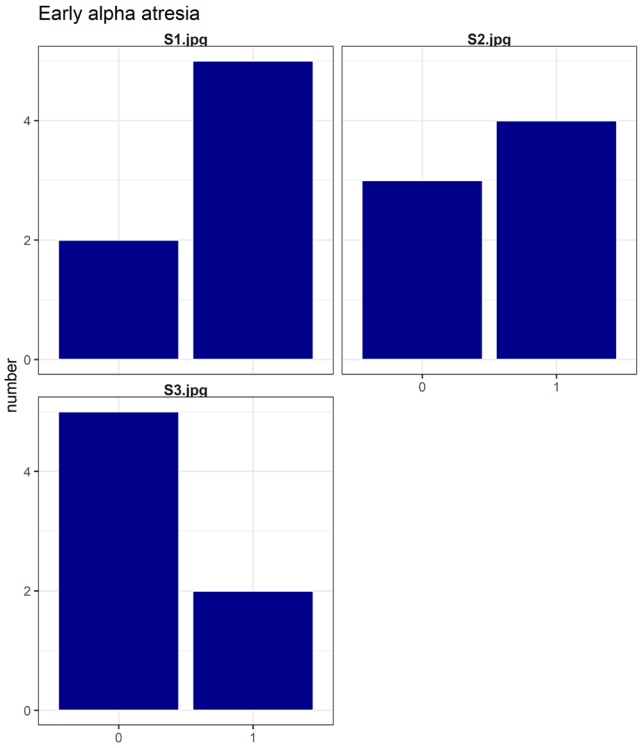


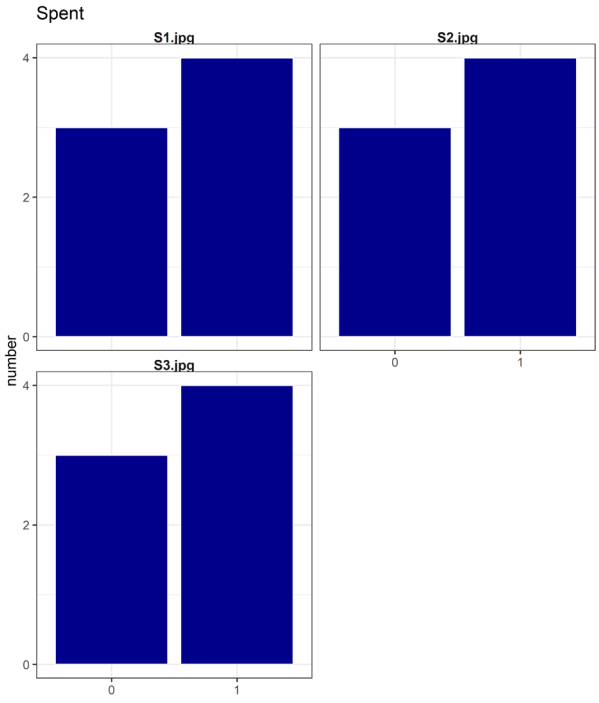
- **Screening**

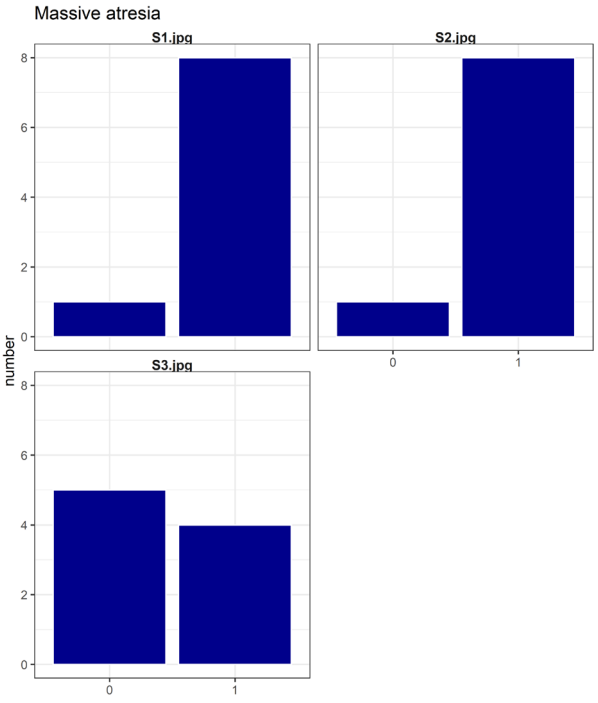




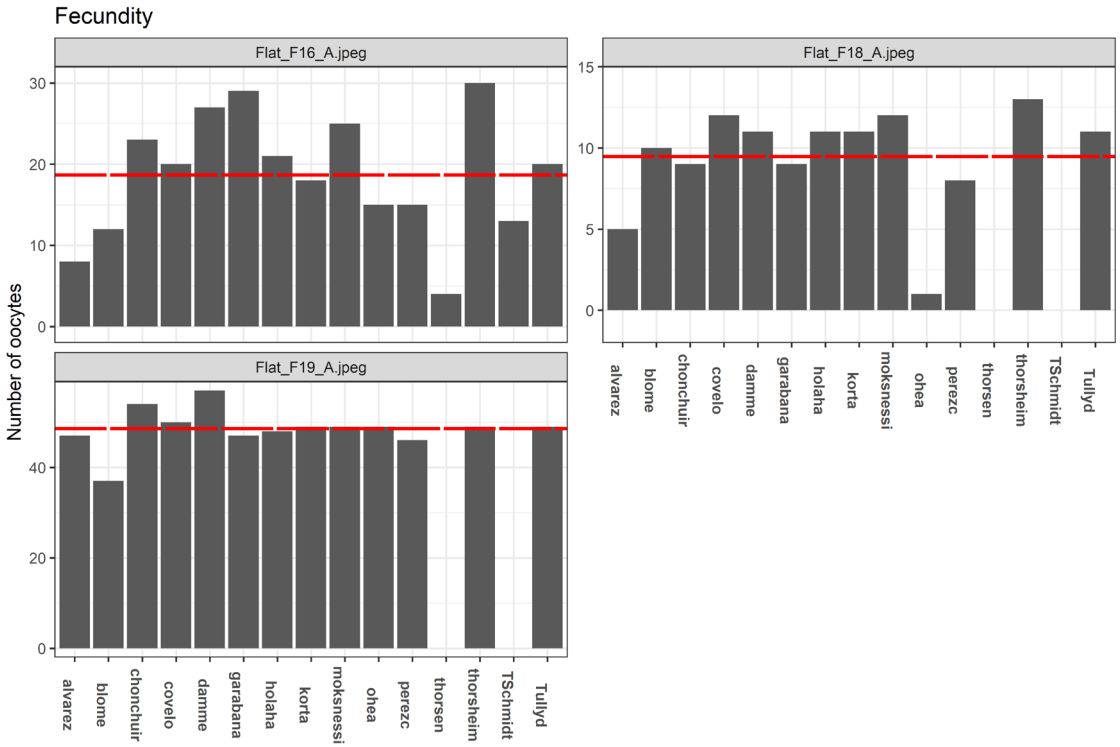

POF
S1.jpg
S2.jpg
S3.jpg
number
0
2
4
6
0
1


Early alpha atresia
S1.jpg
S2.jpg
S3.jpg
number
0
2
4
0
1

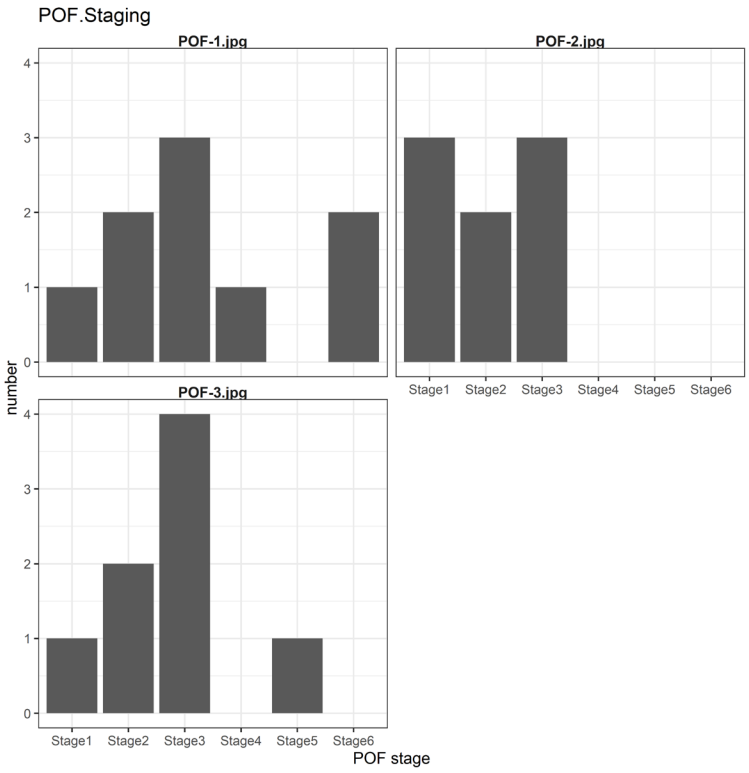

Spent
S1.jpg
S2.jpg
S3.jpg
number
0
2
4
0
1


Massive atresia
S1.jpg
S2.jpg
S3.jpg
number
0
2
4
6
8
0
1

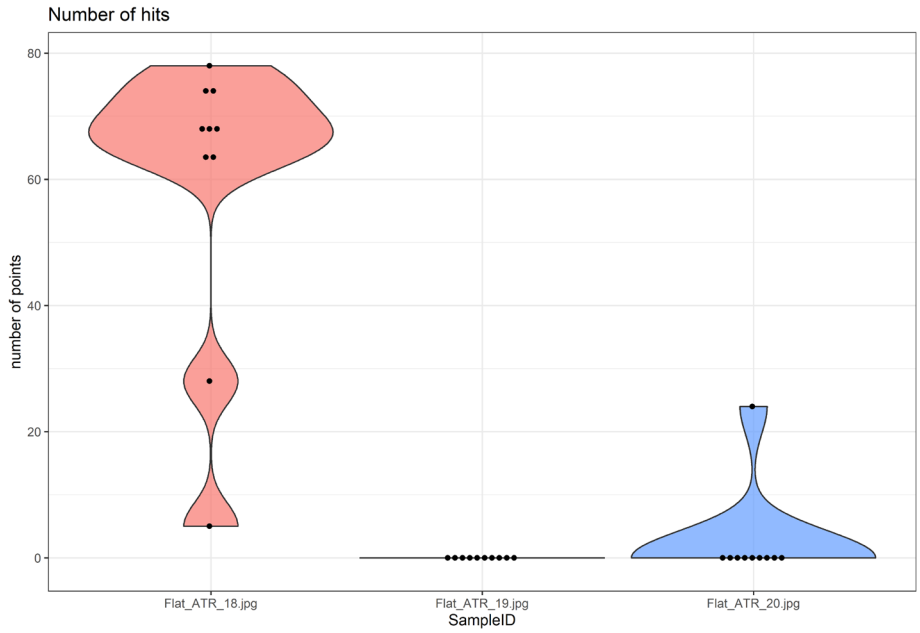
- **Fecundity**

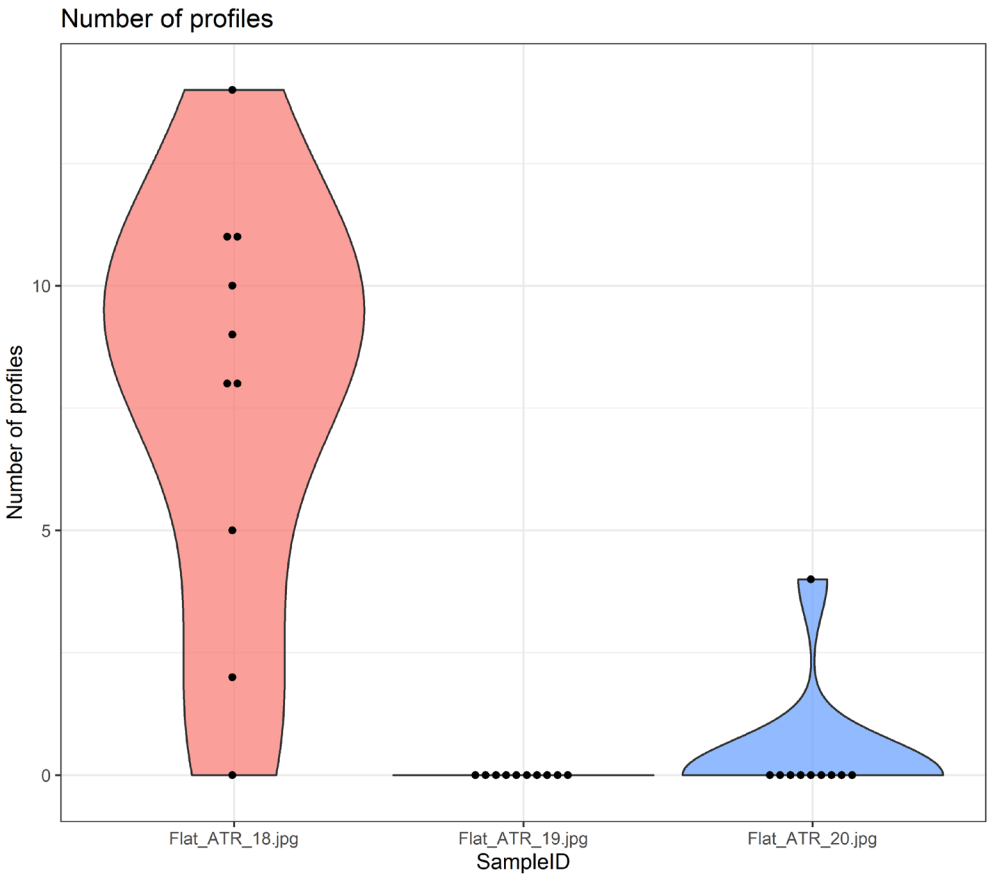


- **POF staging**



- **Atresia**


Number of hits
number of points
80
60
40
20
0
Flat_ATR_18.jpg
Flat_ATR_19.jpg
Flat_ATR_20.jpg
SampleID


Number of profiles
Number of profiles
10
5
0
Flat_ATR_18.jpg
Flat_ATR_19.jpg
Flat_ATR_20.jpg
SampleID

Annex 5: List of participants in the workshop and calibration exercise

Name	Institute	Country (of institute)	Email
Anders Thorsen	IMR	Norway	anders.thorsen@hi.no
Anne Torsvik	IMR	Norway	anne.torsvik@hi.no
Antía Lourido	IEO	Spain	antia.lourido@ieo.es
Antonio Solla	IEO	Spain	antonio.solla@ieo.es
Brendan O'Hea	MI	Ireland	brendan.ohea@marine.ie
Carlos Pinto	ICES	Denmark	carlos@ices.dk
Carlota Pérez	AZTI	Spain	cperez@azti.es
Cindy van Damme	WMR	The Netherlands	cindy.vandamme@wur.nl
Cristina Nunes	IPMA	Portugal	cnunes@ipma.pt
David Tully	MI	Ireland	david.tully@marine.ie
Dolores Garabana	IEO	Spain	dolores.garabana@ieo.es
Erika Koelemij	WMR	The Netherlands	erika.koelemij@wur.nl
Ewout Blom	WMR	The Netherlands	ewout.blom@wur.nl
Gersom Costas	IEO	Spain	gersom.costas@ieo.es
Gráinne Ní Chonchúir	MI	Ireland	grainne.nichonchuir@marine.ie
Grethe Thorsheim	IMR	Norway	grethe.thorsheim@hi.no
Hannah Holah	MSS	Scotland	hannah.holah@gov.scot
Hanz Wiegerinck	WMR	The Netherlands	hanz.wiegerinck@wur.nl
Ine Moksness	IMR	Norway	ine.moksness@hi.no
Ineke Pennock	WMR	The Netherlands	ineke.pennock@wur.n
Joana Ribero	ICES	Denmark	joana.ribeiro@ices.dk
Jonna Tomkiewicz	DTU-Aqua	Denmark	jt@aqua.dtu.dk
Liesbeth van der Vlies	WMR	The Netherlands	liesbeth.vandervlies@wur.nl
Maria Korta	AZTI	Spain	mkorta@azti.es

Marie Gjessing Bruun	DTU-Aqua	Denmark	mgjbr@aqua.dtu.dk
Paula Álvarez	AZTI	Spain	palvarez@azti.es
Thassya dos Santos Schmidt	IMR	Norway	thassya.dos.santos.schmidt@hi.no

From top left to bottom right: Maria Korta, Ine Moksness, Grethe Thorsheim, Anders Thorsen, Paula Álvarez, Antonio Solla, Antía Lourido, Brendan O'Hea, Cindy van Damme, Carlota Pérez, David Tully, Ewout Blom, Gersom Costas, Gráinne Ní Chonchúir, Hannah Holah, Marie Gjessing Bruun, Jonna Tomkiewicz, Lola Garabana, Cristina Nunes, Alondra Sofía Rodríguez and Thassya dos Santos Schmidt.

Annex 6: Resolutions

The **Workshop on Adult Egg Production Methods Parameters estimation in Mackerel and Horse Mackerel (WKAEPM)** chaired by Maria Korta*, Spain, will meet **online**, 22-26 November 2021 to:

a) Inter-calibrate the estimation of adult parameters in egg production methods (Annual and Daily Egg Production Methods), in particular, screening (histological maturity assignment), (batch) fecundity and atresia estimation, and POF staging**; ICES Science plan** 3.1, 3.3, 5.1

b) Harmonize the analysis and interpretation of results with those of previous surveys; **ICES Science plan** 3.1, 3.3, 5.1

c) Review current, previously utilized and newly developed methods and calculations for realised fecundity estimation as well as batch fecundity and spawning fraction estimation, and document changes in procedures and their consequences in a protocol to be stored on the WGMEGS GitHub; **ICES Science plan** 3.1, 3.3, 5.1

d) Review available documentation on adult parameters estimation, both textual and **figures, to redefine the standard protocols and update the survey manual; ICES Science plan** 3.1, 3.3, 5.1

WKAEPDM will report by 11 January 2022 for the attention of EOSG, WGMEGS, WGALES and WGBIOP

Supporting Information

Priority	Data quality, used to provide fisheries advice through WGWIDE, will be impaired if this workshop is not conducted.
Scientific justification	Adult parameters estimation is fundamental for conversion of egg production into spawning stock biomass of western and southern mackerel and horse mackerel stock components. Both (batch) fecundity and atresia estimation as well as spawning fraction estimation are carried out using histological and image analysis methods, and the analysis and interpretation of these materials requires standardization across participating institutes. The standardization in this aspect is carried out in workshops since 2001 which have been extremely helpful for agreed practices among institutes and is recommended that experiences gathered during these workshops be extended during the consecutive workshop in 2021. It is expected that the workshop will refine the developed methodologies and clarify established calculations for these adult parameters estimation to obtain unbiased biomass output from the egg surveys. In this sense, the workshop will also update the manual for the fecundity, atresia, and spawning fraction estimation from sampling to analysis procedures and final calculations, which will improve the agreed MEGS standard survey manual.
Resource requirements	None

Participants	Mainly scientists and technicians (approximately 20) involved in the surveys.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to advisory committees	SCICOM, ACOM
Linkages to other committees or groups	WGMEGS, WGBIOP, WGALES and WGWIDE
Linkages to other organizations	None.

Annex 7: Agenda

- Please note that the time refers to CET
- Coffee Breaks: at 11:30-11:45 and at 15:15-15:30
- SmartDorts: Chrome, Edge, Firefox should be used.

Monday 22 (ToR A: Inter-calibration adult parameters)
10:00 Start of the morning session
- Start of the meeting – Welcome and domestics. General announcements.
- Presentation on ringtest results and discussion (Gersom, Anders, Maria).
- Presentation on fecundity estimation (Thassya).
- Presentation on batch fecundity (Paula).

13:00 Lunch break
14:00 Start of the afternoon session
- Introduction into use of SmartDots for AEPM parameters analysis (Carlos Pinto).
- Ringtest results and discussion (Continue-Plenary).

15:30 Plenary
16:00 End of the day

Tuesday 23 (ToR B: Harmonize sampling and analysis)
10:00 Start of the morning session
- Short plenary.
- Continue results and discussion (Plenary).

13:00 Lunch break
14:00 Start of the afternoon session
- Continue results and discussion (Plenary).
- Introduction to individual analysis of SmartDots event Num. 386

15:30 Plenary
16:00 End of the day

Wednesday 24 (ToR C: Methods and Calculations)
10:00 Start of the morning session
- Presentation on adult parameters sampling AEPM Y DEPM (Lola)
  # Discussion on: Define Periods; PFA samples; NS additional samples
- Recommendations to WKAEPM: WGWIDE 2021.
- Conclusions on calibration results.
- Continue Individual analysis of SmartDots event Num. 386 (If necessary).
  #Counting frame clarification.

13:00 Lunch break
14:00 Start of the afternoon session
- Subgroup works: AEPM; DEPM; Manuals

15:30 Plenary
16:00 End of the day

Thursday 25 (ToR D: Results and Manuals update)

10:00 Start of the morning session

- Presentation: on adult samples tables (Cindy and Merete)
- Continue Individual analysis of SmartDots event Num. 386 (If necessary).
- Subgroup works: AEPM; DEPM; Manuals
- Plenary 12:30.

13:00 Lunch break

14:00 Start of the afternoon session

- Presentation updates on the ICES db (Joana Ribeiro)
- WGBIOP request: Atlantic mackerel reproductive strategy (CRR)
- Subgroup works: AEPM; DEPM; Manuals

15:30 Plenary

16:00 End of the day

Friday 26

09:00 Start of the morning session

- Report and assignations
- WGWIDE recommendation
- Results on the SmartDots event Num. 386.
- Check coffee breaks 10:45-11:00
- Feedback SmartDots sheets

12:30 Plenary and group photo.

13:00 End of the day and end of the wk.

Annex 8: Working documents

Batch fecundity estimation: gravimetric and volumetric method comparison

Paula Alvarez[1] and Maria Korta[1]

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Abstract

The determination of the number of eggs produced by a female in a batch (Bach Fecundity, BF) is a key parameter for the application of the daily egg production method. In MEGS surveys, this parameter is estimated by counting the number of oocytes through image analysis with samples taken with a pipette to collect 100 µl of ovary (volumetric method). This is an alternative to the gravimetric method in which the ovarian sample is taken by cutting three pieces of tissue and counting the number of hydrated oocytes with a binocular. In hydrated ovaries, the question arose whether in the volumetric method hydrated oocytes might be under-sampled due to the size of the oocytes in relation to the size of the pipette. We selected the ovary of 49 females in which BF has been estimated using the volumetric method (BFv) to estimate this parameter again using the gravimetric method (BFg). 7 samples were excluded because not hydrated oocytes were not observed in the ovarian sub-samples. Linear regression models fitted between BFs and ovary weight were statistically significant for both ($p = 0.001$, $R^2 = 0.33$, $gl = 41$). The Kruskal-Wallis test ($X = 0.0033$, $p = 0.954$) indicated that there were no statistically significant differences in BF estimates between methods. Finally, linear regression performed between BFv *vs.* BFg values when the intercept was forced to be 0 revealed a slope = 1.0367 ± 0.039 (Fig. 1, $p = 0.001$, $R^2 = 94\%$, $gl = 39$). The slope was not statistically significantly different from 1. Therefore, based on the analysis performed in this study it can be said that the hypothesis of an underestimation of BF using samples taken with the pipette has not been confirmed.

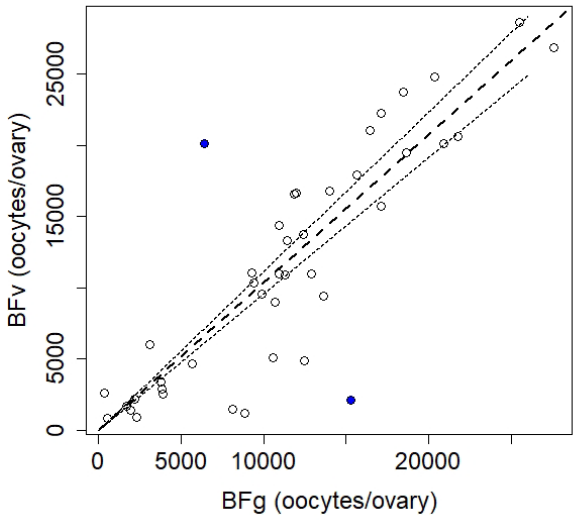

Fig. 1: Plot between BFv (Number of oocytes by ovary) vs. BFg. Dashed line is the regression line and dotted line the 95% confidence interval. The blue dots refer to the values deleted from the analysis.

First thorough assessment of de novo oocyte recruitment in a teleost serial spawner, the Northeast Atlantic mackerel (*Scomber scombrus*) case

Thassya C. dos Santos Schmidt[1], Anders Thorsen, Aril Slotte, Leif Nøttestad, Olav S. Kjesbu

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Abstract

Results of recently published paper on mackerel oocyte recruitment was presented during the workshop. The paper is part of the project Climate and Vital Rates of Marine Stocks (CLIMRATES) Our study demonstrated that the fecundity of the Northeast **Atlantic** mackerel (*Scomber scombrus*) is indeterminate, i. e. a *de novo* oocyte recruitment takes place during spawning, Using different advanced methods, we clarified that the latest phase of previtellogenic oocytes (PVOs) (PVO4c) are *de novo* recruited to the cortical alveoli-vitellogenic pool during the spawning period, resulting in a dome-shaped seasonal pattern in relative fecundity (RF_i). As PVO4c oocytes – currently identified around 230 μm – mackerel fecundity counts should rather use this diameter as the lower threshold instead of historically 185 μm. Any use of a too low threshold value in this context will inevitably lead to an overestimation of RF_i and thereby underestimated spawning stock biomass.