ICES WKMHMES REPORT 2009

ICES LIVING RESOURCES COMMITTEE

ICES CM 2009/LRC:13

REF. WGISUR, WGWIDE, SCICOM

Report of the Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES)

5–9 October 2009 and
1–4 December 2009

IJmuiden, The Netherlands and
San Sebastian, Spain

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

Recommended format for purposes of citation:

ICES. 2009. Report of the Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES), 5–9 October 2009 and 1–4 December 2009, IJmuiden, The Netherlands and San Sebastian, Spain. ICES CM 2009/LRC:13. 90 pp.

Contents

Executive summary **1**

1 Opening of the meeting **2**

1.1 Background 2

1.2 Terms of Reference (ToR's) 2

2 Adoption of the agenda **3**

3 Materials and methods **4**

3.1 Egg sorting trials (referring to ToR a) 4

3.2 Egg staging (referring to ToR b, c and d) 4

3.2.1 Egg staging trials 4

3.2.2 Egg staging criteria 5

3.3 Egg identification (referring to ToR c, d and e) 9

3.3.1 Egg identification trials 9

3.3.2 Egg identification criteria 9

3.3.3 Misclassifiaction of eggs from *Trachurus* spp. and from *Scomber* spp. in ICES Division IXa 13

3.4 Fecundity and atresia estimation (referring to ToR f) 15

3.4.1 Methodology for fecundity estimation 15

3.4.2 Standardisation of potential fecundity analysis 15

3.4.3 Standardisation of mackerel atresia assessment 15

3.4.4 Image analysis for fecundity and atresia estimation with ImageJ 16

3.4.5 Alternative method for mackerel atresia estimation 16

3.4.6 Use of paraffin vs. resin for the histological estimation of atresia 17

4 Results **18**

4.1 Result of egg sorting exercise 18

4.2 Result of egg staging exercise 19

4.3 Result of egg identification exercise 33

4.4 Result of the fecundity and atresia estimation 39

4.4.1 Result of the fecundity analysis 39

4.4.2 Result of the mackerel atresia exercise 41

5 Discussion **44**

5.1 Egg staging exercise 44

5.2 Egg identification exercise 44

6 References **45**

Annex 1: List of participants **47**

Annex 2: Agenda **51**

Annex 3: WKMHMES terms of reference for the next meeting 54

Annex 4: Recommendations.....56

Annex 5: Manual for sampling adult mackerel and horse mackerel and to estimate fecundity and atresia.....58

Executive summary

The Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES) met twice in 2009. The first meeting was from 5–9 October in IJmuiden, The Netherlands, to calibrate egg staging and identification. The second meeting was from 1–4 December in San Sebastian, Spain, to calibrate fecundity and atresia estimations (section 1.2).

Highlights

- A number of excellent presentations were given on the use of image analysis systems (IMAGEJ / ObjectJ) for the automatic measuring of fish egg and oil globule diameters and fecundity and atresia analysis. This imaging technology is advancing rapidly, and participants agreed to use this for the analysis of the 2010 survey samples.
- The 'spray technique' for the removal of fish eggs from preserved plankton samples was again tested and shown to inexperienced participants.
- The majority of the time at the Workshop was spent identifying and staging mackerel, horse mackerel and similar eggs. The results promoted discussion and highlighted specific problem areas. These discussions led to the further development of standard protocols, and enhancements to the species and stage descriptions. The results were very reassuring and similar to those obtained at the 2006 workshop. There was an overestimate of stage 1 mackerel eggs (stages 1a and 1b combined) during the first round of analysis (15%) but this reduced (5%) during the second round. The results for stage 1 horse mackerel eggs were similar to underestimates of –2% and overestimate of 6% respectively. This is particularly re-assuring as it is this stage on which the egg production estimates are based.
- The fecundity and atresia calibration proved beneficial to all participants. After discussion the manual has been improved and there was agreement on identification of vitellogenic and early alpha-atretic oocytes.

1 Opening of the meeting

The Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES) met twice in 2009. The first meeting was from 5–9 October in IJmuiden, The Netherlands, to calibrate egg staging and identification. The second meeting was from 1–4 December in San Sebastian, Spain, to calibrate fecundity and atresia estimations.

1.1 Background

In preparation for the 2010 international ICES coordinated mackerel and horse mackerel egg survey, a workshop was held at IMARES, IJmuiden, The Netherlands for the majority of plankton analysts who would be involved with the 2010 survey. The aims of the workshop were to standardize procedures and produce definitive criteria for the identification and staging of mackerel and horse mackerel eggs. The workshop would also investigate the reasons for individual differences in the identification and staging of mackerel and horse mackerel eggs and attempt to harmonize these. In addition, further evaluation of the 'spray' technique for removing fish eggs from plankton samples, was carried out.

To permit the calculation of the numbers of spawning female fish in a stock by using the Annual Egg Production Method (AEPM. Lockwood *et al.*, 1981, Armstrong *et al.*, 2001) it is essential to correctly identify (both in terms of species and age) the number of freshly spawned eggs, *i.e.* the eggs in development stages Ia and Ib, and to distinguish these from eggs in later stages of development. It is therefore vital that the analysts involved with sorting, identification and staging of mackerel and horse mackerel eggs from the triennial egg surveys (ICES, 2009) are able to accurately identify and stage the eggs of each of the target species. These workshops (WKMHMES) were designed to bring the analysts together to develop consistent criteria for the identification and staging of the eggs, and to discuss how to overcome the practical problems encountered whilst doing so.

Previous workshops (ICES, 2001; 2004; 2006) have developed a comprehensive set of criteria for both mackerel and horse mackerel egg identification and staging. These criteria were to be expanded and developed during the 2009 workshop. In addition, a few inexperienced analysts would be involved for the first time, and it was critical that they became fully aware of the procedures and criteria in advance of the 2010 plankton samples being collected.

In addition to a correct identification of spawned eggs it is vital for the AEPM to have a good estimation of potential fecundity and atresia in order to estimate Spawning Stock Biomass (SSB). In order to calibrate estimations of fecundity and atresia a second workshop took place at AZTI, San Sebastian, Spain. Methods and criteria had been developed in previous workshops (ICES, 2006) and were expanded and further developed during the 2009 workshop. Also inexperienced analysts were taught how to correctly identify vitellogenic and atretic oocytes and how to estimate fecundity and atresia.

1.2 Terms of Reference (ToR's)

The Workshop on Mackerel and Horse Mackerel Egg Staging and Identification [WKMHMES] (Chair: Cindy van Damme*, The Netherlands) will meet twice in IJmuiden, The Netherlands, 5–9 October 2009 and also in San Sebastian, Spain, 30 November – 4 December 2009 to:

a) carry out comparative plankton sorting trials on typical survey samples. This should follow the pattern of trial – analysis – retrial – identification of problem areas (October);

b) carry out a comparative egg staging trial for mackerel and horse mackerel eggs following the pattern used in the 2006 egg staging workshop (October);

c) update a set of standard pictures and descriptions for species identification and egg staging (October);

d) provide a review of any available documentation on identifying eggs to species and define standard protocols (October);

e) provide a review of any information available on other egg identification procedures – particularly DNA probes (October);

f) carry out inter-calibration work on fecundity determination and harmonize the analysis and interpretation of fecundity samples (December).

WKMHMES will report by 1 January 2010 for the attention of the SCICOM, WGISUR and WGWIDE.

2 Adoption of the agenda

An agenda was distributed to all participants a few weeks before the workshop. This agenda, which can be found at Annex 2 of this report, was agreed prior to the workshop commencing.

3 Materials and methods

3.1 Egg sorting trials (referring to ToR a)

As a result of the egg sorting trials conducted during the 2003 and 2006 workshops, all participating institutes are now using the 'spray technique' for routinely removing fish eggs from plankton samples (Eltink, 2007).

In an attempt to standardize and teach inexperienced participants the 'spray technique' three plankton samples (typical plankton from the 2007 survey) were prepared, each containing a total of 100 mackerel and horse mackerel eggs. As many participants as possible (including all inexperienced) were asked to undertake the following procedure to remove and count the eggs from the prepared samples.

The formaldehyde was rinsed from the sample in a 270μm mesh sieve. The plankton was then washed into a plastic funnel, fitted with a tap, with a little seawater. A normal garden spray pump with an attached water vacuum filter pump was used to fill the funnel as much as possible with pressurised water. The spray jet was rotated around the sides of the funnel to limit damage to the plankton. The fine, pressurised spray caused aeration of the sample with many fine bubbles, which gave the sample a cloudy appearance. The sample was then left to stand for one to two minutes whilst the air bubbles became trapped in the parts of the plankton that had projections (legs, antennas etc). The aerated plankton floated to the surface and all smooth particles, including the fish eggs, sank to the bottom. The fish eggs were then drained from the bottom of the funnel, by opening the tap, and collected in a small beaker. The spraying was then repeated until very few eggs were removed from the bottom of the funnel (a maximum of 8 times). It is recommended that the waiting time is increased for each subsequent spraying to allow the more buoyant eggs time to settle out from the rest of the plankton. The sample was then fully sorted using a binocular microscope, to remove any remaining eggs from the plankton.

The numbers of eggs removed after each spraying and those eggs remaining in the plankton were counted, and the results recorded in Table 4.1.1.

3.2 Egg staging (referring to ToR b, c and d)

3.2.1 Egg staging trials

A total of 700 mackerel, horse mackerel, hake (*Merluccius merluccius*, L.) and megrim (*Lepidorhombus whiffiagonis*, Walbaum) and other species, which can be found in egg survey samples, eggs were placed in 20 small, Perspex trays. After the 2006 workshop new trays were developed with deeper wells to avoid eggs moving from one well to the other and to avoid drying of the eggs as a result of evaporation of the liquid. Each tray contained 25 small wells but only the first 20 wells were used to hold one egg each. Each tray was numbered and placed on the stage of a stereo-zoom microscope. The rows and columns of each tray were labelled so that the position of each individual egg could be identified. The first round 400 eggs were staged by participants. It was not possible to obtain 20 microscopes with a bottom light source; therefore those without bottom light were not used for the second round. Only 300 eggs were staged during the second round.

Some of the eggs used were validated (of known species from artificial fertilizations or from natural spawning of captive fish) and others were taken from the 2007 Atlantic and 2008 North Sea mackerel egg surveys. The eggs were mainly those of mackerel and horse mackerel with a few eggs of hake and megrim, which are

morphologically similar to those of the two target species. It was hoped that these definitive eggs, of known parentage, would enable participants' species identification to be judged more consistently than in previous workshops. The eggs were selected at random with the intention of providing the full range of egg stages, but with greater emphasis on stage 1 eggs on which the estimates of SSB are based. The mackerel, hake and megrim eggs in each tray were staged to Ia, Ib, II, III, IV, V and the horse mackerel were staged to Ia, Ib, II, III, IV, as horse mackerel larvae hatch before the eggs reach stage V. Due to the fact that computers can only calculate with numeric values, stage Ia was changed to 0 and stage Ib to 1 in the result tables.

Each participant moved from one microscope to another in order to complete the staging and identification of all 700 eggs. In this way, the results of the egg stage readers were not affected by differences in the quality of the microscopes. There were, however, limitations to the amount of transmitted light provided by some microscopes and only a few were fitted with eyepiece graticules.

Once each participant had staged and identified each of the eggs and the results had been entered into a result spreadsheet, a full discussion on egg staging and identification took place. From the analysis of the first set of results it became apparent which individual eggs had resulted in high or low agreement of allocated stage. Low agreement among participants indicated problems in allocating an egg consistently to one developmental stage. These eggs were then placed under a microscope equipped with a video camera and displayed on a large screen. Discussions then took place on the diagnostic features visible in the egg, which generally led to an agreement on the most likely developmental stage and/or species involved. In this way, the egg staging criteria (ICES, 2006) were revised (see section 3.2.2).

During the course of the first round of analysis several eggs became damaged, or were moved, from one cell to another in the trays. It was not, therefore, possible for all participants to always stage or identify each egg. Before the second round of analysis began, another set of eggs was randomly placed in the trays. This provided a different mix of species and stages and prevented a direct comparison between the first and second round of results. However, the lessons learned during the first round of analysis and subsequent discussions would, hopefully, still be reflected in the second round results.

3.2.2 Egg staging criteria

As a result of discussions following the first round of egg staging the participants decided upon the following definitions of the developmental stages for mackerel, horse mackerel, hake and megrim. The primary characteristics are based on those presented in Lockwood *et al*. (1977) for mackerel (Figure 3.2–1.), but now include some other (secondary) characteristics, which the participants thought were crucial in determining egg stage. Figure 3.2–2 shows the development stages for horse mackerel.

Stage Ia

Primary characteristics**:** From fertilization until cleavage produces a cell bundle in which the individual cells are not visible.

Secondary characteristics**:** There are no signs of a thickening of cells around the edge of the cell bundle. **NB**. In preserved eggs the edge of the cell bundle can sometimes fold over giving the appearance of a 'signet ring' seen in a stage Ib.

Stage Ib

Primary characteristics: Formation of the blastodisc, visible as a 'signet ring' and subsequent thickening a one pole.

Secondary characteristics: The cell bundle has thickened around the edge giving a distinct ring appearance. Cells in the centre of the ring form a progressively thinner layer and eventually disappear. **NB**. At the end of this stage the ring can become very indistinct as it spreads towards the circumference of the egg.

Stage II

Primary characteristics: From the first sign of the primitive streak until closure of the blastopore. By the end of this stage the embryo is half way round the circumference of the egg. However, the tail still tapers to end flattened against the yolk, in this stage.

Secondary characteristics**:** Early in this stage the primitive streak can be difficult to see, only appearing as a faint line in the surface of the yolk. Late in this stage the head is still narrow and the eyes are not well formed.

Stage III

Primary characteristics: Growth of the embryo from half way to three-quarters of the way around the circumference of the egg. The end of the tail has thickened, becoming bulbous in appearance.

Secondary characteristics: Widening of the head and development of the eyes. Pigment spots develop on the embryo, usually close to the posterior end.

Stage IV

Primary characteristics: Growth of the embryo from three-quarters to the full circumference of the egg.

Secondary characteristics: Eyes continue to develop and the lenses become visible. Development of the marginal fin and the tail begins to separate from the yolk. Pigmentation of the body increases.

Stage V

Primary characteristics: Growth of the embryo until the tail is touching the nose or beyond.

Secondary characteristics: Pigmentation develops in the eye.

NB

The preservation of eggs can cause shrinkage and distortion of the embryo. Therefore care should be taken when assessing the length of the embryo, as they do not always remain around the full circumference of the yolk. They may also become distorted giving a false impression of development stage.

Horse mackerel and hake embryos hatch at the end of stage 4.

Figure 3.2–1. Mackerel eggs at the beginning and end of the six development stages.

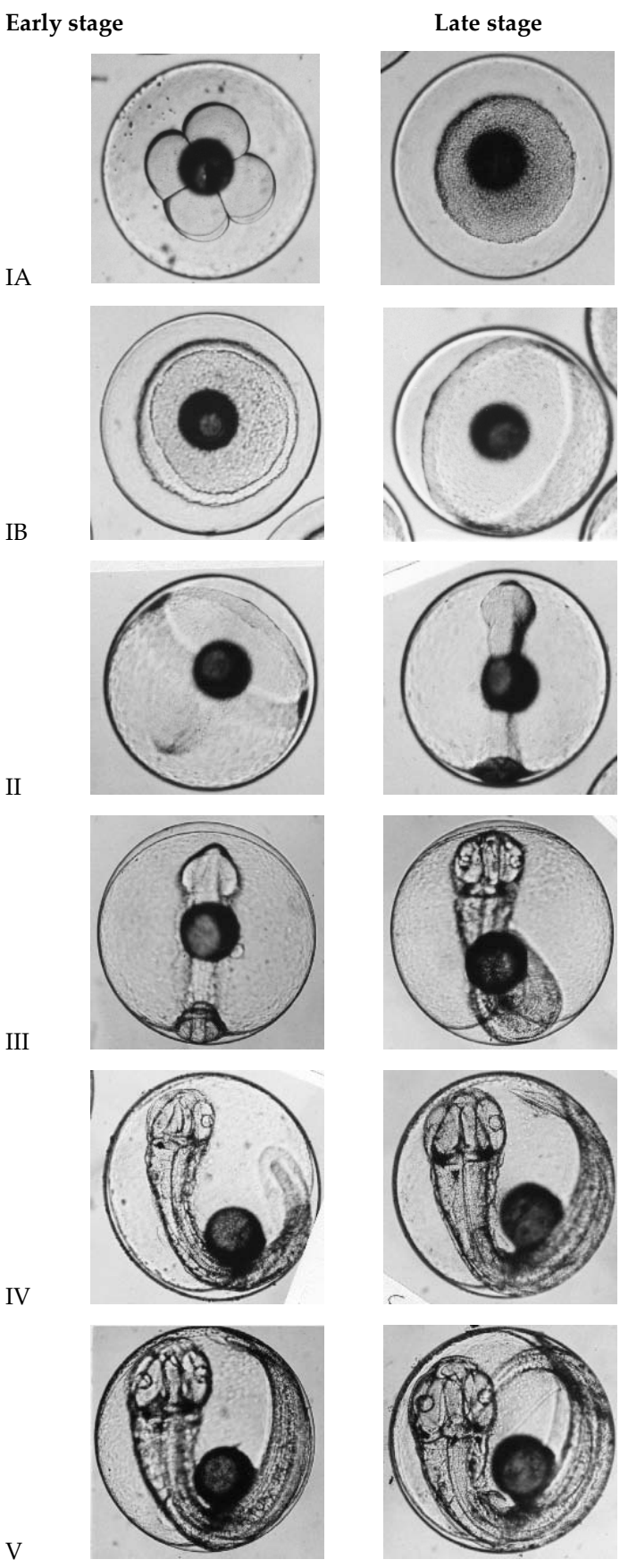
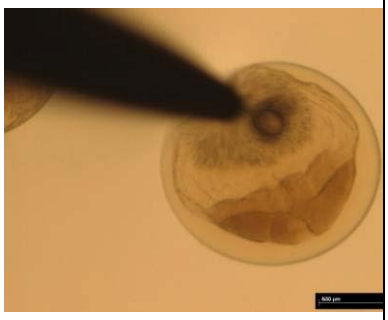
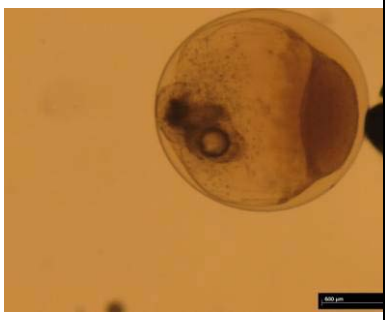
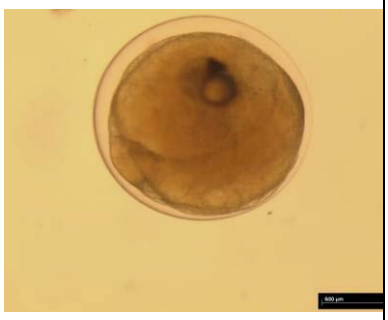
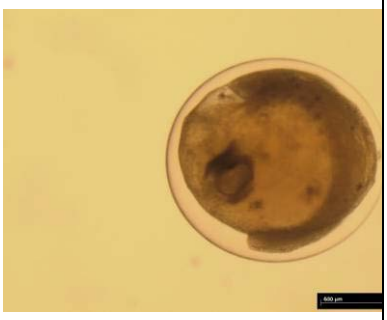
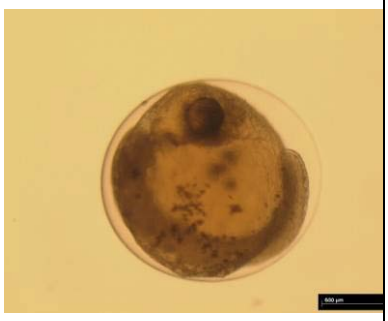
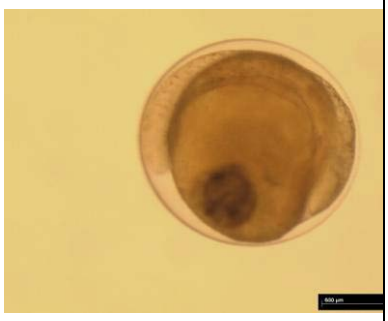
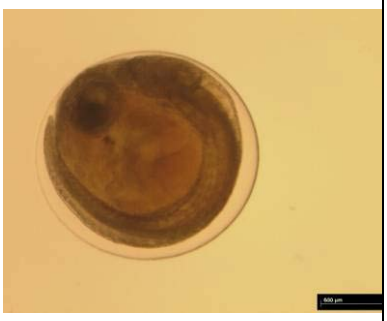
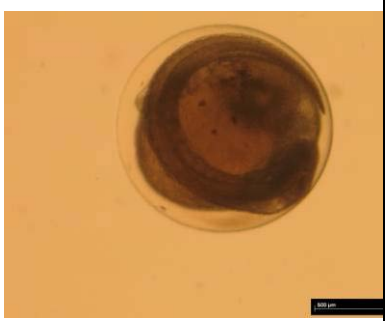
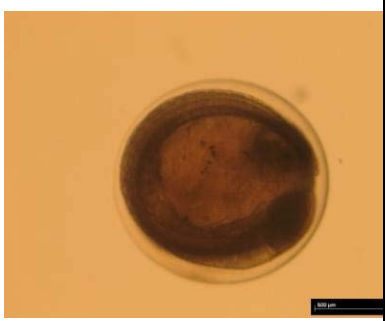


Figure 3.2–2. Development stages of horse mackerel from fertilization experiments.

Stage IA	**Stage IA**	**Stage IB**
		
Stage II	**Stage II**	**Stage II**
		
Stage III	**Stage III**	**Stage IV**
		
Stage IV	**Stage IV**	
		

3.3 Egg identification (referring to ToR c, d and e)

3.3.1 Egg identification trials

The same trays of fish eggs (described in section 3.2 above) were also used for the egg identification exercise. As each participant moved from microscope to microscope they were asked to provide a species identification for each egg, in addition to a development stage. The descriptions of the different species from the 2006 workshop report (ICES, 2006) were available to all participants prior to the first staging round.

The results of the first round of egg identifications were collated and input into spreadsheets at the same time as the results for egg staging. The results were presented and eggs with low agreement in species identification were displayed on a large screen (as described in section 3.2 above). A discussion then took place until a consensus was reached on the most likely species identification for each of these eggs. As a result of these discussions and before the second round of analysis was begun, a review of the egg identification criteria produced by previous WKMHMES participants was carried out.

3.3.2 Egg identification criteria

Table 3.3–1 summarizes published descriptions of mackerel, horse mackerel and other species of eggs with similar morphological features. It particularly concentrates on egg and oil globule sizes, which may vary through the spawning season and from area to area. A complete reference list is given at the end of this report.

In addition to the published descriptions given in Table 3.3–1, various other criteria are used by participants to help with egg identification based their own knowledge and experience. These criteria can be regarded as secondary characteristics and are described for each species below.

Mackerel *(Scomber scombrus)*. (See Lockwood *et al.*, 1977)

- Oil globule often orientated to the top of the egg during analysis with the embryo following the circumference of the egg. However, this is not always the case in preserved eggs.

Horse Mackerel (*Trachurus trachurus*). (See Pipe and Walker, 1987)

- Oil globule easily broken into several smaller pieces. This seems to be more common in eggs found in the southern area, particularly in eggs from the Portuguese coast.
- The oil globule migrates to the head of the embryo after stage 2.
- In stages 3 and 4 the embryo shows very strong pigmentation.

Hake (*Merluccius merluccius)* (See Coombs, 1982)

- Strongly pigmented oil globule.
- Towards the end of its development the embryo begins to show the characteristic post-anal pigmentation of three bars.
- Positive surface adhesion test (SAT) is also used to identify hake eggs (Porebski, 1975) and (Coombs, 1994).

Megrim (Lepidorhombus whiffiagonis)

- Striated appearance of egg membrane.

- Oil globule is closer to egg membrane than in mackerel.
- Embryo thinner than a mackerel embryo.
- Yolk unsegmented and the egg has a small perivitelline space.
- Pigmentation on yolk from stage II onwards.

Longspine snipefish (Macrorhamphosus scolopax)

- Membrane is light amber with grainy reflections.
- Yolk with rose or violet halo depending on viewing light.
- Oil globule is amber / rose in colour.

NB

The striated appearance of megrim eggs is reasonably diagnostic in fresh specimens. However, preserved specimens of other eggs also appear to develop apparent striations on the egg membrane which can therefore lead to misidentification of eggs which have been preserved for some time.

Table 3.3–1. Comparison of the Characteristics of Mackerel, Horse Mackerel, Megrim, Hake and Snipefish Eggs (Details of fixative and concentration unknown).

Species	Diameter (mm)		Other Features Noted	Area	Reference
	Egg	Oil Globule			
Mackerel (*Scomber scombrus)*	1.0–1.38	0.28–0.35	Unsegmented yolk	North Sea, English Channel	Russell, 1976
	1.09–1.36	0.26–0.37	Homogenous yolk	N.W. Atlantic	Fahay, 1983
	0.97–1.38	0.25–0.35		Irish Sea, North Sea	Ehrenbaum, 1905–09
	1.071–1.193	0.285–0.360		Mediterranean	D'Ancona *et al.*, 1956
	0.97–1.38	0.22–0.38	Perivitelline space approx 0.05mm	Mid-Atlantic Bight	Development of Fishes of the Mid-Atlantic Bight, 1978
	1.0–1.38			North Atlantic	
	0.86–1.04			Mediterranean	
	0.97–1.38	?		Isle of Man	Johnstone, Scott and Chadwick, 1934
	1.21–1.33	~0.32		West of Ireland	Holt, 1893
	0.9–1.4	?		NE Atlantic	Froese and Pauly, 2003
	1.16	0.27			IPIMAR, fertilization experiment 2008
Horse Mackerel (*Trachurus trachurus)*	0.81–1.04	0.19–0.28	Segmented yolk	North Sea, English Channel	Russell, 1976
	1.03–1.09	0.26–0.27	Segmented yolk	North Sea	Holt, 1898
	0.81–0.93	0.22–0.23		Plymouth	
	0.84–1.04	0.19–0.24	Totally segmented yolk	North Sea, English Channel	Ehrenbaum, 1905–09
	0.81–1.04	0.19–0.24	Segmented yolk	North Sea, English Channel	D'Ancona *et al.*, 1956
	Max. 0.84	0.24–0.26	Granular yolk	English Channel	Holt, 1893
	0.76–1.07	0.19–0.29	Segmented yolk	Europe	Froese and Pauly, 2003
	0.96	0.24			IPIMAR, fertilization experiment 2008
Megrim (*Lepidorhombus whiffiagonis*)	1.02–1.22	0.25–0.30	Striated membrane. Pigment develops in the yolk, close to the caudal region and under the oil globule as embryo develops	North Sea, Irish Sea	Russell, 1976
	1.07–1.22	0.25–0.30	Fine "meshwork" on inside of membrane. Pigment on oil globule as embryo develops	North Sea	Ehrenbaum, 1905–09
	1.07–1.13	0.30	Striations on inside of membrane	West of Ireland	Holt, 1893
	1.08–1.30	0.29–0.34	Striated membrane	Celtic Sea	Milligan *et al.*, In prep.
	1.02–1.22	0.25–0.3	Slight ridges on inside of membrane	Europe	Froese and Pauly, 2003
Hake	0.94–1.03	0.25–0.28	Pigmented oil globule	North Sea, English Channel, Mediterranean	Russell, 1976

Species	Diameter (mm)		Other Features Noted	Area	Reference
	Egg	Oil Globule			
(*Merluccius merluccius)*	0.94–1.03	~0.27	Black and yellow chromatophores on oil globule	North Sea, English Channel, Mediterranean	Ehrenbaum, 1905–09
	0.94–1.03	~0.27		?	D'Ancona *et al.*, 1956
	1.10–1.16	0.27–0.35		Celtic Sea	Shaw, 2003
	0.94–1.03	0.25–0.28		Europe	Froese and Pauly, 2003
Longspine Snipefish (*Macrorhamphosus scolopax)*	1.00	0.2	Amber/rose single oil globule Membrane is light amber with grainy reflections	Europe	Development of Fishes of the Mid-Atlantic Bight, 1978. US Fish and Wildlife service. FWS/OBS-78/12.

NB

The information inTable 3.3–1 above is based on observations of live or recently preserved eggs. It must be noted that preservation in formaldehyde gradually destroys pigmentation and therefore observation of chromatophores may well be difficult in specimens which have been preserved for any length of time.

3.3.3 Misclassifiaction of eggs from *Trachurus* spp. and from *Scomber* spp. in ICES Division IXa

In the southern part of the area of the triennial mackerel and horse mackerel egg survey different species of mackerel (*Scomber scombrus* and *S. colias*) and horse mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T picturatus*) occur. The species of each genus show overlapping distributions and spawning periods and their eggs are similar in morphology. During the workshop a presentation was presented on this topic. In order to help in the identification of these species, descriptions of morphometric characteristics of these eggs and the most relevant aspects for their identification are given below

Trachurus mediterraneus

- Egg diameter: 1.00–1.04 mm
- Oil globule: 0.24 mm
- Description: Pelagic eggs, spherical, transparent. No perivitelline space. Oil globule colourless. Fine striated membrane (Padoa, 1956).
- Eggs are similar to *Trachurus trachurus*, but a bit bigger.
- Distribution of adults appears in the reports of ICES-WGACEGG.

Trachurus picturatus

No descriptions were found in the literature. Arkhipov and Mamedov (2008) presented maps of *T. picturatus* eggs and larvae in the area of Azores Seamounts, without references on its morphology. There are no references from plankton samples for ICES division IXa although the presence of adults being registered in ICES reports (IBTSWG, WGACEGG).

Scomber colias

- The eggs are spherical, on average ranging in diameter from 1.06–1.14 mm. Similar description was offered by Fahay (1983), with little differences in diameter range, which ranged from 1.06–1.36 mm.
- Oil globule 0.26–0.37 mm in diameter. In the Pacific oil globules diameters varies between 0.25 and 0.32 mm (Fritzsche, 1978).
- Yolk is smooth, transparent and unsegmented and under magnification (x36) can be seen to be filled with a large number of tiny vacuoles. The only difference with *S. scombrus* is that the yolk is pigmented with several melanophores, whereas in *S. scombrus* eggs the yolk is pigmented just before hatching, when a spot per side appears just posterior to the head.
- The perivitelline space is narrow.
- In advanced stage of development both the dorsum of the embryo and the oil globule are pigmented, the latter on the hemisphere facing the head (Kramer, 1960).
- Distribution of adults appears in the reports of ICES-WGACEGG.

Macroramphosus scolopax

- Egg diameter: 1.0 mm
- Oil globule: 0.20 mm
- Description: Pelagic eggs, spherical, transparent, single oil globule. Yolk pigmentation is described as light amber; pigmentation of oil globule is

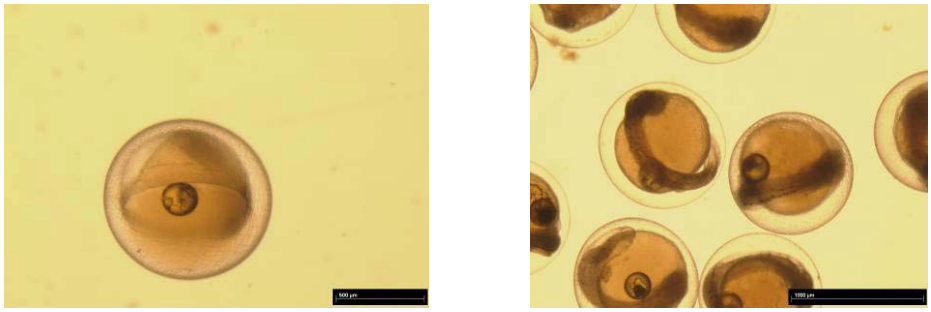
amber-rose (Spartà, 1936). Eggs are similar to those of *Trachurus trachurus* but without yolk segmentation.

- For fish distributions see for example Marques *et al.* (2005).

Boops boops

- Egg diamater: 0.93 mm (based on eggs from artificial fertilization, IPIMAR, 2008, see Figure 3.3–1)
- Oil globule: 0.18 mm (based on eggs from artificial fertilization, IPIMAR, 2008)
- Description: Pelagic eggs, spherical. Single oil globule with melanophores (Gaetani, 1937).
- Fish distribution is mapped in the reports of ICES-WGACEGG.

Figure 3.3–1. Eggs of *Boops boops* from fertilization experiments.



3.4 Fecundity and atresia estimation (referring to ToR f)

3.4.1 Methodology for fecundity estimation

A detailed review was carried out during this Workshop to provide an updated fecundity manual for both species (Annex 5) based on the manual produced after the Fecundity Workshop held at Lowestoft in October 2006. The text table below summarizes the changes in the manual since 2006.

2006	2009
MACKEREL AND HORSE MACKEREL	
Fecundity samples: In 2007 count all oocytes >185 um and measure 1/3 of the oocytes.	Fecundity samples: Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis. Count all the oocytes >185µm in the sample that are not automatically detected.
Every institute used their own image analysis systems.	ImageJ, ObjectJ and macros will be made available to all participants and they should use these for analysis of the samples.
The results of the 2007 survey showed problems with low fecundity estimates, probably due to underestimating the number of oocytes in the samples due to overlap in the trays.	Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.
Spawning markers: hydrated, >5 POF's	Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample.

3.4.2 Standardisation of potential fecundity analysis

Images were prepared from unstained whole mount samples of mackerel ovary tissue. Each analyst attending the meeting scored 8 images whether to include them in the fecundity analysis based on the criteria agreed upon before the exercise (see also Fonn *et al.*, 2009 and table above). If the sample could be used for fecundity analysis each participant carried out the automatic measurements of the diameters and counted the number of normal vitellogenic follicles in each preparation. The results are presented in section 4.4.1.

The whole mount samples that exhibit spawning markers should be discarded from the fecundity analysis. The markers are

- Presence of hydrated oocytes (>800um), or
- The appearance of POF's or
- If all the oocytes in the whole mount sample have a diameter of < 400um.

For mackerel these samples (excluding those samples where all the oocytes are < 400um) should instead be analysed for atresia.

3.4.3 Standardisation of mackerel atresia assessment

The quantification of each early alpha atresia stage follicle classes (yolk vesical, yolk vesical – yolk granule and yolkgranule) stained with heamotoxylin and eosin (H&E) Schiff-Mallory Trichrome (SM) or Toluidine blue (TB) was discussed. Serial sections were produced from mackerel ovary samples and stained with either H&E, SM or TB.

6 images from one mackerel ovary stained with Toluidine Blue where used for the calibration exercise during the workshop. The atretic follicle classification criteria was based on the mackerel / horse mackerel fecundity methods manual. Each participant scored the images using ImageJ and ObjectJ following the mackerel/ horse mackerel manual (Fonn *et al.*, 2009).

3.4.4 Image analysis for fecundity and atresia estimation with ImageJ

During the workshop presentations were presented by Anders Thorsen, IMR, on the development of ImageJ and ObjectJ macros for fecundity and atresia estimation. ImageJ and ObjectJ is freely available for everyone, as are the specially developed macros for the fecundity and atresia analysis. These macros were used during the workshop. Everyone agreed to use this software for the 2010 egg survey.

The macros will be updated with the suggestions made at the workshop and will be made available to all participants prior to the survey in 2010.

3.4.5 Alternative method for mackerel atresia estimation

The method that is used for atresia estimation does not consider the size of the three different atretic development stages (YV, YV-YG and YG). Also for this method one whole lobe of the ovary is fixed in formaldehyde. Especially with large ovaries this often leads to bad fixation of the ovary, causing problems with the sectioning of the samples. The amount of chemicals that is used is very high. IMR has developed an alternative method is which less sample needs to be collected and hence the amount of chemicals used will be reduced considerably.

The alternative method takes the size class of the different stages into account and is based on the following equations:

$N_i = Ov/V_i * V_{Vi}$

in which N_i is Number of i in the ovary, Ov is Ovary volume, V_i is the average volume of each stage and V_{Vi} is the fraction of tissue volume occupied by each stage. V_i is calculated as:

$V_i = 4/3\pi r_i^3$

And V_{vi} is calculated as:

V_{vi} = Number of $hits_{vi}$ / (total points – negative grid)

This alternative method has been tested on a small number of samples. For the 2010 survey the standard method will be used to estimate atresia, but samples (in 24 ml scintillation vials with one teaspoon of ovary tissue fixed in formaldehyde) will be collected by every participant and sent to IMR. IMR will further test the alternative method to:

- Investigate if atretic oocytes are homogenously distributed in the ovary and
- Compare the results of the standard and alternative methods and
- Compare total fecundity estimates from whole mount image analysis to fecundity estimated from histological sections.

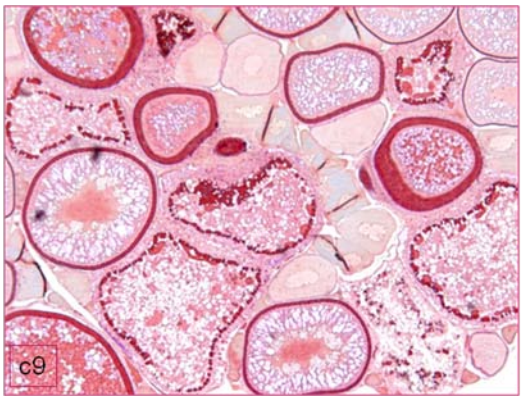
Results will be presented at the 2011 WGMEGS meeting and it will then be decided which method will be used for the 2011 North Sea mackerel survey.

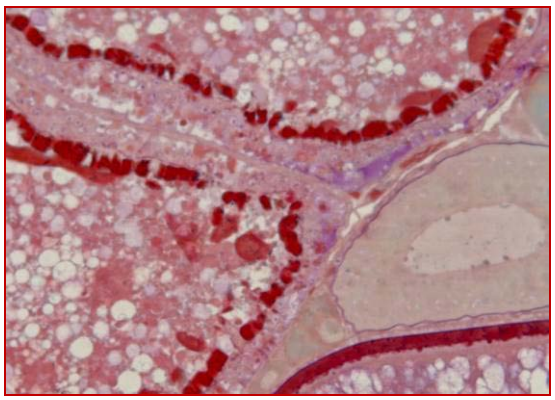
3.4.6 Use of paraffin vs. resin for the histological estimation of atresia

During the workshop a presentation was given by IEO comparing the use of paraffin vs. resin for tissue processing. Paraffin is a cheaper method for histological analysis. IEO has been using this medium for different fish species and has an automatic tissue processor that would be less time consuming for the mackerel atresia estimation.

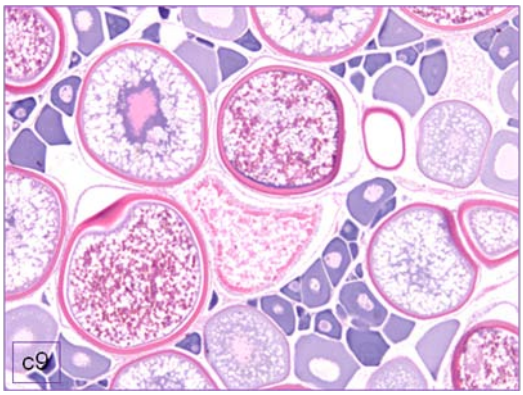
The quality of the images obtained from paraffin sections was tested against the quality of those obtained from resin. The images below show the result of this comparison. Alpha atretic oocytes can be easily identified in both resin and paraffin sections. However, during this exercise, oocyte shrinkage was observed in some of the paraffin sections (see figure below). The cause of this shrinkage is probably the temperature used during the paraffin embedding process. During the 2010 survey IEO will use resin for embedding the mackerel atresia samples. But more tests will be carried out with paraffin embedding at different temperatures to find the cause of the shrinkage of the oocytes. Results will be presented at the workshop in 2012.

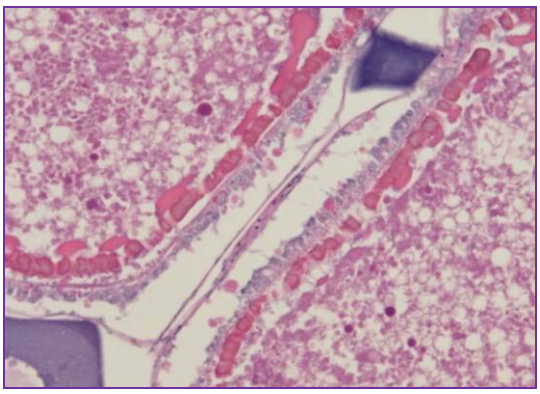
Resin Schiff



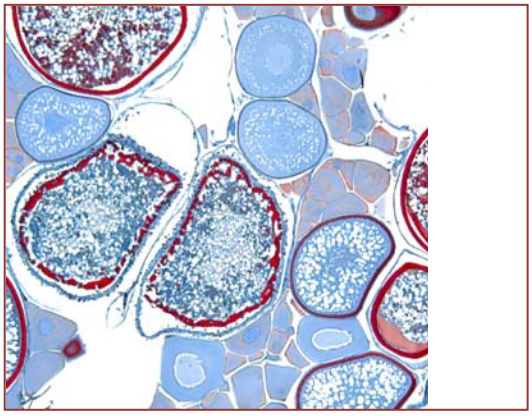


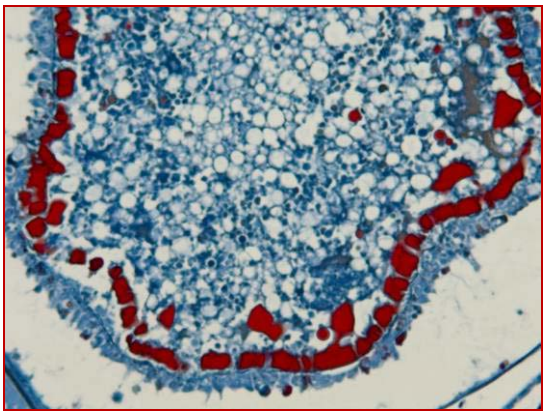
Paraffin H&E



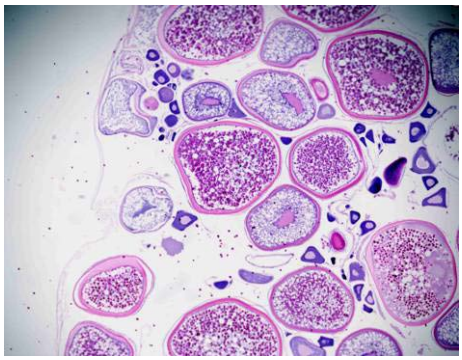


Paraffin Schiff





Paraffin shrinkage



Resin no shrinkage

4 Results

4.1 Result of egg sorting exercise

The results of the egg sorting exercise using the 'spray technique' are given in Table 4.1–1. Four plankton samples were prepared with 100 fish eggs (a mix of mackerel and horse mackerel eggs) present in each. There were widely fluctuating results in determining egg numbers and increasing damage to the eggs whilst using the first prepared sample. After four participants had used the first sample, it was decided to use a second pre-prepared sample until a spillage prevented its further use. Three

participants then used the 'spray technique' to remove eggs from a second pre-prepared sample. The results from the second sample were much more consistent than those from the first sample, as participants discussed the technique and began to resolve the practical problems encountered.

Table 4.1–1 shows the numbers of eggs removed by each use of the spray technique. In the first and second sample more eggs appear to have been removed than originally (100) occurred in the sample. This was due to inexperience of the participants with removing fish eggs from plankton samples. They also removed copepod eggs from the sample, hence the larger numbers of eggs found. Experienced sprayers removed between 81% and 93% of the eggs present in the samples.

Table 4.1–1. Results of the egg sorting exercise.

Sample Nr	Participant	1st	2nd	3rd	4th	Total
1	Solva					153
1	Carlotta					120
1	Eilert					103
1	Jim					60
2	Birgit					130
2	Paula	92	1	0	0	93
2	Jan					81
3	Brendan	83	3	0	0	86
4	Finlay	82	5	0	0	87

4.2 Result of egg staging exercise

The results of the egg staging exercise are given in Tables 4.2–1 to 4.2–6.

Tables 4.2–1 to 4.2–3 presents the results for each participant for the first round of analysis for eggs of all species (Table 4.2–1), for mackerel eggs (Table 4.2–2) and for horse mackerel eggs (Table 4.2–3). Tables 4.2–4 to 4.2–6 presents the results for the second round of analysis in exactly the same way.

The original assessment of each egg, by each participant, for stage (and species), was input into a primary result table (not presented here). Once the results were available from every participant a modal stage could be calculated for each egg that was not validated (from fertilization experiments). This modal assessment of egg stage was presumed to be 'correct' although it does not necessarily mean that this was the true stage. In some cases, eggs were apparently misidentified to species by a few readers before staging. When these 'misidentified' eggs were allocated a stage by a few readers then it was not always possible for a model stage to be calculated. These eggs were then removed from the species / stage analysis in Tables 4.2–2, 4.2–3, 4.2–5 and 4.2–6.

Tables 4.2–1 to 4.2–6 summarize the results into six sub-tables labelled A-F, where the performance of each participant is judged against the modal egg stage.

Sub-tables A show the number of eggs at each modal stage that were assessed by each participant. The numbers at each modal stage will therefore be the same for all participants that read all the eggs.

Sub-tables B show the numbers of eggs at each stage as assessed by each participant.

Sub-tables C show the over / underestimation of stage 1 (1a + 1b) by each participant.

Sub-tables D show how well each participant's assessment of egg stage agrees with the numbers of eggs at each model stage.

Sub-tables E show the percentage agreement of each participant's assessment of eggs in stage 1a+1b against the modal stage 1a+1b.

Sub-tables F show the bias of each participant's egg staging against the modal stage i.e. how much their assessment of each egg stage varies from the modal stage.

By studying the results presented in Tables 4.2–1 to 4.2–6, some encouraging improvements in the consistency of egg staging between participants can be observed from the first to the second round of analysis.

The overall agreement in egg stage for all species of eggs, in all stages of development was 68% in the first round (Table 4.2–1). This increased to 76% agreement in the second round of analysis (Table 4.2–4). The overall agreement for all egg stages, for mackerel, increased from 67% (Table 4.2–2) to 77% (Table 4.2–4), and for horse mackerel was 81% in both rounds (Table 4.2–5 and 4.2–6).

The overall agreement for stage 1 (1a+1b) eggs shows similar improvements from the first to the second round, but with an overall greater level of agreement (≥93%) . This is very re-assuring, as it is this stage upon which the estimates of SSB for both mackerel and horse mackerel are based.

The overall agreement in the assessment of stage 1 (1a+1b) eggs of all species was 93% in the first round (Table 4.2–1). This increased to 96% agreement in the second round of analysis (Table 4.2–4). The overall agreement of stage 1 eggs, for mackerel, increased from 93% (Table 4.2–2) to 97% (Table 4.2–5), and for horse mackerel from 95% (Table 4.2–3) to 97% (Table 4.2–6).

The percentage agreement in allocating eggs to stage 1 (1a+1b) as a percentage over or underestimation, are given in sub-tables C. Although the overall bias was reasonable, particularly after the second round of analysis, some individuals showed surprisingly high levels of bias. In the first round of analysis the overall bias was an overestimate of 17% for eggs of all species but individual bias ranged from an underestimate of 8% to an overestimate of 26% (Table 4.2–1). In the second round this did improve to an overestimate of 3%, demonstrating low overall bias, with a range of individual bias also reduced to range between -10% to 17%.

The overall bias for stage 1 mackerel eggs (Tables 4.2–2 and 4.2–5) was 15% in the first round and 5% in the second round of analysis. However, the bias of individual participants was much greater, ranging from -23% to 29% in the first round, but improving to from -15% to 16% in the second round of analysis. The overall bias for stage 1 horse mackerel eggs (Tables 4.2–3 and 4.2–6) was 6% in the first round to -2% in the second round of analysis. However, the bias of individual participants was again much greater, ranging from -15% to 33% in the first round, but improving to between -17% and 4% in the second round of analysis.

Figures 4.2–1 to 4.2–6 show the egg stage bias plots in which the mean egg stage +/-2 standard deviations of each stage reader and all stage readers combined are plotted against the modal egg stage.

Table 4.2–1. All eggs first staging.

(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.

(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.

(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.

(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.

For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Stage 1a ==>	0	149	155	156	152	141	25	156	150	151	112	139	155	134	153	153	82	114	151	155	144	154	2881
Stage 1b ==>	1	22	22	22	22	16	11	22	22	22	9	22	22	21	22	22	6	20	22	22	22	22	413
Stage 2 ==>	2	51	50	52	51	45	9	53	52	51	30	45	51	45	51	53	32	39	48	53	47	52	960
Stage 3 ==>	3	76	76	76	76	68	28	76	76	76	43	75	76	69	69	73	33	64	76	76	75	76	1433
Stage 4 ==>	4	53	54	54	54	47	14	55	55	54	33	54	54	47	49	55	28	44	55	54	54	54	1021
Stage 5 ==>	5	11	11	11	11	11	6	11	11	11	5	11	11	10	6	11	6	11	11	11	11	11	209
Total	0-5	362	368	371	366	328	93	373	366	365	232	346	369	326	350	367	187	292	363	371	353	369	6917

B

EGG STAGE COMPOSITION

	Stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Stage 1a ==>	0	197	181	177	172	167	25	178	134	142	113	115	173	154	202	200	104	147	154	198	178	149	3260
Stage 1b ==>	1	12	30	33	35	21	19	21	24	66	27	70	43	23	8	14	7	4	43	16	23	53	592
Stage 2 ==>	2	51	31	36	19	26	6	53	90	46	18	33	24	34	39	14	8	27	33	22	24	39	673
Stage 3 ==>	3	56	73	72	48	59	18	64	66	45	35	64	68	68	60	75	20	67	61	67	83	77	1246
Stage 4 ==>	4	40	43	43	71	39	21	39	45	53	32	46	45	27	35	55	40	38	57	48	32	40	889
Stage 5 ==>	5	6	10	10	21	16	4	18	7	13	7	18	16	20	6	9	8	9	15	20	13	11	257
Total	0-5	362	368	371	366	328	93	373	366	365	232	346	369	326	350	367	187	292	363	371	353	369	6917

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
1a+1b	22%	19%	18%	19%	20%	22%	12%	-8%	20%	16%	15%	22%	14%	20%	22%	26%	13%	14%	21%	21%	15%	17%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Stage 1a ==>	0	97%	88%	87%	82%	86%	56%	79%	73%	74%	76%	70%	83%	78%	90%	92%	94%	88%	78%	87%	85%	79%	83%
Stage 1b ==>	1	27%	45%	41%	18%	19%	64%	36%	36%	45%	44%	68%	50%	10%	5%	18%	17%	10%	64%	0%	41%	64%	34%
Stage 2 ==>	2	53%	50%	52%	25%	47%	33%	51%	81%	51%	40%	53%	43%	38%	55%	23%	16%	26%	46%	32%	30%	56%	44%
Stage 3 ==>	3	55%	79%	76%	39%	74%	43%	74%	71%	54%	63%	72%	71%	70%	71%	62%	36%	75%	72%	64%	72%	80%	67%
Stage 4 ==>	4	60%	69%	67%	67%	70%	57%	65%	69%	76%	70%	65%	63%	38%	51%	55%	61%	64%	84%	57%	46%	63%	63%
Stage 5 ==>	5	45%	64%	73%	91%	100%	50%	100%	45%	82%	80%	73%	100%	90%	50%	18%	50%	36%	100%	91%	82%	91%	73%
Weighted mean	0-5	71.0%	75.0%	73.6%	59.6%	72.9%	50.5%	70.0%	70.2%	65.5%	66.8%	67.3%	70.5%	61.0%	69.4%	63.8%	61.5%	65.8%	73.3%	65.2%	66.3%	72.9%	68.1%
RANKING		6	1	2	20	5	21	9	8	15	12	11	7	19	10	17	18	14	3	16	13	4	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
1a+1b	98%	97%	96%	95%	99%	94%	87%	76%	96%	94%	94%	98%	87%	93%	97%	98%	87%	93%	96%	92%	94%	93%
RANKING	3	5	8	10	1	11	18	21	7	12	13	2	20	15	6	4	19	16	9	17	14	

F

BIAS

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Stage 1a ==>	0	0.05	0.16	0.19	0.23	0.17	0.52	0.37	0.50	0.32	0.32	0.38	0.20	0.37	0.21	0.12	0.07	0.28	0.31	0.21	0.26	0.30	0.26
Stage 1b ==>	1	-0.73	-0.55	-0.50	-0.73	-0.81	-0.36	-0.55	0.18	-0.55	-0.56	-0.14	-0.50	-0.19	-0.86	-0.50	-0.50	-0.45	-0.36	-1.00	-0.50	-0.27	-0.49
Stage 2 ==>	2	-0.86	-0.80	-0.77	-0.65	-1.00	-0.33	-0.92	-0.13	-0.63	-0.60	-0.51	-0.78	-0.76	-0.90	-0.74	-0.91	-1.13	-0.75	-0.72	-0.91	-0.63	-0.75
Stage 3 ==>	3	-0.64	-0.45	-0.42	0.12	-0.24	-0.21	-0.57	-0.39	-0.43	-0.33	-0.29	-0.28	-0.51	-0.49	-0.14	0.24	-0.36	-0.21	-0.30	-0.48	-0.30	-0.34
Stage 4 ==>	4	-0.75	-0.43	-0.48	-0.20	-0.36	-0.50	-0.36	-0.58	-0.41	-0.67	-0.15	-0.50	-0.62	-1.04	-0.71	-0.25	-0.32	-0.24	-0.39	-0.81	-0.57	-0.49
Stage 5 ==>	5	-0.55	-0.36	-0.27	-0.09	0.00	-0.50	0.00	-2.00	-0.18	-0.20	-0.27	0.00	-0.10	-0.50	-0.91	-0.50	-0.64	0.00	-0.18	-0.18	-0.09	-0.35

Table 4.2–2. Mackerel eggs first staging.

(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.

(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.

(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.

(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.

For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Stage 1a ==>	0	95	104	89	131	83	21	70	76	70	56	81	82	70	88	82	30	70	60	110	66	77	1611
Stage 1b ==>	1	20	21	23	28	15	11	18	16	17	8	19	22	17	21	19	3	16	17	30	16	19	376
Stage 2 ==>	2	31	32	27	40	22	7	13	19	31	10	27	36	24	25	23	6	16	13	41	22	24	489
Stage 3 ==>	3	62	61	47	68	54	26	54	49	49	31	45	50	60	54	42	13	38	47	66	37	39	992
Stage 4 ==>	4	40	39	34	50	37	15	38	37	27	14	36	33	41	32	23	11	32	35	42	32	26	674
Stage 5 ==>	5	12	13	11	13	13	7	12	10	10	5	7	12	12	8	6	4	8	8	14	7	10	202
Total	0-5	260	270	231	330	224	87	205	207	204	124	215	235	224	228	195	67	180	180	303	180	195	4344

B

EGG STAGE COMPOSITION

	Stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Stage 1a ==>	0	130	129	104	151	103	21	81	51	67	52	60	93	91	121	111	33	90	61	151	86	70	1856
Stage 1b ==>	1	12	23	25	33	12	19	13	20	45	21	53	38	11	3	10	4	1	26	18	15	34	436
Stage 2 ==>	2	32	15	15	14	12	5	17	45	26	8	18	17	18	20	3	3	13	8	16	13	19	337
Stage 3 ==>	3	44	59	47	47	46	18	46	50	29	24	43	39	57	49	39	3	44	37	53	40	41	855
Stage 4 ==>	4	36	34	33	67	36	20	32	34	25	14	26	33	27	29	23	16	23	39	41	20	23	631
Stage 5 ==>	5	6	10	7	18	15	4	16	7	12	5	15	15	20	6	9	8	9	9	24	6	8	229
Total	0-5	260	270	231	330	224	87	205	207	204	124	215	235	224	228	195	67	180	180	303	180	195	4344

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
1a+1b	23%	22%	15%	16%	17%	25%	7%	-23%	29%	14%	13%	26%	17%	14%	20%	12%	6%	13%	21%	23%	8%	15%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Stage 1a ==>	0	96%	88%	83%	83%	86%	48%	77%	64%	67%	71%	58%	72%	76%	90%	89%	90%	89%	78%	85%	86%	75%	80%
Stage 1b ==>	1	30%	48%	48%	32%	13%	64%	33%	50%	53%	63%	74%	50%	12%	10%	21%	33%	0%	71%	20%	50%	74%	39%
Stage 2 ==>	2	35%	31%	33%	23%	45%	14%	31%	74%	35%	40%	44%	44%	25%	48%	9%	33%	25%	46%	29%	36%	58%	36%
Stage 3 ==>	3	53%	75%	79%	47%	72%	46%	78%	82%	57%	71%	80%	70%	68%	74%	55%	8%	74%	74%	67%	70%	85%	68%
Stage 4 ==>	4	75%	72%	76%	80%	76%	60%	82%	78%	74%	79%	67%	82%	46%	66%	48%	45%	56%	94%	62%	59%	77%	71%
Stage 5 ==>	5	42%	54%	55%	92%	85%	43%	100%	60%	80%	80%	100%	92%	92%	63%	33%	100%	50%	100%	93%	86%	80%	76%
Weighted mean	0-5	67.7%	71.1%	70.6%	63.9%	71.9%	48.3%	72.7%	70.5%	60.3%	69.4%	65.1%	67.7%	58.9%	69.7%	59.0%	59.7%	64.4%	78.3%	64.0%	68.9%	75.4%	67.1%
RANKING		11	5	6	16	4	21	3	7	17	9	13	12	20	8	19	18	14	1	15	10	2	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
1a+1b	97%	97%	94%	96%	98%	94%	88%	69%	98%	97%	91%	98%	86%	92%	95%	97%	87%	95%	96%	94%	93%	93%
RANKING	4	7	13	8	2	14	18	21	3	6	17	1	20	16	10	5	19	11	9	12	15	

F

BIAS

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Stage 1a ==>	0	0.08	0.18	0.27	0.22	0.19	0.62	0.39	0.64	0.37	0.30	0.56	0.32	0.43	0.26	0.18	0.13	0.30	0.32	0.24	0.21	0.34	0.30
Stage 1b ==>	1	-0.70	-0.52	-0.43	-0.54	-0.87	-0.36	-0.56	0.56	-0.47	-0.38	-0.16	-0.50	-0.29	-0.90	-0.42	-0.67	-0.63	-0.29	-0.80	-0.38	-0.16	-0.47
Stage 2 ==>	2	-1.16	-1.06	-1.15	-0.65	-1.09	-0.43	-1.38	-0.05	-0.87	-0.80	-0.63	-0.89	-0.79	-1.04	-1.04	-0.83	-1.06	-0.77	-0.88	-1.09	-0.58	-0.88
Stage 3 ==>	3	-0.77	-0.57	-0.38	-0.04	-0.22	-0.38	-0.46	-0.16	-0.49	-0.42	-0.38	-0.40	-0.58	-0.44	-0.38	0.69	-0.39	-0.23	-0.30	-0.73	-0.31	-0.39
Stage 4 ==>	4	-0.20	-0.13	-0.18	-0.02	-0.16	-0.47	0.03	-0.24	-0.19	-0.29	0.06	-0.03	-0.32	-0.50	-0.17	-0.09	-0.28	-0.09	0.00	-0.59	-0.23	-0.18
Stage 5 ==>	5	-0.67	-0.54	-0.45	-0.08	-0.15	-0.57	0.00	-0.80	-0.20	-0.20	0.00	-0.17	-0.08	-0.38	-1.00	0.00	-0.50	0.00	-0.07	-0.14	-0.20	-0.29

Table 4.2–3. Horse Mackerel eggs first staging.

(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.

(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.

(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.

(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.

For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Stage 1a ==>	0	41	32	46	12	45	4	35	31	55	36	52	44	47	48	76	55	37	40	32	62	46	876
Stage 1b ==>	1	1	3	4	0	2	0	2	3	4	1	5	1	5	3	5	5	4	3	1	7	3	62
Stage 2 ==>	2	14	15	9	8	11	1	13	14	18	11	13	5	16	19	22	13	13	11	5	12	14	257
Stage 3 ==>	3	10	10	19	2	9	0	14	10	15	7	23	18	5	10	32	23	24	16	6	31	12	296
Stage 4 ==>	4	7	9	13	2	6	1	9	10	11	6	13	15	6	11	30	17	15	9	3	16	11	220
Stage 5 ==>	5	-	-	1	3	-	-	-	-	1	-	1	-	-	-	1	-	1	-	-	5	-	-
Total	0-5	73	69	92	27	73	6	73	68	104	61	107	83	79	91	166	113	94	79	47	133	86	1724

B

EGG STAGE COMPOSITION

	Stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Stage 1a ==>	0	49	35	48	14	41	4	29	28	47	35	47	48	47	52	84	65	39	38	35	62	40	887
Stage 1b ==>	1	0	6	6	2	6	0	5	1	14	5	14	2	8	5	4	3	3	6	0	7	12	109
Stage 2 ==>	2	12	12	11	4	13	1	18	21	19	7	13	2	16	17	11	5	14	9	4	9	14	232
Stage 3 ==>	3	10	11	18	1	11	-	16	12	12	7	18	22	8	11	36	17	23	16	5	38	12	304
Stage 4 ==>	4	2	5	8	4	1	1	5	6	12	7	15	9	-	6	31	23	15	10	3	12	7	182
Stage 5 ==>	5	-	-	1	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	1	10
Total	0-5	73	69	92	27	73	6	73	68	104	61	107	83	79	91	166	113	94	79	47	133	86	1724

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
1a+1b	17%	17%	8%	33%	0%	0%	-8%	-15%	3%	8%	7%	11%	6%	12%	9%	13%	2%	2%	6%	0%	6%	6%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Stage 1a ==>	0	100%	97%	96%	92%	89%	100%	80%	84%	82%	78%	87%	100%	89%	90%	96%	98%	95%	88%	100%	92%	87%	91%
Stage 1b ==>	1	0%	67%	50%		50%		50%	0%	50%	0%	60%	0%	20%	33%	20%	40%	50%	100%	0%	57%	67%	33%
Stage 2 ==>	2	71%	80%	89%	50%	91%	100%	85%	93%	89%	45%	69%	40%	81%	79%	45%	31%	69%	55%	80%	42%	79%	69%
Stage 3 ==>	3	80%	90%	79%	50%	89%		86%	80%	73%	57%	78%	100%	80%	80%	81%	65%	88%	81%	67%	90%	83%	82%
Stage 4 ==>	4	29%	44%	54%	50%	17%	100%	44%	50%	73%	50%	92%	60%	0%	36%	80%	88%	87%	89%	67%	69%	45%	62%
Stage 5 ==>	5	-	-	100%	33%	-	-	-	-	0%	-	0%	-	-	-	0%	-	0%	-	-	100%	-	-
Weighted mean	0-5	83.6%	84.1%	83.7%	66.7%	82.2%	100.0%	76.7%	76.5%	78.8%	65.6%	81.3%	88.0%	75.9%	78.0%	80.7%	79.6%	85.1%	82.3%	89.4%	82.7%	79.1%	80.6%
RANKING		7	5	6	20	10	1	17	18	15	21	11	3	19	16	12	13	4	9	2	8	14	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
1a+1b	100%	97%	98%	100%	100%	100%	89%	80%	95%	92%	98%	100%	96%	94%	99%	98%	91%	93%	97%	96%	92%	95%
RANKING	1	10	9	1	1	1	20	21	14	17	8	1	12	15	6	7	19	16	11	13	18	

F

BIAS

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Stage 1a ==>	0	0.00	0.03	0.07	0.08	0.11	0.00	0.29	0.32	0.22	0.33	0.13	0.00	0.15	0.15	0.05	0.02	0.11	0.18	0.00	0.15	0.17	0.12
Stage 1b ==>	1	-1.00	-0.33	-0.50	#VALUE!	-0.50	#VALUE!	0.50	0.33	-0.50	-1.00	0.00	-1.00	-0.80	-0.67	-0.80	-0.60	0.00	0.00	-1.00	-0.43	0.33	-0.37
Stage 2 ==>	2	-0.57	-0.40	-0.22	-0.88	0.09	0.00	-0.08	0.07	0.00	-0.18	-0.31	-0.40	-0.06	-0.42	-0.14	-1.08	-0.54	-0.36	-0.40	-0.08	-0.21	-0.28
Stage 3 ==>	3	-0.20	0.10	-0.21	0.50	0.22	#VALUE!	0.00	-0.20	0.13	0.43	-0.04	0.00	-0.20	0.20	0.19	0.26	-0.04	0.06	-0.33	-0.03	0.17	0.04
Stage 4 ==>	4	-2.00	-1.22	-0.92	0.50	-1.33	0.00	-0.78	-0.80	-0.82	-2.00	-0.31	-0.93	-2.17	-1.64	-0.47	-0.12	-0.13	-0.11	-0.33	-0.31	-0.91	-0.75
Stage 5 ==>	5	-	-	0.00	-0.67	-	-	-	-	-1.00	-	-1.00	-	-	-	-1.00	-	-1.00	-	-	0.00	-	-

Table 4.2–4. All eggs second staging.

(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.

(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.

(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.

(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.

For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Stage 1a ==>	0	108	108	108	108	97	105	108	107	106	105	106	108	108	108	108	108	108	99	104	108	2125
Stage 1b ==>	1	18	18	18	18	17	18	18	18	18	18	17	18	18	18	18	18	18	13	17	18	352
Stage 2 ==>	2	50	50	50	50	49	50	50	50	49	46	50	50	50	50	50	50	50	49	50	50	993
Stage 3 ==>	3	51	51	51	50	49	50	50	50	50	44	50	50	49	49	50	51	49	51	49	51	995
Stage 4 ==>	4	24	24	24	24	24	24	24	24	24	22	24	24	24	24	24	24	24	24	23	24	477
Stage 5 ==>	5	49	49	49	49	45	49	49	49	48	43	49	49	48	49	49	49	48	49	49	49	967
Total	0-5	300	300	300	299	281	296	299	298	295	278	296	299	297	298	299	300	297	285	292	300	5909

B

EGG STAGE COMPOSITION

	Stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Stage 1a ==>	0	128	120	115	117	115	111	104	88	90	117	74	128	121	114	134	111	116	93	105	107	2208
Stage 1b ==>	1	6	8	12	11	4	28	19	24	35	12	50	5	10	13	1	37	15	21	20	18	349
Stage 2 ==>	2	53	57	43	25	52	21	65	63	66	31	32	33	56	54	24	24	44	58	32	64	897
Stage 3 ==>	3	54	48	57	71	48	66	44	57	32	40	55	65	52	50	65	48	60	42	67	47	1068
Stage 4 ==>	4	20	23	30	42	21	28	22	40	34	41	37	34	21	36	31	42	26	32	25	37	622
Stage 5 ==>	5	39	44	43	33	41	42	45	26	38	37	48	34	37	31	44	38	36	39	43	27	765
Total	0-5	300	300	300	299	281	296	299	298	295	278	296	299	297	298	299	300	297	285	292	300	5909

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
1a+1b	6%	2%	1%	2%	4%	13%	-2%	-10%	1%	5%	1%	6%	4%	1%	7%	17%	4%	2%	3%	-1%	3%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Stage 1a ==>	0	96%	93%	94%	93%	97%	86%	87%	70%	76%	90%	68%	99%	95%	89%	97%	86%	75%	86%	88%	86%	88%
Stage 1b ==>	1	11%	17%	44%	28%	12%	50%	67%	44%	61%	22%	88%	28%	44%	22%	6%	72%	28%	77%	29%	50%	39%
Stage 2 ==>	2	84%	94%	82%	48%	92%	38%	94%	76%	86%	61%	54%	64%	88%	94%	44%	42%	56%	96%	62%	94%	72%
Stage 3 ==>	3	78%	76%	82%	82%	82%	90%	74%	84%	62%	64%	70%	88%	80%	84%	74%	69%	67%	73%	94%	78%	78%
Stage 4 ==>	4	50%	63%	63%	71%	63%	75%	67%	71%	75%	91%	63%	71%	50%	79%	67%	75%	50%	79%	78%	79%	69%
Stage 5 ==>	5	73%	80%	82%	55%	82%	80%	84%	47%	73%	79%	82%	63%	71%	59%	80%	69%	40%	73%	82%	49%	70%
Weighted mean	0-5	78.7%	81.0%	82.7%	71.6%	82.9%	74.3%	82.6%	68.1%	73.9%	75.2%	68.9%	78.9%	80.8%	79.2%	73.6%	71.3%	59.9%	82.1%	79.1%	77.3%	76.1%
RANKING		10	5	2	16	1	13	3	19	14	12	18	9	6	7	15	17	20	4	8	11	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
1a+1b	100%	98%	98%	98%	99%	98%	94%	82%	94%	97%	97%	99%	98%	98%	98%	98%	80%	97%	98%	95%	96%
RANKING	1	8	4	4	3	12	17	19	18	14	14	2	8	8	8	4	20	13	7	16	

F

BIAS

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Stage 1a ==>	0	0.04	0.14	0.11	0.12	0.04	0.18	0.19	0.47	0.32	0.16	0.35	0.03	0.08	0.16	0.07	0.18	0.73	0.17	0.16	0.23	0.20
Stage 1b ==>	1	-0.89	-0.83	-0.56	-0.72	-0.88	-0.50	-0.33	0.22	-0.39	-0.78	0.00	-0.72	-0.56	-0.78	-0.94	-0.28	-0.50	-0.23	-0.71	-0.50	-0.55
Stage 2 ==>	2	-0.08	-0.06	0.12	0.34	0.02	-0.32	0.00	0.22	-0.16	-0.15	0.24	0.00	-0.18	-0.06	0.00	-0.34	-0.18	-0.02	0.16	-0.12	-0.03
Stage 3 ==>	3	-0.29	-0.24	-0.02	0.14	-0.22	0.02	-0.34	-0.20	-0.30	0.30	0.28	0.04	-0.20	-0.04	0.06	0.10	-0.29	-0.27	-0.02	-0.29	-0.09
Stage 4 ==>	4	-0.33	0.00	-0.17	-0.04	-0.13	0.00	-0.08	-0.25	-0.13	0.00	0.21	-0.08	-0.33	-0.13	0.00	-0.08	-0.50	-0.04	0.04	-0.13	-0.11
Stage 5 ==>	5	-0.49	-0.39	-0.22	-0.51	-0.40	-0.29	-0.22	-0.96	-0.29	-0.26	-0.18	-0.55	-0.54	-0.63	-0.31	-0.51	-1.44	-0.35	-0.29	-0.69	-0.48

Table 4.2–5. Mackerel eggs second staging.

(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.

(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.

(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.

(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.

For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Stage 1a ==>	0	72	77	67	85	71	81	71	61	69	75	70	76	73	76	79	81	71	56	75	69	**1455**
Stage 1b ==>	1	10	10	10	13	9	10	10	10	10	11	9	10	10	10	10	11	12	6	9	10	**200**
Stage 2 ==>	2	43	42	41	44	41	43	38	38	39	38	42	40	41	42	42	33	32	25	42	36	**782**
Stage 3 ==>	3	41	41	41	48	40	49	39	40	40	36	40	38	40	41	41	39	39	40	42	42	**817**
Stage 4 ==>	4	20	20	20	20	20	24	24	12	18	21	20	20	21	23	20	22	17	20	22	21	**405**
Stage 5 ==>	5	46	49	47	47	43	49	48	44	40	42	48	45	45	45	48	41	48	48	49	46	**918**
Total	0-5	**232**	**239**	**226**	**257**	**224**	**256**	**230**	**205**	**216**	**223**	**229**	**229**	**230**	**237**	**240**	**227**	**219**	**195**	**239**	**224**	**4577**

B

EGG STAGE COMPOSITION

	Stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Stage 1a ==>	0	83	83	70	90	79	82	65	50	55	85	42	90	82	81	97	72	72	51	73	69	**1471**
Stage 1b ==>	1	4	6	7	10	2	27	13	10	27	9	41	3	7	8	1	35	13	12	15	13	**263**
Stage 2 ==>	2	48	49	40	25	45	19	50	46	53	24	25	27	47	45	22	18	35	32	32	45	**727**
Stage 3 ==>	3	43	41	42	67	41	59	37	44	25	34	48	48	38	39	51	33	44	35	53	38	**860**
Stage 4 ==>	4	15	16	26	36	16	27	21	31	20	36	26	27	19	33	25	31	20	26	23	33	**507**
Stage 5 ==>	5	39	44	41	29	41	42	44	24	36	35	47	34	37	31	44	38	35	39	43	26	**749**
Total	0-5	**232**	**239**	**226**	**257**	**224**	**256**	**230**	**205**	**216**	**223**	**229**	**229**	**230**	**237**	**240**	**227**	**219**	**195**	**239**	**224**	**4577**

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
1a+1b	**6%**	**2%**	**0%**	**2%**	**1%**	**20%**	**-4%**	**-15%**	**4%**	**9%**	**5%**	**8%**	**7%**	**3%**	**10%**	**16%**	**2%**	**2%**	**5%**	**4%**	**5%**

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Stage 1a ==>	0	96%	92%	93%	93%	97%	80%	87%	74%	71%	93%	57%	100%	96%	91%	99%	77%	76%	86%	88%	88%	**87%**
Stage 1b ==>	1	10%	20%	30%	38%	11%	60%	80%	30%	50%	18%	89%	30%	50%	20%	10%	55%	50%	83%	33%	60%	**40%**
Stage 2 ==>	2	88%	93%	95%	55%	95%	40%	100%	84%	85%	61%	55%	65%	85%	95%	48%	39%	59%	96%	74%	92%	**75%**
Stage 3 ==>	3	78%	78%	88%	88%	88%	90%	82%	90%	63%	69%	80%	89%	75%	85%	73%	67%	64%	78%	93%	76%	**80%**
Stage 4 ==>	4	45%	55%	75%	70%	55%	75%	67%	75%	72%	90%	55%	70%	57%	83%	60%	73%	35%	75%	77%	76%	**67%**
Stage 5 ==>	5	78%	80%	81%	57%	86%	80%	83%	52%	83%	81%	83%	69%	76%	64%	81%	85%	44%	75%	82%	50%	**73%**
Weighted mean	0-5	**79.7%**	**81.2%**	**85.4%**	**74.3%**	**85.7%**	**73.8%**	**85.2%**	**72.2%**	**73.1%**	**77.6%**	**67.2%**	**80.3%**	**80.9%**	**81.9%**	**75.0%**	**69.6%**	**59.8%**	**81.5%**	**82.0%**	**76.3%**	**77.2%**
RANKING		**10**	**7**	**2**	**14**	**1**	**15**	**3**	**17**	**16**	**11**	**19**	**9**	**8**	**5**	**13**	**18**	**20**	**6**	**4**	**12**	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
1a+1b	**100%**	**98%**	**99%**	**99%**	**99%**	**98%**	**95%**	**82%**	**95%**	**99%**	**99%**	**100%**	**99%**	**99%**	**99%**	**99%**	**78%**	**98%**	**99%**	**99%**	**97%**
RANKING	**1**	**16**	**13**	**3**	**10**	**15**	**17**	**19**	**18**	**6**	**11**	**1**	**9**	**6**	**5**	**4**	**20**	**14**	**8**	**11**	

F

BIAS

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Stage 1a ==>	0	0.04	0.13	0.12	0.08	0.04	0.22	0.18	0.43	0.35	0.07	0.44	0.00	0.05	0.11	0.03	0.25	0.75	0.16	0.13	0.17	**0.18**
Stage 1b ==>	1	-0.90	-0.80	-0.70	-0.62	-0.89	-0.40	-0.20	0.10	-0.50	-0.64	-0.11	-0.70	-0.50	-0.80	-0.90	-0.45	-0.17	-0.17	-0.67	-0.40	**-0.53**
Stage 2 ==>	2	-0.09	-0.07	0.05	0.32	-0.02	-0.49	0.00	0.21	-0.15	-0.18	0.19	-0.02	-0.22	-0.10	-0.07	-0.42	-0.09	-0.08	0.07	-0.17	**-0.06**
Stage 3 ==>	3	-0.27	-0.27	-0.02	0.04	-0.13	-0.06	-0.23	-0.05	-0.43	0.19	0.15	0.00	-0.30	-0.10	0.12	0.13	-0.18	-0.13	-0.02	-0.29	**-0.09**
Stage 4 ==>	4	-0.35	0.00	0.05	-0.10	-0.15	0.00	-0.08	-0.33	-0.06	0.00	0.25	-0.05	-0.24	-0.04	0.00	-0.09	-0.47	-0.05	0.05	-0.14	**-0.08**
Stage 5 ==>	5	-0.37	-0.39	-0.23	-0.49	-0.19	-0.29	-0.23	-0.57	-0.18	-0.21	-0.17	-0.47	-0.40	-0.51	-0.29	-0.17	-1.33	-0.33	-0.29	-0.65	**-0.39**

Table 4.2–6. Horse Mackerel eggs second staging.

(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.

(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.

(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.

(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.

For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Stage 1a ==>	0	28	28	30	19	24	25	26	27	26	25	25	25	27	26	27	30	29	29	26	30	532
Stage 1b ==>	1	8	8	8	5	7	1	5	8	6	7	7	5	8	8	8	3	3	7	8	10	130
Stage 2 ==>	2	8	8	8	1	8	1	6	3	4	8	8	9	8	8	7	6	5	6	8	14	134
Stage 3 ==>	3	6	7	7	5	7	6	8	7	7	6	7	7	8	6	7	16	16	7	6	8	154
Stage 4 ==>	4	4	4	4	3	4	-	-	4	3	1	5	4	3	1	6	10	6	3	1	5	71
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-
Total	0-5	54	55	57	33	50	33	45	49	46	47	53	50	54	49	55	65	60	52	49	67	1023

B

EGG STAGE COMPOSITION

	Stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Stage 1a ==>	0	36	34	33	24	29	25	26	23	22	30	21	29	31	28	35	31	29	28	30	34	578
Stage 1b ==>	1	1	2	5	1	2	1	4	6	8	3	9	2	3	5	0	2	2	7	5	5	73
Stage 2 ==>	2	5	8	3	-	7	2	8	8	6	5	6	5	9	9	2	6	8	7	-	17	121
Stage 3 ==>	3	9	6	14	4	7	5	6	8	6	5	7	9	11	6	12	15	15	6	13	6	170
Stage 4 ==>	4	3	5	2	4	5	-	1	4	4	4	9	5	-	1	6	11	5	4	1	4	78
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	1	3
Total	0-5	54	55	57	33	50	33	45	49	46	47	53	50	54	49	55	65	60	52	49	67	1023

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
1a+1b	3%	0%	0%	4%	0%	0%	-3%	-17%	-6%	3%	-6%	3%	-3%	-3%	0%	0%	-3%	-3%	3%	-3%	-2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Stage 1a ==>	0	100%	96%	100%	95%	96%	100%	92%	85%	81%	100%	84%	100%	96%	85%	96%	100%	90%	90%	88%	97%	93%
Stage 1b ==>	1	13%	13%	63%	0%	14%	100%	40%	63%	83%	43%	86%	40%	38%	25%	0%	67%	33%	71%	25%	40%	40%
Stage 2 ==>	2	50%	100%	25%	0%	75%	100%	100%	100%	100%	63%	50%	56%	100%	88%	29%	100%	100%	100%	0%	100%	67%
Stage 3 ==>	3	83%	86%	71%	80%	71%	83%	75%	100%	86%	50%	43%	71%	100%	83%	86%	88%	88%	86%	100%	75%	81%
Stage 4 ==>	4	75%	100%	0%	100%	100%	-	-	100%	100%	100%	100%	75%	0%	0%	100%	90%	83%	100%	100%	80%	83%
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	100%	-	-	-	-	-	100%	-	-	-	-
Weighted mean	0-5	75.9%	83.6%	73.7%	75.8%	78.0%	97.0%	84.4%	85.7%	84.8%	78.7%	75.5%	80.0%	83.3%	73.5%	72.7%	93.8%	86.7%	88.5%	65.3%	85.1%	81.1%
RANKING		14	9	17	15	13	1	8	5	7	12	16	11	10	18	19	2	4	3	20	6	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
1a+1b	100%	100%	100%	100%	100%	100%	100%	83%	94%	100%	94%	100%	97%	97%	97%	100%	94%	97%	100%	100%	97%
RANKING	1	1	1	1	1	1	1	20	17	1	17	1	14	16	14	1	19	13	1	1	

F

BIAS

	MODAL stage	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Stage 1a ==>	0	0.00	0.04	0.00	0.05	0.04	0.00	0.08	0.30	0.27	0.00	0.20	0.00	0.07	0.19	0.11	0.00	0.21	0.14	0.12	0.03	0.09
Stage 1b ==>	1	-0.88	-0.88	-0.38	-1.00	-0.86	0.00	-0.20	0.38	-0.17	-0.57	0.14	-0.60	-0.63	-0.75	-1.00	-0.33	-0.67	-0.29	-0.75	-0.40	-0.52
Stage 2 ==>	2	0.13	0.00	0.75	-2.00	0.25	0.00	0.00	0.00	0.00	0.00	0.50	0.11	0.00	0.13	0.71	0.00	0.00	0.00	0.63	0.00	0.17
Stage 3 ==>	3	-0.17	0.14	0.29	0.20	0.00	-0.17	0.00	0.00	0.14	0.50	0.57	0.29	0.00	0.17	-0.43	0.13	-0.25	0.14	0.00	-0.25	0.05
Stage 4 ==>	4	-0.25	0.00	-1.25	0.00	0.00	-	-	0.00	0.00	0.00	0.00	-0.25	-1.00	-2.00	0.00	-0.10	-0.33	0.00	0.00	0.20	-0.20
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	0.00	-	-	-	-	-	0.00	-	-	-	-

Figure 4.2-1 All eggs first staging

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

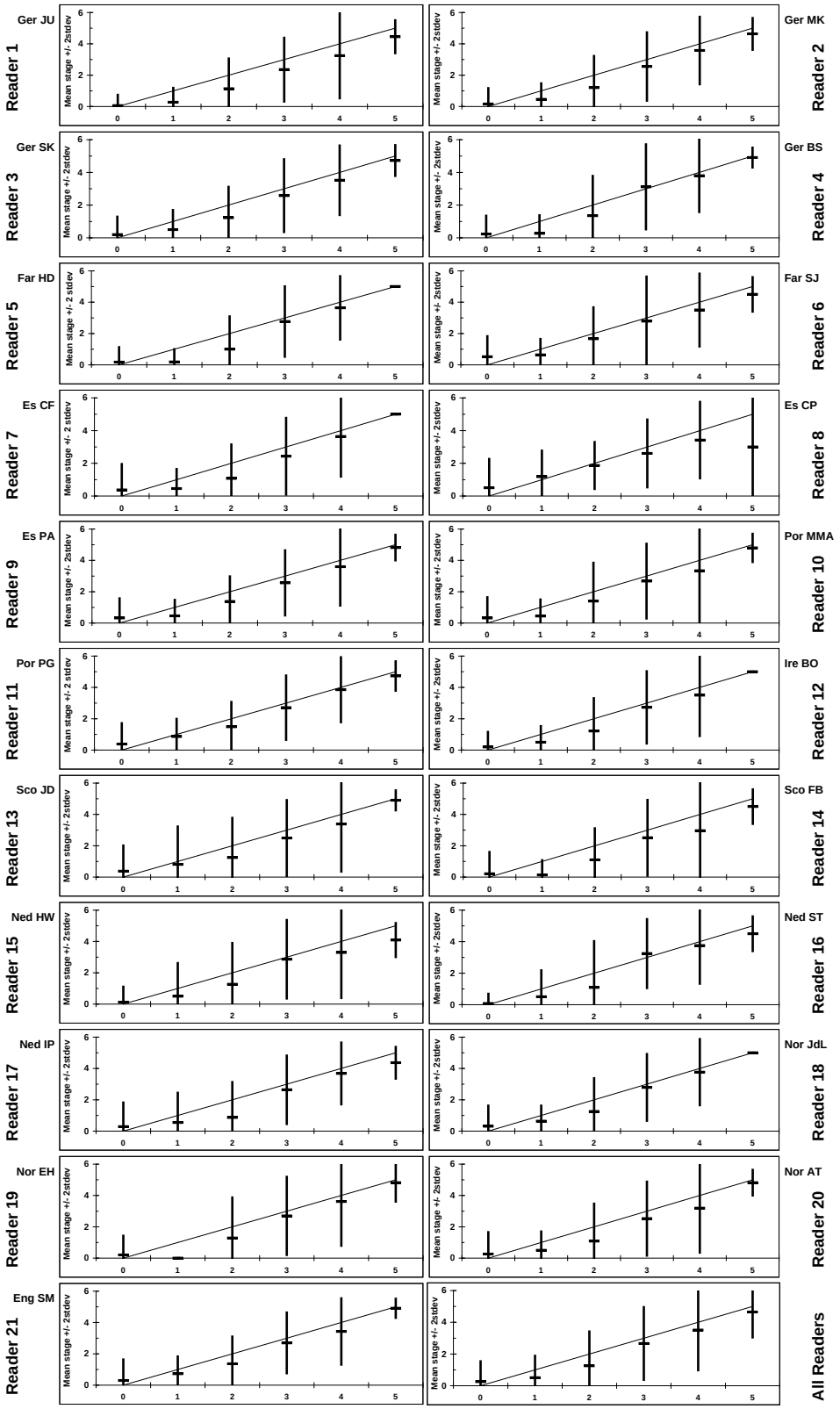


Figure 4.2-2 Mackerel eggs first staging

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

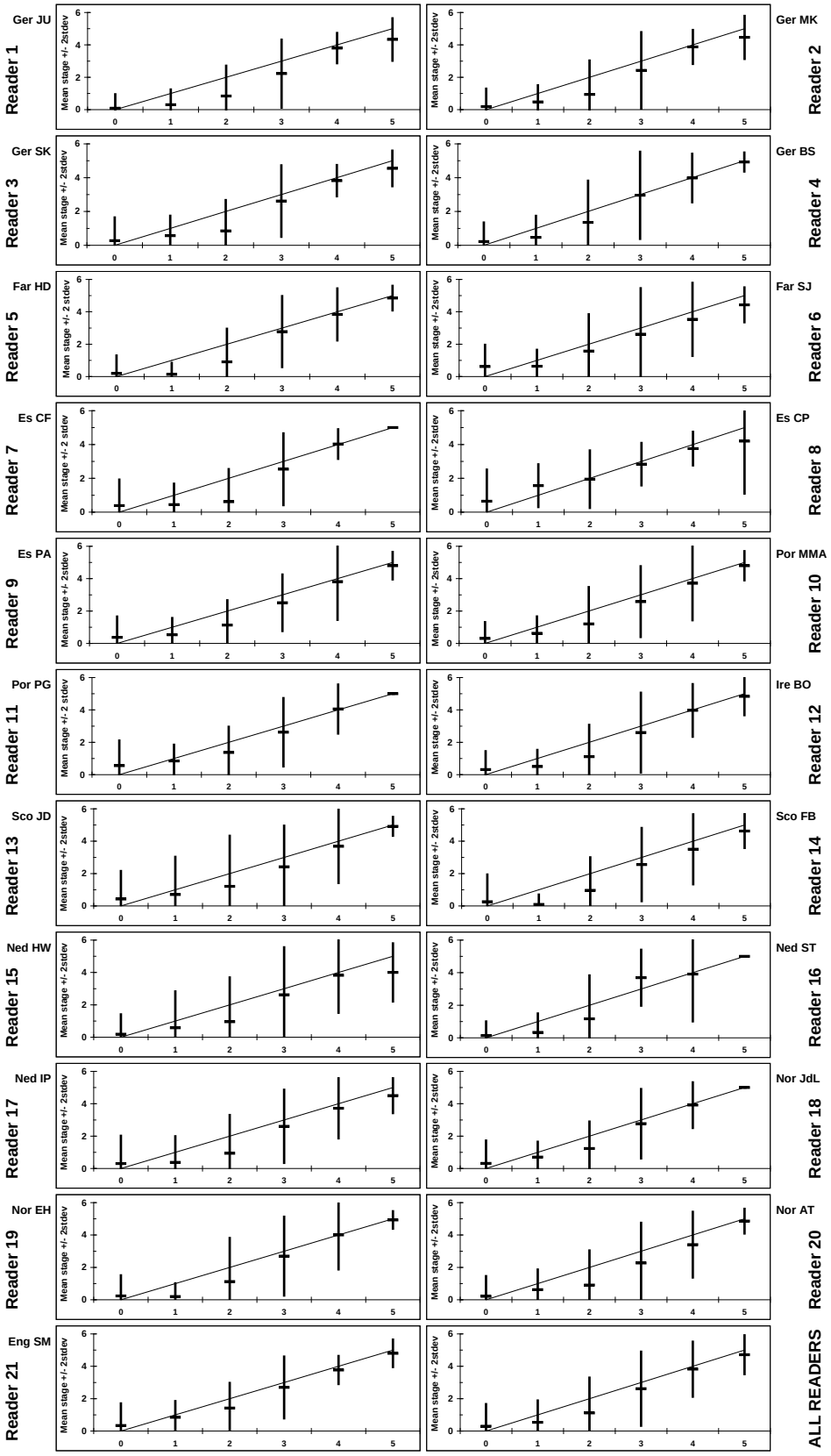


Figure 4.2-3. Horse mackerel eggs first staging

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

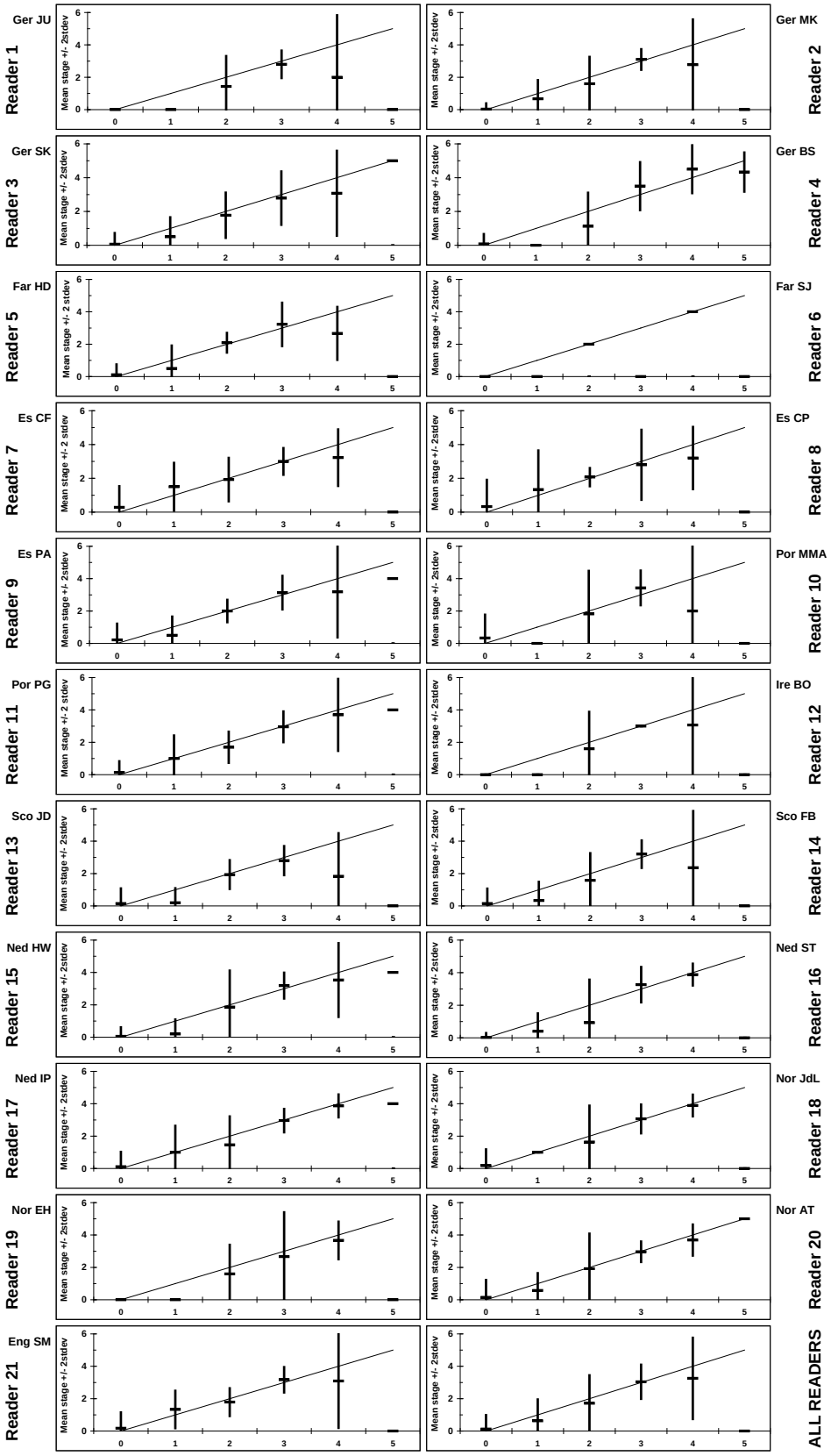


Figure 4.2-4. All eggs second staging

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

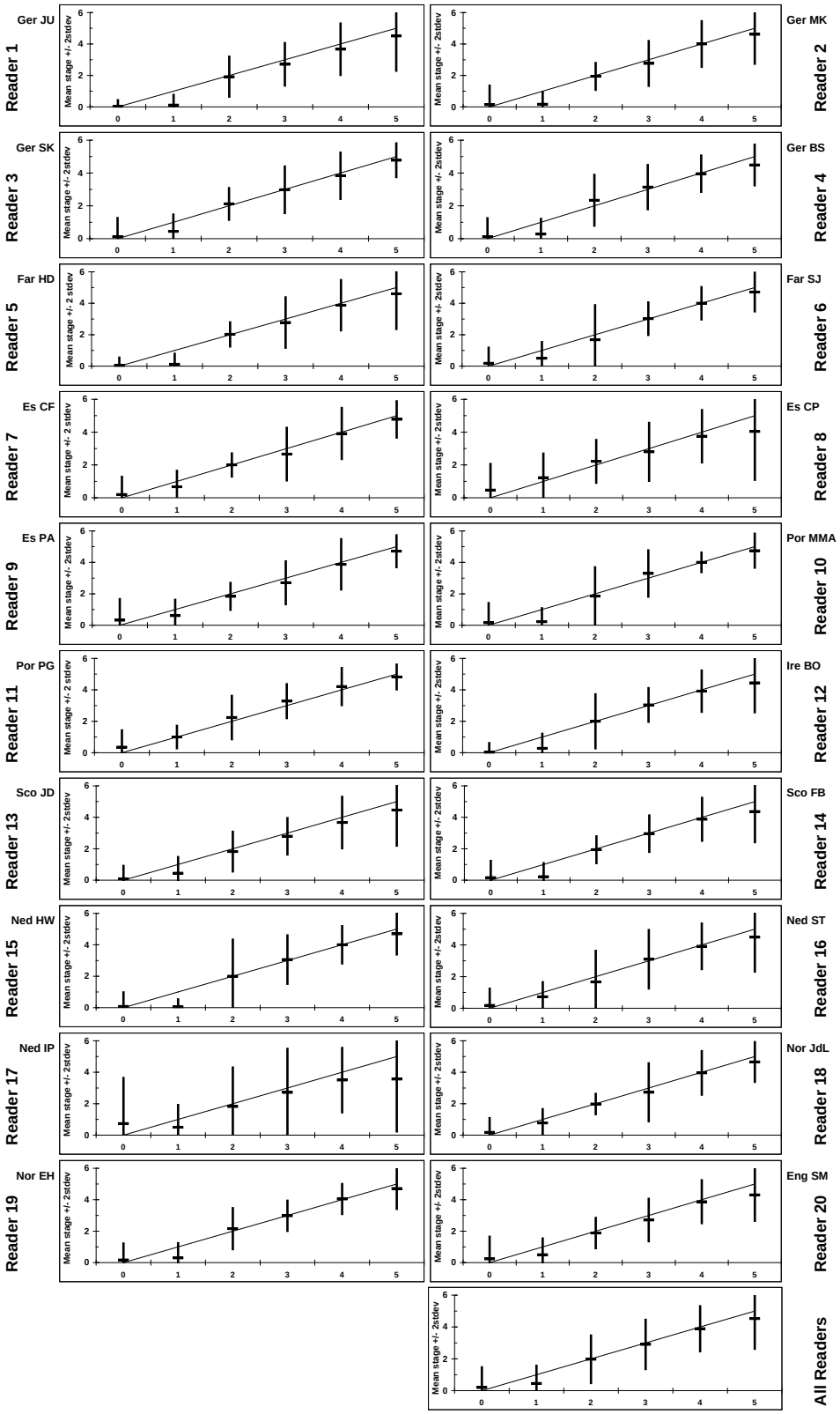


Figure 4.2-5 Mackerel eggs second staging

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

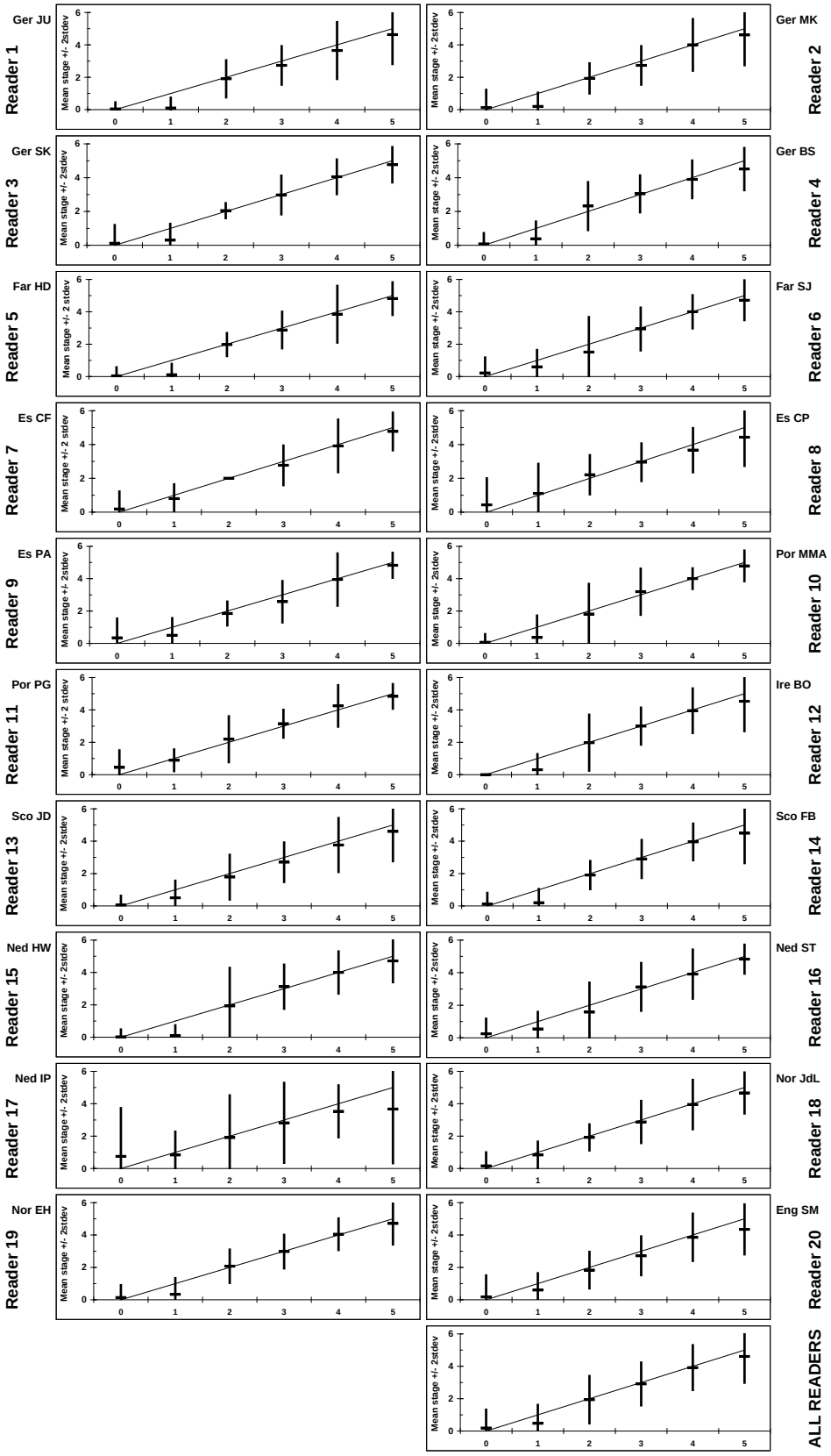
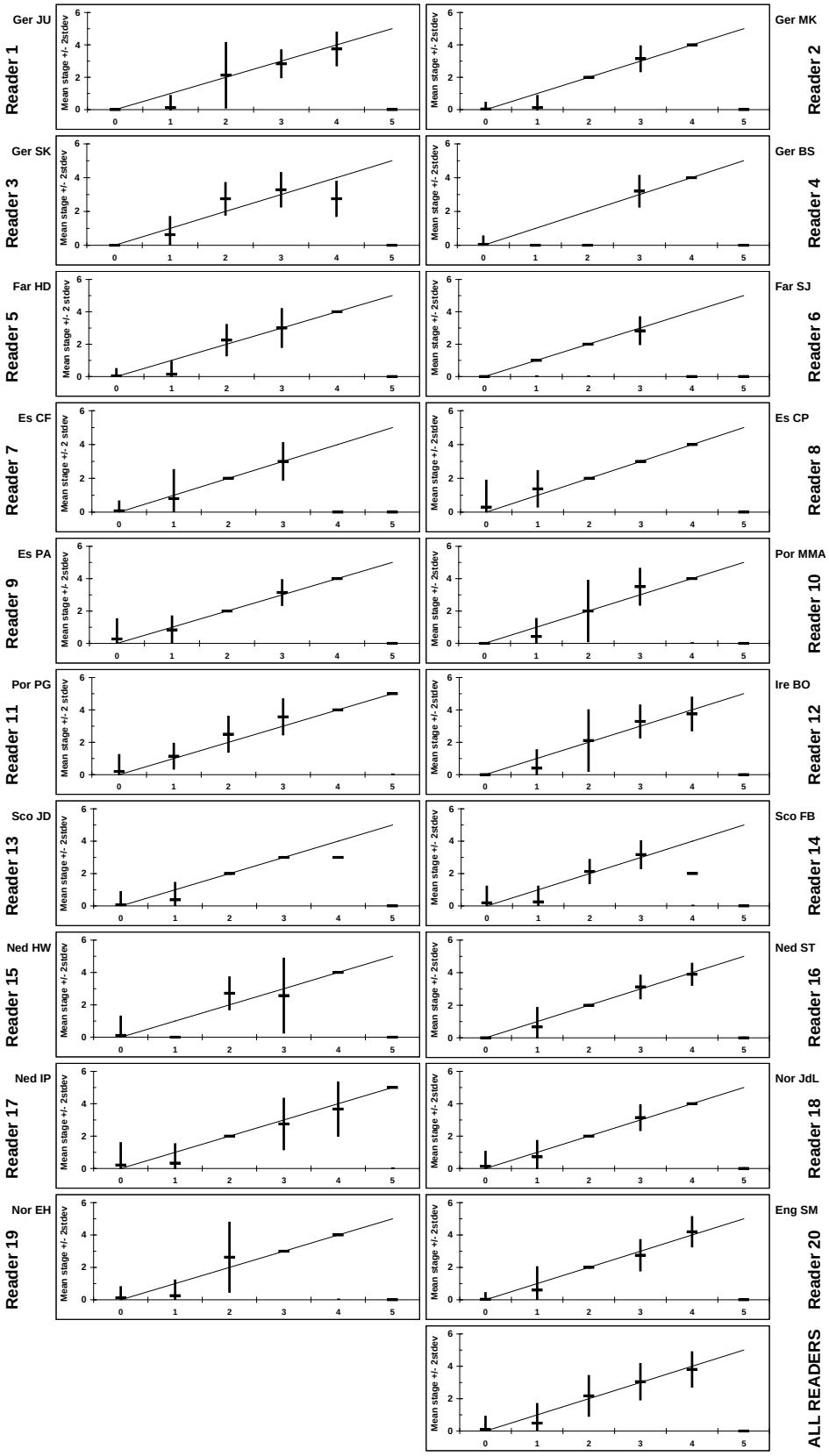


Figure 4.2-6. Horse mackerel eggs second staging

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



4.3 Result of egg identification exercise

The same trays of eggs, which were used for egg staging, were also used for the egg identification exercise. Some of the eggs used were from artificial fertilizations and so the species of those eggs was definitely known. It was hoped that by using eggs of known species any problems associated with identification would be highlighted clearly and better descriptions of each species could be prepared.

The original assessment of species identification for each egg, by each participant, was put into a primary result table (not presented here). Once the results were available from every participant two methods of analysis were conducted. The results were initially compared with the actual or modal species of egg. The second table shows the results for the actual species, which should have been present in the wells of each tray. Both sets of results from are presented below. It is possible that most of the differences between these tables can be accounted for by movement of eggs from one well to another.

Summaries of the results from the two rounds of egg species determination are presented in Tables 4.3–1 to 4.3–4. Each of these tables are divided into four sub-tables labelled A-D, where the performance of each participant is judged against the actual species and modal species determination.

Sub-tables A show the number of eggs at each actual or modal species that were assessed by each participant. The numbers at each modal species will therefore be the same for all participants that read all the eggs.

Sub-tables B show the numbers of eggs of each species as assessed by each participant.

Sub-tables C show the percentage under or overestimation by each participant for each species.

Sub-tables D show the percentage agreement in species identification between the assessment of each participant and the actual or modal species.

Tables 4.3–1 and 4.3–2 show differences in the results from the first round of analysis, where modal and actual species of eggs were used (Table 4.3–1) and where actual determinations (Table 4.3–2) were used to compare with participants' assessment of species. The differences between these tables probably reflect the extent to which some eggs were unintentionally moved between cells during the first round of analysis. This is apparent when comparing the results in sub-tables C and D (Tables 4.3–1 and 4.3–2) and is particularly highlighted by the difference between 'actual' and 'modal' species determinations for 'other species'. If participants are judged against 'actual' species they appear to have overestimated 'other species' by 7% but if comparisons are made with modal species they appear to have overestimated 'other species' by 26%.

The results of the second round of analysis also show a high difference between the use of 'actual' or 'modal' species determination (Tables 4.3–3 and 4.3–4). If participants are judged against 'actual' species they appear to have overestimated 'other species' by 91% but if comparisons are made with modal species they appear to have overestimated 'other species' by 72%.

The results show significant improvements in the allocation of eggs to mackerel and horse mackerel, from the first to the second round of analysis. However, they also highlight the difficulties in being able to positively identify eggs where there are few distinguishing features other than the size of egg and oil globule diameters. After the

first round of analysis there was some discussion on the features which aid fish egg identification. Some references and criteria were produced (see section 3.3.2) to help with the identification of eggs which are similar to those of mackerel and horse mackerel. These discussions and criteria helped to improve the mean percentage agreement between participants' identification of eggs to species (Tables 4.3–1D, 4.3–2D, 4.3–3D and 4.3–4D). For mackerel eggs the percentage agreement increased from 80% to 95% with modal/actual species and from 76% to 95% with actual species. For horse mackerel the improvement rose from 72% to 84% for modal/actual species and slightly decreased from 68% to 64% for the actual species. Overall, the percentage agreement rose from 67% ('actual' spp.) and 75% (modal spp.) in the first round to 85% and 89% in the second round of analysis. These results were very re-assuring particularly as most of the microscopes were not fitted with eyepiece graticules to enable measurement of egg or oil globule diameters.

Table 4.3-1. Species identification with actual/modal species, first determination

The species compositions based on modal/actual species reflecting the best estimates based on only those eggs that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percentages over- and underestimation (C) and the percentages agreement with modal species or actual species (D)are shown per species by participant and for the whole group that took part in the species identification excercise on fish eggs. A weighted mean percent agreement is given by person and all persons combined.

A **Species compositions using modal/actual species (second last column input table)**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Mackerel	1	262	263	264	263	234	78	265	264	262	151	252	264	234	244	263	127	226	262	274	252	264	4968
Horse Mackerel	2	75	76	77	76	71	10	79	74	76	59	73	76	66	77	77	45	48	75	77	73	76	1436
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hake	4	6	9	9	8	6	-	9	9	7	9	4	9	8	9	9	4	4	8	9	8	9	153
Other species	5	19	20	21	19	17	5	20	19	20	13	17	20	18	20	18	11	14	18	20	20	20	369
Total	1-5	362	368	371	366	328	93	373	366	365	232	346	369	326	350	367	187	292	363	392	353	369	6938

B **Species compositions as estimated per participant and whole group**

Species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Mackerel	1	260	270	231	330	224	87	205	207	204	124	215	235	224	228	195	67	180	180	303	180	195	4344
Horse Mackerel	2	73	69	92	27	73	6	73	68	104	61	107	83	79	91	166	113	94	79	47	133	86	1724
Megrim	3	3	2	10	7	7	-	21	35	-	20	3	14	10	15	-	-	1	44	18	7	30	247
Hake	4	9	5	4	-	-	-	12	1	21	7	13	-	-	-	-	-	4	22	1	28	31	158
Other species	5	17	22	34	2	24	-	62	55	36	20	8	37	13	16	6	7	13	38	23	5	27	465
Total	1-5	362	368	371	366	328	93	373	366	365	232	346	369	326	350	367	187	292	363	392	353	369	6938

C **Percentage overestimation / underestimation**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Mackerel	1	-1%	3%	-13%	25%	-4%	12%	-23%	-22%	-22%	-18%	-15%	-11%	-4%	-7%	-26%	-47%	-20%	-31%	11%	-29%	-26%	-13%
Horse Mackerel	2	-3%	-9%	19%	-64%	3%	-40%	-8%	-8%	37%	3%	47%	9%	20%	18%	116%	151%	96%	5%	-39%	82%	13%	20%
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hake	4	50%	-44%	-56%	0%	0%	-	33%	-89%	200%	-22%	225%	0%	0%	0%	0%	0%	0%	175%	-89%	250%	244%	3%
Other species	5	-11%	10%	62%	-89%	41%	0%	210%	189%	80%	54%	-53%	85%	-28%	-20%	-67%	-36%	-7%	111%	15%	-75%	35%	26%

D **Percentage agreement in species identification per species**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Mackerel	1	97%	97%	83%	97%	85%	99%	77%	78%	77%	80%	78%	84%	85%	92%	73%	37%	68%	68%	96%	53%	72%	80%
Horse Mackerel	2	89%	82%	71%	24%	69%	50%	75%	85%	89%	88%	70%	68%	76%	92%	99%	64%	42%	67%	40%	53%	84%	72%
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hake	4	0%	0%	0%	0%	0%	-	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Other species	5	53%	50%	38%	11%	59%	0%	65%	79%	50%	62%	35%	50%	33%	50%	17%	45%	29%	67%	50%	5%	60%	45%
Weighted mean	1-5	91.4%	88.6%	76.0%	75.4%	79.0%	88.2%	73.7%	77.9%	76.7%	78.0%	73.4%	76.7%	77.9%	87.1%	73.6%	43.3%	60.6%	66.4%	77.3%	49.0%	72.4%	74.7%
	RANKING	1	2	12	13	5	3	14	8	10	6	16	11	7	4	15	21	19	18	9	20	17	

Table 4.3-2. Species identification with actual species, first determination

The species compositions based on actual species reflecting the best estimates based on only those eggs that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percentages over- and underestimation (C) and the percentages agreement with modal species or actual species (D)are shown per species by participant and for the whole group that took part in the species identification excercise on fish eggs. A weighted mean percent agreement is given by person and all persons combined.

A **Species compositions using modal/actual species (second last column input table)**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Mackerel	1	102	101	102	103	88	30	103	102	101	52	92	102	93	102	101	41	86	101	112	92	102	1908
Horse Mackerel	2	13	13	13	13	13	-	13	13	13	10	13	13	11	13	13	6	10	13	13	12	13	244
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hake	4	6	9	9	8	6	-	9	9	7	9	4	9	8	9	9	4	4	8	9	8	9	153
Other species	5	7	7	7	7	4	5	7	7	7	2	6	7	7	7	7	2	2	7	7	7	7	126
Total	1-5	128	130	131	131	111	35	132	131	128	73	115	131	119	131	130	53	102	129	152	119	131	2442

B **Species compositions as estimated per participant and whole group**

Species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	TOTAL
Mackerel	1	96	100	88	117	79	35	69	67	74	35	79	85	81	89	71	17	69	67	125	62	66	1571
Horse Mackerel	2	18	18	20	8	22	-	14	16	34	12	30	22	25	28	58	36	21	24	13	33	24	476
Megrim	3	3	2	8	5	5	-	20	32	-	18	1	13	8	14	-	-	1	12	7	5	30	184
Hake	4	7	5	4	0	0	-	9	0	5	1	4	0	0	0	0	0	3	12	1	15	10	76
Other species	5	4	5	11	1	5	0	20	16	15	7	1	11	5	0	1	0	8	14	6	4	1	135
Total	1-5	128	130	131	131	111	35	132	131	128	73	115	131	119	131	130	53	102	129	152	119	131	2442

C **Percentage overestimation / underestimation**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Mackerel	1	-6%	-1%	-14%	14%	-10%	17%	-33%	-34%	-27%	-33%	-14%	-17%	-13%	-13%	-30%	-59%	-20%	-34%	12%	-33%	-35%	-18%
Horse Mackerel	2	38%	38%	54%	-38%	69%	-	8%	23%	162%	20%	131%	69%	127%	115%	346%	500%	110%	85%	0%	175%	85%	95%
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hake	4	17%	-44%	-56%	-100%	-100%	-	0%	-100%	-29%	-89%	0%	-100%	-100%	-100%	-100%	-100%	-25%	50%	-89%	88%	11%	-50%
Other species	5	-43%	-29%	57%	-86%	25%	-100%	186%	129%	114%	250%	-83%	57%	-29%	-100%	-86%	-100%	300%	100%	-14%	-43%	-86%	7%

D **Percentage agreement in species identification per species**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Nor AT Reader 20	Eng SM Reader 21	ALL
Mackerel	1	94%	95%	79%	98%	82%	100%	66%	66%	72%	67%	80%	76%	75%	86%	68%	34%	73%	66%	95%	43%	62%	76%
Horse Mackerel	2	100%	100%	38%	54%	77%	-	54%	100%	77%	100%	85%	46%	64%	100%	100%	100%	10%	38%	15%	17%	92%	68%
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hake	4	0%	0%	0%	0%	0%	-	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Other species	5	0%	0%	0%	14%	25%	0%	0%	43%	0%	50%	17%	43%	0%	0%	0%	0%	50%	29%	0%	0%	0%	10%
Weighted mean	1-5	85.2%	83.8%	65.6%	83.2%	74.8%	85.7%	56.8%	63.4%	64.8%	63.0%	74.8%	66.4%	64.7%	77.1%	63.1%	37.7%	63.7%	57.4%	71.1%	35.3%	57.3%	66.7%
RANKING		2	3	10	4	7	1	19	14	11	16	6	9	12	5	15	20	13	17	8	21	18	

Table 4.3-3. Species identification with actual/modal species, second determination

The species compositions based on modal/actual species reflecting the best estimates based on only those eggs that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percentages over- and underestimation (C) and the percentages agreement with modal species or actual species (D)are shown per species by participant and for the whole group that took part in the species identification excercise on fish eggs. A weighted mean percent agreement is given by person and all persons combined.

A **Species compositions using modal/actual species (second last column input table)**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Mackerel	1	227	227	227	226	217	224	226	225	222	205	223	226	224	225	226	227	224	217	221	227	4466
Horse Mackerel	2	54	54	54	54	52	53	54	54	54	54	54	54	54	54	54	54	54	50	53	54	1072
Megrim	3	12	12	12	12	9	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	237
Hake	4	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	18
Other species	5	6	6	6	6	3	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	116
Total	1-5	300	300	300	299	281	296	299	298	295	278	296	299	297	298	299	300	297	285	292	300	5909

B **Species compositions as estimated per participant and whole group**

	Species	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Mackerel	1	232	239	226	257	224	256	230	205	216	223	229	229	230	237	240	227	219	195	239	224	4577
Horse Mackerel	2	54	55	57	33	50	33	45	49	46	47	53	50	54	49	55	65	60	52	49	67	1023
Megrim	3	0	0	0	2	2	7	6	20	10	0	0	0	1	0	0	0	3	22	0	1	74
Hake	4	0	0	1	1	-	0	9	0	1	0	0	7	4	0	0	0	0	10	0	2	35
Other species	5	14	6	16	6	5	0	9	24	22	8	14	13	8	12	4	8	15	6	4	6	200
Total	1-5	300	300	300	299	281	296	299	298	295	278	296	299	297	298	299	300	297	285	292	300	5909

C **Percentage overestimation / underestimation**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Mackerel	1	2%	5%	0%	14%	3%	14%	2%	-9%	-3%	9%	3%	1%	3%	5%	6%	0%	-2%	-10%	8%	-1%	2%
Horse Mackerel	2	0%	2%	6%	-39%	-4%	-38%	-17%	-9%	-15%	-13%	-2%	-7%	0%	-9%	2%	20%	11%	4%	-8%	24%	-5%
Megrim	3	-100%	-100%	-100%	-83%	-78%	-42%	-50%	67%	-17%	-100%	-100%	-100%	-92%	-100%	-100%	-100%	-75%	83%	-100%	-92%	-69%
Hake	4	-100%	-100%	0%	0%	-	-100%	800%	-100%	0%	-100%	-100%	600%	300%	-100%	-100%	-100%	-100%	-	-100%	100%	94%
Other species	5	133%	0%	167%	0%	67%	-100%	50%	300%	267%	33%	133%	117%	33%	100%	-33%	33%	150%	0%	-20%	0%	72%

D **Percentage agreement in species identification per species**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Mackerel	1	98%	100%	98%	99%	99%	98%	93%	91%	94%	99%	99%	96%	97%	98%	99%	86%	84%	88%	100%	88%	95%
Horse Mackerel	2	98%	100%	100%	61%	96%	55%	72%	89%	83%	87%	93%	89%	94%	89%	98%	67%	54%	86%	92%	80%	84%
Megrim	3	0%	0%	0%	0%	22%	0%	17%	67%	67%	0%	0%	0%	8%	0%	0%	0%	17%	0%	0%	0%	10%
Hake	4	0%	0%	100%	0%	-	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	-	0%	0%	6%
Other species	5	100%	100%	100%	50%	100%	0%	100%	100%	100%	83%	100%	100%	83%	100%	67%	67%	17%	100%	80%	100%	82%
Weighted mean	1-5	94.0%	95.3%	94.3%	86.6%	96.1%	83.8%	86.3%	89.3%	90.8%	91.4%	93.2%	90.3%	92.6%	92.3%	94.0%	78.7%	74.4%	83.9%	93.8%	83.0%	89.2%
	RANKING	4	2	3	14	1	17	15	13	11	10	7	12	8	9	5	19	20	16	6	18	

Table 4.3-4. Species identification with actual species, second determination

The species compositions based on actual species reflecting the best estimates based on only those eggs that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percentages over- and underestimation (C) and the percentages agreement with modal species or actual species (D)are shown per species by participant and for the whole group that took part in the species identification excercise on fish eggs. A weighted mean percent agreement is given by person and all persons combined.

A **Species compositions using modal/actual species (second last column input table)**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Mackerel	1	146	146	146	145	136	145	145	144	141	129	142	145	144	145	145	146	145	140	142	146	2863
Horse Mackerel	2	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	14	15	16	317
Megrim	3	12	12	12	12	9	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	237
Hake	4	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	18
Other species	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	1-5	175	175	175	174	161	174	174	173	170	158	171	174	173	174	174	175	174	166	170	175	3435

B **Species compositions as estimated per participant and whole group**

	Species	Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	TOTAL
Mackerel	1	151	159	146	160	141	171	154	125	138	145	148	148	150	157	157	145	141	125	159	153	2973
Horse Mackerel	2	16	16	18	9	16	0	7	12	8	11	15	14	15	13	17	27	19	14	11	20	278
Megrim	3	0	0	0	2	2	3	6	20	10	0	0	0	1	0	0	0	3	17	0	1	65
Hake	4	0	0	1	0	-	0	5	0	0	0	0	6	4	0	0	0	0	10	0	1	27
Other species	5	8	0	10	3	2	0	2	16	14	2	8	6	3	4	0	3	11	0	0	0	92
Total	1-5	175	175	175	174	161	174	174	173	170	158	171	174	173	174	174	175	174	166	170	175	3435

C **Percentage overestimation / underestimation**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Mackerel	1	3%	9%	0%	10%	4%	18%	6%	-13%	-2%	12%	4%	2%	4%	8%	8%	-1%	-3%	-11%	12%	5%	4%
Horse Mackerel	2	0%	0%	13%	-44%	0%	-100%	-56%	-25%	-50%	-31%	-6%	-13%	-6%	-19%	6%	69%	19%	0%	-27%	25%	-12%
Megrim	3	-100%	-100%	-100%	-83%	-78%	-75%	-50%	67%	-17%	-100%	-100%	-100%	-92%	-100%	-100%	-100%	-75%	42%	-100%	-92%	-73%
Hake	4	-100%	-100%	0%	-100%	-	-100%	400%	-100%	-100%	-100%	-100%	500%	300%	-100%	-100%	-100%	-100%	-	-100%	0%	50%
Other species	5	7%	-1%	9%	2%	1%	-1%	1%	15%	13%	1%	7%	5%	2%	3%	-1%	2%	10%	-1%	-1%	-1%	91%

D **Percentage agreement in species identification per species**

Modal or actual species		Ger JU Reader 1	Ger MK Reader 2	Ger SK Reader 3	Ger BS Reader 4	Far HD Reader 5	Far SJ Reader 6	Es CF Reader 7	Es CP Reader 8	Es PA Reader 9	Por MMA Reader 10	Por PG Reader 11	Ire BO Reader 12	Sco JD Reader 13	Sco FB Reader 14	Ned HW Reader 15	Ned ST Reader 16	Ned IP Reader 17	Nor JdL Reader 18	Nor EH Reader 19	Eng SM Reader 20	ALL
Mackerel	1	98%	100%	97%	98%	99%	98%	93%	87%	93%	98%	99%	93%	97%	97%	99%	82%	87%	86%	100%	89%	95%
Horse Mackerel	2	100%	100%	100%	56%	100%	0%	13%	69%	50%	69%	88%	75%	81%	75%	100%	19%	25%	57%	73%	38%	64%
Megrim	3	0%	0%	0%	0%	22%	0%	17%	67%	67%	0%	0%	0%	8%	0%	0%	0%	17%	0%	0%	0%	10%
Hake	4	0%	0%	100%	0%	-	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	-	0%	0%	6%
Other species	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	1-5	90.9%	92.6%	90.9%	86.8%	94.4%	81.6%	79.9%	83.2%	86.5%	87.3%	90.6%	84.5%	88.4%	87.9%	92.0%	70.3%	75.9%	77.7%	90.0%	77.7%	85.4%
	RANKING	4	2	4	11	1	15	16	14	12	10	6	13	8	9	3	20	19	18	7	17	

4.4 Result of the fecundity and atresia estimation

4.4.1 Result of the fecundity analysis

Of the 8 samples, four (A09, A81, A85, C49) should be discarded because the images were either too crowded, oocytes were not completely separated or there was overlapping (Table 4.4–1). Initially not every participant followed the protocol and started measurements and counting without first deciding whether the image could be used for fecundity analysis. However, after discussion everyone agreed that these four images should not be used for fecundity analysis. Figure 4.4–1 shows an image that should not be used for fecundity analysis.

The remaining four images were scored by the participants for fecundity analysis. For the images A113, A125 and A97 there was general agreement on the number of vitellogenic oocytes (Table 4.4–1 and 4.4–2). However for the sample A109 agreement was low. This sample contained a lot of small vitellogenic oocytes around 185 um. This image showed the importance of a good calibration of the image analysis system and the use of a good reference circle for the threshold of 185 um. After discussion there was agreement on which oocytes to include as vitellogenic. Figure 4.4–2 shows an image that should be used for fecundity estimation.

Initially it was also thought that the low agreement on sample A109 was because the oocytes were too crowded in the image, however for sample A97, which had similar oocyte size distribution and where more of the sample had to be counted manually, agreement was much higher on the manual and hence the total count. It was decided that no limits could be placed on the number of oocytes per image as this depended on the sample as demonstrated above. It was decided just to emphasize that the 25 ul samples are spread out in the trays/images. If the image is too crowded the count of vitellogenic will be lower because oocytes are hidden under each other. As a result, fecundity will be underestimated. If the sample has lots of (small) oocytes, then it is advisable to use multiple images for the count.

Table 4.4–1. Results of the whole mount analysis: Total number of vitellogenic oocytes counted.

Participant	A09	A109	A113	A125	A81	A85	A97	C49
Alex		318	228	290			340	
Antonio	197	302	222					
AZTI	199	296	235	293	380	278	336	
Bente		313	224	288			330	
CEFAS		290		206			320	
Cindy		308	227	290			331	
Finlay		297	219	286			293	
Ineke		275	210	277		296	302	
Lola		305	223	287				
Merete		313		298			340	
Selene		295		283			318	

Table 4.4–2. Results of the whole mount analysis: Total number of vitellogenic oocytes counted manually.

Participant	A09	A109	A113	A125	A81	A85	A97	C49
Alex		92	78	69			138	
Antonio	73	72	72					
AZTI	75	66	85	72	142	100	134	
Bente		86	74	68			128	
CEFAS		61		68			118	
Cindy		78	77	69			129	
Finlay		69	69	65			91	
Ineke		45	60	56		118	100	
Lola		75	73	66				
Merete		85		78			138	
Selene		66		62			116	

Table 4.4–3. Results of the whole mount analysis: Mean oocyte diameter of vitellogenic oocytes.

Participant	A09	A109	A113	A125	A81	A85	A97	C49
Alex		412	423	425			388	
Antonio	584	507	546					
AZTI	584	507	546	500	283	525	526	
Bente		507	546	501			526	
CEFAS		507		501			527	
Cindy		507	546	500			526	
Finlay		507	546	500			526	
Ineke		507	547	500		525	526	
Lola		507	546	500				
Merete		507		501			526	
Selene		507		500			924	

Table 4.4–4 .Results of the whole mount analysis: Maximum oocyte diameter of vitellogenic oocytes.

Participant	A09	A109	A113	A125	A81	A85	A97	C49
Alex		787	775	771			810	
Antonio	918	787	775					
AZTI	918	787	775	771	733	944	810	
Bente		787	775	771			810	
CEFAS		787		771			810	
Cindy		787	775	771			810	
Finlay		787	775	771			810	
Ineke		787	775	771		944	810	
Lola		787	775	770				
Merete		787		771			810	
Selene		787		771			1025	

Table 4.4–5. Results of the whole mount analysis: Minimum oocyte diameter of vitellogenic oocytes.

Participant	A09	A109	A113	A125	A81	A85	A97	C49
Alex		258	252	251			264	
Antonio	262	258	252					
AZTI	262	258	252	251	250	274	264	
Bente		258	252	251			264	
CEFAS		258		251			264	
Cindy		258	252	251			264	
Finlay		258	252	251			264	
Ineke		257	251	251		274	264	
Lola		257	251	251				
Merete		258		251			264	
Selene		229		251			824	

4.4.2 Result of the mackerel atresia exercise

A comparison of the early alpha atresia counts during the 2006 workshop showed that staining method was not a significant factor in the results (ICES, 2006). During this workshop images with different staining were discussed and all participants agreed that staining method did not influence the atresia estimation. It was agreed that during the 2010 survey every institute will use its preferred staining.

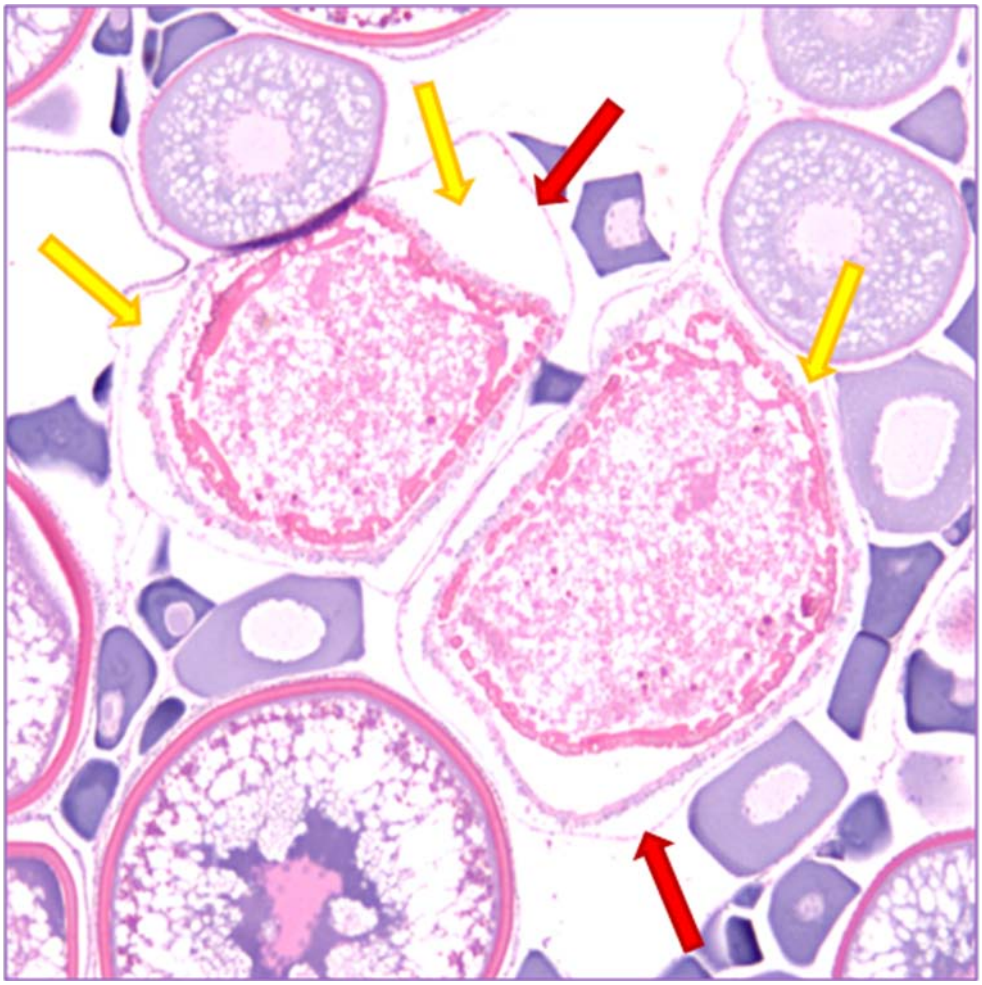
During the calibration exercise all participants assessed atresia on 6 images from one female using the protocol described in the manual (Fonn *et al.*, 2009). Results are shown in Table 4.4–6). In general there was good agreement between the participants. Some participants did not follow the manual and also included in the point counts the oocytes that touched the red line of the superimposed frame. Also there was some discussion on which points of the Weibel grid should be included in the oocytes and in the Grid Negative.

Table 4.4–6. Results of the calibration exercise for the early alpha atresia estimation in mackerel. (Stages, YV = Yolk vesical, YV-YG = Yolk vesical – Yolk Granule, YG = Yolk Granule, YV-p = point count for the Yolk vesical stage etc.)

Image	Stage	Antonio	AZTI	Bente	Cefas	Cindy	ineke	lola	Marlab	Merete	Selene
c57 1.tif	YV										
	YV-YG										
	YG	13	13	13	13	11	12	12	13	13	11
	NegGrid	6	5	5	21	5	8		23	5	5
	YV-p										
	YV-YG-p										
	YG-p	1	1	1	1	1	1	1	1	1	1
c57 2.tif	YV										
	YV-YG				7		6				
	YG	7	9	26	19	24	17	7	30	28	27
	NegGrid						31		35		
	YV-p										
	YV-YG-p				1		1				
	YG-p	1	1	1		1	1	1	2	1	1
c57 3.tif	YV										
	YV-YG										
	YG		9								
	NegGrid						15				
	YV-p										
	YV-YG-p										
	YG-p		1								
c57 4.tif	YV										
	YV-YG										9
	YG	19	28	24	41	41	26	8	42	24	20
	NegGrid				22		7		16		
	YV-p										
	YV-YG-p										16
	YG-p	1	2	1	2	2	3	1	2	1	1
c57 5.tif	YV										
	YV-YG										
	YG	12	13	13	32	13	30	12	29	13	31
	NegGrid				32		20		29		
	YV-p										
	YV-YG-p										
	YG-p	1	1	1	1	1	2	1	1	1	2
c57 6.tif	YV										
	YV-YG				12		9				11
	YG	16	20	20	9	19	9	16	21	20	9
	NegGrid				21		15		21		
	YV-p										
	YV-YG-p				1		1				1
	YG-p	2	2	2	2	2	1	2	2	2	1

After discussion it was agreed that the theca and follicle layers should also be included for point counting. The area delimited by the follicle layer is considered to be occupied by the oocyte. Grid points lying over the follicle layer and those lying inside the area delimited by the follicle layer must be considered as hitting the oocyte. In some atretic oocytes this area can be very large. It is supposed that this wide space between the oocyte and the follicle layer does not exist in the fresh ovary, but appears as an artifact created by fixation and/or tissue processing. Figure 4.4–3 shows the follicle layer and the wide space inside alpha atretic oocytes.

Figure 4.4–1. The follicle layer (thin purple line marked by the red arrows) and the wide space inside (yellow arrows) in two alpha atretic YG oocytes. These areas should be included for point counting.



After discussion it was agreed, following the manual (Fonn *et al*., 2009), that only points outside the ovary tunica wall should be marked as Grid Negative. Cracks inside the ovary, which appear due to fixation or sectioning, should not be included in the Grid Negative counts.

5 Discussion

5.1 Egg staging exercise

The criteria for staging mackerel eggs (Lockwood *et al.*, 1977) and .horse mackerel eggs (Pipe and Walker, 1987) have been used by WGMEGS participants since the instigation of the triennial surveys. Following discussions at previous egg-staging workshops in 2000, 2003 and 2006 (ICES, 2001; 2004; 2007), and further consultations at this workshop, these egg staging criteria have been further enhanced (section 3.2.2). These characteristics are the result of many years of personal experience (from various participants) in staging preserved fish eggs from plankton samples. These characteristics proved invaluable to less experienced participants during this workshop, particularly during the second round of analysis when much greater levels of agreement on egg stages were obtained (section 4.2.1).

A weakness of the analytical method previously used for assessing the results is that the modal stage is not necessarily the true stage. In some difficult cases with a low percentage of agreement the majority of the group could be incorrect in its judgement and only a minority of participants (often the most experienced) could be correct in their assessment of egg stage. This would lead to the modal stage being 'incorrect', and therefore the assessment made by the more experienced readers would appear to be wrong. This problem is difficult to overcome unless eggs of validated stages are available for these exercises.

These results (Tables 4.2–1 to 4.2–6) certainly highlight the need to conduct regular quality assurance workshops and the very valuable benefit, which can be gained by bringing practitioners together to discuss problems and clarify procedures.

5.2 Egg identification exercise

The eggs used for species identification were the same as those used for the egg staging exercise. The exercise proved to be extremely valuable, not least in the production of some egg identification criteria (section 3.3.2) from both published sources and from the experience gained by several participants over many years. The benefits are highlighted by the increase in the mean percentage agreement in the identification of each species (Tables 4.3–1 to 4.3–3). For mackerel agreement increased from 80% in the first round to 86% in the second round. For horse mackerel agreement increased from 72% to 84%. These results are comparable to those obtained at the 2006 workshop where the percentage agreement in species identification after the second round of analysis were 90% for mackerel, 96% for horse mackerel. This is very encouraging, particularly given the number of inexperienced participants at this workshop

The levels of agreement seen in these results (both for stage and species) are probably lower than in the analysis of real survey samples. There were a number of inexperienced participants at this workshop who were identifying and staging fish eggs for the first time. These analysts benefited greatly from participating in the workshop and from the knowledge gained from other, more experienced, participants. They will be able to utilize this knowledge when they begin to process plankton samples collected on the 2010 surveys. The accidental movement of eggs from one well to another, also caused problems. This led to low levels of agreement (both in staging and identification) between participants as they were sometimes analysing different eggs, which had been moved between wells. The eggs also became more and more damaged during the course of each round of analysis as all participants manipulated each egg to look for the salient features. Because of the movement of eggs and the

damage incurred to some eggs it was decided to replace all the eggs prior to the second round. The condition of the eggs in the second round was generally thought to be better. This could have affected the increase in agreement both in species and stage. However it remains unclear if the thought of better condition of the egg could also be due to the higher confidence of the participants after the first round.

Discussion among participants was difficult to prevent whilst the eggs were being analysed. Independent assessment of the eggs is critical to prevent the introduction of bias or incorrect assignment of modal stages/species. All discussions should be reserved for the plenary sessions to enable every participant to comment fully on the features observed.

Some participants entered their own results into the spreadsheets for analysis. Discussion occurred during the data entering and this could have led to changes in staging and identification after the initial decision of species and stage. For the next workshop this should be prevented.

The participants were unfamiliar with the microscopes used for the analysis. This did lead to some problems at the beginning of the analysis where the lighting on some microscopes was not adjusted to its optimum settings. In addition, few of the microscopes were fitted with eye-piece graticules which would have made the speciation of mackerel and horse mackerel eggs easier, as horse mackerel eggs are generally slightly smaller.

6 References

Arkhipov, A.G., and Mamedov, A. A. 2008. Icthyoplankton of the Azores Seamounts. Journal of Ichthyology, Vol 8(3): 259–267.

Armstrong, M.J., Connolly, P., Nash, R.D.M., Pawson, M., Alesworth, E., Coulahan, P.J., Dickey-Collas, Milligan, S.P., O'Neill, M., Witthames, P.R., and Woolner, L. 2001. An application of the annual egg production method to estimate the spawning biomass of cod (*Gadus morhua* L.), plaice (*Pleuronectes platessa* L.) and sole (*Solea solea* L.) in the Irish Sea. ICES Journal of Marine Science, 58: 183 - 203.

Coombs, S.H. 1994. Identification of eggs of hake, *Merluccius merluccius.* J. March biol. Ass.UK (1994), 74, 449–450.

Coombs, S.H., and Mitchell, C.E. 1982. The development rate of eggs and larvae of the hake, *Merluccius merluccius* (L.) and their distribution to the west of the British Isles. J. Cons. Int. Explor. Mer, 40: 119–126.

D'Ancona *et al.* 1956. Fauna e Flora del Golfo di Napoli, Monographia 38: Uova, larve e stadi giovanili di Teleosti, Pubblicata dalla Stazione Zoologica di Napoli, 4 vols.

Ehrenbaum, E. 1905–1909. Eier und Larven von Fischen. *Nordisches Plankton*, 1, 413pp. Lipsius & Tischer, Leipzig.

Eltink, G. 2007. The spray technique: a new method for an efficient separation of fish eggs from plankton. Journal of Plankton Research, 29, 871–880.

Fahay, M. P. 1983. Guide to the early stages of marine fishes occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf, Journal of Northwest Atlantic Fisheries Science, 4, 423pp.

Froese, R., and Pauly, D. (Eds.) 2003. FishBase, WWW Pulication, www.fishbase.org , version 12 October 2003

Fonn, M., Damme, C. van, and Thorsen, A. 2009. A manual for: Sampling at sea mackerel and horse mackerel, Estimation of fecundity and atresia in mackerel and fecundity in horse mackerel. Version 9, 30pp.

Gaetani, D. 1937. Uova, sviluppo embrionale e stadi post-embrionali negli *Sparidi* 5. *Box boops* L. Memoria R. Comitato Talassografico Italiano 241: 1–14.

Holt, E. W. L. 1893. Survey of fishing grounds, west coast of Ireland, 1890–91: on the eggs and larval and post-larval stages of teleosteans, Scientific Transactions of the Royal Dublin Society, Ser. 2, 5, 121pp.

Holt, E. W. L. 1898. Notes on the reproduction of teleostean fishes in the south-western district, J.M.B.A., 5:107–155.

ICES. 2001. Mackerel and Horse Mackerel Egg Staging and Histology Workshop. ICES CM 2001/G:01.

ICES. 2004. Workshop on Mackerel and Horse Mackerel Egg Staging and Identification. ICES CM 2004/G:13.

ICES. 2005. Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys. ICES CM 2005/G:09.

ICES. 2006. Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS). ICES CM 2006/LRC:09.

ICES. 2006. Workshop on Mackerel and Horse Mackerel Egg Staging and Identification. ICES CM 2006/LRC:17.

Johnstone, J., Scott, A., and Chadwick, H. C. 1934. The Marine Plankton, Hodder and Stoughton Limited, London, 194pp.

Kramer, D. 1960. Development of eggs and larvae of the Pacific Mackerel and distribution and abundance of larvae 1952–56. Fish. Bull. US, 60: 393–438.

Lockwood, S.J., Nichols, J.H. and Coombs, S.H. 1977. The development rates of mackerel (*Scomber scombrus* L.) eggs over a range of temperature. ICES CM 1977/J:13, 8pp.

Lockwood, S.J., Nichols, J.H., and Dawson, W.A. 1981. The estimation of a Mackerel (*Scomber scombrus* L.) spawning stock size by plankton survey. J. Plankton Research, No 3 (2): 217–233p.

Marques V., Chaves C., Morais A., Cardador F., Stratoudakis Y. 2005. Distribution and abundance of snipefish (*Macroramphosus* spp.) of Portugal (1998–2003). Sci. Mar. 69:563–576.

Olivar, M-P., and Fortuño, J-M. 1991. Guide to Ichthyoplankton of the Southeast Atlantic (Benquela Current Region). SCI. MAR., 55(1): 1–383.

Padoa, E. 1956. *in* Lo Bianco, S. Uova, larvae e stadi giovanili di Teleoste – Divisione: Carangiformes, Fauna e flora del golfo di Napoli, Stazione Zoologica di Napoli, Monografia 38 and 3ª Puntata, 2ª parte: 548 – 576.

Pipe, R.K., and Walker, P. 1987. The effect of temperature on the development and hatching of scad (*Trachurus trachurus,* L.) eggs. J. Fish Biol., 31: 675–682p.

Porebski, J. 1975. Application of the surface adhesion test to identify the eggs of the hake Merluccius spp. Colln. Scient Papers: Int Commission for South East Atlantic Fisheries 1975. Vol 2 102–106p.

Russell, F.S. 1976. The eggs and planktonic stages of British marine fishes. Academic Press Inc. (London) Ltd., 524 p.

Shaw, M. D. 2003. *Personal communication.*

Spartà, A. 1936. Contributo alla conoscenza di uova, stadi embrionali e post-embrionali in *Macroramphosus scolopax* L. R. Com. Talassog. Ital. Mem. 225: 14 pp.

Annex 1: List of participants

NAME	ADDRESS	PHONE/FAX	EMAIL
Paula Alverez 1 and 2	AZTI FOUNDATION Marine Research Division Herrera kaia portualdea z/g 20110 Pasaia (Gipuzkoa) Basque country. Spain	Phone: +34 943 004 800 Fax: +34 943 004 801	palvarez@pas.azti.es
Linda Berntzen 1	Hjelpemiddelseutralen I Buskerva NAV HMS Norway	00 47 32 218590	Linda.berntzen@gmail.com
Britt Bjoro 1	Hjelpemiddelseutralen I Hordaland NAV HMS Norway	00 47 32 218590	brittbjoro@yahoo.com
Finlay Burns 1 and 2	Marine Services Marine Laboratory PO Box 101 375 Victoria Road Aberdeen AB11 9DB UK	Tel: +44 (0)1224-295416 Fax: +44 (0)1224-295511	burnsf@marlab.ac.uk
Cindy van Damme 1and 2	IMARES Institute for Marine Research and Ecosystem Studies Haringkade 1 PO Box 68 NL-1970 AB IJmuiden The Netherlands	Tel: 31 317 487078 Fax: 31 317 48 73 26	cindy.vandamme@wur.nl
Høgni Debes 1	Havstovan, Nóatún 1 FO-110 Tórshavn, Faroe Islands	Tel: +298 353900 Fax: +298 353901	Hoegnid@frs.fo
Jim Drewery 1	Marine Services Marine Laboratory PO Box 101 375 Victoria Road Aberdeen AB11 9DB UK	Tel: +44 (0)1224-295416 Fax: +44 (0)1224-295511	J.Drewery@marlab.ac.uk
Alex Ederidge 2	Marine Services Marine Laboratory PO Box 101 375 Victoria Road Aberdeen AB11 9DB UK	Tel: +44 (0)1224-295416 Fax: +44 (0)1224-295511	edridgea@marlab.ac.uk
Merete Fonn 2	Institute of Marine Research Nordnesgaten 50 Postboks 1870 Nordnes N-5817 Norway	004755238444	merete.fonn@imr.no

Name	Address	Phone/Fax	Email
Concha Franco 1	Instituto Español de Oceanografía Corazón de María, 8 28002 Madrid Spain	Tel: +34 91 3473736-35 Fax: +34 91 4135597	concha.franco@md.ieo.es
Dolores Garabana 2	Paseo Marítimo Alcalde Francisco Vázquez nº 10 15001 A Coruña (Spain)		dolores.garabana@co.ieo.es
Patricia Gonçalves 1	Instituto Portugues de Investigação Maritima (IPIMAR) Avenida Brasilia 1409-006 Lisbon Portugal	Tel: 00 351 121 302 7190 Fax: 00 351 21 301 5948	patricia@ipimar.pt
Lorraine Greenwood 2	Cefas Lowestoft Laboratory Pakefield Road, Lowestoft Suffolk NR33 0HT	00441502524290	lorraine.greenwood@cefas.co.uk
Eilert Hermansen 1	Institute of Marine Research Nordnesgaten 50 Postboks 1870 Nordnes N-5817 Norway	004755238444	eilert.hermansen@imr.no
Selene Hoey 2	Fisheries Science Services Marine Institute Rinville Oranmore Galway Ireland	0035391730402	selene.hoey@marine.ie
Sólvá Jacobsen 1	Havstovan, Nóatún 1 FO-110 Tórshavn, Faroe Islands	Tel: +298 353900 Fax: +298 353901	solvaj@hav.fosolvaj@hav.fo
Matthias Kloppmann 1	vTi Institut für Seefischerei Palmaille 9 22757 Hamburg Germany	Tel +49 (0)40 38905 217 Fax +49 (0)40 38905 263	matthias.kloppmann@vti.bund.de
Sakis Kroupis 1	vTi Institut für Seefischerei Palmaille 9 22757 Hamburg Germany	Tel +49 (0)40 38905 217 Fax +49 (0)40 38905 263	sakis.kroupis@ vti.bund.de
Jan de Lange 1	Institute of Marine Research Nordnesgaten 50 Postboks 1870 Nordnes N-5817 Norway	004755238444	jan.de.lange@imr.no

NAME	ADDRESS	PHONE/FAX	EMAIL
Maria Manuel Angelico 1	Instituto Portugues de Investigação Maritima (IPIMAR) Avenida Brasilia 1409-006 Lisbon Portugal	Tel: 00 351 121 302 7190 Fax: 00 351 21 301 5948	angelico@ipimar.pt
Stephen Milligan 1	Cefas Lowestoft Laboratory Lowestoft Suffolk NR33 0HT UK	Tel+44 1502 562244 Fax +44 1502 513865	steve.milligan@cefas.co.uk
Antonio Solla 2	IEO, Subida A Radio Faro 50-52, 36390 Vigo, Spain	Tel +34986462926 Fax +34986498626	antonio.solla@vi.ieo.es
Bent Njøs Strand 2	Institute of Marine Research Nordnesgaten 50 Postboks 1870 Nord-nes N-5817 Norway	004755238444	bente.njoes.strand@imr.no
Anders Thorsen 1 and 2	Institute of Marine Research Nordnesgaten 50 Postboks 1870 Nord-nes N-5817 Norway	004755238444	anders.thorsen@imr.no
Brendan O'Hea 1	Fisheries Science Services Marine Institute Rinville Oranmore Galway Ireland	Tel. +353 91 387200 Fax. +353 91 387201	brendan.ohea@marine.ie
Ineke Pennock 1 and 2	IMARES Institute for Marine Research and Ecosys-tem Studies Haringkade 1 PO Box 68 NL-1970 AB IJmuiden The Netherlands	Tel: 31 317 487078 Fax: 31 317 48 73 26	ineke.pennock@wur.nl
Carlota Pérez 1 and 2	AZTI FOUNDATION Marine Research Divi-sion Herrera kaia portual-dea z/g 20110 Pasaia (Gipuz-koa) Basque country. Spain	Phone: +34 943 004 800 Fax: +34 943 004 801	
Samuel Roslyn 2	Cefas Lowestoft Labo-ratory Pakefield Road, Lowestoft Suffolk NR33 0HT		Samuel.Roslyn@cefas.co.uk

NAME	ADDRESS	PHONE/FAX	EMAIL
Birgit Suer 1	vTi Institut für Seefischerei Palmaille 9 22757 Hamburg Germany	Tel +49 (0)40 38905 217 Fax +49 (0)40 38905 263	birgit.suer@vti.bund.de
Silja Tribuhl 1	IMARES Institute for Marine Research and Ecosystem Studies Haringkade 1 PO Box 68 NL-1970 AB IJmuiden The Netherlands	Tel: 31 317 487078 Fax: 31 317 48 73 26	silja.tribuhl@wur.nl
Jens Ulleweit 1	vTi Institut für Seefischerei Palmaille 9 22757 Hamburg Germany	Tel +49 (0)40 38905 217 Fax +49 (0)40 38905 263	jens.ulleweit@vti.bund.de
Hanz Wiegerinck 1	IMARES Institute for Marine Research and Ecosystem Studies Haringkade 1 PO Box 68 NL-1970 AB IJmuiden The Netherlands	Tel: 31 317 487078 Fax: 31 317 48 73 26	hanz.wiegerinck@wur.nl
Luisa Iglesias 2	IEO, Subida A Radio Faro 50-52, 36390 Vigo, Spain		
Denis 2	AZTI FOUNDATION Marine Research Division Herrera kaia portualdea z/g 20110 Pasaia (Gipuzkoa) Basque country. Spain	Phone: +34 943 004 800 Fax: +34 943 004 801	

[1] **Participant at the egg workshop**

[2] **Participant at the fecundity workshop**

Annex 2: Agenda

2.1 Agenda for the first workshop on mackerel and horse mackerel egg staging and identification

Monday 5 October

- Start meeting at 10.00.
- Introduction
- Presentations Matthias, Cindy
- Lunch 12.30–13.30
- Afternoon: ImageJ and ObjectJ by Norbert Vischer (UVA) and working with ImageJ

Tuesday 6 October

- 1st round of egg identification and staging
- Spray method
- Pipette sampling for fecundity and atresia
- Write report
- Lunch 12.30–13.30

Wednesday 7 October

- Finish 1st round of egg identification and staging
- Discussing results of 1st round
- Spray method
- Write report
- Lunch 12.30–13.30

Thursday 8 October

- 2nd round of egg identification and staging
- Spray method
- Write report
- Lunch 12.00–13.00

Friday 9 October

- Discuss results
- Write report
- Lunch 12–30–13.30

2.2 Agenda for the second workshop on mackerel and horse mackerel fecundity and atresia estimation

Things that need to be discussed:

- Whole mount calibration and standardization of whole mount pictures: for this everyone should bring (and put a copy on the WKMHMES sharepoint Data/fecundity/whole mount pictures 2007 survey) of some of their whole mount pictures used during the 2007 survey.
- Histology calibration: discussion of the different structures and use of different stainings. Everyone should stain the slides Merete prepared and sent round with the staining they will be using and bring (and put a copy on the share point Data/fecundity/histology pictures of) the pictures of the stained slides to the workshop.
- Decision on which histology method to be used: Merete to give a presentation on the developments of the histology method. Dolores to give a presentation on comparison between paraffin and resin.
- Developments in ImageJ / ObjectJ: Anders to give a presentation.
- Pipette sampling fish: Paula to arrange fresh fish and Cindy to take pipettes and tubes to the workshop
- Update manuals for fecundity and atresia sampling
- Update sampling scheme for adult sampling
- Suggested name change to include fecundity: Workshop on Mackerel and Horse Mackerel Egg and Fecundity Determination, WKMHMEFD.

Tuesday 1 December

- Welcome and introduction
- Presentation: Anders development ImageJ / ObjectJ for whole mount analysis
- Whole mount analysis
- Pipette sampling

Wednesday 2 December

- Presentation: Merete histology methods, Dolores use of paraffin vs. resin for histological sectioning, Anders development ImageJ / ObjectJ for histological analysis
- Histology calibration
- Which histology method to use

Thursday 3 December

- Suggested name change
- Update manual
- Update sampling scheme
- Report writing
- Pipette sampling

Friday 4 December

- Recommendations
- Update manual
- Update sampling scheme
- Report writing

Annex 3: WKMHMES terms of reference for the next meeting

The **Workshop on Mackerel and Horse mackerel Egg staging and Identification (**WKMHMES) chaired by Cindy van Damme*, The Netherlands, will be renamed **Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel** (WKFATHOM) and will meet twice in autumn 2012 to:

a) Carry out comparative plankton sorting trials on typical survey samples. This should follow the pattern of trial – analysis – retrial – identification of problem areas;

b) Carry out a comparative egg staging trial for mackerel and horse mackerel eggs following the pattern used in the 2009 egg staging workshop;

c) Update a set of standard pictures and descriptions for species identification and egg staging;

d) Provide a review of any available documentation on identifying eggs to species and define standard protocols;

e) Carry out inter-calibration work on fecundity determination and harmonize the analysis and interpretation of fecundity samples;

WKFATHOM will report by January 2013 (via SSGEST) for the attention of SCICOM, WGISUR, WGMEGS and WGWIDE.

Supporting Information

Priority:	Information quality, used to provide fishery advice through WGMHSA, will be impaired if this workshop is not conducted.

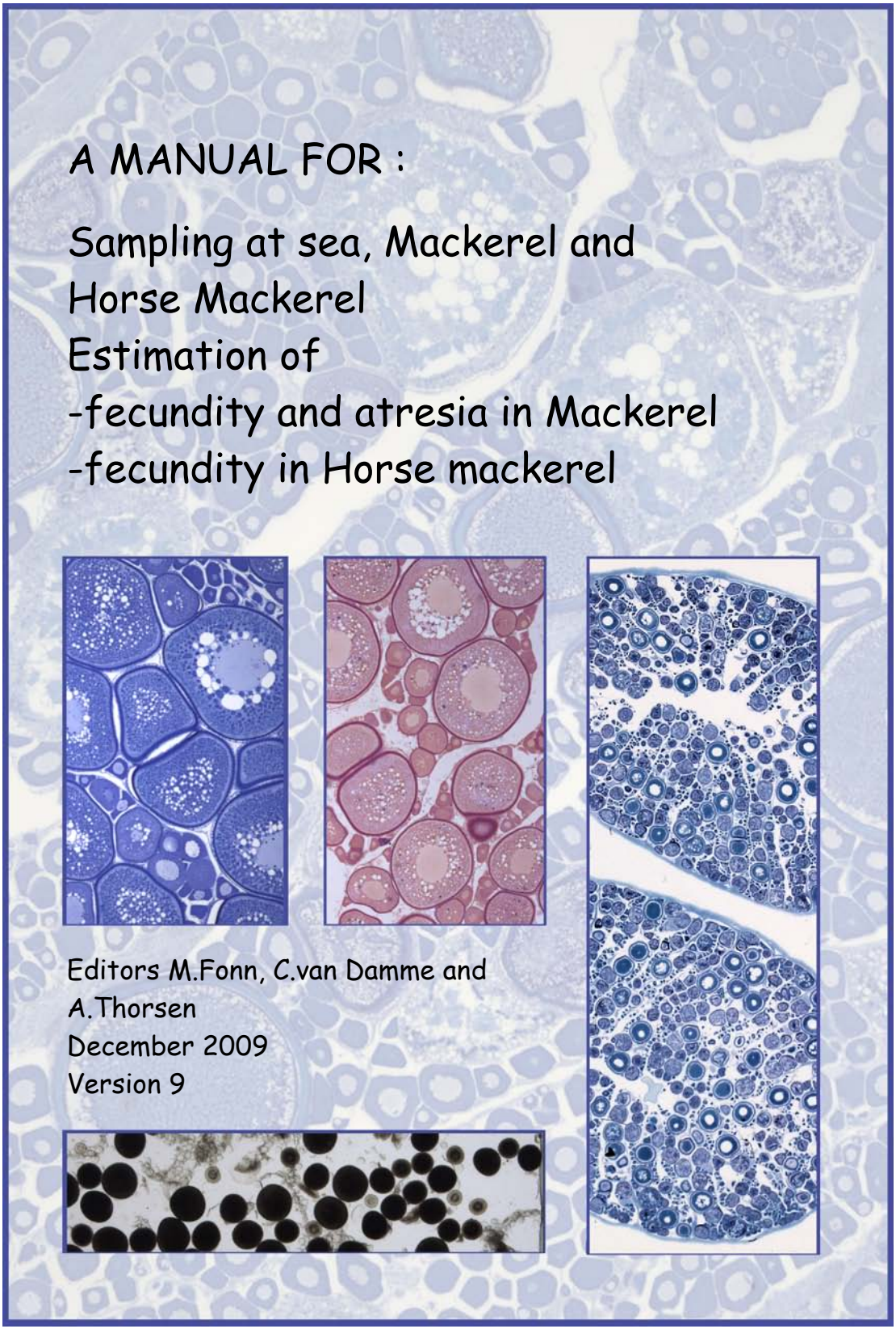
Scientific justification	Sorting eggs from plankton samples, Identification of eggs to species and the staging of those eggs remains one of the key areas in the execution of the mackerel and horse mackerel egg surveys. As this process is carried out by a number of different operators in many different countries, then the data combined, it is vital that the process be standardized. WGMHSA and WGMEGS strongly feel that this is best done through the mechanism of sample exchange programmes and regular workshops to compare results. In the context of the triennial egg surveys it would seem appropriate to hold a workshop prior to every survey to standardize approaches and methodologies in the run-up to the surveys. This will have the advantage of training new operators as well as harmonizing the approach of experienced operators. Egg staging workshops were held in 2000, 2003and 2006 and were very successful in achieving these aims. It is proposed that these be used as a model for the proposed workshop in 2009. It is expected that the workshop will use the proven method of carrying out a set of sorting trials, analysing the results and identifying problems, then repeating the trials on the basis of the new understanding. The workshop will also be tasked to update a standard manual of descriptions and photographs to assist in the plankton sample handling procedure. This material was assembled into an agreed standard manual at previous workshops. In the context of these surveys, fecundity estimation is very important for conversion of egg production to biomass. Fecundity estimation is carried out using histological methods and the analysis and interpretation of this material also requires standardization across participating institutes. Standardization of this aspect of the work will be included in the workshop. Goal 1. Understand the physical, chemical, and biological functioning of marine ecosystems Modernise technologies and sampling designs for collecting, measuring, and enumerating marine organisms, and improve the precision and accuracy of resource surveys. Goal 4. Advise on the sustainable use of living marine resources and protection of the marine environment Develop quality assurance protocols to enhance confidence in scientific advice.
Resource requirements	None
Participants	Mainly scientists (approximately 20) involved in the surveys.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to advisory committees	ACOM
Linkages to other committees or groups	WGMEGS and WGWIDE.
Linkages to other organizations:	None.

Annex 4: Recommendations

RECOMMENDATION	FOR FOLLOW UP BY:
1. WKMHMES recommends to change the name to WKFATHOM Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel to also recognize the fecundity and atresia work that is undertaken in this workshop.	SCICOM
2. It is recommended that all participants carry out artificial fertilizations of any species, which have eggs similar to those of mackerel and horse mackerel. It would be useful if egg and oil globule diameters are measured and that photographs are taken of as many stages as possible. It would also be beneficial if the eggs were preserved at various stages of development and any morphological changes noted following fixation. These eggs should be made available for analysis during the next workshop (scheduled for 2009).	All participants to consider providing eggs for analysis at the next workshop.
3. The group reiterates the need to continue with the egg identification/staging and fecundity workshop prior to the egg surveys as they are essential to quality assurance of the mackerel and horse mackerel egg surveys. It is almost impossible to organize and run workshops such as this without some financial assistance. Without access to central financial resources, each participant is wholly reliant on funding from their own institute for travel and subsistence. Therefore, WGMHMES recommends PGCCDBS and STECF/SGRN to consider the including of the workshop into the list of eligible meetings within the Data Collection Framework.	PGCCDBS/EU STECF-SGRN/EU RCM-NA
4. It is recommended that all microscopes at the next workshop are fitted with eyepiece graticules. These graticules should be calibrated to the same standard i.e. that one eyepiece unit (epu) should be equivalent to the same number of millimetres, regardless of microscope used.	Chair to consider before next workshop in 2012.
5. The Spray technique should be included as a method for sorting eggs from the rest of the plankton during the 2010 triennial surveys. Following the use of the 'Spray Technique' to remove the eggs, each sample should subsequently be resorted by hand to remove any remaining eggs.	All participants.
6. All participants are reminded that the procedures described in the WGMEGS survey manual should be followed during the 2010 surveys. Particularly that 4% formaldehyde, buffered with sodium acetate tri-hydrate, is the standard survey fixative and that plankton samples should never come into contact with formaldehyde of a concentration greater than 4%. All participants are encouraged to check the pH of their fixative on a regular basis.	All participants.

7. Based on the experiences at the workshop a recommended binocular microscope should have the following features: Options for a black or white stage plate for use with incident (top) light. A transparent stage plate for transmitted (bottom) light. Dark field illumination for contrast. Adjustable brightness. Magnification with click stops. Magnification should be at least 1.6x. A choice of 10x and 20x eyepieces. Adjustable binocular head and ergonomic design to allow flexibility of movement. Adjustable focus on all eyepieces. Calibrated eyepiece graticules. Double (fibre optic) cold light source, with adjustable focus, to avoid shadows. Mechanical stages to position samples easily in the field of view and to hold the samples firmly.	Chair to consider before next workshop in 2012.
8. All participants should try to collect reference eggs from different species during the 2010 egg survey and keep them for the next workshop in 2012.	All Participants.
9. WGMEGS should consider whether stage 1A and 1B could be amalgamated into a single stage both for the survey samples and future workshops. These stages are combined for the TAEP estimate. Not all participants separate these two stages.	WGMEGS.
10. All analysts who are engaged in the analysis of fecundity and atresia of mackerel and horse mackerel samples must complete the intercalibration exercise before starting the analysis of the 2010 Triennial survey samples.	Members of WGMEGS participating in the 2010 Triennial survey.
11. It is recommended that more data are collected for the comparison of the standard method and the alternative method for atresia estimation. All participants of the 2010 survey should collect an extra sample of the mackerels and send these to IMR.	All participants to collect mackerel samples and IMR to carry out the testing.
12. It is recommended that more calibrations are carried out to test the difference in shrinkage of the oocytes between paraffin and resin.	IEO to carry out the comparison.

Annex 5: Manual for sampling adult mackerel and horse mackerel and to estimate fecundity and atresia



Version 9, December 2009

Ftp-server for data exchange: ftp://ftp.imr.no/

Contents **Page**

Changes in fecundity and atresia estimation methods …….. 3

Walsh scale …… 5

Mackerel

Diagram for Mackerel procedures …… 6

Procedure 1

Mackerel sampling procedure at sea 2007……. 7

Procedure 2

Fecundity whole mount analysis procedure for Mackerel ……. 10

2.1 Spawning markers…….. 10

2.2 Potential fecundity…….. 10

2.3 Relative potential fecundity…… 10

Procedure 3

Atresia analysis for Mackerel ……. 11

3.1 Embedding, sectioning and staining……... 11

3.2 Atresia analysis……... 15

3.3 Calculation of atresia…….. 26

3.4 Calculation of mean atretic loss…….. 26

Horse mackerel

Procedure 4

Horse mackerel sampling procedure at sea …….. 27

Procedure 5

Fecundity whole mount analysis procedure for Horse mackerel 30

5.1 Spawning markers…….. 30

5.2 Potential fecundity…….. 30

5.3 Relative potential fecundity…… 30

Changes in fecundity and atresia estimation methods for Mackerel and Horse mackerel since 2001 (Version 1 of the manual Witthames, 2001).

2001	2007	2010
Mackerel		
On-board ovaries were collected whole and fixed in Gilson's fluid (for potential fecundity) and formaldehyde solution (for assessing spawning status and atresia)	On-board ovaries are weighed and pipette subsamples of known volume and weight taken and fixed in formaldehyde solution	
Potential fecundity Count follicles > 130 μm after Gilson digestion	Gravimetric fecundity estimation Sub samples preserved in 3.6% buffered formaldehyde. F = O * C * S (F = fecundity, O = Ovary weight, C = count follicles > 185 μm in subsample, S = subsample weight) (Hunter *et al.*, 1989)	
Atresia Stereometric method	Stereometric method	
PAS stained sections	H&E -PAS – Toluidine blue	
Mackerel and Horse mackerel		
	Fecundity samples: In 2007 count all oocytes >185 um and measure 1/3 of the oocytes.	Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis. Count all the oocytes >185μm in the sample that are not automatically detected.
		ImageJ and macros will be made available during the wk to all participants and they should use this for analysis of the samples.
		Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.
		For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.
	Spawning markers: hydrated, >5 POF's	Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample
Horse mackerel		

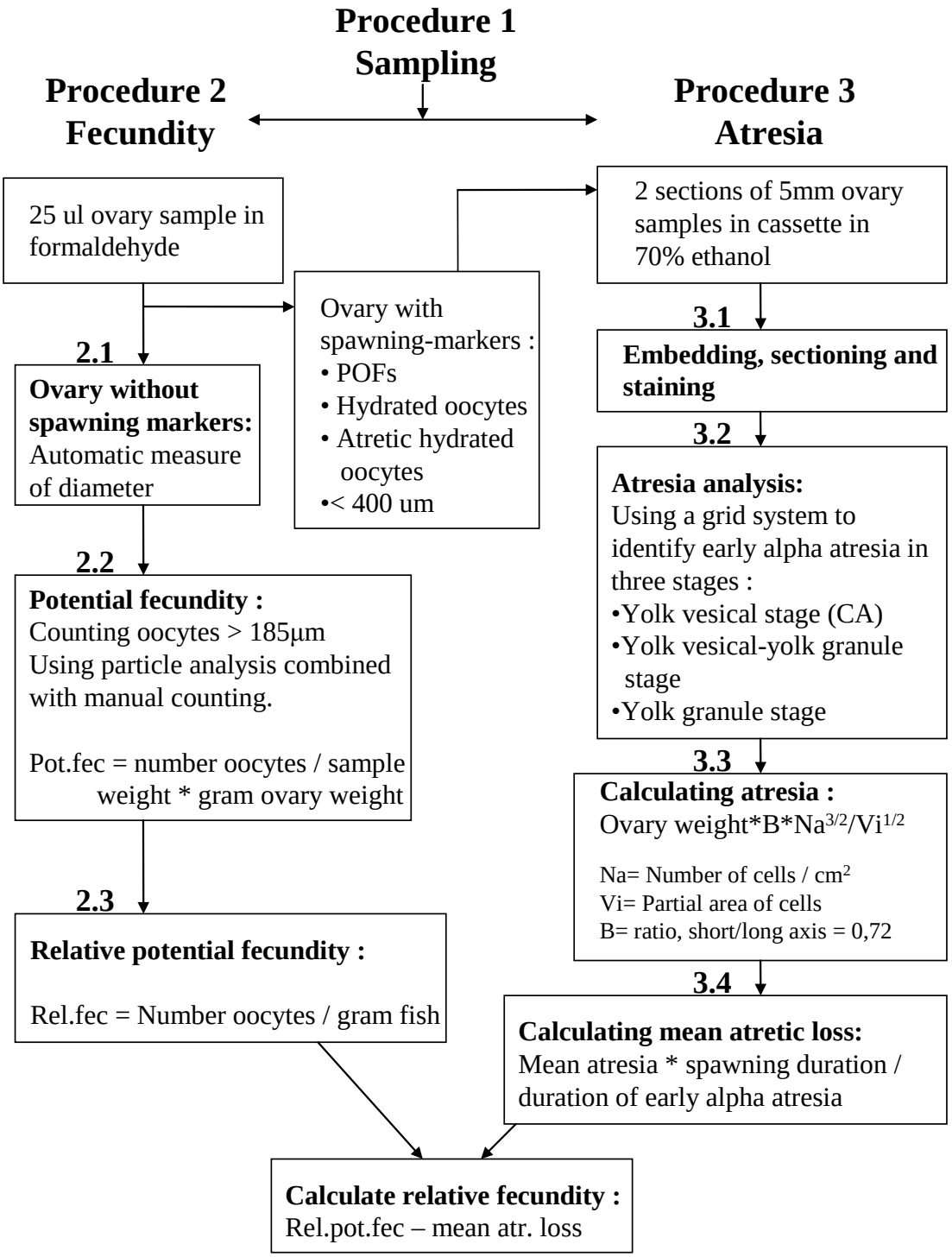
2001	2007	2010
Potential fecundity Stereometric method	Gravimetric fecundity estimation Sub samples preserved in 3.6% buffered formaldehyde. F = O * C * S (F = fecundity, O = Ovary weight, C = count follicles > 185 μm in subsample, S = subsample weight) (Hunter *et al*., 1989)	
	On-board ovaries are weighed and pipette subsamples of known volume and weight taken and fixed in formaldehyde solution	
		IPIMAR will perform a DEPM survey for horse mackerel. Batch fecundity: Gravimetric method. Take whole fixed ovary to the lab, take 3 subsamples, weigh and count all the hydrated oocytes in subsample. Spawning fraction: migratory nucleus, hydrated, POF's

Standard and Walsh mature scale for mackerel and horse mackerel maturity staging.

Standard*	Walsh	Mature/ Immature	State	Female	Male
1	1	Immature	Immature	Gonads small. Ovaries wine red and clear, torpedo shaped.	Gonads small. Males pale, flattened and transparent.
2	2	Mature	Maturing	Gonads occupying 1/4 to 3/4 body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish colouration, largest eggs without oil globule.	Gonads occupying 1/4 to 3/4 body cavity. Testes off-white, milt not running.
	3	Mature	Maturing	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange. Largest eggs may have oil globules.	Gonads occupying 3/4 to almost filing body cavity. Testes creamy white.
3	4	Mature	Spawning	Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Ovary size variable from full to 1/4.	Testes filling body cavity, milt freely running.
	5	Mature	Spawning	Gonads occupying 3/4 to < 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot.	Gonads occupying 3/4 to < 1/4 body cavity. Testes with free running milt and shrivelled at anus end.
4	6	Mature	Spent/ Recovery	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs.	Gonads occupying 1/4 or less of body cavity. Testes opaque with brownish tint and no trace of milt.

* **Standard scale as proposed by the WKMSMAC 2007.**

MACKEREL


Procedure 1
Sampling
Procedure 2
Fecundity
Procedure 3
Atresia
25 ul ovary sample in formaldehyde
2 sections of 5mm ovary samples in cassette in 70% ethanol
Ovary with spawning-markers :
• POFs
• Hydrated oocytes
• Atretic hydrated oocytes
•< 400 um
2.1
Ovary without spawning markers: Automatic measure of diameter
3.1
Embedding, sectioning and staining
3.2
Atresia analysis:
Using a grid system to identify early alpha atresia in three stages :
•Yolk vesical stage (CA)
•Yolk vesical-yolk granule stage
•Yolk granule stage
2.2
Potential fecundity :
Counting oocytes > 185µm
Using particle analysis combined with manual counting.
Pot.fec = number oocytes / sample weight * gram ovary weight
3.3
Calculating atresia :
Ovary weight*B*Na3/2/Vi1/2
Na= Number of cells / cm2
Vi= Partial area of cells
B= ratio, short/long axis = 0,72
2.3
Relative potential fecundity :
Rel.fec = Number oocytes / gram fish
3.4
Calculating mean atretic loss:
Mean atresia * spawning duration / duration of early alpha atresia
Calculate relative fecundity :
Rel.pot.fec – mean atr. loss

Procedure 1

Mackerel sampling procedure at sea

Before the cruise:

Procure 25–50 µl capillary pipettes (Table 3.3.1) Test performance of the pipette by practice, taking 25 µl water samples.

IMR and IMARES will send around labels to all the institutes participating in the survey. Fill the labelled 2.5 ml Nunc tubes (with screw on lids) with 1.2 ml of 3.6% buffered (sodium phosphate) formaldehyde (see excel-file on the ftp-sever: Buffered formaldehyde).

During the cruise:

Measure the weight of the whole catch and randomly select a subsample of 100 fish and measure the total weight of the subsample where suitable.

Measure total length, weight, maturity (Walsh scale) and sex of each fish in the subsample.

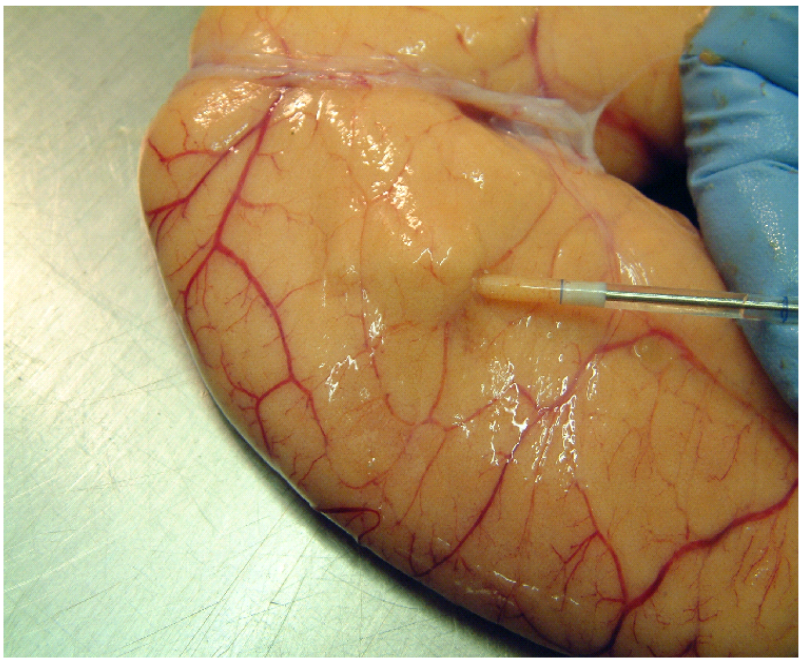
Select females in maturity stages 3–6 from the subsample of 100 (if less than 100 fish are in the catch, sample all the mackerel) for fecundity and atresia analysis. If possible divide the total quota of females equally into the 4 weight categories: < 250g, 251–400g, 401–550g and >550g. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes.

Measurements:

- Total length
- Total weight
- Maturity
- Otoliths
- Weight of ovary (If it is not possible to take these weights at sea, take the pipette and atresia samples and fix the remainder of the ovary and weigh the ovary in the lab. The fixed and frozen weights should be corrected to fresh weights.)

Fecundity sampling:

- From one lobe of the ovary take 3 samples of each 25µl with a pipette and immediately put each sample in individual coded Nunc tubes. Ensure all oocytes are immersed in 3.6% buffered formaldehyde solution. Rinse the pipette with seawater and dry it with a paper towel prior to sampling another fish.



Method to use a capillary pipette to remove an ovary sample.

Atresia sampling:

- For atresia: Place the other lobe of the ovary in a labelled bottle (100–250 ml with wide opening) filled with 3.6% buffered (sodium phosphate) formaldehyde. From the lobe where the pipette samples are taken, also take with a small teaspoon a 2 to 3 grammes sample and put this in a 3.6% buffered formaldehyde filled vial.
- Make sure that all the ovary samples are covered with formaldehyde

After the cruise:

All the ovary samples should remain fixed in formaldehyde for at least two weeks before sections are taken and put in ethanol. From the fixed ovary lobe, cut two 5mm thick slices and put them in a coded cassette. Write the code with a pencil on the outside the cassette. If the ovary is very big you may have to use 2 cassettes. Separate the cassettes into 4 colour coded leak proof bottles filled with 70% ethanol. Pack the consignments for each country with a maximum volume of 1000 ml solution in each package. On the outer cover of the package indicate the volume of fixative and that it is within the limits for unclassified transport. Send the cassettes and nunc samples for analysis to the different institutes referring to table 2.

Send the extra samples to IMR in 70% ethanol.

Table 2.

Colour code	Country	Institute and address	Responsible person	Labcode for ImageJ
Blue	Norway	IMR, Nordnesgaten 50, 5005 Bergen, Norway	Merete Fonn	IMR
Red	Ireland	MI, Rinville, Oranmore, Co.Galway, Ireland	Selene Hoey	MII
Yellow	Scotland	Marine Scotland Science, Marine Laboratory, Victoria Road, Torry, Aberdeen, AB9 8DB, Scotland	Alex Edridge	MSS
White- Even numbers	Spain	IEO, Subida A Radio Faro 50-52, 36390 Vigo, Spain	Antonio Solla	IEO
White- Uneven numbers	Spain	AZTI, Foundation Herrera Kaia, Portualde z/ g20110 Pasaia, Basque Country, Spain	Paula Alvarez / Maria Korta	AZT

Procedure 2

Fecundity whole mount analysis in the lab procedure for mackerel

2.1 Spawning markers and atretic oocytes

Transfer the unstained sample to a tray and try to separate the oocytes.

Under the microscope check for spawning markers, if there are hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample, it should not be analysed for fecundity. For mackerel the samples with spawning markers should be analysed for atresia, but excluding the samples with oocytes diameter <400 um.

2.2 Potential fecundity

Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.

Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis.

Count all the oocytes >185μm in the sample that are not automatically detected.

Save the pictures using the standard code: e.g. J000_A_IMR, build up as: Samplecode_number for the pictures_institute initials (three letters, see Table 2).

Potential fecundity:

Pot.fec. = number of oocytes / weight of the pipette sample (0.026 g) * fresh ovary weight

2.3 Relative potential fecundity

Relative potential fecundity:

Rel.pot.fec. = Pot. fec. / total fish weight

Procedure 3

Atresia analysis in the lab for mackerel

3.1 Embedding, sectioning and staining

Preparing resin blocks

Use the two 5 mm sections in the cassettes, following these steps:

Procedure used by IMR, CEFAS and IMARES

Step	Infiltration solution	Duration	Process temperature
1	90% ethanol	2 hours	Room temperature
2	Pour out the liquid and add fresh 90% ethanol	1 hour	Room temperature
3	Pour out the liquid and add fresh 96% ethanol	1 hour	Room temperature
4	96% ethanol + Technovit 7100 (1:1 ratio) prepared by diluting Technovit 7100 (from used in steps 4).	overnight	Store cool (+5°C) after the orbital shaker
5	Replace the liquid with Technovit 7100 (from step 5).	3 days	Store cool (+5°C) after the orbital shaker
6	Replace the liquid with freshly prepared Technovit 7100.	2 days	Store cool (+5°C) after the orbital shaker
7	Transfer the sections from the cassettes to the moulds.		Cooling plate (-5°C)
8	Polymerise by adding Technovit 7100: hardener (15:1) at cooling plate (-5°C).	6 hours	Cooling plate (-5°C)
9	Leave overnight	overnight	Store cool (+5°C)
10	Block up using Technovit universal.	15 minutes	Room temperature
11	Store the blocks in a box containing 70% glycerol.		

Procedure used by IEO

Step	Infiltration solution	Duration	Temperature
1	70% ethanol 70%	1 day	Room temperature
2	90% ethanol 90%	1 day	Room temperature
3	96% ethanol 96%	1 day	Room temperature
4	96% ethanol 96% + activated resin (technovit 7100) (1:1)	2 days	Store cool (+5°C) with several slight shakes
5	100% activated resin (technovit 7100)	2,5 days	Store cool (+5°C) with several slight shakes

Procedure used by AZTI

STEP	INFILTRATION SOLUTION	DURATION	PROCESS TEMPERATURE
1	70% ethanol	32 hours	Room temperature
2	90% ethanol	16 hours	Room temperature
3	96% ethanol	8 hours	Room temperature
4	96% ethanol + Resin activated (1:1 ratio)	2 days	Store cool (+5°C)
5	Resin activated	2–3 days	Store cool (+5°C)
6	Transfer the tissue from the cassettes to the moulds.		Store cool (+5°C) after the orbital shaker
7	Cover the tissue with resin activated and hardener (15:1) and put the block	1 day	Room temperature

Disposal of waste resin (in the fume cupboard)

After step 3 the 1:1 resin mix should be put in an aluminium tray and left in the fume cupboard over a few days to allow the EMS to evaporate from the resin. Use about 1 g hardener to 100g resin to polymerise and wrap the block in a poly bag for disposal. Caution the reaction is exothermic and potentially hazardous if too much hardener is added.

Sectioning the blocks

Use a microtome to cut 5 µm sections and dry at 100°C.

Staining the sections

Recipe 2% Toluidine blue

2% Toluidine blue and 1% Sodium tetraborat (Borax). The borax is dissolved in the distilled water then the dye added under constant stirring. Filter the solution before use.

For individual slides: Cover the section with a few drops of 2% Toluidine blue and pour the excess back in the bottle and rinse the section with hot (60°C) tap water for 20 seconds. Dry on a 60°C hot plate. Cover the section with a cover slip using two drops of mountex.

Hematoxylin and Eosin (H&E) as used by IEO

STEP	REAGENT	TIME (MIN:SEC)	EXACT
1	Tap water	2:00	No
2	Harris hematoxylin	10:00	Yes
3	Tap water	2:00	No
4	Alcohol acid	0:05	Yes
5	Tap water	1:00	No
6	Lithium carbonate	1:30	Yes
7	Tap water	1:00	No
8	70% Ethanol	1:00	No
9	Eosin-Floxin b	3:30	Yes
10	96% Ethanol	2:00	No
11	100% Ethanol	2:00	No
12	OTTIXCLEAR*	5:00	No
13	OTTIXCLEAR*	3:00	No
Exit	OTTIXCLEAR*		

Hematoxylin and Eosin (H&E) as used by AZTI

Cover the sections following the protocol:

- 5 minutes in Hematoxiline
- 5 minutes in running tap water.
- 5 minutes in 1% eosine (1 gr/100 ml)
- Clean the rest of eosine with running water.

Schiff-Mallory Trichrome

STEP	REAGENT	TIME (MIN:SEC)	EXACT
1	5% Periodic acid	4:30	Yes
2	Distilled water	00:10	No
3	Schiffs	60:00	Yes
4	Tap water	10:00	No
5	1%Acid Fuchsin	1:00	Yes
6	Distilled water	00:30	Yes
7	Distilled water	00:30	Yes
8	1% Phospho Molybdic acid	1:00	Yes
9	Distilled water	00:10	Yes
10	Mallory Trichrome	00:15	Yes
11	Distilled water	00:10	Yes
12	90% Ethanol	00:05	Yes
13	100% Ethanol	00:05	Yes
14	100% Ethanol	00:05	Yes
15	1:1 100% Ethanol – OTTIXCLEAR*	00:05	Yes
16	OTTIXCLEAR*	00:05	Yes
17	OTTIXCLEAR*	00:05	Yes
Exit	Exit	.	

3.2 Atresia analysis

Classification of atretic oocytes is based mainly on the breakdown of the two chorion layers, but other changes also occur. Subdivision of the alpha stage into early alpha and late alpha atresia is based on the size of breaks and position of the thicker chorion layer. If any nick or breakdown in the thicker inner chorion layer is observed and if the breaks are smaller than twice the width of the chorion thickness, the oocyte is classed as early alpha atretic. If the thicker inner chorion layer has breaks more than twice its width and the fragments are displaced inwards from the outer follicle boundary the ooctye is classed as late alpha. After the chorion has disappeared the breakdown progresses from the alpha into the beta stage and the oocyte is now much reduced in size, highly vacuolated and with no yolk contents visible.

For mackerel we score only the early alpha atretic stage.

The oocytes are divided into 3 different stages:

YV (yolk vesical stage): arises from the smallest vitellogenic oocytes making up the potential fecundity ranging in size from 175 (appearance of corticale alveolie) to 325μm when a complete ring of vacuoles extends throughout the oocyte cytoplasm.

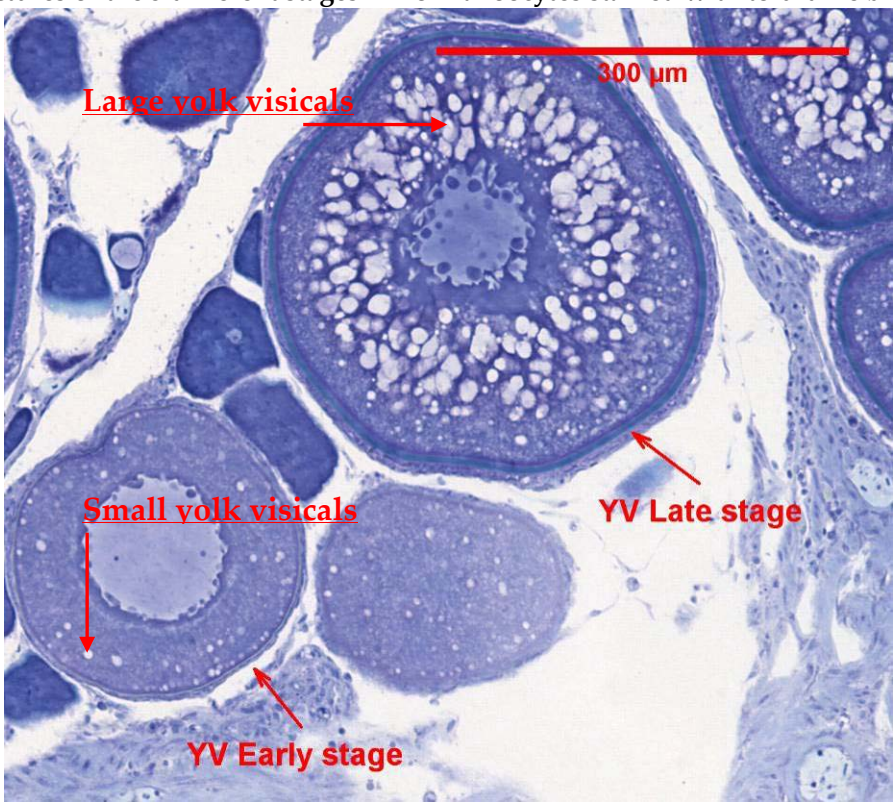
YV-YG (yolk vesical to yolk granule stage): the oocytes range in size from 325 to 525μm and contain yolk granules that slowly enlarge and start to fill the cytoplasm.

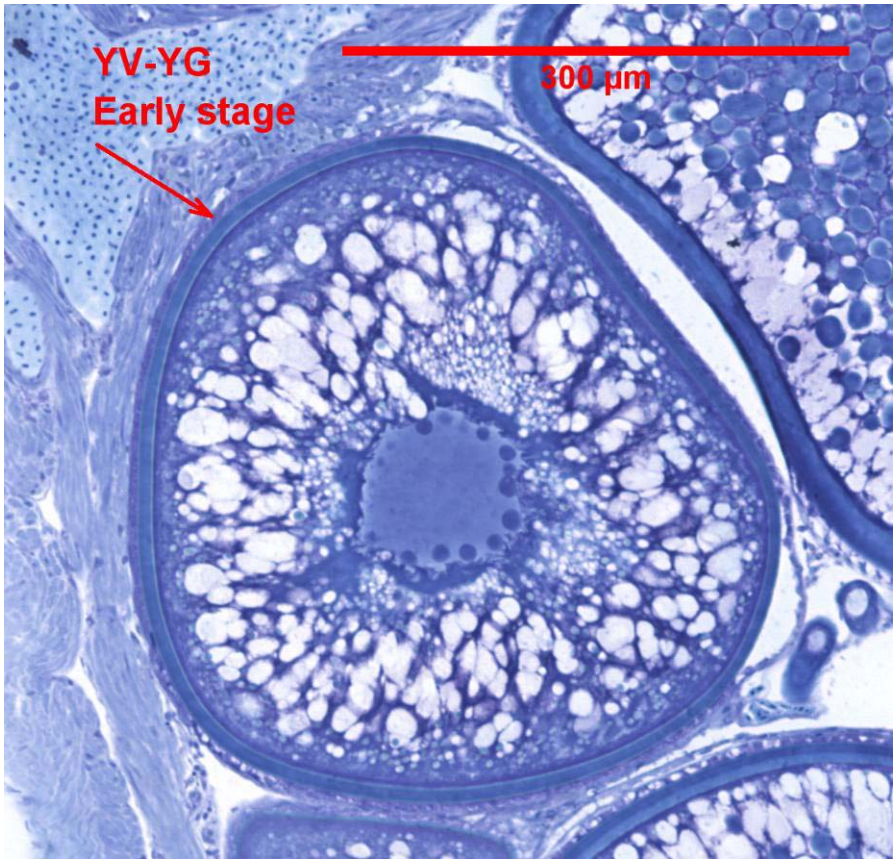
YG (yolk granules): yolk granules occur throughout the full depth of the cytoplasm. This stage also includes the largest oocytes making up the potential fecundity up to oil droplet formation and the migratory nucleus stage.

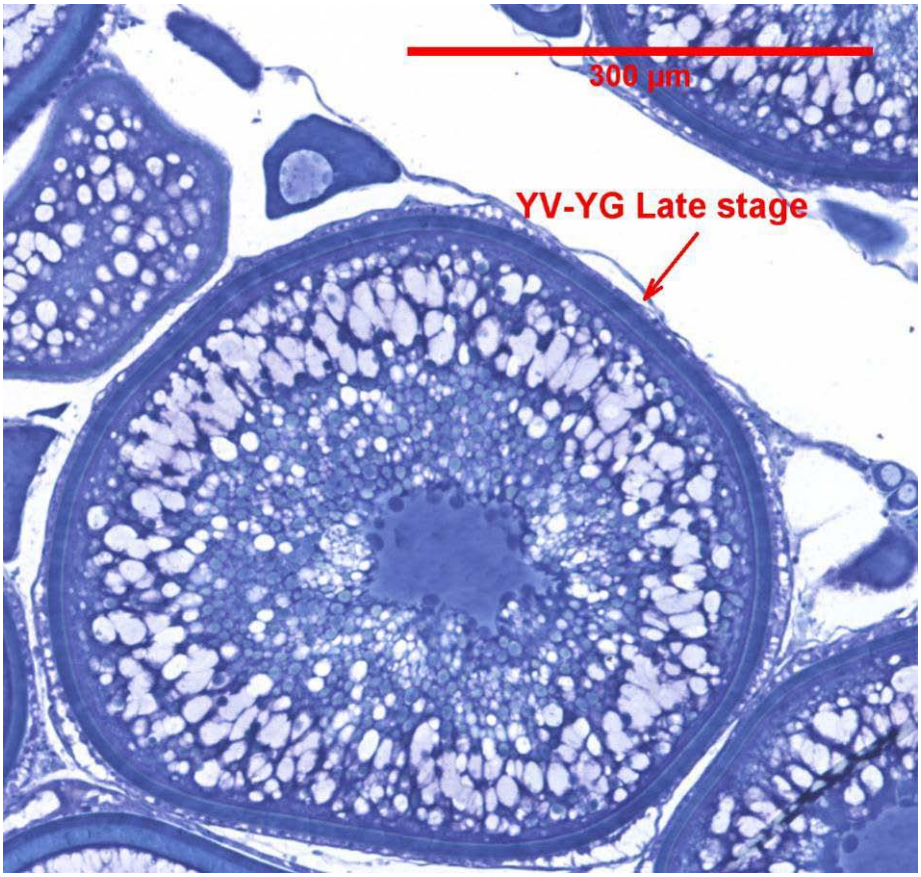
Oocyte Stage Classification

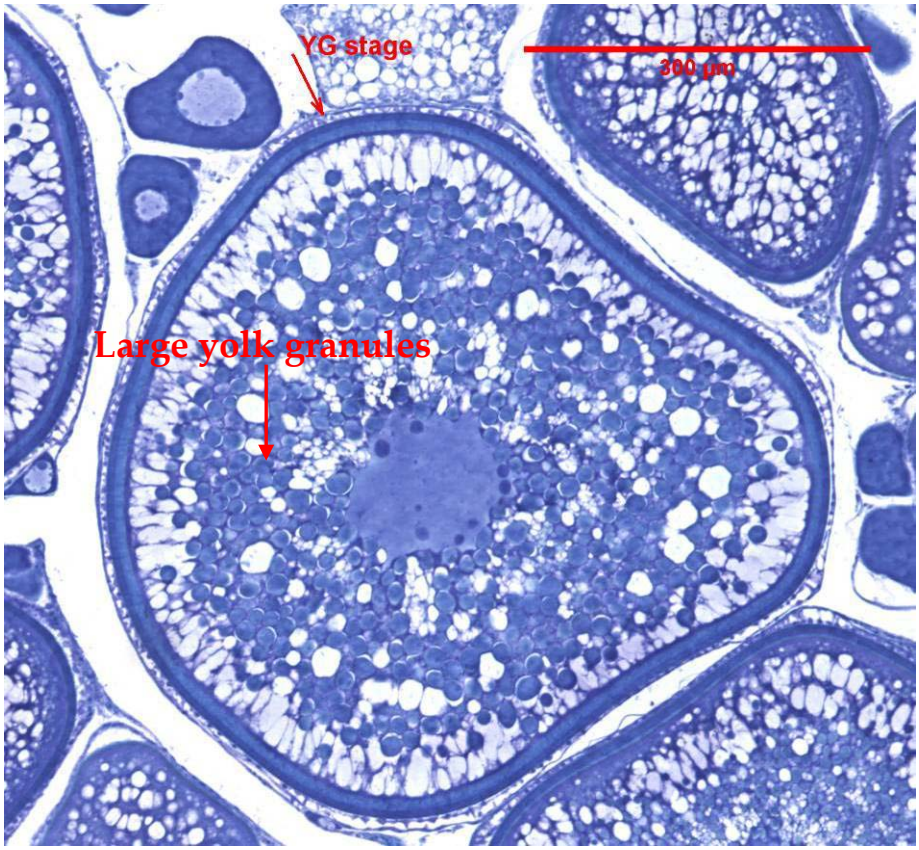
YV (YOLK VESICAL STAGE)	• **SMALLEST VITELLOGENIC STAGE** • **WHITE VACUOLES VISIBLE, RANGING IN SIZE FROM VERY SMALL TO RELATIVELY LARGE** • **185–325μM**
YV-YG (yolk vesical to yolk granule)	• Yolk vacuoles still present. • Yolk granules (blue particles in Tolluidine blue) begin to enlarge throughout the oocytes • 325–525μm
YG (yolk granule)	• Yolk granules begin to fill whole cytoplasm • In the late YG stage oil droplets will appear • In the late YG stage migratory nucleus is also present • >525μm

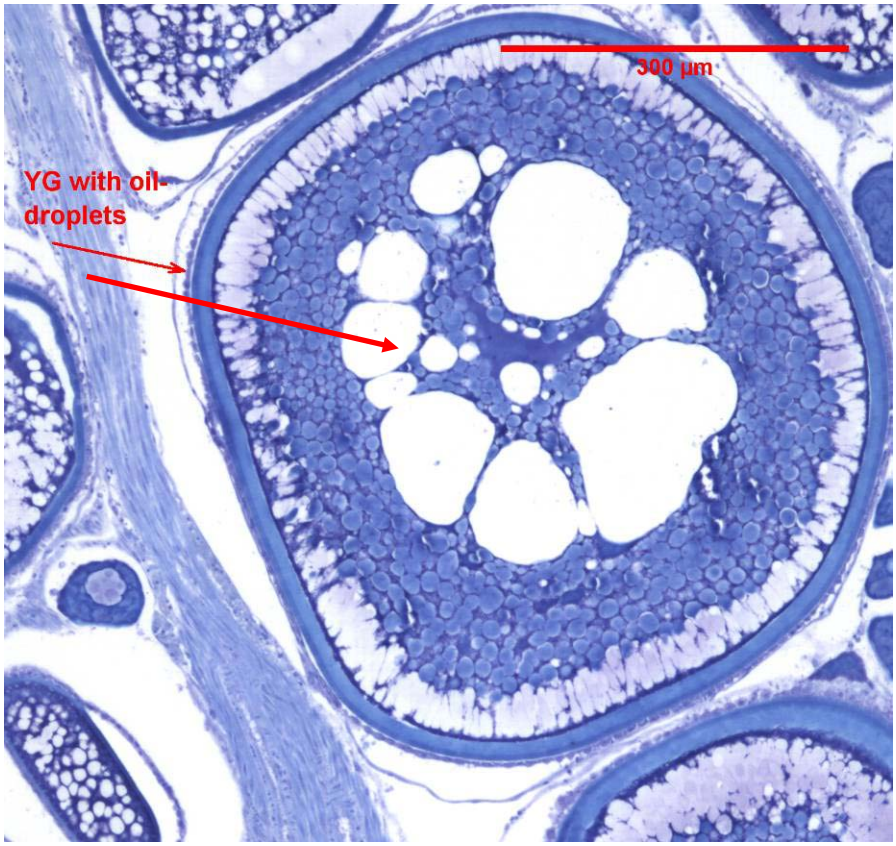
Pictures of the 3 different stages in normal oocytes stained with toluidine blue.

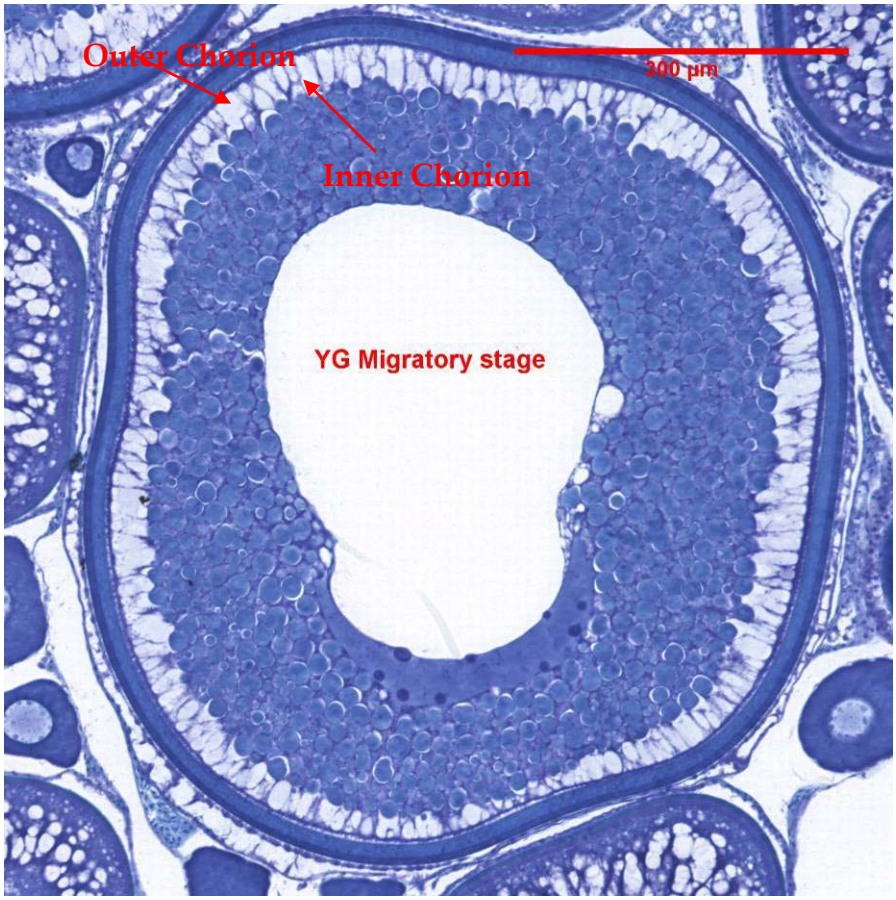




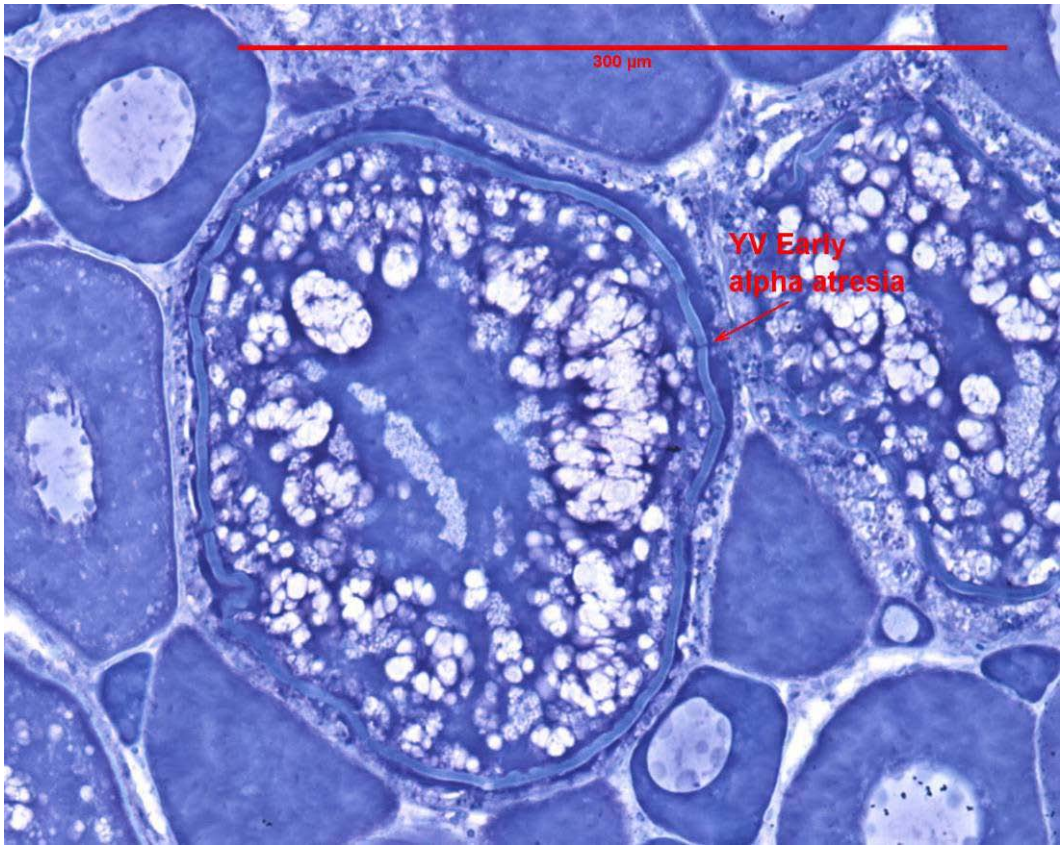

300 µm
YV-YG Late stage

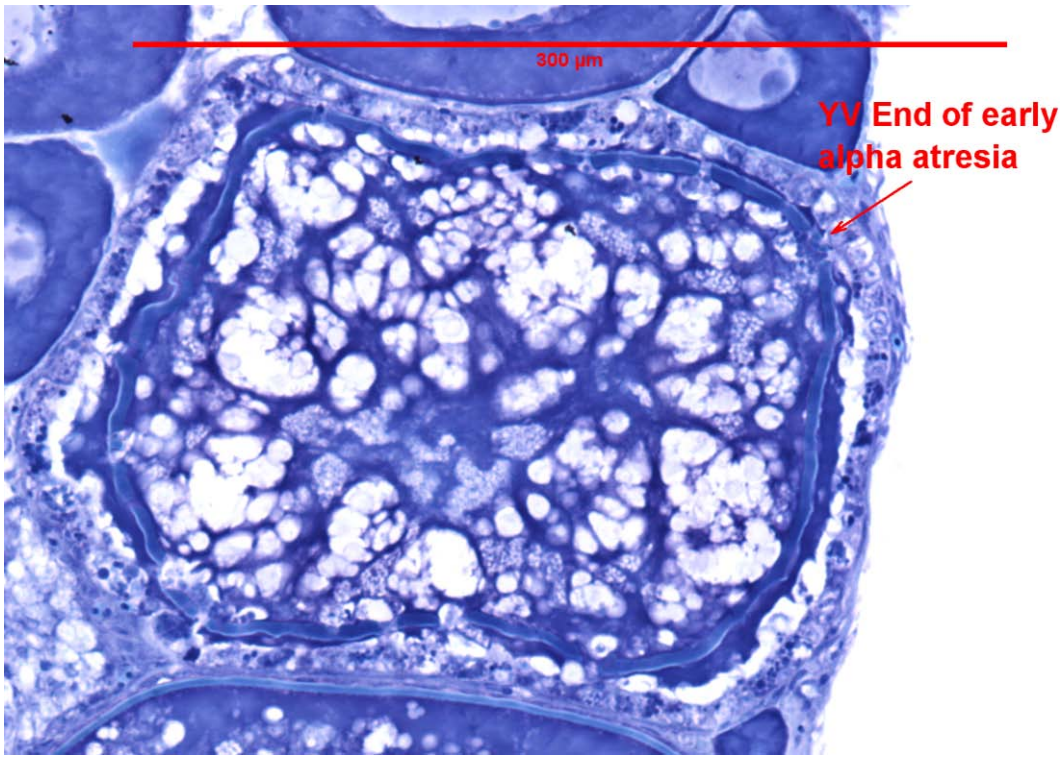

YG stage
300 µm
Large yolk granules

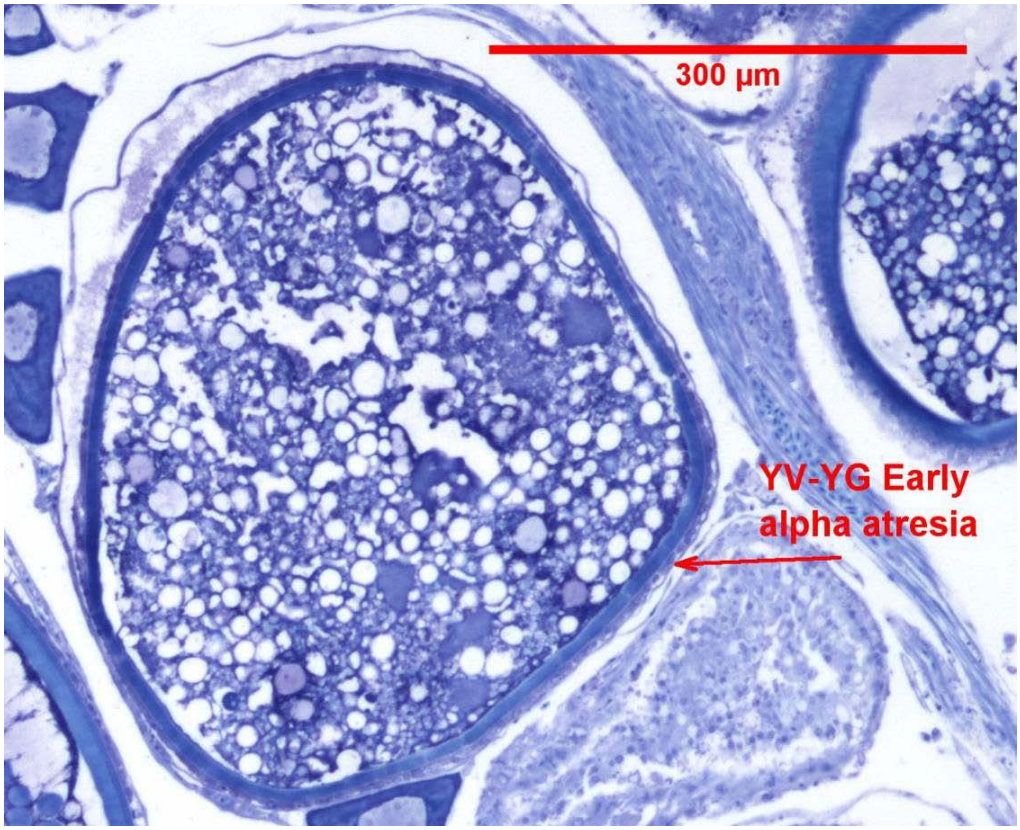

300 µm
YG with oil-droplets


Outer Chorion
300 µm
Inner Chorion
YG Migratory stage

Pictures of the 3 different stages in early alpha atretic oocytes stained with toluidine blue.

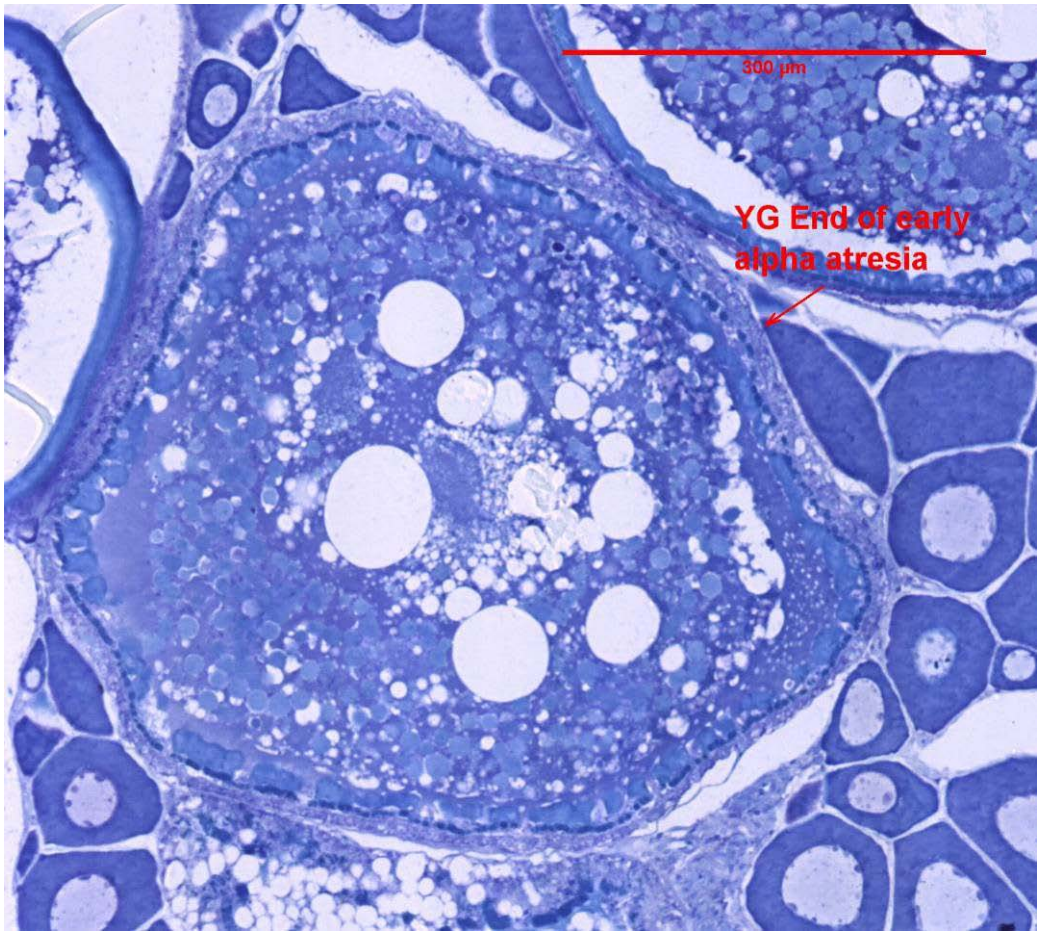





300 µm
YV-YG Early
alpha atresia


300 µm
YV-YG End of
early alpha atresia


300 µm
YG Early
alpha atresia


300 µm
YG End of early
alpha atresia

Measurement of Vi:

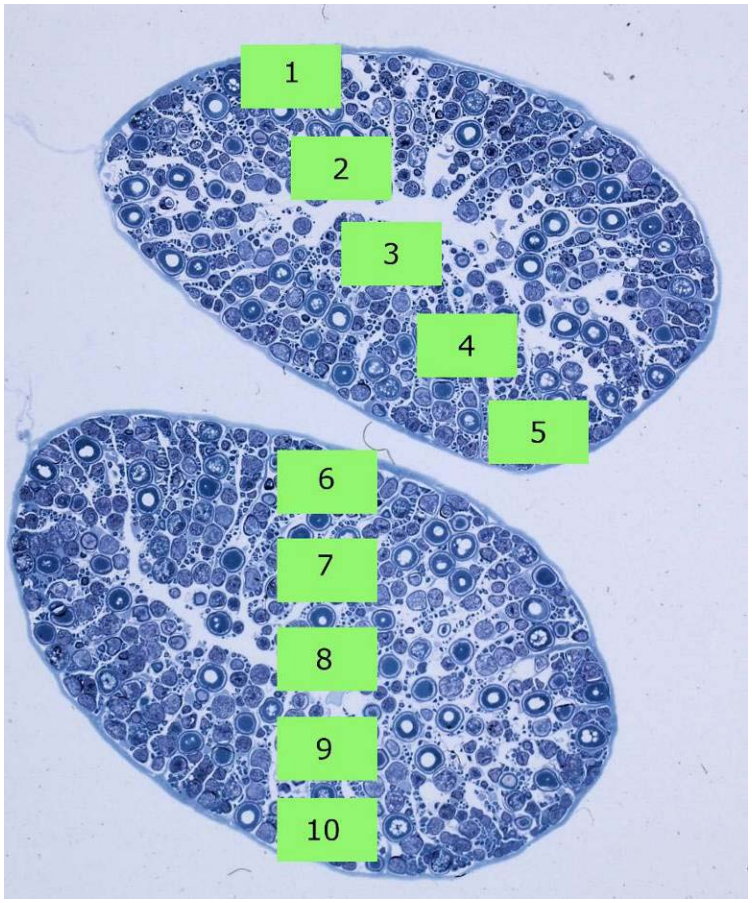
Vi = partial area of vitellogenic atretic oocytes in the histological section.

A number of frames are superimposed across both ovary sections at regular intervals in order to estimate the mean Na and Vi for the fish. The area analysed should be proportional to the ovary weight.

A Weibel grid made up of test points is superimposed on the section in order to estimate the partial area of early alpha atretic oocytes as a proportion of the total surface area in the sample frame. The test points are located at the ends of the lines in a grid.

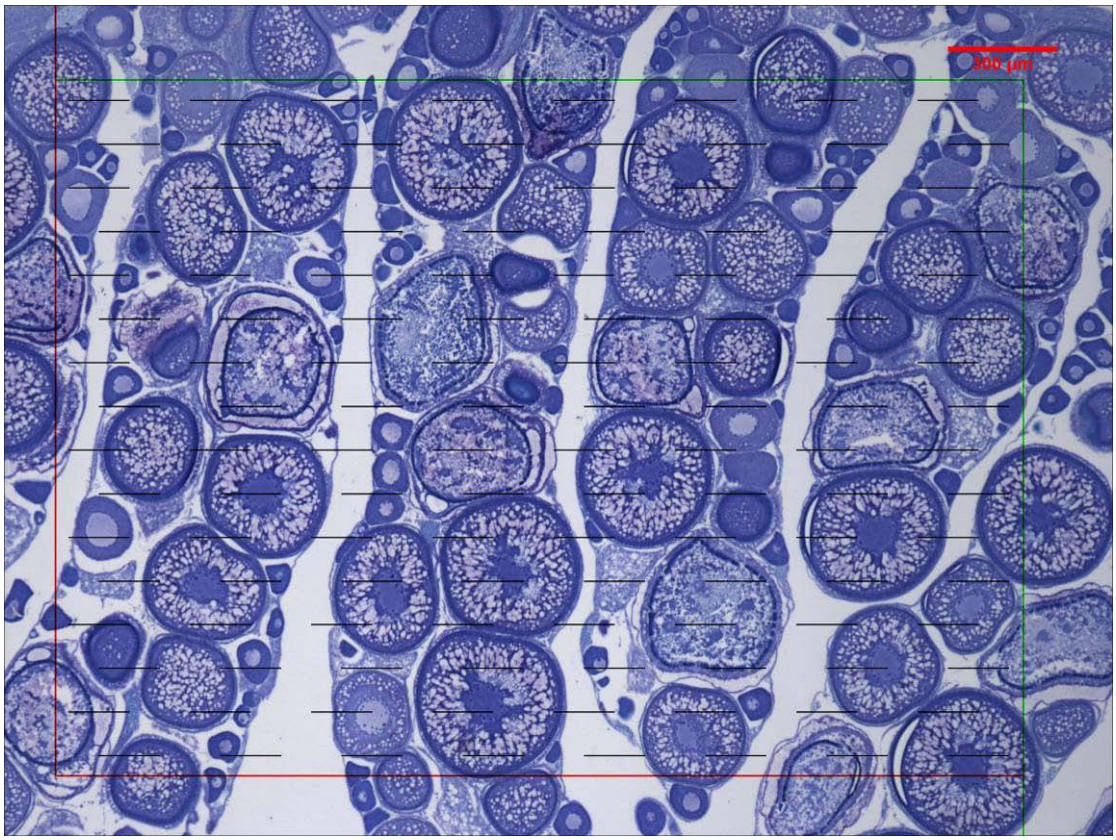
Ovary weight (g)	Approximate area to be analysed	Number of fields to be analysed if the area is 0,05 cm^2
2–9	0,3 cm^2	6
10–19	0,4 cm^2	8
20–29	0,6 cm^2	12
>30	0,7 cm^2	14

The outer grids should include area occupied by the ovary tunica.



The grid should have about 5000 points per cm^2 to cover the field.

In the example below the area inside the frame is 0,050 cm^2 and there are 256 points, which means that there are 5120 points per cm^2.



Count the point that hit early alpha atretic oocyte in each of the three stages: YV, YV-YG, YG. All points inside and on the follicle layer should be includes in the point counts. Points lying outside the ovary tunica wall should be discounted (negative grid).

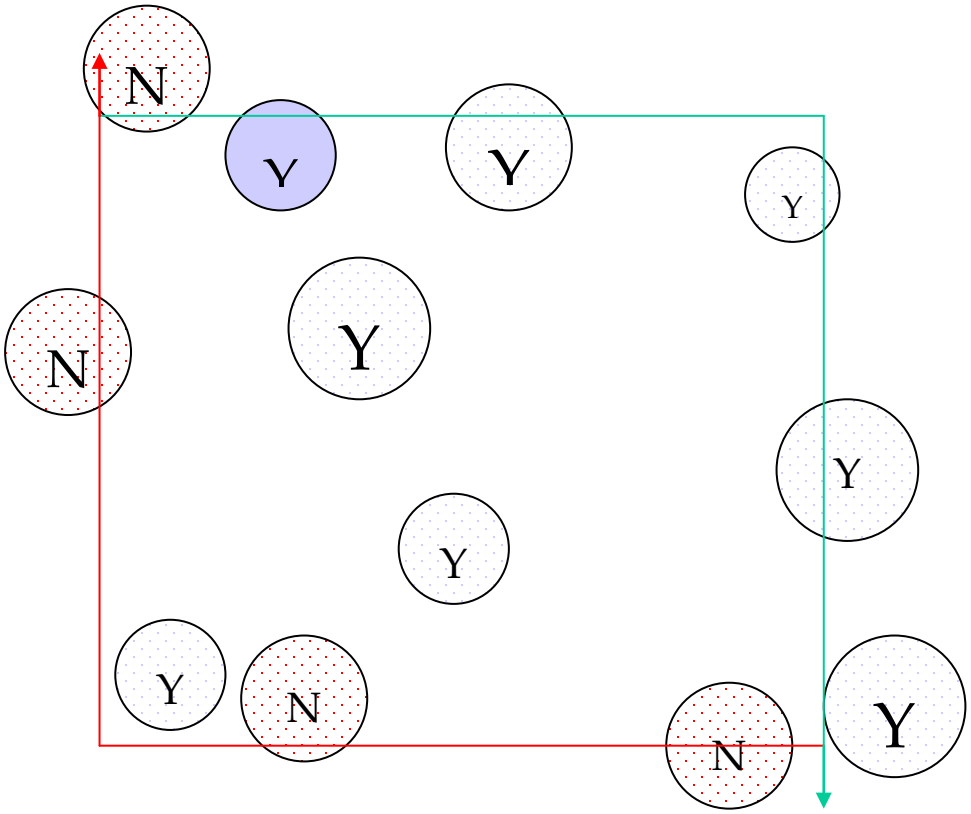
Calculate Vi for each stage using the following equation:

Vi = Number of hits / (total points – negative grid)

Measurement of Na:

Na = number of vitellogenic atretic oocyte transactions per unit area.

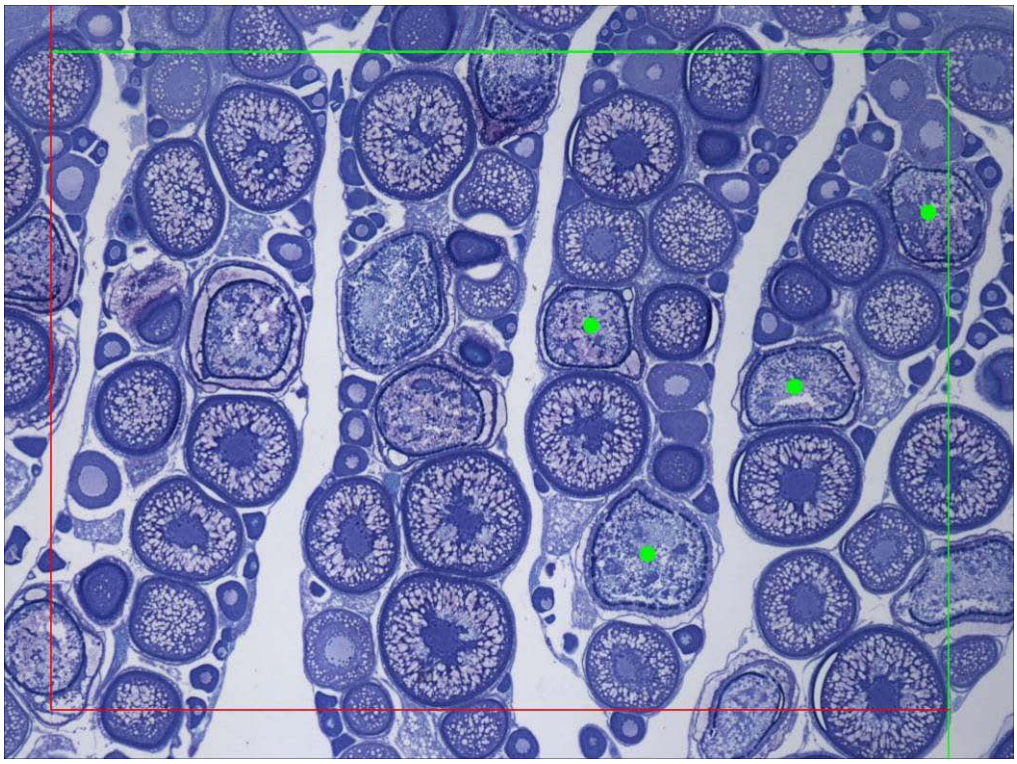
A frame is superimposed over the section and the number of early alpha atretic cells in each class of oocyte counted using the rules shown in the illustration below. Oocytes touching the forbidden line (red) or extended red line will not be counted (N). Oocytes inside the frame or touching only the green line should be counted (Y).



Calculate Na for each stage using the following equation:

Na = Number of profiles / field area

In the example below 4 early alpha atresia cells in the stage (YV-YG) are counted. The area inside the frame is 0,053 cm^2, Na for YV-YG will be 4 / 0,053 = 75.5 profiles / cm^2.



Saving of results and pictures

For each fish create a separate folder, containing the ObjectJ (J000.ojj) file and the pictures for the fish J000. Save the pictures using the standard code: e.g. J000_A_IMR, build up as: Samplecode_number for the pictures_institute initials (three letters). There will be an example of the folders on the ftp-site.

3.3 Calculation of atresia

To estimate the number of atretic oocytes in the gonad we use the following equation:

$F_{atr} = Ov * B * K * Na^{3/2} / Vi^{1/2} = Ov * 0{,}72 * Na^{3/2} / Vi^{1/2}$

Ov = ovary weight in gram

B = 0,72 (constant value, ratio between the longest and shortest axis of the oocytes transected)

K = 1 (constant value for atretic oocytes)

Calculate relative atresia:

Rel.atr. = F_{atr} / fish weight (this is the number that should be entered into the data-base)

Summarize F_{atr} for the 3 stages

Calculate the mean atresia from all the fish examined.

3.4 Calculation of mean atretic loss

To estimate the mean atretic loss we use the following equation:

Mean atr. loss = mean atresia * spawning duration / duration of early alpha atresia

Spawning duration = 60 days

Duration of early alpha atresia = 7.5 days

Procedure 4

Horse mackerel sampling procedure at sea

Before the cruise:

IMARES will send around labels to all the institutes participating in the survey. Fill the labelled 2.5 ml nunc tubes with 1,2 ml of 3.6% buffered (sodium phosphate) formaldehyde (see excel-file on the ftp-server: Buffered formaldehyde).

During the cruise:

Measure the weight of the whole catch and randomly select a subsample of 100 fish and measure the total weight of the subsample.

Measure total length, weight, maturity (Walsh scale) and sex of each fish in the subsample.

Select females in maturity stages 3–5 (Walsh scale) from the subsample for fecundity analysis. If possible divide the total quota of females equally into the 4 weight categories: < 150g, 151–250g, 251–350g and >350g. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes.

Measure or take:

- Total length
- Total weight
- Maturity
- Otoliths for age reading
- Weight of ovary: If it is not possible to take these weights at sea, take the pipette and atresia samples and fix the remainder of the ovary and weigh the ovary in the lab. The fixed weights should be corrected to fresh weights.)

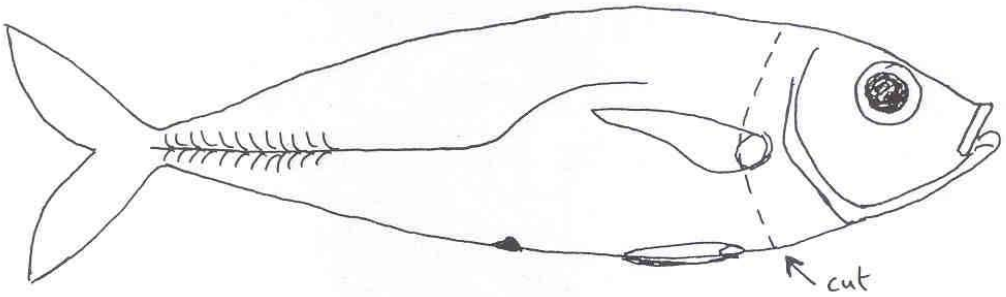
Ovary sampling:

- From the ovary take 3 * 25µl samples with a pipette and immediately put each sample in individual coded nunc tubes.
- Make sure that all the ovary samples are covered with formaldehyde.

Figure 4.1 Method to remove undamaged ovaries from horse mackerel

Removal of horse mackerel (*Trachurus trachurus)* ovaries
(A technique that was found to work well during Ciro 2/00)

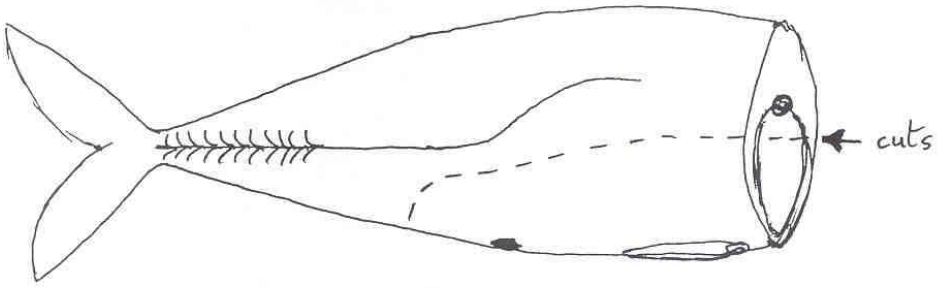
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral column.



3) Remove and discard the head and as much gut as you can carefully pull out with it. Ascertain the sex and maturity and if appropriate then continue.

NB All work is now carried out with blunt nosed scissors.

4) Make a cut either side of the fish high along the body cavity wall to a point about 2cm beyond the vent and join these two cuts through the keel of the fish.



5) Hold the body of the fish allowing the ovary, remaining gut and severed body cavity wall to hang down. Working from one side, the ovary may now be teased away from the body. If fat depositions are heavy some may be removed during this part of the process. Beyond the vent, two heavy vertical bones will be encountered separating the posterior lobes of the ovary. These should be cut. It should now be possible to separate the ovary, remaining gut and body cavity wall from the body. Discard the body.

After the cruise:

Send the nunc samples for analysis to the different institutes referring to the Table 3 below.

Table 3.

Colour code	Country	Institute and address	Responsible person	Code for ImageJ
Blue	Norway	IMR, Nordnesgaten 50, 5005 Bergen, Norway	Merete Fonn	IMR
Pink	Ireland	MI, Rinville, Oranmore, Co.Galway, Ireland	Selene Hoey	MII
Green	Netherlands	IMARES, Haringkade 1, 1976 CP IJmuiden, Netherlands	Cindy van Damme	IMA
White	Spain	IEO, Subida A Radio Faro 50-52, 36390 Vigo, Spain	Jose Ramon Perez	IEO
White	Spain	AZTI, Foundation Herrera Kaia, Portualdez/ g20110 Pasaia, Basque Country, Spain	Paula Alvarez / Maria Korta	AZT

Procedure 5

Fecundity whole mount analysis procedure for Horse mackerel

5.1 Spawning markers

Transfer the unstained sample to a tray and try to separate the oocytes.

Under the microscope check for spawning markers, if there are hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample, it should not be analysed for fecundity

5.2 Potential fecundity

Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.

Measure the oocyte diameters automatically using ImageJ software provide for the fecundity analysis.

Count all the oocytes >185µm in the sample that are not automatically detected.

Save the pictures using the standard code: e.g. J000_A_IMR, build up as: Samplecode_number for the pictures_institute initials (three letters, see Table 3).

Potential fecundity:

Pot.fec. = number of oocytes / weight of the pipette sample (0.026 g) * ovary weight

5.3 Relative potential fecundity

Relative potential fecundity:

Rel.pot.fec. = Pot. fec. / total fish weight