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Report of the Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM)

12–16 October 2015 and 9–12 November 2015

Hamburg, Germany and

Bergen Norway

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CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM) chaired by Cindy van Damme, Netherlands, met twice in 2015. The first meeting was from 12–16 October 2015 in Hamburg, Germany, to calibrate egg sorting, staging and identification. The second meeting was from 9–12 November 2015 in Bergen, Norway, to calibrate fecundity and atresia estimation and standardize analysis for the DEPM method.

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 2012 workshop. For the experts there was an underestimate of stage 1 mackerel eggs (stages 1a and 1b combined) during the first round of analysis (-3%) and (-4%) during the second round. The results for stage 1 horse mackerel eggs reduced from an overestimate of 5% to 3% underestimate. This is particularly reassuring as it is at this stage on which the egg production estimates are based.

The pipette sampling for fecundity samples was again shown to the participants. A trial during the workshop showed that all participants take the pipette samples correct as weight of the samples were close to the assumed weight.

The screening, fecundity and atresia calibration proved beneficial to all participants. Agreement in fecundity estimates is very high. For atresia problems occurred which sparked discussion and improved the description of early alpha atresia stages. After discussion, the manual has been improved and there was agreement on identification of vitellogenic and early alpha atretic oocytes.

POF staging remains difficult, but the plenary session on POF staging clarified the POF stages and assessing POF stage for the whole sample.

As the mackerel and horse mackerel egg surveys are carried out once every three years, these workshops are a refresher for expert survey participants and a first acquaintance with new participants in the sample analyses. It should however be realized that two weeks of workshops are not enough to train new participants. Institutes should allow newcomers to be trained properly before the survey.

1 Opening of the meeting

The Working Group on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM) chaired by Cindy van Damme, Netherlands, met 12–16 October 2015 in Hamburg, Germany and 9–12 November 2015 in Bergen, Norway. Twenty-one participants from nine countries (representing 10 different institutes) participated in the October meeting. Sixteen participants from 10 countries (representing 11 different institutes) participated in the November meeting. The participant lists can be found in Annex 1.

1.1 Background

In preparation for the 2016 international ICES coordinated mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) egg survey, two workshops were held to standardize and calibrate the identification and staging of eggs and the estimation of fecundity.

The first workshop was held at TI-SF, Hamburg, Germany, for the majority of plankton analysts who will be involved in the 2016 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 also investigated the reasons for individual differences in the identification and staging of mackerel and horse mackerel eggs and attempted to harmonize these. Evaluation of the use of the 'spray' technique, for removing fish eggs from plankton samples, was carried out.

To allow the calculation of the numbers of spawning female fish in a stock by the Annual Egg Production Method (AEPM; Lockwood *et al.*, 1981, Armstrong *et al.*, 2001) or Daily Egg Production Method (DEPM; Lasker, 1985) 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 are able to accurately identify and stage the eggs of each of the target species (ICES, 2015). These workshops (WKFATHOM) 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 whereas doing so. Previous workshops (ICES, 2001; 2004; 2006; 2009; 2012) have developed a comprehensive set of criteria for both mackerel and horse mackerel egg identification and staging. These criteria were updated during the 2015 workshop. In addition, inexperienced analysts were involved for the first time, and it was critical that they became fully aware of the procedures and criteria in advance of the 2016 surveys.

In addition to the correct identification of spawned eggs, it is vital for egg production methods (EPM) to have a good estimation of potential fecundity, batch fecundity, atresia and spawning fraction in order to estimate Spawning-Stock Biomass (SSB). In order to calibrate estimations of fecundity and atresia a second workshop took place at IMR, Bergen, Norway. Methods and criteria developed in previous workshops (ICES, 2006; 2009; 2012) were expanded and further developed during this workshop. In addition, inexperienced analysts were taught how to identify correctly vitellogenic and atretic oocytes and how to estimate fecundity and atresia.

1.2 Terms of Reference

The terms of reference for the meetings were:

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 2012 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.

2 Adoption of the agenda

The agendas addressed all ToRs and were adopted without changes. The agendas can be found in Annex 2.

3 Material and methods

3.1 Egg sorting trials (ToR a)

Because of the egg sorting trials conducted during the previous workshops, most 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' two plankton samples (typical plankton from the 2013 survey) were prepared, each containing a known number of fish eggs. All participants 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 280-µm mesh sieve. The plankton was then washed into a glass beaker with a little seawater. A normal garden spray pump was used to fill the funnel as much as possible with pressurized water. The spray jet was rotated around the sides of the beaker to limit damage to the plankton. The fine, pressurized 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 two to three minutes whereas 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 floating plankton was then drained from the beaker, and collected in a sieve. The sunken eggs were removed from the beaker and kept separately. The spraying was then repeated until very few eggs were removed from the plankton. 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.

3.2 Egg staging (ToR b, c and d)

3.2.1 Egg staging trials

A total of 475 mackerel, horse mackerel and hake (*Merluccius merluccius*) eggs as well as other species, which can be found in egg survey samples, were placed in 18 small, Perspex trays. Each tray contained 25 small wells but only the first 10–15 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. During the first round, 205 eggs were staged by participants, while the second round the number of eggs was increased to 270. No validated eggs were available for the first round, but in 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 2013 Atlantic and 2015 North Sea mackerel egg surveys. Some of the validated eggs also had known stages. The eggs were mainly those of mackerel and horse mackerel with a few hake eggs, which are morphologically similar to those of the two target species. It was hoped that these definitive eggs, of known parentage, would allow participants' species identification to be judged more consistently. 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. All participants were asked to stage all eggs, irrespective of species. The mackerel eggs in each tray were staged to IA, IB, II, III, IV, V and the horse mackerel and hake eggs were staged to IA, IB, II, III, IV, as horse mackerel and hake 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 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 not all were fitted with eyepiece graticules.

Once each participant had staged and identified each of the eggs, 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, 2012) were revised (see Section 3.2.2).

During the course of both rounds of analysis several eggs became damaged, or were moved, from one cell to another in the trays. It was therefore not, 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

3.2.2.1 Egg staging criteria for mackerel and horse mackerel (Western stock)

Because of discussions following the first and second 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 (Figures 3.1 and 3.2), but now include some other (secondary) characteristics, which the participants thought were crucial in determining egg stage. Figures 3.3 and 3.4 show the development stages for horse mackerel and Figure 3.5 provides some development stages for hake eggs.

Participants should be aware that both horse mackerel and hake hatch at the end of stage 4.

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 at 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, which begins as a cleft in the cell bundle, until closure of the blastopore. Towards the end of this stage the tail tapers and is flattened against the yolk. Also at the end of this stage, the embryo should be half way around the circumference of the egg.

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

Stage III

Primary characteristics: The end of the tail has thickened, becoming bulbous in appearance, and may have lifted clear of the yolk sac. Growth of the embryo is from half way to three-quarters of the way around the circumference of the egg.

Secondary characteristics: Widening of the head and development of the eyes. Pigment spots develop on the embryo.

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 separates from the yolk. Pigmentation on the embryo increases compared to stage 3.

Stage V

Primary characteristics: The tail of the embryo is touching the nose or beyond and circumnavigates the egg following the inner margin of the membrane.

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 egg. The embryo may also become distorted giving a false impression of development stage.

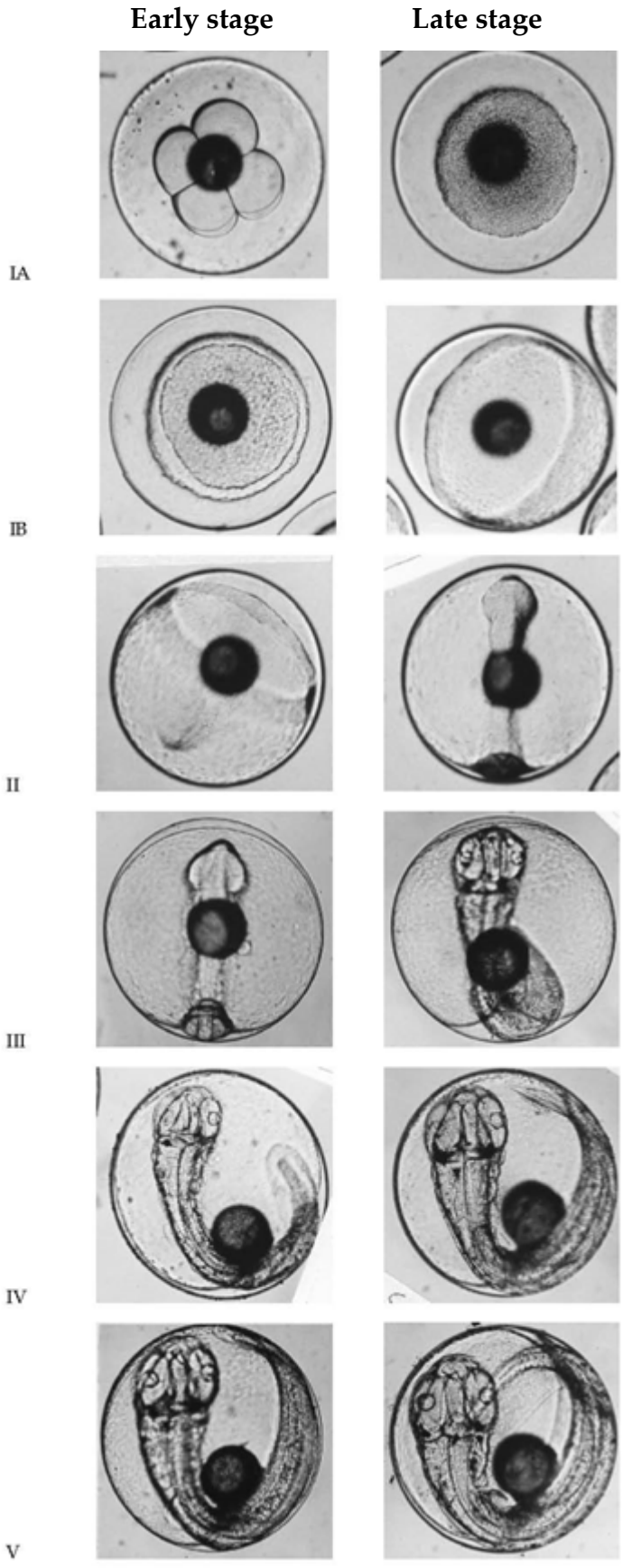
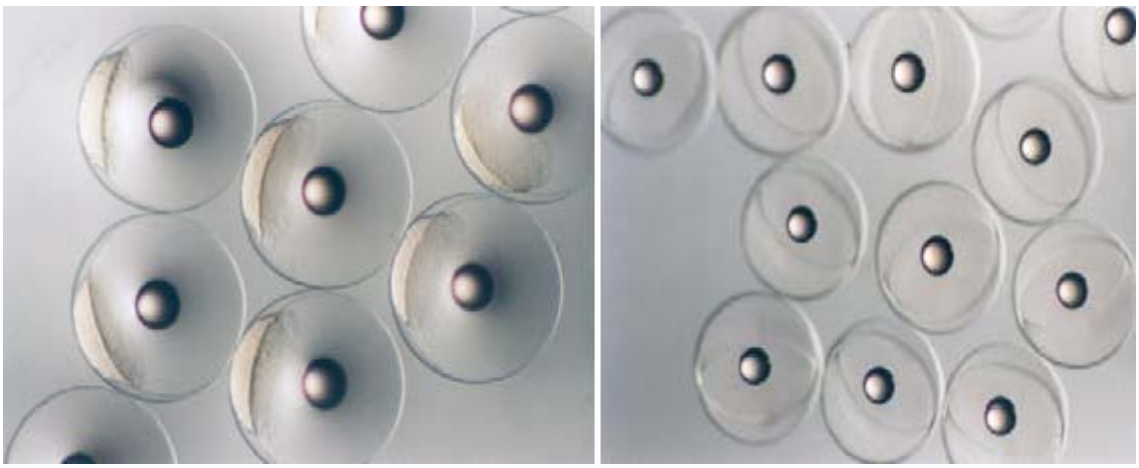
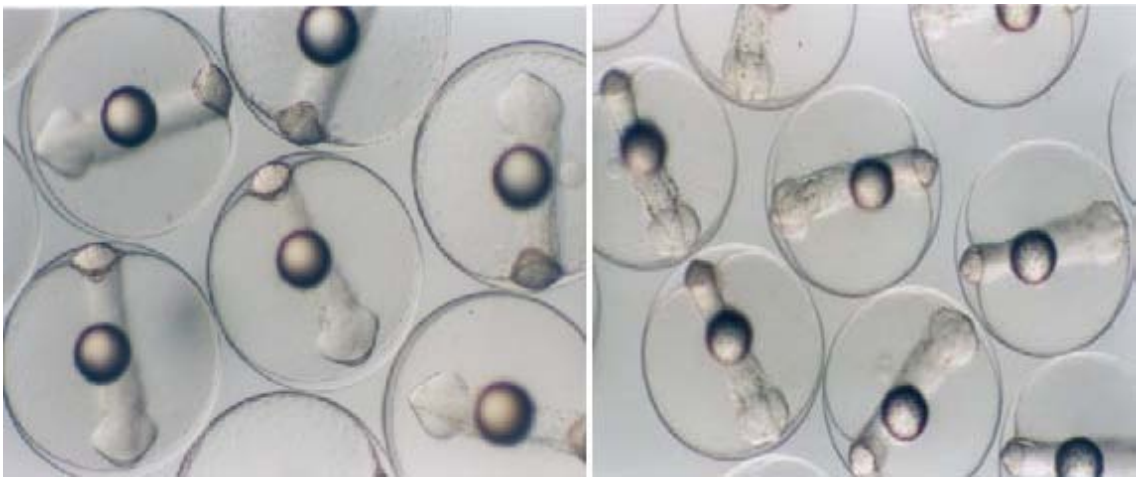


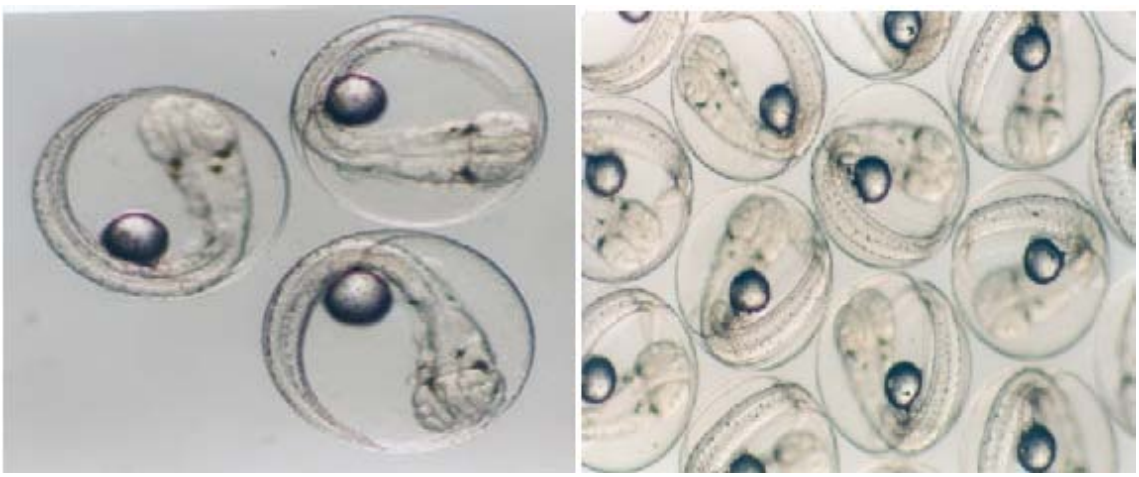
Figure 3.1. Mackerel eggs at the beginning and end of the six development stages.



Stage 1A **Stage 1B**



Stage II **Stage III**



Stage IV **Stage V**

Figure 3.2. Development stages of mackerel from fertilization experiments.

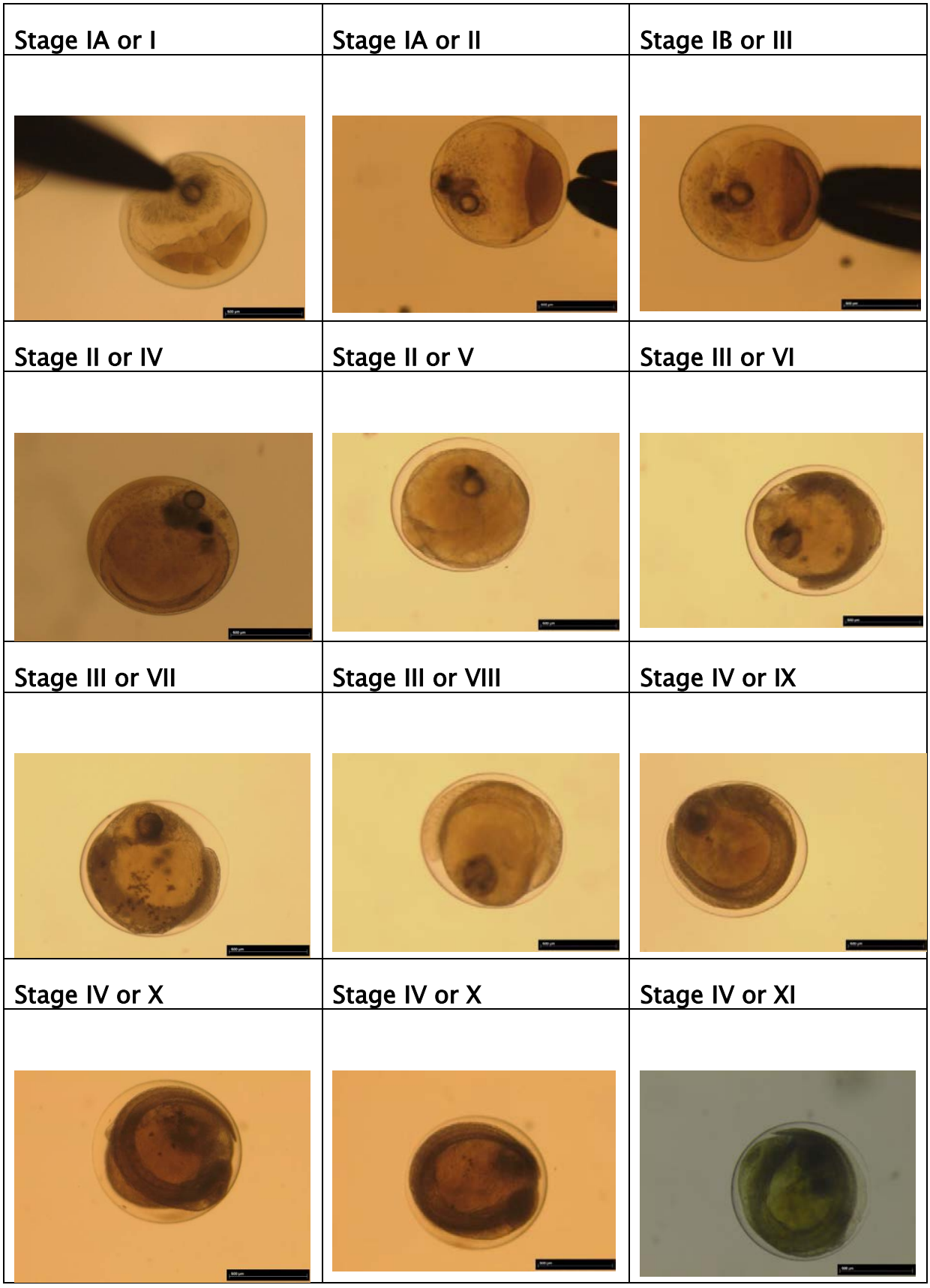
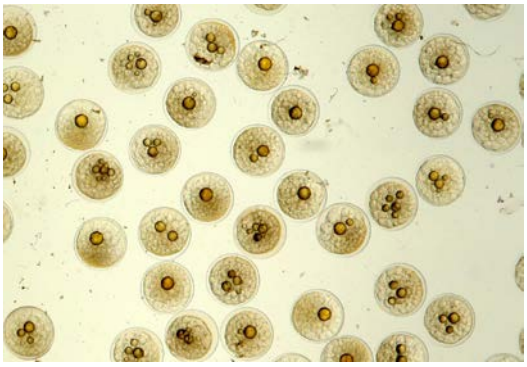
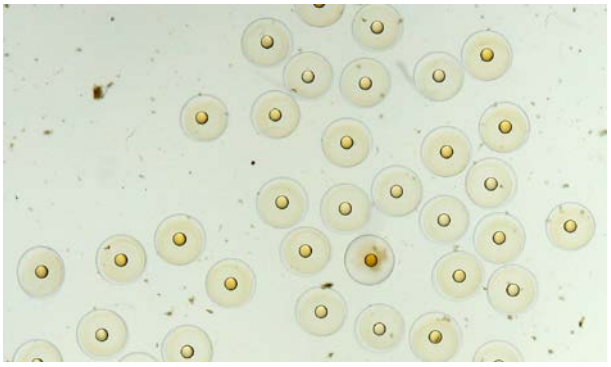


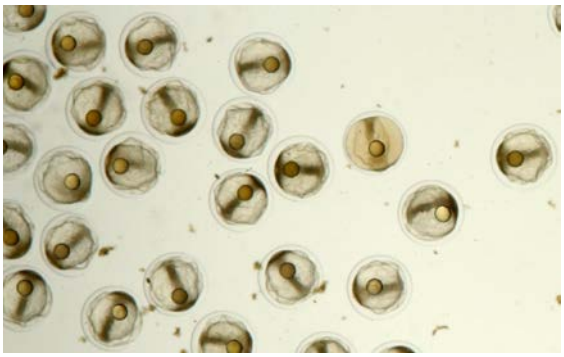
Figure 3.3. Development stages of horse mackerel from fertilization experiments. First stage number is the stage development used for the Western stock, second number is the stage development used for the Southern stock.



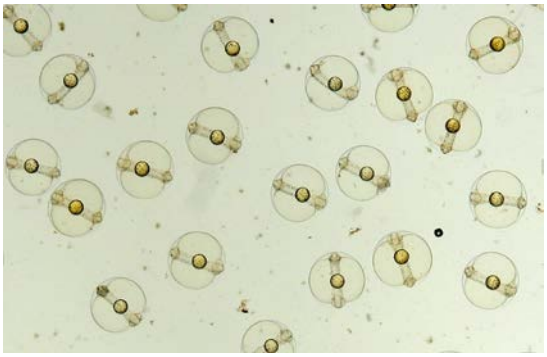
Stage IA



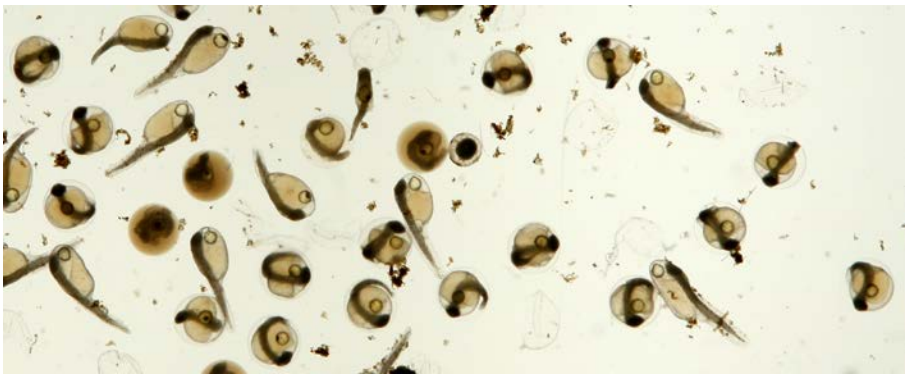
Stage IB



Late stage II

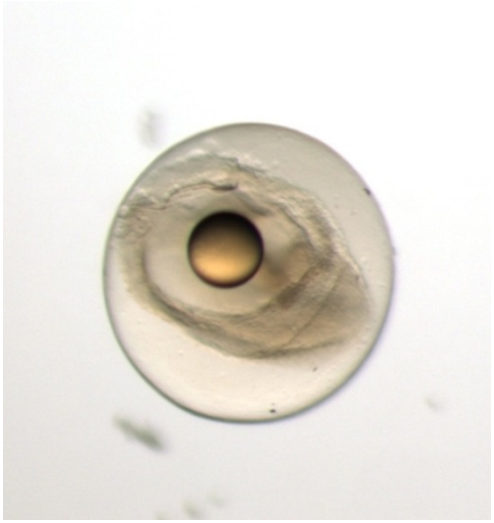


Early stage III

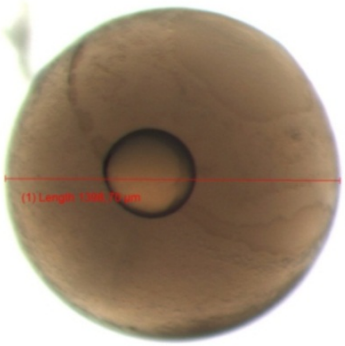


Late stage IV and hatching

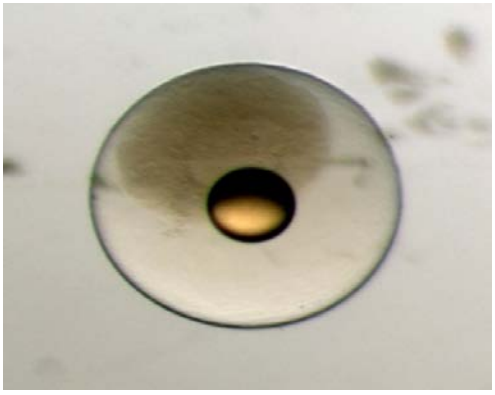
Figure 3.4 Development stages of horse mackerel from fertilization experiments.



Stage 1A

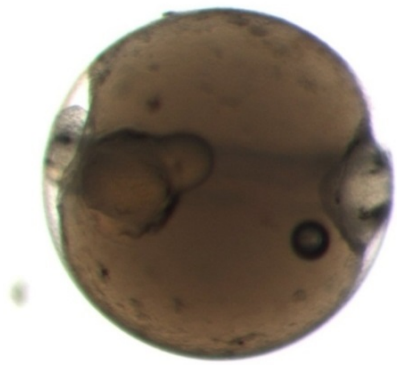


Stage 1A



Stage 1B

Stage II



Stage III

Stage III

Figure 3.5. Development stages of hake eggs from fertilization experiments.

3.2.2.2 Egg staging for horse mackerel (southern stock)

Since 2007, the horse-mackerel southern stock, monitored by Portugal, IPMA, has been surveyed according to a DEPM methodology. For the implementation of this method an egg development scale with 11 stages has been developed (Cunha *et al*., 2008). A revised version of that classification is now in use (Figure 3.3).

Stage I

First segmentation, which, under dim reflected light, is easily visible. This stage lasts until individual cells are easily distinguishable from each other, and counting is possible Equivalent to stages IA of Pipe and Walker (1987) and 1 of King *et al*. (1977). The unfertilized eggs are included in this stage (however, they are difficult to distinguish).

Stage II

Cleavage proceeds until a blastodermal cap is formed, counting of individual cells is no longer possible although visible. Equivalent to stages IA of Pipe and Walker (1987) and 1 of King *et al*. (1977).

Stage III

Development of the blastocoele. First appearance of the germinal ring, where the embryonic shield starts to develop. Equivalent to stages IB of Pipe and Walker (1987) and 1 of King *et al*. (1977).

Stage IV

First appearance of the embryonic axis. The outline of the embryo is clearly defined in the median line of the embryonic shield. The embryo develops, but the head and tail are not yet discernible. Equivalent to stages II of Pipe and Walker (1987) and 2 of King *et al*. (1977). The blastopore is still large.

Stage V

The cephalic region becomes apparent and an outline of the optic vesicles may be discerned. The body of the embryo is glued to the yolk but without having thickened. Blastodermal cap development proceeds around the yolk and the blastopore diminishes. Equivalent to stages II of Pipe and Walker (1987) and 2 of King *et al*. (1977). In this stage, it is possible to see the somites, although not so clearly, and pigmentation may begin to appear.

Stage VI

The embryo becomes bulbous. However the angle formed by the tail and yolk is >= 90°. The closure of the blastopore occurs during this stage. Equivalent to stages II of Pipe and Walker (1987) and 2 and 3 of King *et al*. (1977).

Stage VII

The embryo tail begins to separate from the yolk mass. The angle formed by the tail and the yolk is < 90° and this stage lasts until the free tail reaches the same length as the head size. The pupils can be discerned in the eyes. The pigment spots appear clearly in two rows along the dorsal body contour. Equivalent to stages III of Pipe and Walker (1987) and 3 and 4 of King *et al*. (1977).

Stage VIII

Growth of the tail still short of three-quarters of the egg circumference. Equivalent to stages III of Pipe and Walker (1987) and 4 of King *et al*. (1977).

Stage IX

The embryo length exceeds 3/4 of the length around the yolk and grows until it reaches 7/8 of its circumference. Equivalent to stages III of Pipe and Walker (1987) and 4 of King *et al*. (1977).

Stage X

The embryo length exceeds 7/8 of the circumference around the yolk and grows until the tail is close to the head but without touching it. Equivalent to stages IV of Pipe and Walker (1987) and 5 of King *et al*. (1977).

Stage XI

The tail touches the head and may grow beyond it. At the end of this stage, the embryo hatches. Equivalent to stages IV of Pipe and Walker (1987) and 5 of King *et al*. (1977).

3.3 Egg identification (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 2012 workshop report (ICES, 2012) was available to 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 WKFATHOM participants was carried out. The survey manual was also updated with these changes in egg identification criteria.

3.3.2 Egg identification criteria

Egg and oil globule size are the primary criteria used in identify eggs. Mackerel eggs range in size from 0.97 mm to 1.38 mm with the oil globule ranging from 0.22 to 0.38 mm. Horse mackerel eggs range from 0.81 to 1.04 mm with an oil globule ranging from 0.19 to 0.28 mm.

Table 3.1 summarizes published descriptions of mackerel, horse mackerel and other species of eggs that contain similar morphological features. It provides validated observed egg and oil globule diameters for each species as well as the diagnostic features and criteria used by the participants to help with egg identification. It should be noted that the diameter of the egg and oil globule within a species can and may vary through the spawning season and from area to area. Variation in egg size for the same species can also be observed within the same sample

Eggs may also show regional variations in pigmentation and this should not therefore be used as a primary characteristic for identification. Due to this variation, egg identification should be carried out only by experienced staff that have participated in the WKFATHOM egg identification and staging workshops carried out in the year prior to the survey year.

Table 3.1. Comparison of the Characteristics of Mackerel, Horse Mackerel, Blue Jack Mackerel, Megrim, Hake and Snipefish Eggs (Details of fixative and concentration unknown). NB The information 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.

Species	Diameter (mm)		Reference	Area	Diagnostic Features
	Egg	Oil Globule			
Mackerel (*Scomber scombrus*) (See Lockwood *et al.*, 1977)	1.0–1.38	0.28–0.35	Russell, 1976	North Sea, English Channel	• Unsegmented/ Homogenous yolk • Perivitelline space approx. 0.05mm • Oil globule often orientated to the top of the egg
	1.09–1.36	0.26–0.37	Fahay, 1983	N.W. Atlantic	
	0.97–1.38	0.25–0.35	Ehrenbaum, 1905–09	Irish Sea, North Sea	
	1.24	?	Mendiola *et al.*, 2006	Biscay	
	0.97–1.38	0.22–0.38	Development of Fishes of the Mid-Atlantic Bight, 1978	Mid-Atlantic Bight	
	1.0–1.38			North Atlantic	
	0.97–1.38	?	Johnstone, Scott and Chadwick, 1934	Isle of Man	
	1.21–1.33	~0.32	Holt, 1893	West of Ireland	
	1.16	0.27	IPIMAR, fertilization experiment 2008		
Horse Mackerel (*Trachurus trachurus*) (See Pipe and Walker, 1987)	0.81–1.04	0.19–0.28	Russell, 1976	North Sea, English Channel	• Granular / segmented yolk, although this may not be as obvious at the southern end of the species range. • The oil globule migrates towards the head of the embryo after stage 2. • In stages 3 and 4 the embryos show stronger pigmentation compared to
	1.03–1.09	0.26–0.27	Holt, 1898	North Sea	
	0.81–0.93	0.22–0.23		Plymouth	
	0.84–1.04	0.19–0.24	Ehrenbaum, 1905–09	North Sea, English Channel	

Species	Diameter (mm) Egg	Oil Globule	Reference	Area	Diagnostic Features
	Max. 0.84	0.24–0.26	Holt, 1893	English Channel	mackerel. However, the pigmentation is not as strong as in hake. • 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.
Blue Jack Mackerel (*Trachurus picturatus*)	0.98–1.10	0.19–0.31	IPIMAR, fertilization experiment 2010 (Gonçalves *et al.*, 2012)	W Portugal	• Segmented yolk
Megrim (*Lepidorhombus whiffiagonis*)	1.02–1.22	0.25–0.30	Russell, 1976	North Sea, Irish Sea	• Striated appearance of egg membrane*. (See below and Figure 3.7) • 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. • Pigment on oil globule as embryo develops ***Striations can be observed on the membranes of preserved eggs of other species. This can lead to misidentification of eggs which have been preserved for some time.**
	1.07–1.22	0.25–0.30	Ehrenbaum, 1905–09	North Sea	
	1.07–1.13	0.30	Holt, 1893	West of Ireland	
	1.08–1.30	0.29–0.34	Cefas unpublished data	Celtic Sea	
Hake	0.94–1.03	0.25–0.28	Russell, 1976	North Sea, English Channel, Mediterranean	• Positive surface adhesion test (SAT) is used to identify hake eggs (Porebski, 1975) and (Coombs, 1994).

Species	Diameter (mm)		Reference	Area	Diagnostic Features
	Egg	Oil Globule			
(*Merluccius merluccius*) (See Coombs, 1982)	0.94–1.03	~0.27	Ehrenbaum, 1905–09	North Sea, English Channel, Mediterranean	• From stage III onwards embryos display strong pigmentation along the embryo. Towards the end of its development, the embryo begins to show the characteristic post-anal pigmentation of three bars.
	0.94–1.03	~0.27	D'Ancona *et al*., 1956	?	
	1.10–1.16	0.27–0.35	Shaw, 2003	Celtic Sea	
Longspine Snipefish *(Macrorhamphosus scolopax)*	1.00	0.2	Development of Fishes of the Mid-Atlantic Bight, 1978. US Fish and Wildlife service. FWS/OBS-78/12.	Europe	• Membrane is light amber with grainy reflections • Yolk with rose or violet halo depending on viewing light. • Oil globule is amber/rose in colour
Lings (Molva spp.)	0.97 – 1.13	0.28 – 0.31	Russell, 1976	North Sea	• Unsegmented yolk • Pigmented oil globule • Pigmentation in later stage embryo is concentrated into 2 distinct lines that run all the way along the back.

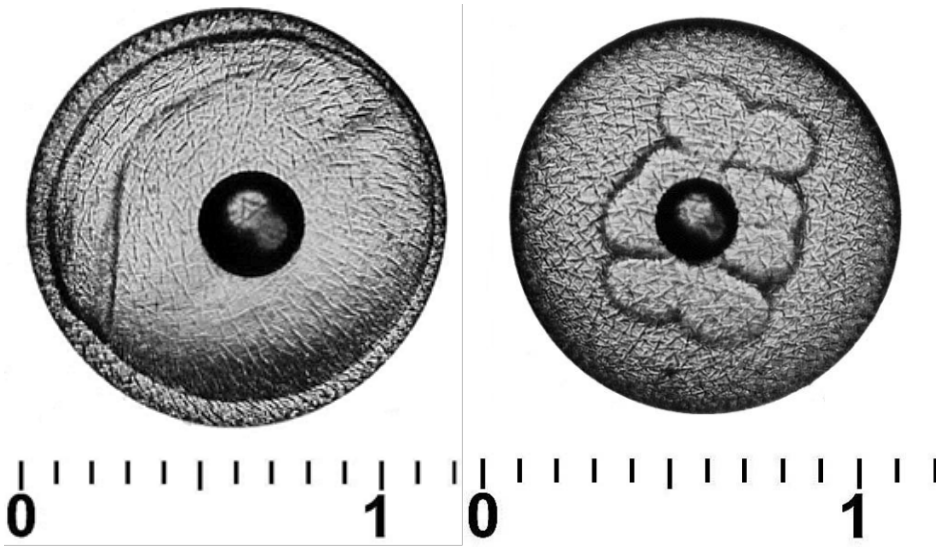


Figure 3.6. Eggs of megrim, showing the striations on the membrane.

3.3.3 Misclassification of mackerel and horse mackerel eggs 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. 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

Description and measurements based on eggs from a single artificial fertilization experiment carried out in 2010 by IPMA (Figure 3.7).

- Pelagic, spherical and transparent eggs with a small perivitelline space. The yolk sac is segmented. A single yellow oil globule is located towards the posterior portion of the yolk. In the early embryo, two rows of spots appear along the dorsal body contour.
- Eggs are very similar to the eggs of *Trachurus trachurus*. The *T. picturatus* eggs from the 2010 fertilization experiment were slightly larger than the eggs of *T. trachurus* described in the literature and exhibited a more intense pigmentation.
- Egg diameter: 0.98 – 1.10 mm
- Oil globule: 0.19 – 0.31 mm

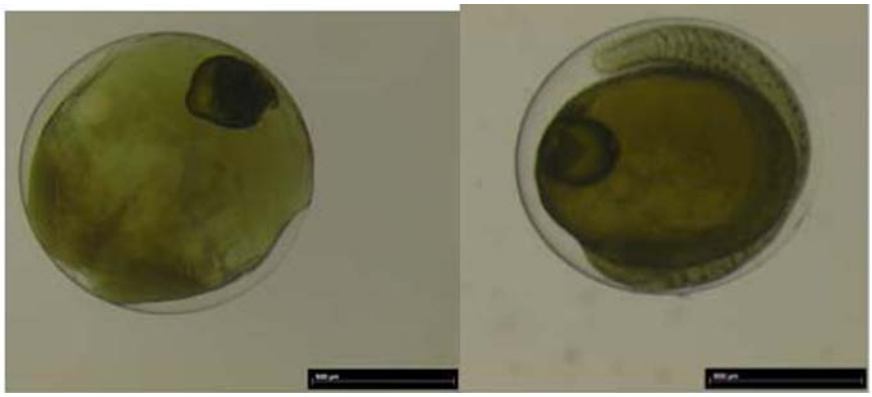


Figure 3.7. Eggs of *Trachurus picturatus* from a fertilization experiment (IPMA, 2010).

Scomber colias

- The eggs are spherical, on average ranging in diameter from 1.06–1.14 mm. A 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, while 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 diameter: 0.93 mm (based on eggs from artificial fertilization, IPMA, 2008, see Figure 3.8).
- Oil globule: 0.18 mm (based on eggs from artificial fertilization, IPMA, 2008).
- Description: Pelagic eggs, spherical. Single oil globule with melanophores (Gaetani, 1937).
- Fish distribution is mapped in the reports of ICES-WGACEGG.

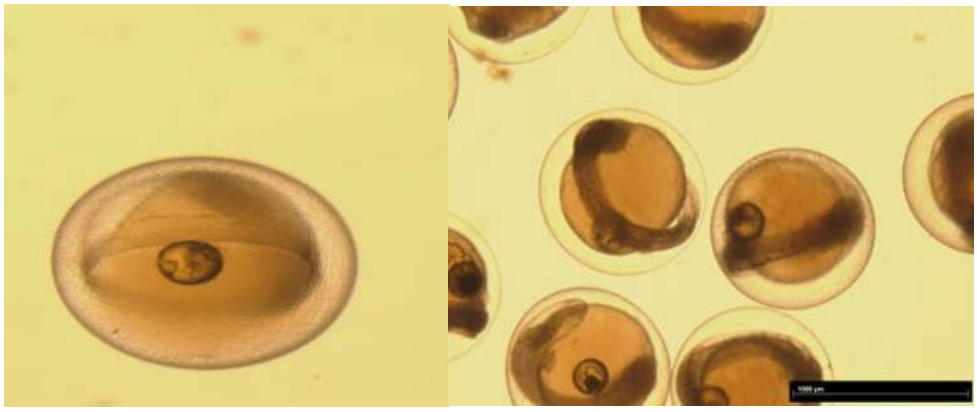


Figure 3.8. Eggs of *Boops boops* from fertilization experiments (IPMA).

3.4 Fecundity and atresia estimation (ToR f)

3.4.1 Methodology for realized fecundity and DEPM estimation

A detailed review was carried out during this Workshop to provide an updated fecundity manual for both species. The table below summarizes the changes in the manual since 2010.

Table 3.2. Changes to the fecundity manual since 2010.

2010	2013	2016
Mackerel		
	Samples are taken for screening for spawning markers and atresia. The results from the histology are used to decide which samples will be analysed for fecundity and which for atresia. Only samples that contain spawning markers and early alpha atresia will be embedded from the cassettes for further atresia analyses.	
	Each cruise will collect 10 samples of one fish (stages 3 to 6) for the fecundity ring test.	
	Ovary lobes need to be pierced with a fine needle before fixation in formaldehyde.	Cut off both ends (1–2 cm depending on the size of the ovary) before fixation in formaldehyde.
Mackerel and Horse mackerel		

2010	2013	2016
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.	Measure the oocyte diameters automatically using ImageJ software provided for the batch fecundity analysis. Count and measure all the oocytes >500 that are not automatically detected.	If possible try using an ultrasound pen to separate the oocyte in whole mounts.
ImageJ and macros will be made available during the wk to all participants and they should use this for analysis of the samples.		Look into the screening slide for the most advanced ovary stage, POFs, hyaline eggs, early alpha atresia, massive atresia, if it is spent and if it should be discarded.
Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.		For 5 mackerel slides would be provided for POF staging calibration between institutes.
For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.		Oocyte development stages is change to stage 1–5. Hyaline eggs is taken out of the oocyte stage as well as the spent stage.
Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample		New screening and POFs staging template
Horse mackerel		
	From 2013 and onwards no samples for potential fecundity are collected. Only DEPM adults parameter samples will be collected.	
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		

3.4.2 Standardization of pipette sampling and picture taking

A correct analysis of the fecundity and atresia samples can only be gained if the pipette samples are taken correctly, and if the pictures from the samples are taken in the right way.

Pipette sampling was shown to all participants at both workshops. At the fecundity workshop, a trial was also done with all participants taking samples of water and

weighing these to check the amount of sample collected. This exercise can be carried out easily at the individual institutes before going on the survey. Water was used because fresh fish were not available for the exercise. (When taking water the weight of the sample will be 25 or 50 mg (depending on the pipette measure), instead of 26 mg or 52 mg for the oocyte samples!)

For the picture, taking of fecundity samples it is important that all oocytes, especially the small oocytes are well separated before taking the picture. IMR carried out a trial with separation of oocytes using ultra sound bath or pen or a vibration pen. The ultra sound bath did not separate the oocytes, however both pens did. Both the ultrasound and vibration pen can be used with the sample in formaldehyde in the original tube. Both pens separate the oocytes well, but the ultrasound pen takes only a few seconds, while the vibration pens takes a few minutes. The procurement cost of a vibration pen is a 100–200 euros but the ultrasound pen is 1000–5000 euros (depending on the country).

Every participant brought fecundity and atresia pictures taken from the 2013 survey samples to be used for the analysis.

3.4.3 Standardization of screening the ovary samples

In 2013 the samples of the survey were first screened using a histological preparation of a small (2–3 gr) ovary sample to decide if the sample should be used for fecundity, batch fecundity or atresia analysis. It is difficult to estimate the maturity stage macroscopically. In 2013, 1163 samples were screened. The histological preparation is time consuming but the results of the screening were good and allowed for an excellent selection of samples to be analysed for (batch) fecundity, atresia and POF analyses. Fewer samples needed to be analysed later on and a lot of time was saved with the analyses of these samples.

The screening samples need be embedded immediately after the survey and screened under a compound microscope. If the most advanced oocyte development stage is previtellogenic or early vitellogenic the samples will not be analysed. Samples with vitellogenic oocytes and containing no migratory nucleus and hydrated oocytes, hyaline eggs or postovulatory follicles (POFs) will be analysed for potential fecundity estimation. Samples with migratory nucleus or hydrated oocytes will be analysed for batch fecundity. If the samples contain vitellogenic, migratory nucleus or hydrated oocytes or hyaline eggs or POFs, these will be used for the atresia analysis. However, only if the sample contains early alpha atretic oocytes will they be embedded and sectioned (Fonn *et al*., 2015). Samples with POFs will be used to estimate spawning frequency through POF staging.

Pictures of seven females from the 2013 survey were analysed during the workshop. Results are presented in Section 4.4.2.

3.4.4 Standardization of mackerel potential fecundity analysis

Images were prepared from 25 µl unstained whole mount samples of mackerel ovary tissue. Each participant scored images of five females for potential fecundity analysis. A different institute photographed each sample, so the picture quality differed per sample. Each participant carried out the automatic measurements of the diameters and counted the number of normal vitellogenic follicles >185 µm in each preparation. Each participant scored the images using ImageJ and ObjectJ following the manual (Fonn *et al*., 2015). The results are presented in Section 4.4.3.

3.4.5 Standardization of mackerel and horse mackerel batch fecundity analysis

Again, batch fecundity samples will be collected during the survey for both mackerel and horse mackerel in order to carry out the DEPM. Batch fecundity analysis will be done on 100 µl whole mount samples. In batch fecundity samples all oocytes > 500 µm are measured and counted (Fonn *et al*., 2015). Images of 1 hydrated female were analysed by all workshop participants. Each participant scored the images using ImageJ and ObjectJ following the manual (Fonn *et al*., 2015). Results of the batch fecundity analysis are presented in Section 4.4.4.

3.4.6 Standardization of atresia estimation for mackerel

The quantification of each early alpha atresia stage follicle classes (yolk vesical, yolk vesical – yolk granule and yolk granule) during the 2013 survey will be carried out from sections stained with haematoxylin and eosin (H&E) Schiff-Mallory Trichrome (SM) or Toluidine blue (TB; as preferred by each institute; Fonn *et al*., 2015). For the workshop, exercise samples with the different staining were available. Everyone analysed all the samples, regardless of the staining they were used to. Images from 3 mackerel ovaries containing atretic oocytes were used for the calibration exercise during the workshop. The atretic follicle classification criteria were based on the mackerel fecundity methods manual. Each participant scored the images using ImageJ and ObjectJ following the manual (Fonn *et al*., 2015). Results of the atresia calibration exercise are presented in Section 4.4.5.

3.4.7 Standardization of Post Ovulatory Follicle (POF) staging

In 2013 the DEPM sampling and analyses was carried out for the first time. WGMEGS decided that for 2016 this method would be carried out again for mackerel and horse mackerel in conjunction with the AEPM for mackerel.

All participants in the 2013 survey found the POF staging very difficult and were unsure. Therefore, a whole day at the workshop was spent on POF staging. First two introductory presentations were shown, presenting descriptions of the 7 POF stages, possible structures that can be confused with POFs, results from 2013 for mackerel and horse mackerel and results of the DEPM for southern horse mackerel.

From the discussions, it became clear that the degeneration of the cells and lumen should be the primary characteristic and size should only be used as a secondary characteristic. POFs are not round structures as are oocytes, hence in a histological slide it is unclear what part of the POF is seen. Thus, it is possible that the POF appears as a small structure, while it is still young and in an early degeneration stage.

A large POF is always in an early stage, while a small POF can be either a young or an old stage. For a correct estimation of POF stage and size, the POFs in the whole sample should be screened. The largest POF is usually the best indicator of the POF size. However, there are indications from the 2013 samples that mackerel spawn batches daily. In some samples, hydrated oocytes and day 0 POFs are found. Thus, it is possible to find multiple POF stages in mackerel samples.

If multiple stages are found in the sample the analyst should decide what the POF stage of the whole sample is. This decision should be based on the following rule: If there is a dominant stage the POF stage of the sample is the dominant stage. If the numbers of POFs of the different stages are all similar, the sample POF stage is the youngest stage.

For the 2016 sample analyses for each sample presence or absence of all 7 POF stages found in the sample will be recorded. The analyst should also present a final POF stage for the whole sample (based on the above rule) and in the comment field it should be noted why the final stage is assessed.

Care should be taken when assessing size on how the sample was collected or the histological section prepared. POFs are expanded in spoon samples compared to a slice of the whole ovary. Also in paraffin sections POFs are expanded, while in resin POFs keep their original size.

Because all participants were uncertain of the POF stages the samples were analysed in plenary, rather than individually. Results of the POF staging discussions are presented in Section 4.4.6.

4 Results

4.1 Result of egg sorting exercise

Two plankton samples were prepared with a known number of fish eggs (a mix of mackerel and horse mackerel and other eggs typical for survey samples) present in each. There were widely fluctuating results in determining egg numbers and increasing damage to the eggs. Table 4.1 shows the numbers of eggs removed by each use of the spray technique. Most participants appeared to have removed more eggs than originally occurred in the sample. During the survey, participants remove all items that appear like fish eggs and during the actual egg identification under the microscope these items are subsequently removed again.

4.2 Result of egg staging exercises

The results of the egg staging exercises are given in Tables 4.2.1 to 4.2.12.

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). Half of the participants at the workshop were inexperienced; hence, results of the expert readers only are also presented separately (Table 4.2.4 – 4.2.6). Tables 4.2.7 to 4.2.12 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 unvalidated egg (i.e. those not 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 4.2.3 – 4.2.6 and 4.2.9 – 4.2.12.

Tables 4.2.1 to 4.2.12 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.

Table 4.1. Results of the egg sorting exercise. (Original is the number of eggs that were put in the sample.)

Participant	Sprayer 1	Sprayer 2	Sprayer 3	Sprayer 4	Sprayer 5	Sprayer 6	Sprayer 7	Sprayer 8	Sprayer 9	Sprayer 10	Sprayer 11
Sample number	**173**	**173**	**173**	**173**	**173**	**173**	**173**	**173**	**173**	**173**	**173**
Spray 1	147	131	126	139	128	134	125	113	127	108	120
Spray 2	5	13	7	3	5	10	4	6	3	19	3
Spray 3											
Spray 4											
Eggs left	3	6		0	6	3				0	1
Total	155	150	133	142	139	147	129	119	130	127	124
Original	118	118	118	118	118	118	118	118	118	118	118

Participant	Sprayer 12	Sprayer 13	Sprayer 14	Sprayer 15	Sprayer 16
Sample number	**162**	**162**	**162**	**162**	**162**
Spray 1	126	118	126	121	119
Spray 2	3	8	5	4	6
Spray 3	0	1	1	1	0
Spray 4					
Eggs left	1	0	0	0	1
Total	130	127	132	126	126
Original	111	111	111	111	111

By studying the results presented in Tables 4.2.1 to 4.2.12, 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 64% in the first round (Table 4.2.1). This increased to 72% agreement in the second round of analysis (Table 4.2.7). The agreement between the expert readers was higher compared to overall and increased from 74% to 82% (Table 4.2.2 and 4.2.8). The overall agreement for all egg stages, for mackerel, increased from 65% (Table 4.2.3) to 76% (Table 4.2.9), and for horse mackerel increased from 69% (Table 4.2.5) to 72% (Table 4.2.11). For the experts agreement for all egg stages, for mackerel, increased from 74% (Table 4.2.4) to 86% (Table 4.2.10), and for horse mackerel remained at the same level 82% (Table 4.2.6 and 4.2.12).

The overall agreement for stage 1 (1a+1b) eggs also shows improvements but with an overall greater level of agreement, from 88% in the first round to 90% in the second round. 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 88% in the first round (Table 4.2.1). This increased to 90% agreement in the second round of analysis (Table 4.2.7). Agreement between the experts was somewhat higher to overall agreement, 92%, but this did not change from the first to the second round (Tables 4.2.2 and 4.2.8). The overall agreement of stage 1 eggs, for mackerel, increased from 88% (Table 4.2.3) to 92% (Table 4.2.9), and for horse mackerel from 87% (Table 4.2.5) to 88% (Table 4.2.11). For experts agreement of stage 1 eggs, for mackerel, increased from 93% (Table 4.2.4) to 94% (Table 4.2.10), and for horse mackerel remained at the same level 82% (Table 4.2.6 and 4.2.12).

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 underestimate of 3% for eggs of all species but individual bias ranged from an underestimate of 30% to an overestimate of 16% (Table 4.2.1). In the second round, this remained the same, but the range of individual bias reduced to between -20% to 11% (Table 4.2.7). For the experts the overall bias was an overestimate of 1% for eggs of all species but individual bias ranged from an underestimate of 28% to an overestimate of 14% (Table 4.2.2). In the second round this was an underestimate of 2%, but the range of individual bias reduced to a between -17% to 15% (Table 4.2.8).

The overall bias for stage 1 mackerel eggs (Tables 4.2.3 and 4.2.9) was -3% in the first round and -4% in the second round of analysis. However, the bias of individual participants was much greater, ranging from -27% to 16% in the first round, but improving to from -26% to 6% in the second round of analysis. For experts the overall bias for mackerel stage 1 was -3% in the first round and -4% in the second (Tables 4.2.4 and 4.2.10). Individual bias ranged from -23% to 14% and improved to -24% to 7% in the second round. The overall bias for stage 1 horse mackerel eggs (Tables 4.2.5 and 4.2.11) was 5% in the first round to -1% in the second round of analysis. However, the bias of individual participants was again much greater, ranging from -56% to 38% in the first round, but improving to between -28% and 16% in the second round of analysis. For experts the overall bias for horse mackerel stage 1 was -3% in both rounds (Tables 4.2.6 and 4.2.12). Individual bias for horse mackerel in the first round ranged from -75% to 14% and improved from -26% to 13%.

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	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	113	116	120	121	121	120	122	102	114	120	120	118	121	121	112	119	115	113	2108
Stage 1b ==>	1	9	11	11	11	11	10	11	9	10	11	11	11	11	11	9	10	11	11	189
Stage 2 ==>	2	32	32	32	32	32	30	32	29	30	32	32	32	32	32	31	32	32	32	568
Stage 3 ==>	3	20	21	21	21	21	21	21	20	19	21	21	21	21	21	21	21	21	21	374
Stage 4 ==>	4	4	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	88
Stage 5 ==>	5	6	7	7	7	7	7	7	6	7	7	7	6	7	7	7	7	7	7	123
Total	0-5	184	192	196	197	197	193	198	170	185	196	196	193	197	197	185	194	191	189	3450

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	82	101	92	78	120	130	87	69	44	57	124	128	106	101	85	68	99	49	1620
Stage 1b ==>	1	4	17	49	37	18	16	55	49	62	47	6	3	25	26	36	81	41	47	619
Stage 2 ==>	2	61	38	34	57	28	15	25	27	36	54	41	32	43	33	23	22	32	46	647
Stage 3 ==>	3	27	25	8	14	15	14	18	20	22	21	15	14	17	21	21	10	8	39	329
Stage 4 ==>	4	7	9	8	4	16	10	10	5	14	13	4	9	3	10	12	12	6	4	156
Stage 5 ==>	5	3	2	5	7	-	8	3	-	7	4	6	7	3	6	8	1	5	4	79
Total	0-5	184	192	196	197	197	193	198	170	185	196	196	193	197	197	185	194	191	189	3450

C

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

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	-30%	-7%	8%	-13%	5%	12%	7%	6%	-15%	-21%	-1%	2%	-1%	-4%	0%	16%	11%	-23%	-3%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	70%	72%	73%	60%	87%	91%	63%	53%	38%	47%	95%	98%	72%	80%	71%	55%	75%	42%	69%
Stage 1b ==>	1	22%	36%	73%	45%	27%	30%	45%	67%	50%	45%	27%	9%	36%	55%	56%	100%	82%	73%	49%
Stage 2 ==>	2	81%	38%	59%	91%	69%	30%	56%	31%	43%	81%	84%	84%	38%	81%	55%	31%	53%	56%	59%
Stage 3 ==>	3	95%	48%	38%	62%	57%	33%	62%	65%	58%	71%	62%	62%	43%	67%	62%	33%	24%	90%	57%
Stage 4 ==>	4	100%	60%	80%	60%	100%	40%	100%	25%	20%	80%	60%	80%	20%	80%	60%	80%	40%	60%	64%
Stage 5 ==>	5	50%	29%	57%	86%	0%	86%	43%	0%	43%	43%	71%	100%	43%	71%	86%	14%	57%	57%	52%
Weighted mean	0-5	72.3%	59.9%	66.3%	65.0%	74.6%	70.5%	61.1%	48.8%	41.1%	55.6%	84.2%	86.5%	58.9%	77.2%	66.5%	50.0%	64.4%	52.4%	64.3%
	RANKING	5	12	8	9	4	6	11	17	18	14	2	1	13	3	7	16	10	15	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	68%	79%	96%	80%	98%	98%	98%	85%	80%	76%	93%	97%	83%	92%	95%	100%	96%	70%	88%
RANKING	18	15	7	13	3	4	2	11	14	16	9	5	12	10	8	1	6	17	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	0.60	0.55	0.29	0.55	0.17	0.13	0.39	0.64	0.82	0.74	0.09	0.02	0.46	0.25	0.33	0.45	0.32	0.96	0.43
Stage 1b ==>	1	0.11	-0.09	0.27	0.36	-0.73	-0.70	-0.18	0.11	0.30	0.55	-0.36	-0.55	-0.27	0.00	-0.22	0.00	-0.18	0.18	-0.08
Stage 2 ==>	2	0.19	-0.56	-0.53	-0.19	-0.31	-0.67	-0.34	-1.00	0.23	0.09	-0.28	-0.22	-0.84	0.13	0.19	-0.66	-0.66	0.06	-0.30
Stage 3 ==>	3	-0.05	-0.52	-0.52	-0.38	-0.24	-0.19	-0.43	-0.35	0.32	0.19	-0.38	0.10	-0.67	-0.05	0.10	-0.48	-1.10	-0.14	-0.27
Stage 4 ==>	4	0.00	-1.00	0.20	0.00	0.00	-0.80	0.00	-1.50	0.80	-0.20	0.00	0.20	-1.00	0.20	0.40	-0.20	-0.40	-1.00	-0.23
Stage 5 ==>	5	-0.50	-1.14	-0.86	-0.71	-1.00	-0.14	-0.57	-2.33	-0.57	-0.57	-0.57	0.00	-1.57	-0.29	-0.14	-0.86	-0.57	-0.86	-0.73

Table 4.2.2. All eggs first staging, expert readers only.
(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	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ⟹	0	118	126	127	127	126	128	126	124	127	**1129**
Stage 1b ⟹	1	1	2	2	2	2	2	2	2	2	**17**
Stage 2 ⟹	2	37	37	37	37	34	37	37	37	37	**330**
Stage 3 ⟹	3	18	19	19	19	19	19	19	19	19	**170**
Stage 4 ⟹	4	5	6	6	6	6	6	6	6	6	**53**
Stage 5 ⟹	5	5	6	6	6	6	6	6	5	6	**52**
Total	0-5	**184**	**196**	**197**	**197**	**193**	**198**	**196**	**193**	**197**	**1751**

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ⟹	0	82	92	78	120	130	87	124	128	101	**942**
Stage 1b ⟹	1	4	49	37	18	16	55	6	3	26	**214**
Stage 2 ⟹	2	61	34	57	28	15	25	41	32	33	**326**
Stage 3 ⟹	3	27	8	14	15	14	18	15	14	21	**146**
Stage 4 ⟹	4	7	8	4	16	10	10	4	9	10	**78**
Stage 5 ⟹	5	3	5	7	-	8	3	6	7	6	**45**
Total	0-5	**184**	**196**	**197**	**197**	**193**	**198**	**196**	**193**	**197**	**1751**

C

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

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	**-28%**	**10%**	**-11%**	**7%**	**14%**	**9%**	**2%**	**4%**	**-2%**	**1%**

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ⟹	0	69%	69%	57%	87%	90%	60%	94%	98%	79%	**78%**
Stage 1b ⟹	1	100%	50%	100%	100%	0%	0%	100%	0%	100%	**59%**
Stage 2 ⟹	2	78%	62%	89%	65%	32%	51%	81%	78%	76%	**68%**
Stage 3 ⟹	3	100%	42%	63%	63%	37%	63%	68%	58%	68%	**62%**
Stage 4 ⟹	4	80%	67%	50%	100%	50%	100%	50%	67%	83%	**72%**
Stage 5 ⟹	5	40%	50%	100%	0%	100%	50%	83%	100%	83%	**67%**
Weighted mean	0-5	**73.4%**	**64.3%**	**65.5%**	**78.2%**	**73.1%**	**59.1%**	**87.8%**	**88.1%**	**77.7%**	**74.1%**
	RANKING	**5**	**8**	**7**	**3**	**6**	**9**	**2**	**1**	**4**	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	**70%**	**96%**	**83%**	**98%**	**98%**	**98%**	**95%**	**99%**	**93%**	**93%**
RANKING	**9**	**5**	**8**	**2**	**3**	**4**	**6**	**1**	**7**	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ⟹	0	0.62	0.33	0.57	0.17	0.13	0.43	0.10	0.02	0.28	**0.29**
Stage 1b ⟹	1	0.00	0.50	0.00	0.00	-1.00	-1.00	0.00	-1.00	0.00	**-0.29**
Stage 2 ⟹	2	0.14	-0.49	-0.14	-0.43	-0.65	-0.41	-0.32	-0.19	0.08	**-0.26**
Stage 3 ⟹	3	0.00	-0.47	-0.37	-0.16	-0.11	-0.42	-0.32	0.11	0.00	**-0.19**
Stage 4 ⟹	4	0.20	0.33	-0.67	0.00	-0.67	0.00	-0.33	0.33	0.17	**-0.08**
Stage 5 ⟹	5	-0.60	-1.00	0.00	-1.00	0.00	-0.50	-0.17	0.00	-0.17	**-0.38**

Table 4.2.3. 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	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	85	89	90	80	64	88	100	80	76	79	79	88	77	90	27	81	91	68	1432
Stage 1b ==>	1	10	10	10	9	8	10	11	8	8	8	10	10	9	9	9	12	13	8	172
Stage 2 ==>	2	26	20	26	26	23	21	22	15	25	13	25	26	18	20	24	26	25	17	398
Stage 3 ==>	3	13	14	15	14	16	13	14	10	16	6	15	16	12	15	17	16	17	8	247
Stage 4 ==>	4	3	4	3	3	5	3	3	4	4	2	3	3	4	3	4	5	4	1	61
Stage 5 ==>	5	4	2	5	5	5	6	3	3	7	2	5	5	4	4	7	5	6	4	82
Total	0-5	141	139	149	137	121	141	153	120	136	110	137	148	124	141	88	145	156	106	2392

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	65	78	66	45	64	99	66	37	29	38	84	98	61	74	26	44	83	34	1091
Stage 1b ==>	1	4	13	41	29	12	10	51	45	46	32	4	2	15	21	11	64	30	28	458
Stage 2 ==>	2	46	28	26	44	17	12	15	21	23	26	28	23	31	22	16	19	28	27	452
Stage 3 ==>	3	19	14	7	11	15	6	14	14	19	8	14	13	12	14	18	8	5	13	224
Stage 4 ==>	4	5	6	5	3	13	6	5	3	12	3	1	7	2	7	9	10	6	-	103
Stage 5 ==>	5	2	-	4	5	-	8	2	-	7	3	6	5	3	3	8	-	4	4	64
Total	0-5	141	139	149	137	121	141	153	120	136	110	137	148	124	141	88	145	156	106	2392

C

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

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	-27%	-8%	7%	-17%	6%	11%	5%	-7%	-11%	-20%	-1%	2%	-12%	-4%	3%	16%	9%	-18%	-3%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	73%	74%	70%	55%	84%	93%	58%	44%	37%	48%	96%	99%	70%	78%	70%	53%	79%	49%	69%
Stage 1b ==>	1	20%	30%	80%	44%	38%	20%	45%	75%	63%	63%	30%	10%	44%	44%	33%	100%	85%	88%	51%
Stage 2 ==>	2	81%	45%	65%	96%	70%	43%	45%	47%	44%	77%	84%	73%	50%	90%	58%	42%	64%	59%	64%
Stage 3 ==>	3	100%	43%	47%	71%	81%	46%	57%	70%	50%	67%	73%	56%	33%	67%	65%	44%	24%	75%	58%
Stage 4 ==>	4	100%	75%	67%	100%	100%	67%	100%	50%	25%	50%	33%	100%	0%	100%	75%	80%	50%	0%	67%
Stage 5 ==>	5	50%	0%	60%	100%	0%	100%	67%	0%	57%	100%	100%	100%	50%	75%	100%	0%	67%	100%	66%
Weighted mean	0-5	73.0%	62.6%	67.1%	66.4%	75.2%	75.9%	56.2%	47.5%	41.9%	54.5%	85.4%	83.8%	58.9%	76.6%	64.8%	53.1%	69.9%	56.6%	65.4%
	RANKING	6	11	8	9	5	4	14	17	18	15	1	2	12	3	10	16	7	13	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	70%	79%	95%	80%	96%	96%	98%	85%	84%	79%	94%	98%	80%	91%	93%	100%	95%	74%	88%
RANKING	18	16	7	14	5	4	2	11	12	15	8	3	13	10	9	1	6	17	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	0.53	0.53	0.32	0.59	0.22	0.11	0.42	0.75	0.80	0.73	0.09	0.01	0.52	0.27	0.37	0.47	0.30	0.82	0.43
Stage 1b ==>	1	0.20	-0.10	0.20	0.33	-0.63	-0.40	-0.18	0.25	0.13	0.38	-0.50	-0.70	-0.11	0.00	-0.22	0.00	-0.15	0.13	-0.09
Stage 2 ==>	2	0.19	-0.60	-0.42	0.04	-0.30	-1.00	-0.14	-0.40	0.28	0.23	-0.16	-0.08	-0.50	0.10	0.13	-0.62	-0.60	0.06	-0.21
Stage 3 ==>	3	0.00	-0.71	-0.40	-0.29	-0.06	-0.23	-0.50	-0.40	0.38	0.33	-0.27	0.06	-0.50	-0.07	0.12	-0.44	-1.18	-0.38	-0.26
Stage 4 ==>	4	0.00	-1.00	0.33	0.00	0.00	0.33	0.00	-0.75	0.75	-0.50	0.00	0.00	-0.50	0.00	0.25	-0.20	-0.75	-4.00	-0.20
Stage 5 ==>	5	-0.50	-1.50	-1.00	0.00	-1.00	0.00	-0.33	-2.33	-0.43	0.00	0.00	0.00	-0.75	-0.25	0.00	-1.00	-0.33	0.00	-0.45

Table 4.2.4. Mackerel eggs first staging, expert readers only.
(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	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ⟹	0	89	95	83	67	90	101	84	91	94	794
Stage 1b ⟹	1	1	2	3	3	3	3	2	3	2	22
Stage 2 ⟹	2	29	28	30	25	21	26	28	30	24	241
Stage 3 ⟹	3	13	15	13	16	13	14	15	16	14	129
Stage 4 ⟹	4	3	3	3	4	2	3	3	3	3	27
Stage 5 ⟹	5	3	5	5	5	5	3	5	5	4	40
Total	0-5	138	148	137	120	134	150	137	148	141	1253

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ⟹	0	65	65	45	64	96	64	84	98	74	655
Stage 1b ⟹	1	4	41	29	12	10	50	4	2	21	173
Stage 2 ⟹	2	45	26	44	17	10	15	28	23	22	230
Stage 3 ⟹	3	18	7	11	15	6	14	14	13	14	112
Stage 4 ⟹	4	5	5	3	12	6	5	1	7	7	51
Stage 5 ⟹	5	1	4	5	-	6	2	6	5	3	32
Total	0-5	138	148	137	120	134	150	137	148	141	1253

C

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

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	-23%	9%	-14%	9%	14%	10%	2%	6%	-1%	1%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ⟹	0	73%	65%	54%	84%	91%	55%	95%	99%	78%	77%
Stage 1b ⟹	1	100%	50%	67%	67%	0%	33%	100%	33%	100%	55%
Stage 2 ⟹	2	86%	64%	97%	60%	33%	42%	79%	70%	88%	70%
Stage 3 ⟹	3	100%	47%	77%	75%	38%	64%	80%	63%	64%	67%
Stage 4 ⟹	4	100%	67%	100%	100%	100%	100%	33%	100%	100%	89%
Stage 5 ⟹	5	33%	60%	100%	0%	100%	67%	100%	100%	75%	73%
Weighted mean	0-5	78.3%	62.8%	68.6%	74.2%	75.4%	54.7%	89.1%	87.8%	78.7%	74.2%
	RANKING	4	8	7	6	5	9	1	2	3	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
	1a+1b	73%	96%	83%	97%	98%	98%	96%	100%	94%	93%
	RANKING	9	5	8	4	3	2	6	1	7	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ⟹	0	0.52	0.37	0.59	0.22	0.14	0.47	0.11	0.01	0.27	0.30
Stage 1b ⟹	1	0.00	0.50	0.33	-0.33	-1.00	-0.67	0.00	-0.67	0.00	-0.27
Stage 2 ⟹	2	0.14	-0.43	0.03	-0.40	-1.00	-0.38	-0.36	-0.30	0.13	-0.27
Stage 3 ⟹	3	0.00	-0.40	-0.23	-0.13	-0.31	-0.43	-0.20	0.13	-0.07	-0.18
Stage 4 ⟹	4	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
Stage 5 ⟹	5	-0.67	-1.00	0.00	-1.00	0.00	-0.33	0.00	0.00	-0.25	-0.35

Table 4.2.5. 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	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	9	6	9	8	8	12	14	11	15	24	7	10	20	14	21	8	5	19	220
Stage 1b ==>	1	-	2	1	-	-	-	-	-	4	3	-	-	4	-	6	-	-	3	-
Stage 2 ==>	2	3	4	3	2	2	3	7	5	4	9	2	1	7	4	5	3	-	14	78
Stage 3 ==>	3	-	5	-	-	-	4	1	3	-	6	-	-	6	-	1	-	-	6	-
Stage 4 ==>	4	-	-	1	-	-	-	1	-	-	3	1	1	2	-	1	-	-	2	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	12	17	14	10	10	19	23	19	23	45	10	12	39	18	34	11	5	44	365

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	4	3	9	4	9	11	13	10	10	12	7	10	23	11	24	6	3	7	176
Stage 1b ==>	1	-	2	2	1	-	1	3	2	6	10	-	-	5	3	6	5	2	8	56
Stage 2 ==>	2	8	5	2	5	1	1	5	4	6	13	2	1	6	4	2	-	-	14	79
Stage 3 ==>	3	-	6	-	-	-	5	1	3	1	7	-	-	4	-	1	-	-	13	41
Stage 4 ==>	4	-	1	1	-	-	1	1	-	-	3	1	1	1	-	1	-	-	2	13
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	12	17	14	10	10	19	23	19	23	45	10	12	39	18	34	11	5	44	365

C

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

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	-56%	-38%	10%	-38%	13%	0%	14%	9%	-16%	-19%	0%	0%	17%	0%	11%	38%	0%	-32%	5%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	44%	50%	89%	50%	100%	92%	86%	91%	53%	46%	86%	100%	90%	79%	100%	63%	60%	37%	73%
Stage 1b ==>	1	-	50%	100%	-	-	-	-	-	50%	67%	-	-	50%	-	67%	-	-	33%	-
Stage 2 ==>	2	100%	75%	67%	100%	50%	33%	57%	60%	75%	78%	50%	100%	29%	75%	40%	0%	-	50%	65%
Stage 3 ==>	3	-	60%	-	-	-	75%	0%	100%	-	83%	-	-	50%	-	100%	-	-	100%	-
Stage 4 ==>	4	-	-	100%	-	-	-	100%	-	-	100%	100%	100%	50%	-	100%	-	-	100%	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	58.3%	58.8%	85.7%	60.0%	90.0%	78.9%	73.9%	84.2%	56.5%	62.2%	80.0%	100.0%	66.7%	77.8%	85.3%	45.5%	60.0%	52.3%	69.3%
	RANKING	15	14	3	12	2	7	9	5	16	11	6	1	10	8	4	18	12	17	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	44%	56%	100%	63%	100%	100%	100%	93%	85%	83%	88%	100%	92%	93%	100%	100%	100%	63%	87%
RANKING	18	17	1	16	1	1	1	9	13	14	12	1	11	9	1	1	1	15	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	1.11	1.00	0.11	0.88	0.00	0.08	0.14	0.18	0.73	0.75	0.29	0.00	0.20	0.29	0.00	0.38	0.40	1.11	0.43
Stage 1b ==>	1	-	1.00	0.00	-	-	-	-	-	0.00	-0.33	-	-	-0.50	-	-0.33	-	-	0.67	-
Stage 2 ==>	2	0.00	0.25	-0.67	0.00	-1.00	0.67	-0.29	-0.40	-0.50	0.22	-1.00	0.00	-0.86	-0.25	-0.80	-1.33	-	0.21	-0.24
Stage 3 ==>	3	-	0.00	-	-	-	0.25	-1.00	0.00	-	-0.17	-	-	-0.83	-	0.00	-	-	0.00	-
Stage 4 ==>	4	-	-	0.00	-	-	-	0.00	-	-	0.00	0.00	0.00	-1.00	-	0.00	-	-	0.00	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.6. Horse mackerel eggs first staging, expert readers only.
(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	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ==>	0	4	7	8	8	8	9	7	9	12	72
Stage 1b ==>	1	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>	2	2	3	2	2	-	3	2	1	2	17
Stage 3 ==>	3	-	-	-	-	-	-	-	-	-	-
Stage 4 ==>	4	-	1	-	-	-	-	1	1	-	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-
Total	0-5	6	11	10	10	8	12	10	11	14	92

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ==>	0	1	8	4	9	7	8	7	9	10	63
Stage 1b ==>	1	-	-	1	-	1	2	-	-	3	7
Stage 2 ==>	2	5	2	5	1	-	2	2	1	1	19
Stage 3 ==>	3	-	-	-	-	-	-	-	-	-	-
Stage 4 ==>	4	-	1	-	-	-	-	1	1	-	3
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-
Total	0-5	6	11	10	10	8	12	10	11	14	92

C

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

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
	1a+1b	-75%	14%	-38%	13%	0%	11%	0%	0%	8%	-3%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ==>	0	25%	100%	50%	100%	88%	89%	86%	100%	83%	83%
Stage 1b ==>	1	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>	2	100%	67%	100%	50%	-	67%	50%	100%	50%	71%
Stage 3 ==>	3	-	-	-	-	-	-	-	-	-	-
Stage 4 ==>	4	-	100%	-	-	-	-	100%	100%	-	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	50.0%	90.9%	60.0%	90.0%	87.5%	83.3%	80.0%	100.0%	78.6%	81.5%
	RANKING	9	2	8	3	4	5	6	1	7	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
	1a+1b	25%	100%	63%	100%	100%	100%	86%	100%	100%	91%
	RANKING	9	1	8	1	1	1	7	1	1	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ==>	0	1.50	0.00	0.88	0.00	0.13	0.11	0.29	0.00	0.17	0.26
Stage 1b ==>	1	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>	2	0.00	-0.67	0.00	-1.00	-	-0.33	-1.00	0.00	-0.50	-0.47
Stage 3 ==>	3	-	-	-	-	-	-	-	-	-	-
Stage 4 ==>	4	-	0.00	-	-	-	-	0.00	0.00	-	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-

Table 4.2.7. 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	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	160	152	151	165	164	164	165	164	161	161	163	161	165	152	152	165	165	162	2892
Stage 1b ==>	1	1	2	2	2	2	2	2	2	1	1	2	1	2	2	2	2	2	2	32
Stage 2 ==>	2	31	31	31	30	31	31	31	30	31	31	31	31	31	29	24	31	31	30	546
Stage 3 ==>	3	50	42	49	48	48	51	50	50	48	48	48	45	51	46	47	48	51	51	871
Stage 4 ==>	4	19	18	18	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	340
Stage 5 ==>	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	36
Total	0-5	263	247	253	266	266	269	269	267	262	262	265	259	270	250	246	267	270	266	4717

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	155	149	99	106	163	169	143	106	163	94	139	151	157	135	29	81	163	130	2332
Stage 1b ==>	1	3	4	30	27	4	15	20	30	15	52	8	4	6	9	126	87	21	32	493
Stage 2 ==>	2	37	33	58	71	34	13	35	54	7	38	55	43	74	34	31	62	36	34	749
Stage 3 ==>	3	53	49	39	44	35	49	41	59	38	53	38	45	25	51	32	23	41	54	769
Stage 4 ==>	4	14	11	25	17	28	21	28	14	33	22	23	15	7	19	27	14	7	14	339
Stage 5 ==>	5	1	1	2	1	2	2	2	4	6	3	2	1	1	2	1	-	2	2	35
Total	0-5	263	247	253	266	266	269	269	267	262	262	265	259	270	250	246	267	270	266	4717

C

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

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	-2%	-1%	-16%	-20%	1%	11%	-2%	-18%	10%	-10%	-11%	-4%	-2%	-6%	1%	1%	10%	-1%	-3%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	94%	88%	65%	61%	96%	91%	85%	62%	89%	58%	83%	91%	87%	86%	16%	47%	92%	77%	76%
Stage 1b ==>	1	0%	0%	50%	50%	100%	50%	100%	50%	100%	100%	50%	100%	0%	50%	50%	50%	50%	50%	53%
Stage 2 ==>	2	84%	39%	90%	87%	81%	29%	74%	50%	13%	65%	87%	94%	68%	76%	50%	65%	55%	43%	64%
Stage 3 ==>	3	90%	79%	78%	79%	65%	65%	70%	84%	54%	79%	69%	87%	31%	87%	40%	33%	63%	86%	69%
Stage 4 ==>	4	58%	44%	89%	74%	84%	47%	89%	47%	79%	68%	95%	79%	32%	74%	53%	53%	37%	68%	65%
Stage 5 ==>	5	50%	0%	100%	50%	100%	100%	100%	100%	100%	100%	100%	50%	50%	100%	50%	0%	100%	50%	72%
Weighted mean	0-5	88.6%	75.7%	72.3%	67.7%	87.6%	75.8%	81.8%	63.7%	73.3%	63.7%	81.9%	89.6%	69.6%	83.6%	27.6%	46.8%	77.8%	74.1%	72.4%
	RANKING	2	9	12	14	3	8	6	16	11	15	5	1	13	4	18	17	7	10	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
	1a+1b	93%	89%	81%	74%	96%	99%	93%	78%	97%	86%	86%	92%	90%	90%	90%	93%	99%	91%	90%
	RANKING	5	13	16	18	4	2	6	17	3	14	15	8	12	10	11	6	1	9	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	0.12	0.24	0.52	0.63	0.08	0.09	0.19	0.60	0.15	0.58	0.31	0.17	0.22	0.26	0.95	0.61	0.08	0.31	0.34
Stage 1b ==>	1	-1.00	0.00	-0.50	0.50	0.00	-0.50	0.00	-0.50	0.00	0.00	-0.50	0.00	-1.00	-0.50	0.50	0.50	-0.50	-0.50	-0.22
Stage 2 ==>	2	-0.23	-0.52	0.00	0.13	-0.29	-0.71	0.03	0.13	-0.71	0.16	-0.03	-0.03	-0.55	-0.07	-0.13	-0.45	-0.74	-0.37	-0.25
Stage 3 ==>	3	-0.06	-0.21	0.10	-0.21	0.15	0.00	0.10	0.04	0.23	0.17	-0.15	-0.13	-0.75	0.09	-0.11	-0.60	-0.43	-0.10	-0.11
Stage 4 ==>	4	-0.47	-0.72	-0.17	-0.63	-0.32	-0.68	-0.16	-0.53	-0.05	-0.47	-0.11	-0.53	-1.00	-0.42	-0.89	-0.68	-0.89	-0.26	-0.50
Stage 5 ==>	5	-0.50	-2.00	0.00	-2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-2.50	-1.00	0.00	-0.50	-1.50	0.00	-1.00	-0.64

Table 4.2.8. All eggs second staging, expert readers only.
(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	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ==>	0	154	146	158	157	157	158	156	155	145	1386
Stage 1b ==>	1	2	3	3	3	3	3	3	2	3	25
Stage 2 ==>	2	38	37	38	39	39	39	39	38	37	344
Stage 3 ==>	3	45	45	44	44	46	45	44	42	41	396
Stage 4 ==>	4	22	20	21	21	22	22	21	20	22	191
Stage 5 ==>	5	2	2	2	2	2	2	2	2	2	18
Total	0-5	263	253	266	266	269	269	265	259	250	2360

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ==>	0	155	99	106	163	169	143	139	151	135	1260
Stage 1b ==>	1	3	30	27	4	15	20	8	4	9	120
Stage 2 ==>	2	37	58	71	34	13	35	55	43	34	380
Stage 3 ==>	3	53	39	44	35	49	41	38	45	51	395
Stage 4 ==>	4	14	25	17	28	21	28	23	15	19	190
Stage 5 ==>	5	1	2	1	2	2	2	2	1	2	15
Total	0-5	263	253	266	266	269	269	265	259	250	2360

C

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

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
	1a+1b	1%	-13%	-17%	4%	15%	1%	-8%	-1%	-3%	-2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ==>	0	95%	67%	63%	97%	92%	88%	87%	94%	88%	86%
Stage 1b ==>	1	0%	33%	67%	67%	33%	100%	67%	50%	67%	56%
Stage 2 ==>	2	82%	78%	89%	74%	23%	69%	87%	92%	65%	73%
Stage 3 ==>	3	96%	78%	86%	68%	65%	78%	75%	90%	88%	80%
Stage 4 ==>	4	50%	85%	71%	86%	55%	95%	95%	75%	73%	76%
Stage 5 ==>	5	50%	100%	50%	100%	100%	100%	100%	50%	100%	83%
Weighted mean	0-5	88.6%	71.9%	71.4%	88.0%	73.6%	84.4%	85.7%	91.1%	83.2%	82.0%
	RANKING	2	8	9	3	7	5	4	1	6	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
	1a+1b	97%	83%	77%	98%	99%	97%	90%	96%	93%	92%
	RANKING	4	8	9	2	1	3	7	5	6	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ==>	0	0.10	0.51	0.58	0.04	0.08	0.16	0.24	0.11	0.20	0.22
Stage 1b ==>	1	0.00	0.00	0.33	-0.33	-0.67	0.00	-0.33	-0.50	-0.33	-0.20
Stage 2 ==>	2	-0.37	-0.03	0.05	-0.44	-0.82	-0.08	-0.08	-0.08	-0.14	-0.22
Stage 3 ==>	3	-0.04	0.04	-0.14	0.14	-0.09	0.00	-0.11	-0.10	0.02	-0.03
Stage 4 ==>	4	-0.55	-0.20	-0.71	-0.14	-0.45	-0.09	-0.05	-0.40	-0.27	-0.32
Stage 5 ==>	5	-0.50	0.00	-2.50	0.00	0.00	0.00	0.00	-2.50	0.00	-0.61

Table 4.2.9. 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	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	106	66	45	60	51	83	79	89	61	66	56	59	69	68	63	75	96	90	1282
Stage 1b ==>	1	4	1	1	1	1	4	1	5	-	-	1	-	1	1	3	1	4	3	32
Stage 2 ==>	2	15	10	12	12	10	9	9	9	10	9	9	10	10	10	2	10	13	14	183
Stage 3 ==>	3	30	11	10	12	12	13	10	18	13	12	13	11	16	11	6	13	23	20	254
Stage 4 ==>	4	10	2	2	2	4	5	3	8	4	4	2	2	3	2	4	3	6	8	74
Stage 5 ==>	5	1	2	2	1	2	2	2	2	2	2	1	-	1	2	-	2	1	1	26
Total	0-5	166	92	72	88	80	116	104	131	90	93	82	82	100	94	78	104	143	136	1851

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	105	66	35	32	50	85	69	53	52	42	53	58	63	56	15	49	98	73	1054
Stage 1b ==>	1	2	3	5	13	2	5	9	24	9	20	1	-	3	6	53	29	8	13	205
Stage 2 ==>	2	19	9	18	31	12	5	11	21	4	8	14	10	25	11	2	17	16	18	251
Stage 3 ==>	3	30	11	10	9	7	12	8	20	12	17	11	11	7	17	5	7	15	22	231
Stage 4 ==>	4	9	2	2	2	7	7	5	9	9	3	2	3	1	2	3	2	5	8	81
Stage 5 ==>	5	1	1	2	1	2	2	2	4	4	3	1	-	1	2	-	-	1	2	29
Total	0-5	166	92	72	88	80	116	104	131	90	93	82	82	100	94	78	104	143	136	1851

C

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

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	-3%	3%	-13%	-26%	0%	3%	-3%	-18%	0%	-6%	-5%	-2%	-6%	-10%	3%	3%	6%	-8%	-4%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	94%	91%	78%	53%	96%	95%	86%	60%	80%	62%	91%	97%	86%	82%	22%	64%	95%	80%	79%
Stage 1b ==>	1	0%	0%	100%	100%	100%	25%	100%	60%	-	-	0%	-	0%	100%	100%	100%	50%	67%	58%
Stage 2 ==>	2	80%	40%	100%	100%	90%	33%	78%	44%	10%	56%	89%	80%	80%	70%	0%	50%	62%	71%	67%
Stage 3 ==>	3	90%	64%	100%	75%	58%	62%	70%	83%	46%	92%	69%	91%	31%	100%	33%	31%	65%	90%	71%
Stage 4 ==>	4	80%	0%	100%	100%	100%	80%	100%	75%	75%	50%	100%	100%	33%	100%	50%	33%	83%	88%	76%
Stage 5 ==>	5	100%	0%	100%	100%	100%	100%	100%	100%	100%	100%	100%	-	100%	100%	-	0%	100%	100%	85%
Weighted mean	0-5	89.2%	77.2%	86.1%	64.8%	90.0%	83.6%	84.6%	63.4%	67.8%	65.6%	86.6%	93.9%	74.0%	84.0%	26.9%	56.7%	85.3%	80.9%	76.3%
	RANKING	3	11	5	15	2	9	7	16	13	14	4	1	12	8	18	17	6	10	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	94%	94%	83%	71%	96%	100%	93%	81%	94%	90%	91%	97%	89%	87%	95%	96%	100%	91%	92%
RANKING	8	7	16	18	4	1	10	17	9	13	11	3	14	15	6	5	1	12	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	0.11	0.15	0.36	0.73	0.06	0.05	0.19	0.57	0.28	0.52	0.21	0.08	0.25	0.32	0.84	0.40	0.05	0.28	0.29
Stage 1b ==>	1	0.25	-1.00	0.00	0.00	0.00	-0.75	0.00	0.40	-	-	-1.00	-	-1.00	0.00	0.00	0.00	-0.50	-0.33	-0.19
Stage 2 ==>	2	-0.40	-0.60	0.00	0.00	-0.20	0.11	0.00	0.56	-0.20	0.22	-0.11	0.00	-0.40	0.30	0.00	-0.60	-0.69	0.29	-0.11
Stage 3 ==>	3	-0.10	-0.36	0.00	-0.25	0.08	-0.23	-0.10	0.17	0.62	0.08	-0.46	-0.09	-0.81	0.00	-1.00	-0.69	-0.43	0.00	-0.18
Stage 4 ==>	4	-0.20	0.00	0.00	0.00	0.00	-0.20	0.00	0.25	0.25	-0.75	0.00	0.00	-0.67	0.00	-1.00	-0.67	-0.33	0.13	-0.16
Stage 5 ==>	5	0.00	-2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	-	-1.50	0.00	0.00	-0.27

Table 4.2.10. Mackerel eggs second staging, expert readers only.
(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	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ==>	0	84	43	58	49	74	76	54	56	66	560
Stage 1b ==>	1	-	1	1	1	1	1	1	-	1	-
Stage 2 ==>	2	15	15	16	14	13	12	13	13	14	125
Stage 3 ==>	3	10	7	10	10	8	9	11	10	9	84
Stage 4 ==>	4	4	2	2	4	3	2	2	2	2	23
Stage 5 ==>	5	1	2	1	2	2	2	1	-	2	13
Total	0-5	114	70	88	80	101	102	82	81	94	812

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ==>	0	81	35	32	50	77	69	53	57	56	510
Stage 1b ==>	1	2	5	13	2	3	8	1	-	6	40
Stage 2 ==>	2	16	17	31	12	5	11	14	10	11	127
Stage 3 ==>	3	10	9	9	7	11	8	11	11	17	93
Stage 4 ==>	4	4	2	2	7	3	4	2	3	2	29
Stage 5 ==>	5	1	2	1	2	2	2	1	-	2	13
Total	0-5	114	70	88	80	101	102	82	81	94	812

C

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

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	-1%	-9%	-24%	4%	7%	0%	-2%	2%	-7%	-2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ==>	0	95%	81%	55%	96%	96%	89%	94%	100%	85%	89%
Stage 1b ==>	1	-	100%	100%	100%	0%	100%	0%	-	100%	-
Stage 2 ==>	2	93%	80%	100%	79%	23%	75%	77%	77%	57%	74%
Stage 3 ==>	3	90%	100%	90%	70%	63%	67%	82%	100%	100%	85%
Stage 4 ==>	4	75%	100%	100%	100%	67%	100%	100%	100%	100%	91%
Stage 5 ==>	5	100%	100%	100%	100%	100%	100%	100%	-	100%	100%
Weighted mean	0-5	93.9%	84.3%	69.3%	90.0%	82.2%	86.3%	89.0%	96.3%	83.0%	86.1%
	RANKING	2	6	9	3	8	5	4	1	7	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	98%	89%	76%	98%	100%	97%	95%	100%	93%	94%
RANKING	4	8	9	3	1	5	6	1	7	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ==>	0	0.10	0.30	0.69	0.06	0.04	0.13	0.13	0.00	0.26	0.18
Stage 1b ==>	1	-	0.00	0.00	0.00	-1.00	0.00	-1.00	-	0.00	-
Stage 2 ==>	2	-0.13	0.07	0.00	-0.43	-0.08	0.08	-0.15	0.08	0.43	-0.02
Stage 3 ==>	3	-0.10	0.00	-0.10	0.30	-0.63	-0.11	-0.18	0.00	0.00	-0.08
Stage 4 ==>	4	-0.25	0.00	0.00	0.00	-0.33	0.00	0.00	0.00	0.00	-0.09
Stage 5 ==>	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00

Table 4.2.11. 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	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	18	76	61	66	61	60	80	36	86	72	68	63	75	66	47	76	54	37	1102
Stage 1b ==>	1	1	1	2	2	2	3	2	1	2	2	2	2	2	2	2	2	2	-	32
Stage 2 ==>	2	12	19	17	18	18	16	23	13	20	22	18	18	17	19	15	19	15	10	309
Stage 3 ==>	3	7	19	30	30	27	26	34	22	29	30	29	28	24	29	24	30	22	11	451
Stage 4 ==>	4	3	10	15	14	12	13	13	8	15	15	13	12	10	16	8	11	10	6	204
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	41	125	125	130	120	118	152	80	152	141	130	123	128	132	96	138	103	64	2098

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Stage 1a ==>	0	19	75	38	41	64	62	70	28	95	38	55	58	76	66	11	28	56	33	913
Stage 1b ==>	1	1	1	17	8	2	9	11	4	6	27	7	4	3	3	39	52	9	8	211
Stage 2 ==>	2	11	21	25	35	16	5	23	13	3	27	30	23	30	19	18	36	14	6	355
Stage 3 ==>	3	8	21	24	32	22	31	31	33	26	31	23	28	15	30	16	14	22	13	420
Stage 4 ==>	4	2	7	21	14	16	11	17	2	20	18	15	10	4	14	12	8	2	4	197
Stage 5 ==>	5	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	2
Total	0-5	41	125	125	130	120	118	152	80	152	141	130	123	128	132	96	138	103	64	2098

C

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

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
1a+1b	5%	-1%	-13%	-28%	5%	13%	-1%	-14%	15%	-12%	-11%	-5%	3%	1%	2%	3%	16%	11%	-1%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	89%	89%	61%	62%	98%	88%	86%	72%	97%	53%	81%	89%	89%	89%	17%	34%	89%	86%	76%
Stage 1b ==>	1	0%	0%	0%	50%	50%	67%	100%	0%	50%	100%	100%	50%	0%	50%	50%	50%	50%	-	48%
Stage 2 ==>	2	75%	47%	82%	72%	78%	25%	74%	54%	15%	68%	89%	89%	59%	68%	53%	63%	47%	40%	62%
Stage 3 ==>	3	86%	79%	77%	87%	70%	77%	79%	100%	72%	77%	69%	89%	38%	86%	46%	33%	64%	82%	72%
Stage 4 ==>	4	67%	50%	93%	86%	83%	46%	100%	13%	80%	87%	92%	83%	30%	75%	50%	45%	20%	50%	68%
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	80.5%	77.6%	70.4%	71.5%	86.7%	72.0%	84.2%	70.0%	78.9%	64.5%	80.8%	87.8%	69.5%	83.3%	33.3%	39.1%	69.9%	75.0%	72.1%
	RANKING	6	8	12	11	2	10	3	13	7	16	5	1	15	4	18	17	14	9	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
	1a+1b	86%	88%	81%	66%	93%	98%	91%	79%	99%	81%	82%	87%	93%	93%	85%	89%	100%	95%	88%
	RANKING	12	10	16	18	6	3	8	17	2	15	14	11	7	5	13	9	1	4	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Stage 1a ==>	0	0.17	0.24	0.59	0.67	0.02	0.12	0.21	0.53	0.03	0.65	0.31	0.17	0.20	0.18	0.98	0.76	0.11	0.16	0.34
Stage 1b ==>	1	-1.00	1.00	0.00	0.50	-0.50	-0.33	0.00	-1.00	-0.50	0.00	0.00	-0.50	-1.00	-0.50	0.50	0.50	-0.50	-	-0.19
Stage 2 ==>	2	-0.25	-0.53	0.00	0.17	-0.28	-0.75	0.09	0.08	-0.85	0.14	0.11	-0.06	-0.65	-0.47	-0.40	-0.47	-0.93	-0.50	-0.29
Stage 3 ==>	3	-0.14	-0.21	0.07	0.00	0.15	0.15	-0.09	0.00	0.28	0.07	-0.10	-0.11	-0.67	-0.07	0.04	-0.60	-0.45	0.00	-0.09
Stage 4 ==>	4	-0.33	-0.70	-0.07	-0.14	-0.17	-0.54	0.00	-0.88	-0.13	-0.13	-0.08	-0.17	-1.00	-0.25	-0.88	-0.73	-0.80	-0.50	-0.36
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.12. Horse mackerel eggs second staging, expert readers only.
(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	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ⟹	0	16	58	63	58	57	75	66	61	63	517
Stage 1b ⟹	1	1	3	3	3	3	3	3	3	3	25
Stage 2 ⟹	2	11	18	20	20	15	21	19	19	19	162
Stage 3 ⟹	3	7	28	28	25	23	30	27	26	27	221
Stage 4 ⟹	4	2	16	16	14	15	15	15	14	18	125
Stage 5 ⟹	5	-	-	-	-	-	-	-	-	-	-
Total	0-5	37	123	130	120	113	144	130	123	130	1050

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Stage 1a ⟹	0	17	38	41	64	60	69	55	58	66	468
Stage 1b ⟹	1	1	17	8	2	8	9	7	4	3	59
Stage 2 ⟹	2	11	24	35	16	5	21	30	23	17	182
Stage 3 ⟹	3	7	24	32	22	29	29	23	28	30	224
Stage 4 ⟹	4	1	20	14	16	11	16	15	10	14	117
Stage 5 ⟹	5	-	-	-	-	-	-	-	-	-	-
Total	0-5	37	123	130	120	113	144	130	123	130	1050

C

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

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	6%	-10%	-26%	8%	13%	0%	-10%	-3%	5%	-3%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ⟹	0	94%	64%	65%	98%	88%	89%	83%	90%	92%	84%
Stage 1b ⟹	1	0%	33%	33%	33%	33%	67%	100%	67%	33%	48%
Stage 2 ⟹	2	91%	72%	80%	65%	27%	76%	89%	89%	63%	73%
Stage 3 ⟹	3	86%	79%	93%	72%	74%	87%	70%	92%	89%	82%
Stage 4 ⟹	4	50%	94%	81%	86%	40%	93%	87%	71%	72%	78%
Stage 5 ⟹	5	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	86.5%	71.5%	74.6%	84.2%	69.0%	86.8%	82.3%	87.8%	83.1%	80.4%
	RANKING	3	8	7	4	9	2	6	1	5	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
1a+1b	100%	83%	70%	97%	98%	95%	86%	93%	94%	90%
RANKING	1	8	9	3	2	4	7	6	5	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Stage 1a ⟹	0	0.06	0.55	0.60	0.02	0.12	0.17	0.27	0.16	0.13	0.25
Stage 1b ⟹	1	-1.00	0.00	0.67	-0.67	-0.67	0.33	0.00	-0.33	0.00	-0.12
Stage 2 ⟹	2	-0.18	0.00	0.10	-0.40	-0.87	-0.10	0.11	-0.21	-0.58	-0.22
Stage 3 ⟹	3	-0.14	-0.04	0.00	0.04	0.17	-0.13	-0.15	-0.08	-0.11	-0.05
Stage 4 ⟹	4	-0.50	-0.06	-0.19	-0.14	-0.60	-0.07	-0.13	-0.29	-0.28	-0.22
Stage 5 ⟹	5	-	-	-	-	-	-	-	-	-	-

4.3 Result of egg identification exercises

The same trays of eggs, which were used for egg staging, were also used for the egg identification exercises. 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).

Summaries of the results from the two rounds of egg species determination are presented in Tables 4.3.1 to 4.3.4. Half of the participants at the workshop were inexperienced; hence, results of the expert readers only are also presented separately. Each of these tables is 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.

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 4.3.1, 4.3.2, 4.3.3 and 4.3.4). For mackerel eggs, the percentage agreement increased from 80% to 88% with modal/actual species and for expert readers from 89% to 90%. For horse mackerel, the improvement rose from 58% to 78% for modal/actual species and for experts from 70% to 85%.

Table 4.3.1. Species identification with modal species, first identification.
(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 **Species compositions using modal/actual species**

	Modal species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Mackerel	1	150	159	163	162	162	160	163	145	158	161	161	162	162	162	156	162	159	160	**2867**
Horse Mackerel	2	6	7	7	7	7	5	7	6	5	7	7	7	7	7	5	6	6	7	**116**
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Hake	4	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	**35**
Other species	5	26	24	24	26	26	26	26	17	20	26	26	23	26	26	22	24	24	20	**432**
Total	1-5	**184**	**192**	**196**	**197**	**197**	**193**	**198**	**170**	**185**	**196**	**196**	**193**	**197**	**197**	**185**	**194**	**191**	**189**	**3450**

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

	Species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Mackerel	1	141	139	149	137	121	141	153	122	139	110	137	148	124	141	92	145	156	107	**2402**
Horse Mackerel	2	13	23	14	10	10	22	27	29	35	60	11	12	44	20	90	11	5	58	**494**
Megrim	3	-	1	1	2	-	4	5	3	4	13	-	-	-	-	1	14	4	1	**53**
Hake	4	4	11	12	22	35	11	5	6	6	7	17	4	12	12	-	-	2	17	**183**
Other species	5	26	18	20	26	31	15	8	10	1	6	31	29	17	24	2	24	24	6	**318**
Total	1-5	**184**	**192**	**196**	**197**	**197**	**193**	**198**	**170**	**185**	**196**	**196**	**193**	**197**	**197**	**185**	**194**	**191**	**189**	**3450**

C **Percentage overestimation / underestimation**

	Modal species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Mackerel	1	-6%	-13%	-9%	-15%	-25%	-12%	-6%	-16%	-12%	-32%	-15%	-9%	-23%	-13%	-41%	-10%	-2%	-33%	**-16%**
Horse Mackerel	2	117%	229%	100%	43%	43%	340%	286%	383%	600%	757%	57%	71%	529%	186%	1700%	83%	-17%	729%	**326%**
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Hake	4	100%	450%	500%	1000%	1650%	450%	150%	200%	200%	250%	750%	300%	500%	500%			0%	750%	**423%**
Other species	5	0%	-25%	-17%	0%	19%	-42%	-69%	-41%	-95%	-77%	19%	26%	-35%	-8%	-91%	0%	0%	-70%	**-26%**

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

	Modal species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Mackerel	1	91%	85%	90%	85%	74%	83%	90%	74%	77%	66%	85%	91%	70%	87%	46%	86%	92%	61%	**80%**
Horse Mackerel	2	100%	14%	86%	86%	71%	20%	86%	17%	20%	71%	71%	57%	29%	100%	40%	83%	17%	43%	**58%**
Megrim	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Hake	4	100%	0%	100%	100%	50%	0%	100%	0%	50%	100%	100%	100%	0%	100%	0%	0%	0%	0%	**49%**
Other species	5	85%	67%	71%	92%	100%	50%	27%	18%	0%	23%	100%	100%	46%	88%	9%	79%	71%	30%	**61%**
Weighted mean	1-5	**90.8%**	**79.2%**	**87.2%**	**85.8%**	**77.2%**	**76.2%**	**81.8%**	**65.3%**	**67.0%**	**61.2%**	**86.7%**	**91.2%**	**64.5%**	**87.8%**	**41.1%**	**84.5%**	**86.4%**	**56.6%**	**76.3%**
	RANKING	**2**	**10**	**4**	**7**	**11**	**12**	**9**	**14**	**13**	**16**	**5**	**1**	**15**	**3**	**18**	**8**	**6**	**17**	

Table 4.3.2. Species identification with modal species, first identification, expert readers only.
(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 **Species compositions using modal/actual species**

	Modal species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Mackerel	1	143	156	155	155	153	156	154	155	155	**1382**
Horse Mackerel	2	11	12	12	12	10	12	12	12	12	**105**
Megrim	3	-	-	-	-	-	-	-	-	-	**-**
Hake	4	2	2	2	2	2	2	2	1	2	**17**
Other species	5	28	26	28	28	28	28	28	25	28	**247**
Total	1-5	**184**	**196**	**197**	**197**	**193**	**198**	**196**	**193**	**197**	**1751**

B **Species compositions as estimated per participant**

	Species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Mackerel	1	141	149	137	121	141	153	137	148	141	**1268**
Horse Mackerel	2	13	14	10	10	22	27	11	12	20	**139**
Megrim	3	-	1	2	-	4	5	-	-	-	**12**
Hake	4	4	12	22	35	11	5	17	4	12	**122**
Other species	5	26	20	26	31	15	8	31	29	24	**210**
Total	1-5	**184**	**196**	**197**	**197**	**193**	**198**	**196**	**193**	**197**	**1751**

C **Percentage overestimation / underestimation**

	Modal species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Mackerel	1	-1%	-4%	-12%	-22%	-8%	-2%	-11%	-5%	-9%	**-8%**
Horse Mackerel	2	18%	17%	-17%	-17%	120%	125%	-8%	0%	67%	**32%**
Megrim	3	-	-	-	-	-	-	-	-	-	**-**
Hake	4	100%	500%	1000%	1650%	450%	150%	750%	300%	500%	**618%**
Other species	5	-7%	-23%	-7%	11%	-46%	-71%	11%	16%	-14%	**-15%**

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

	Modal species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Mackerel	1	92%	93%	88%	77%	85%	92%	88%	95%	90%	**89%**
Horse Mackerel	2	45%	75%	83%	83%	30%	58%	75%	83%	83%	**70%**
Megrim	3	-	-	-	-	-	-	-	-	-	**-**
Hake	4	100%	100%	100%	50%	0%	100%	100%	100%	100%	**82%**
Other species	5	79%	69%	93%	100%	50%	29%	100%	100%	82%	**78%**
Weighted mean	1-5	**87.0%**	**88.8%**	**88.8%**	**80.2%**	**76.2%**	**81.3%**	**89.3%**	**95.3%**	**88.3%**	**86.1%**
	RANKING	**6**	**4**	**3**	**8**	**9**	**7**	**2**	**1**	**5**	

Table 4.3.3. Species identification with modal species, second identification.
(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 **Species compositions using modal/actual species**

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Mackerel	1	86	88	76	88	89	89	89	89	87	85	89	84	89	88	80	89	89	87	1561
Horse Mackerel	2	124	117	127	125	124	127	126	126	123	124	124	121	127	112	122	124	127	127	2227
Megrim	3	5	-	5	5	5	5	5	4	3	5	5	5	5	5	5	5	5	5	82
Hake	4	30	26	29	30	30	30	30	29	30	30	30	30	30	29	28	30	30	29	530
Other species	5	16	14	14	16	16	16	17	17	17	16	16	17	17	14	9	17	17	16	282
Total	1-5	263	247	253	266	266	269	269	267	262	262	266	259	270	250	246	267	270	266	4718

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

	Species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	TOTAL
Mackerel	1	194	92	73	88	80	118	104	143	90	93	82	83	103	94	80	105	148	148	1918
Horse Mackerel	2	41	127	127	130	120	122	153	81	152	142	132	123	128	132	103	139	103	66	2121
Megrim	3	-	-	-	5	5	-	2	3	1	-	5	5	11	-	-	1	-	-	38
Hake	4	14	12	38	26	40	12	4	8	3	16	22	19	14	13	62	12	4	35	354
Other species	5	14	16	15	17	21	17	6	32	16	11	25	29	14	11	1	10	15	17	287
Total	1-5	263	247	253	266	266	269	269	267	262	262	266	259	270	250	246	267	270	266	4718

C **Percentage overestimation / underestimation**

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Mackerel	1	126%	5%	-4%	0%	-10%	33%	17%	61%	3%	9%	-8%	-1%	16%	7%	0%	18%	66%	70%	23%
Horse Mackerel	2	-67%	9%	0%	4%	-3%	-4%	21%	-36%	24%	15%	6%	2%	1%	18%	-16%	12%	-19%	-48%	-5%
Megrim	3	-100%	-	-100%	0%	0%	-100%	-60%	-25%	-67%	-100%	0%	0%	120%	-100%	-100%	-80%	-100%	-100%	-54%
Hake	4	-53%	-54%	31%	-13%	33%	-60%	-87%	-72%	-90%	-47%	-27%	-37%	-53%	-55%	121%	-60%	-87%	21%	-33%
Other species	5	-13%	14%	7%	6%	31%	6%	-65%	88%	-6%	-31%	56%	71%	-18%	-21%	-89%	-41%	-12%	6%	2%

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

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	ALL
Mackerel	1	92%	88%	86%	95%	88%	89%	88%	81%	93%	94%	85%	86%	92%	94%	69%	92%	96%	84%	88%
Horse Mackerel	2	28%	85%	91%	98%	90%	69%	96%	52%	98%	91%	94%	93%	84%	97%	52%	85%	62%	46%	78%
Megrim	3	0%	-	0%	100%	100%	0%	0%	0%	0%	0%	100%	100%	100%	0%	0%	0%	0%	0%	30%
Hake	4	0%	0%	76%	80%	70%	0%	3%	0%	0%	23%	47%	43%	17%	34%	7%	10%	7%	3%	24%
Other species	5	44%	93%	93%	88%	100%	94%	35%	53%	82%	56%	100%	94%	53%	71%	11%	47%	53%	63%	69%
Weighted mean	1-5	46.0%	76.5%	85.0%	93.6%	86.8%	67.3%	76.6%	55.1%	82.4%	79.8%	85.7%	84.6%	77.0%	84.8%	49.6%	74.5%	64.8%	53.4%	73.5%
	RANKING	18	11	4	1	2	13	10	15	7	8	3	6	9	5	17	12	14	16	

Table 4.3.4. Species identification with modal species, second identification, expert readers only.
(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 **Species compositions using modal/actual species**

	Modal or actual species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Mackerel	1	85	75	87	88	88	88	88	83	87	**769**
Horse Mackerel	2	122	125	123	122	125	124	122	119	110	**1092**
Megrim	3	5	5	5	5	5	5	5	5	5	**45**
Hake	4	32	31	32	32	32	32	32	32	31	**286**
Other species	5	17	15	17	17	17	18	17	18	15	**151**
Total	1-5	**263**	**253**	**266**	**266**	**269**	**269**	**266**	**259**	**250**	**2361**

B **Species compositions as estimated per participant**

	Species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	TOTAL
Mackerel	1	194	73	88	80	118	104	82	83	94	**916**
Horse Mackerel	2	41	127	130	120	122	153	132	123	132	**1080**
Megrim	3	-	-	5	5	-	2	5	5	-	**22**
Hake	4	14	38	26	40	12	4	22	19	13	**188**
Other species	5	14	15	17	21	17	6	25	29	11	**155**
Total	1-5	**263**	**253**	**266**	**266**	**269**	**269**	**266**	**259**	**250**	**2361**

C **Percentage overestimation / underestimation**

	Modal or actual species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Mackerel	1	128%	-3%	1%	-9%	34%	18%	-7%	0%	8%	**19%**
Horse Mackerel	2	-66%	2%	6%	-2%	-2%	23%	8%	3%	20%	**-1%**
Megrim	3	-100%	-100%	0%	0%	-100%	-60%	0%	0%	-100%	**-51%**
Hake	4	-56%	23%	-19%	25%	-63%	-88%	-31%	-41%	-58%	**-34%**
Other species	5	-18%	0%	0%	24%	0%	-67%	47%	61%	-27%	**3%**

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

	Modal or actual species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 11	Reader 12	Reader 14	ALL
Mackerel	1	92%	87%	97%	88%	89%	89%	86%	87%	95%	**90%**
Horse Mackerel	2	28%	91%	98%	91%	69%	97%	96%	95%	98%	**85%**
Megrim	3	0%	0%	100%	100%	0%	0%	100%	100%	0%	**44%**
Hake	4	0%	74%	78%	69%	3%	6%	50%	47%	35%	**40%**
Other species	5	41%	93%	88%	100%	88%	33%	100%	94%	73%	**79%**
Weighted mean	1-5	**45.2%**	**85.4%**	**94.0%**	**87.2%**	**66.9%**	**76.6%**	**86.8%**	**85.7%**	**85.2%**	**79.2%**
	RANKING	**9**	**5**	**1**	**2**	**8**	**7**	**3**	**4**	**6**	

4.4 Result of the fecundity and atresia estimation

4.4.1 Results of pipette sampling and picture taking

The balance available at the workshop weighed down to 1 mg. (When doing this exercise ideally, a more precise balance should be used but this was not available in the meeting room.) All participants' samples were very close to 25 and 50 mg.

Fecundity pictures from the 2013 survey were checked. The quality of the fecundity pictures were OK for all institutes. Only one institute did not carry out the white balance before taking the picture. This makes it easier to look at the pictures, but it does not affect the result of the fecundity analyses. Not all institutes have LED illumination available on the microscope. Because of this, the light on the fecundity pictures taken without LED illumination is not even distributed. The pictures are darker at the edges. This does not influence the results of the fecundity analyses.

For the atresia pictures, not all pictures were clear, either because they were not sharp enough or because of the staining.

4.4.2 Results of screening analyses

Pictures of seven different females from the 2013 survey were analysed by the participants at the workshop. For most images, participants were in agreement (Table 4.4). Three fish with low agreement were discussed in plenary.

M005: Participants were confusing the oocyte stage. However, when the sample was shown in plenary it was immediately clear the something had gone wrong when taking the sample or with the fixation of the oocyte sample. Due to this, the oocytes were distorted and it was difficult to identify the development stage. This discussion led to the decision to add a column to the screening datasheet where the reader puts in the information if a sample should be discarded or not.

M029: After the discussion on oocyte stages participants agree the oocyte stage should be 4. Everyone agrees this is not massive atresia.

M089: A sample shows massive atresia if 90% or more of the vitellogenic oocytes are atretic. Thus, this sample is not massive atresia. No POF can be seen in the sample. The start of alpha atresia is defined as a cut in the inner chorion. Do not mix up the vitelline membrane with the inner chorion.

If yolk plates are visible in an oocyte, this is the hydrating stage 5, even if the migrating nucleus is visible. If migrating nucleus is visible but no yolk plates this is stage 4 migratory nucleus.

Table 4.4. Results of the screening analyses exercise.

Reader	Expertise	Sample	POF	Oocyte stage	Early alpha atresia	Massive atresia	Sample	POF	Oocyte stage	Early alpha atresia	Massive atresia
1	Trainee	A013	0	3	0	0	M089	0	4	0	1
2	Trainee		0	3	0	0		0	4	0	0
3	Expert		0	3	0	0		0	4	1	0
4	Expert		0	3	0	0		0	4	1	0
5	Expert		0	3	0	0		0	4	0	0
6	Expert		1	3	0	0		0	4	0	0
7	Expert		1	3	0	0		0	4	0	0
8	Expert		1	3	0	0		1	4	0	0
9	Trainee		0	3	0	0		0	4	0	1
10	Expert		0	3	0	0		0	4	0	0
11	Expert			3	0	0			4	1	0
12	Trainee		0	3	0	0		0	4	0	1
13	Trainee		0	3	0	0		0	4	0	1
1	Trainee	A021	1	6	0	0	M185	0	5	1	0
2	Trainee		1	5	0	0		1	5	0	0
3	Expert		1	5	0	0		0	6	1	0
4	Expert		1	5	0	0		0	5	1	0
5	Expert		1	6	0	0		0	5	0	0
6	Expert		1	5	0	0		1	5	1	0
7	Expert		1	5	0	0		0	4	1	0
8	Expert		1	5	0	0		0	5	0	0
9	Trainee		0	5	0	0		1	6	1	0
10	Expert		1	6	0	0		1	5	1	0
11	Expert		1	5	0	0			5	1	0
12	Trainee		0	5	0	0		0	5	1	0
13	Trainee		1	6	0	0		0	5	1	0
1	Trainee	M005	0	3	1	0	M217	1	6	1	0
2	Trainee		1	6	0	0		1	6	1	0
3	Expert		1	5	0	0		1	6	1	0
4	Expert		1	5	0	0		1	6	1	0
5	Expert		0	3	0	0		1	6	1	0
6	Expert		1	3	1	0		1	5	1	0
7	Expert		0	3	1	0		1	5	1	0
8	Expert		0	5	1	0					
9	Trainee		1	5	0	0		1	6	1	0
10	Expert		0	3	1	0		1	6	1	0
11	Expert		1	4	1	0		1	5	1	0
12	Trainee		1	4	0	0		1	6	0	0
13	Trainee		0	3	1	0		1	6	1	0
1	Trainee	M029	1	4	1	0					
2	Trainee		1	4	1	0					
3	Expert		1	4	1	0					
4	Expert		1	4	1	0					
5	Expert		1	4	1	0					
6	Expert		1	4	1	1					
7	Expert		1	4	1	0					
8	Expert		1	5	1	0					
9	Trainee		1	5	0	0					
10	Expert		1	4	1	0					
11	Expert		1	4	1	0					
12	Trainee		0	4	1	0					
13	Trainee		1	4	1	0					

4.4.3 Results of the potential fecundity analyses

The images of 5 females (prepared by different institutes) were scored by the participants for fecundity analysis. Total oocyte counts of the samples showed differences among the participants (Table 4.5.1), also when only expert readers were analysed. No differences in the measurements of oocytes were found between participants (Table 4.5.2). After the analyses, the pictures were discussed in plenary.

Table 4.5.1. Results of the whole mount fecundity analysis: Total number of vitellogenic oocytes counted.

Reader	1	2	3	4	5	6	7	8	9	10	11	12	All		
Expertise	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Trainee	Expert	Min	Max	St Dev
A011	118	123	121	100	125	118	115	121	128	122	121	119	100	128	6.96
A014	394	556	502	374	449	469	285		464	515	513	523	285	556	79.60
A035	92	114	114	101	102	95	99	164	115	115	113	103	92	164	18.75
C009	234	290	302	271	282	260	261	399	317	281	287	276	234	399	40.80
K137	151	167	178	150	160	162	177	200	175	161	177		150	200	14.45

Table 4.5.2. Results of the whole mount fecundity analysis: Oocyte diameters measured.

	Reader	1	2	3	4	5	6	7	8	9	10	11	12	All		
Sample	Expertise	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Trainee	Expert	Min	Max	St Dev
A011	N	90	92	90	94	92	90	91	93	90	92	92	94	90	94	1.50
	Mean	537	534	538	530	534	538	530	532	538	534	534	530	530	538	3.04
	StDev	130	130	129	132	130	129	131	132	129	130	131	132	129	132	0.85
	P95	703	703	703	703	703	703	703	703	703	703	703	703	703	703	0.00
A014	N	224	224	227	231	230	221	231		230	230	229	228	221	231	3.35
	Mean	514	515	515	512	513	514	512		513	512	512	513	512	515	1.07
	StDev	140	140	140	140	141	142	140		141	141	141	141	140	142	0.62
	P95	729	729	729	729	729	729	729		729	729	729	729	729	729	0.00
A035	N	65	66	66	64	66	66	66	66	66	66	65	67	64	67	0.75
	Mean	569	566	566	562	566	566	566	566	566	566	569	572	562	572	2.30
	StDev	173	173	173	173	173	173	173	173	173	173	173	177	173	177	1.04
	P95	792	792	792	792	792	792	792	792	792	792	792	796	792	796	1.15
C009	N	189	189	189	189	189	189	189	189	189	189	189	189	189	189	0.00
	Mean	433	433	433	433	433	433	433	433	433	433	433	433	433	433	0.00
	StDev	103	103	103	103	103	103	103	103	103	103	103	103	103	103	0.00
	P95	596	596	596	596	596	596	596	596	596	596	596	596	596	596	0.00
K137	N	113	113	115	115	112	108	113	114	112	113	112		108	115	1.90
	Mean	437	435	433	436	436	438	435	437	437	435	433		433	438	1.45
	StDev	100	99	99	100	99	96	99	100	98	99	97		96	100	1.37
	P95	609	605	605	609	605	600	605	609	605	605	600		600	609	3.12

The oocyte should completely fill the ring to be included in the manual count.

Each participant should analyse the picture. After the first analyses the sample needs to be checked completely (preferably on a different day and by a different analyst) to check if any oocytes have been missed.

It is very important that the oocytes in the sample are separated and no clumps left in the sample (see Section 3.4.2). After separation, the oocytes should be spread out carefully without any overlap of the oocytes.

Pictures should be taken with a good light source in order to get a good contrast in the picture. This is especially important for the small transparent vitellogenic oocytes. The resolution of the pictures should be at least 0.2 pixels/μm.

In addition, advanced atretic oocytes should be included because the number of atretic cells will be subtracted in the calculations later on.

If, when checking the image, the automatic size measurement is found to be wrong, this measurement should be removed from the database and that particular oocyte should be counted manually. It is important that the settings of the computer and ImageJ write the category for an automatically measured oocyte as -1 and for a manually counted oocyte as 1. Only automatically measured oocytes can be used for the analyses of oocyte size.

4.4.4 Results of the batch fecundity analyses

Since the actual sample analyses for batch fecundity is very similar to the potential fecundity analyses, only one female was analysed by all participants at the workshop. The results of the batch fecundity analysis for counts and diameter measurements were very similar between the participants (Table 4.6.1 and 4.6.2). Two participants used a lower threshold for the minimum oocyte size than the 500 µm. Their counts are much higher and the average oocyte size much lower than the other participants (Table 4.6.1 and 4.6.2)

Table 4.6.1. Results of the batch fecundity analysis: Total number of vitellogenic oocytes counted (two participants used a lower threshold).

Reader	1	2	3	4	5	6	7	8	9	10	11	12	All		
Expertise	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Trainee	Expert	Min	Max	St Dev
C115	90	90	97	92	92	88	92	92	188	98	91	181	88	188	36.07

Table 4.6.2. Results of the batch fecundity analysis: Oocyte diameters measured (two participants used a lower threshold).

	Reader	1	2	3	4	5	6	7	8	9	10	11	12	All		
Sample	Expertise	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Expert	Expert	Trainee	Trainee	Expert	Min	Max	St Dev
C115	N	79	78	84	82	82	84	80	84	132	84	79	152	78	152	23.99
	Mean	639	639	639	634	636	637	638	637	558	637	638	548	548	639	33.02
	StDev	87	86	85	86	85	85	86	85	120	85	87	118	85	120	12.92

If the automatic measurement is much larger compared to the maximum size of the oocytes, it should be discarded from the picture and the oocyte only counted manually.

4.4.5 Results of the atresia estimation exercise

During the calibration exercise, all participants assessed atresia on images from three females using the protocol described in the manual (Fonn *et al*., 2015). Only a few participants were able to analyse all the females, thus only the results of the first two females are shown (Table 4.7). There were considerable differences among the participants, even among the experts (Table 4.7). Half of the images from the first fish were therefore discussed in plenary.

In the images, it was difficult to distinguish YV and YG, thus participants used size instead. This explains the large differences in the development stage assessment.

If the theca and follicle layers are split from the chorion, these still belong to the oocyte and should be included in the grid count. The area delimited by the follicle layer is considered to be occupied by 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 artefact created by fixation and/or tissue processing.

If an oocyte is counted for profile, it should always be counted for the grid points as well. If a grid point in the oocytes is counted in the grid it should only be counted for profile if the oocyte does not hit the red line. However, if grid points do not hit the atretic cell there is a profile count but no grid count.

Table 4.7. 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.)

Reader	Expertise	Sample_ref	No_Pictures	YV	YV_YG	YG	Neg_grid	Extra	Total	YV_P	YV_YG_P	YG_P	Total
1	**Trainee**	C052	14	0	132	9	0	0	141	0	43	0	43
2	**Trainee**		14	0	17	14	0	0	31	0	6	3	9
3	**Expert**		14	4	52	0	0	0	56	0	11	0	11
4	**Expert**		14	69	0	0	0	0	69	14	0	0	14
5	**Expert**		14	0	67	63	0	0	130	0	11	12	23
6	**Expert**		14	67	102	34	0	0	203	16	13	2	31
7	**Expert**		14	0	16	40	0	0	56	0	6	11	17
8	**Trainee**		14	14	148	12	0	0	174	0	41	0	41
9	**Expert**		14	7	158	11	0	0	176	0	28	2	30
10	**Trainee**		14	0	29	22	0	0	51	0	11	6	17
11	**Trainee**		14	53	2	0	0	0	55	13	0	0	13
12	**Trainee**		14	0	153	13	2	0	168	1	40	2	43
Min				**0**	**0**	**0**	**0**	**0**	**31**	**0**	**0**	**0**	**9**
Max				**69**	**158**	**63**	**2**	**0**	**203**	**16**	**43**	**12**	**43**
St Dev				**28**	**62**	**19**	**1**	**0**	**62**	**6**	**16**	**4**	**13**
2	**Trainee**	E009	6	0	28	11	449	0	488	0	18	5	23
3	**Expert**		6	8	90	0	408	0	506	8	49	0	57
4	**Expert**		6	0	71	0	451	0	522	1	35	0	36
5	**Expert**		6	40	60	0	446	0	546	18	24	0	42
7	**Expert**		6	0	1	62	443	0	506	0	1	35	36
8	**Trainee**		2	0	22	3	172	0	197	0	15	0	15
10	**Trainee**		5	0	23	35	415	0	473	0	16	21	37
Min				**0**	**0**	**0**	**0**	**0**	**31**	**0**	**0**	**0**	**9**
Max				**69**	**158**	**63**	**451**	**0**	**546**	**18**	**49**	**35**	**57**
St Dev				**24**	**53**	**23**	**218**	**0**	**214**	**7**	**17**	**11**	**15**

If the lines or markers are in the way of seeing, you can remove the markers and lines in the view in ImageJ.

Cuts and breaks in the chorion are primary characteristic. If in doubt as a secondary characteristic one can look for disorder in the YV and YG.

If a breakdown or cut in the chorion is visible then the oocyte is atretic. If there is only damage to the chorion this is not atresia. If the break in the chorion is more than twice the width of the chorion, it is not alpha atresia. To be certain of this the chorion should be measured. However, the thinnest part of the chorion should be used as the reference as the chorion thickens during the atretic process.

4.4.6 Results of the POF staging plenary exercise

Three different samples were discussed in plenary. Slides under a compound microscope where shown on the screen.

1) IMR slide with Toluidine blue staining

POF is in stage 6, but general feeling is stage 5. Cellular structure looks like stage 5, but there are few POF's in the sample thus this is considered a stage 6. The number of POFs in a sample decreases with increasing age of the POFs. Size of POFs in the sample is similar to previtellogenic oocytes, thus small.

Other POF is a bit bigger, and in the younger stage 3. Question is if the first POF is only a partly removed, from a stage 3 POF. Looks like there are more cuts of stage 3 POFs.

Hydrated oocytes are also visible in the sample.

There are few vacuoles, few picnotic nuclei, lumen is visible and folds still recognizable, thus POF stage 3.

Overall this sample is stage 3, because stage 3 is the dominant stage.

2) IEO slide Haematoxylin and Eosin (H&E) staining

Recent POFs but unsure what stage. Line of granulosa is visible. Nuclei visible, but no vacuoles. Looks like recent POF. Stage 2 or 3.

Many strange hyaline eggs and normal hydrated oocytes.

More recent POFs

POF stage 1, but the cells do not look like normal cells. However, around the normal oocytes the theca and follicle layer cells look abnormal as well.

Probably a problem with the preparation of this sample.

3) IEO slide H&E staining

Better preparation. Some vacuoles, some picnotic nuclei. Not open lumen. Theca is very close. Early stage 3.

Stage 5: not compact, thus stage 5.

More stage 5 POFs are found in the sample

Overall, this sample is POF stage 3. It is possible that the stage 5 POF are an artefact of the cut of the preparation. Thus, the stage 5 could be a small cut of a stage 3 POF.

5 Discussion

In a plenary session it was discussed what the results of the workshop represent and if results could be used in the assessment of the total egg productions. The goal of WKFATHOM is to refresh the analysts participating in the mackerel and horse mackerel egg surveys. The surveys are carried out triennially and for most survey participant's egg identification, staging, and fecundity estimation are only carried out in the survey year. Hence, it is necessary for survey participants to prepare before going on the survey. Therefore, the results of these workshops should not be used as an indication of the actual egg identification and staging and fecundity estimation. For this ring, tests should be carried out during the survey to assess the performance of survey participants.

For new participants to the survey, the WKFATHOM workshops can be a first acquaintance with egg identification and staging and fecundity analyses. However, it should be realized that one week of egg staging and identification and one week of fecundity and atresia estimation is not a full course to create experts in these fields. It is the responsibility of the individual participating institutes that (new) survey participants get the required training.

5.1 Egg sorting exercise

During the survey, participants remove all items that appear like fish eggs (e.g. copepod eggs) and during the actual egg identification under the microscope these items are subsequently removed again. This might explain the larger number of eggs collected from the samples.

The Spray technique should be included where appropriate as a method for sorting eggs from the rest of the plankton during the 2016 triennial surveys. Following the removal of the eggs, each sample should subsequently be resorted after at least 12 hours of fixation by hand to remove any remaining eggs. It is important that the resorting is done after at least 12 hours of fixation with formaldehyde. Before this period, the eggs might still be transparent because the fixation is not complete.

5.2 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 (ICES, 2001; 2004; 2007; 2009; 2012), 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).

A weakness of the analytical method 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. When experts' only results are compared to the results from all participants, in some cases the modal stage is different for the experts is different from the ones for all participants. This problem is difficult to overcome unless only eggs of validated stages are available for these exercises.

During this workshop, some of the eggs used in the second round were validated in order to minimize the problem discussed above. However, it became apparent during the discussion after both rounds that eggs had been moved between wells, as some wells contained two eggs and others none. This leads to participants disagreeing with the validated stage while their assessment of the switched egg is actually 'correct'. In this type of exercise, this problem is difficult to overcome.

These results (Tables 4.2.1 to 4.2.12) 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.

5.3 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 4.3.4). Overall agreement for mackerel and horse mackerel increased from the first to the second round, but was lower (specifically for horse mackerel) compared to previous workshops. This year the number of trainees at the workshop was much higher compared to previous. Most trainees did not have experience identifying fish eggs and this was probably the reason for the lower agreement.

For the experts mackerel agreement increased from 89% in the first round to 90% in the second round. For horse mackerel agreement increased from 70% to 85%. These results are comparable to those obtained at the 2012 workshop where the percentage agreement in species identification after the second round of analysis were 72% for mackerel, 95% for horse mackerel.

The levels of agreement seen in these results (for both 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 2016 surveys.

The accidental movement of eggs from one well to another, also caused problems. This led to low levels of agreement (in both 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.

5.4 Fecundity and atresia estimation

5.4.1 Pipette sampling and picture taking

All participants carried out pipette sampling correctly. The sample weight of the samples was very close to the expected weight of 25 or 50 mg.

All institutes should procure an ultrasound or vibration pen to separate the oocytes in the sample. When analysing pictures always check the sample under the microscope to decide if more separation is necessary. Use a good screen and 100% view for the analysis.

Pictures should be taken with a good light source in order to get a good contrast in the picture. This is especially important for the small transparent vitellogenic oocytes. The resolution of the pictures should be at least 0.2 pixels/μm.

5.4.2 Screening analyses

If in the screening it is clear that the sample cannot be used for analyses due to sampling or fixation of the sample, the whole sample should be discarded from any analyses. In the screening datasheet, a column has been added to note whether the sample should be discarded or not. However, if a reader decides to discard a sample the comment field should always be filled in with the reason behind the decision.

The most advanced oocyte stage will always be used for deciding the oocyte development stage regardless if the most advanced oocyte is atretic or not.

Massive atresia does not occur often in mackerel and horse mackerel. Only if 90% or more of the vitellogenic oocytes are atretic should a sample be classed as having massive atresia.

The data sheet used for the 2013 survey confused oocyte, egg and ovary stage in one column. This has been corrected in the sheet for the 2016 survey. Presence of hyaline

eggs and the spent stage of the ovary are to be reported in separate columns of the datasheet.

5.4.3 Potential and batch fecundity analyses

The oocyte should completely fill the ring to be included in the manual count.

Each picture of a sample should be analysed completely. After that the pictures need to be checked completely (preferably by another person or on another day) to check if any oocytes have been missed.

Always use a 100% view of the picture for the fecundity analyses.

If, when checking the image, the automatic measurement is found to be wrong, this measurement should be discarded from the database and the oocyte only counted manually.

In addition, advanced atretic oocytes should be included because the number of atretic cells will be substracted from the calculations later on.

LED illumination gives a very even light distribution on the image, however the contrast on the picture is lower compared to an ordinary light source. Participants using LED illumination should try to add some oblique (not too much) to enhance contrast.

In ImageJ the pictures should have the correct scaling, this is important for the threshold size of the oocytes.

Potential fecundity includes all oocytes from 185 μm. For batch fecundity the analyses should only include the oocytes of 500 μm and larger.

5.4.4 Atresia estimation

Big mackerel ovaries can be difficult to fix properly in the formaldehyde. Therefore, all ovary lobes should be punctured with a fine needle in the ends and middle without breaking the ovary wall before putting them in the formaldehyde solution. Formaldehyde will be able to penetrate inside the ovary immediately.

If any breaks or cuts are visible in the chorion, the oocyte is considered atretic. However, care should be taken when the chorion is damaged due to preparation of the sample. If the chorion is damaged the oocyte is not necessarily atretic. The start of alpha atresia is if there is a cut in the inner chorion. During the analyses, one should not mix up the vitelline membrane with the inner chorion.

When in doubt the chorion thickness should be measured to estimate the size of the break. The width of the chorion should always be measured at the thinnest part because the chorion expands during the atretic process.

5.4.5 POF staging

Degeneration of the cells should be the primary characteristic for the assessment of POF stage. Size should only be used as a secondary characteristic.

In sardine only one POF stage is found in a sample. However, the period between spawning of two batches in sardine is probably longer compared to mackerel. In multiple mackerel samples both hydrated oocytes and young POF stages are found in the same sample. This is an indication that mackerel can spawn a batch per day. Thus, day 0 and day 1 POFs can be found simultaneously in a sample.

If multiple stages are found in the sample, an overall POF stage for the whole sample is assessed. If there is a dominant stage, the overall POF stage of the sample is the dominant stage. If the numbers of POFs in the different stages are all similar, the sample POF stage is the youngest stage.

IEO will prepare serial section slides from 5 POF samples. The sections will be stained with H&E. The slides will be sent to the POF analysing institutes for a ring test to calibrate POF staging. The ring test will be carried out in first quarter of 2016 before the survey samples will be analysed.

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Annex 1: List of participants

Annex 1a. List of participants for the WKFATHOM-egg identification meeting in Hamburg, Germany, 12-16 October 2015.

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From left to right: Björn, Paula, Beatriz (front row), Cindy, Lynette, Helga, Sakis, Ineke, Inger, Clare, Linford (second row), Timo, Matthias, Ewout, Brendan, Finlay, Birgit, Luc, Jim (back row).

Annex 1b. List of participants for the WKFATHOM-fecundity estimation meeting in Bergen, Norway, 9-12 November 2015.

Name	Address	Phone/Fax	E-mail
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From left to right: Monica, Inger, Monica, Antonio, Maria, Merete, Paula, Brendan, Anne, Grethe, Dolores, Cindy, Natalie, Anders.

Annex 2: Agenda

Annex 2a. Agenda for the WKFATHOM-egg identification meeting in Hamburg, Germany, 12-16 October 2015

Monday 12 October

10:00 Start of the meeting, practical stuff etc.

10:15 Introduction round, practicalities, division of tasks, etc.

10:30

- Pipette sampling for fecundity – Cindy van Damme
- 1st round of egg identification and staging (ToR b)

13:30

- 1st round of egg identification and staging (ToR b)
- Spray method (ToR a)

Tuesday 13 October

9:00

- Continue 1st round of egg identification and staging (ToR b)
- Spray method (ToR a)

13:30

- Spray method (ToR a)
- Update pictures and descriptions and review available information on species identification and egg staging (ToR c and d)
- Update survey manual and standard protocols (ToR d)
- Write report

15:00 Discussion of results of 1st round of egg identification and staging (ToR b)

Wednesday 14 October

9:00

- 2nd round of egg identification and staging (ToR b)
- Spray method (ToR a)

13:30

- 2nd round of egg identification and staging (ToR b)
- Spray method (ToR a)

Thursday 15 October

9:00

- Planning for the 2016 survey – Brendan O'Hea
- Presentation on blue whiting larvae - Matthias Kloppmann
- Discussion on WGALES recommendation for a general egg and larvae staging workshop

- Discussion: action needed if staging or identification is below average/modal
- Discussion on EU call for 2017
- Discussion on mackerel benchmark issue list

11:00

- Continue 2nd round of egg identification and staging (ToR b)
- Update pictures and descriptions and review available information on species identification and egg staging (ToR c and d)
- Update survey manual and standard protocols (ToR d)
- Write report

13:30

- Continue 2nd round of egg identification and staging (ToR b)
- Spray method (ToR a)
- Update pictures and descriptions and review available information on species identification and egg staging (ToR c and d)
- Update survey manual and standard protocols (ToR d)
- Write report

Friday 16 October

9:00

- Discussion of results of 2nd round of egg identification and staging (ToR b)
- Discussion of results of the spray method
- Finalize standard pictures and descriptions set for both species (ToR c and d)
- Finalize survey manual and standard protocols (ToR d)
- Finalize report, recommendations etc.

13:30 End of the meeting

Annex 2b. Agenda for the WKFATHOM-fecundity estimation meeting in Bergen, Norway, 9-12 November 2015.

Monday 9 November

9:00 Start of the meeting, practical stuff etc.

9:15 Introduction round

9.30 Presentations

- Lessons learned from last survey (Cindy)
- Sampling at sea (Brendan)
- How to take fecundity samples (Maria)
- Separating oocytes in fecundity samples (Anders)
- Taking pictures from fecundity samples and slides (Anders)

Lab practice

- pipette sampling
- taking pictures of fecundity samples and slides (everyone to bring pictures)

13:00

Lab practice

- pipette sampling (if fresh fish available otherwise water)
- taking pictures of fecundity samples and slides (everyone to bring pictures)

Discuss

- pipette and picture results
- modifications needed in the manual
- Fecundity and Atresia database at ICES: preparing datasets and vocabulary lists

Tuesday 10 November

9:00 Presentations

- Introduction to screening analysis (Merete)
- Introduction to image analysis and data sheets of screening analysis (Anders/Merete)

Practice:

- screening analysis from pictures

Discuss:

- results of screening

13:00 Presentations

- Introduction to atresia analysis (Lola)
- Introduction to image analysis and data sheets of atresia analysis (Anders/Merete)

Practice

- atresia analysis from pictures (from pictures from IMR, AZTI and IEO)

Discuss

- results of screening and atresia analysis
- modifications needed in the manual
- Fecundity and Atresia database at ICES: preparing datasets and vocabulary lists

Wednesday 11 November

9:00 Presentations:

- Introduction to fecundity and batch fecundity analysis (Maria/Paula)
- Introduction to image analysis and data sheets of fecundity and batch fecundity analysis (Anders/Merete)

Practice:

- fecundity analysis from pictures

13:00

Practice:

- batch fecundity analysis from pictures

Discuss

- results of fecundity and batch fecundity analysis
- modifications needed in the manual
- Fecundity and Atresia database at ICES: preparing datasets and vocabulary lists

Thursday 12 November

9:00 Presentations

- Introduction to POF analyses, ageing and spawning fraction estimation (Antonio/Lola)
- POFs in southern horse mackerel (Cristina)
- Lessons learned from last survey (everyone to prepare (a) slide(s)
- Introduction to data sheets of POF analysis (Antonio/Lola)

Practice

- POF analysis and ageing from pictures

13:00

Practice

- POF analysis and ageing from pictures

Discuss

- Results of POF analysis
- Modifications needed in the manual

- Fecundity and Atresia database at ICES: preparing datasets and vocabulary lists

After the workshop

Everyone will take a pipette sample and slide of the ring test home to take pictures with their own microscope and camera setup. The two samples needs to be analysed following the manual. The pictures and data should be uploaded to the ftp-site before the **1 December 2015**.

Annex 3: WKFATHOM terms of reference for the next meeting

The **Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel** (WKFATHOM) **chaired by** Matthias Kloppmann*, Germany and Maria Korta*, Spain will meet twice in autumn 2018 (dates and venues to be decided at the WGMEGS 2017 meeting) 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 2015 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 1 January 2019 to the attention of SCICOM, WGMEGS and WGBIOP.

Supporting Information

Priority	Information quality, used to provide fisheries advice through WGWIDE, 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, and 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, and 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, WGWIDE, WGALES and WGBIOP
Linkages to other organizations	None.

Annex 4: Recommendations

RECOMMENDATION	ADRESSED TO
1. It is recommended that all WGMEGS 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 2018).	WGMEGS
2. It is recommended that all microscopes at the next workshops 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. All workshop participants should bring a calibration micrometre to the workshop in 2018.	WGMEGS, WKFATHOM
3. All survey participants are reminded that the procedures described in the WGMEGS survey manual (Kloppmann *et al.*, 2015) should be followed during the 2016 surveys. Participants are particularly reminded 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.	WGMEGS
4. 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.	WGMEGS, WKFATHOM Chair to consider before next workshop in 2018
5. All survey participants are requested to measure formaldehyde preserved egg diameters and oil droplet diameters of 100 hake, 100 mackerel and 100 horse mackerel eggs during each individual cruise, to identify changes in egg diameter over spawning time and area. Also the development stage should be reported.	WGMEGS

RECOMMENDATION	ADRESSED TO
6. All survey participants are requested to investigate genetic analyses of fish eggs to aid species identification.	WGMEGS, WGALES
7. WKFATHOM recommends that egg and larvae identification workshops are only organized to address specific questions in relation to that particular survey. Workshops should be organized by the respective survey WG.	WGALES
8. WKFATHOM recommends that institutes provide continuity of staff to carry out the plankton identification and staging to ensure high quality standard of the survey. It is the institutes responsibility to provide appropriate training for new staff in advance of the survey. This should be done through institute workshops, as one week of WKFATHOM is not enough to turn trainees into experts.	WGMEGS, national institutes
9. WKFATHOM encourages exchanges of staff between participating institutes, to allow exchange of knowledge and increase expertise among survey participants.	WGMEGS
10. All survey participants should take pictures of mackerel, horse mackerel and also species with similarly sized eggs in the different development stages of formaldehyde fixed eggs.	WGMEGS
11. The spray samples in the 2018 workshop should contain a number of validated mackerel, horse mackerel and hake eggs. After spraying participants should carry out the SAT to identify the eggs.	WKFATHOM Chair to consider before next workshop in 2018