

WORKSHOP ON MACKEREL, HORSE MACKEREL AND HAKE EGGS IDENTIFICATION AND STAGING (WKMACHIS)

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

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

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Editors

Matthias Kloppmann

Authors

Paula Alvarez • Maria Manuel Angélico • Beatriz Beldarrain • Ewout Blom • Valeska Borges
Finlay Burns • Gersom Costas • Thassya dos Santos Schmidt • Jim Drewery • Sólva Káradóttir Eliassen
Merete Fonn • Dolores García • Elisabete Henriques • Hannah Holah • Bastian Huwer • Luisa Iglesias
Mette Kjellerup Schiønning • Erika Koelemij • Anne-Mette Kroner • Sakis Kroupis • Karin Krüger
Linford Mann • Bahar Mozfar • Grainne NiChonchuir • Brendan O'Hea • James Pettigrew
Isabel Riveiro • Durita Sørensen • Birgit Suer • Anders Thorsen • Grethe Thorsheim
Frødis Tousgaard Rist • Dave Tully • Jens Ulleweit • Javier Valtierra • Cindy van Damme • Rob van Ree



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i Executive summary

The Workshop on Mackerel, Horse Mackerel and Hake Egg Identification and Staging (WKMACHIS) is part of a series of workshops (WKMHMES, WKFATHOM) that aim to standardise the process of fish egg identification and staging. Since 2000, this workshop is held in autumn of each year prior to the triennial mackerel and horse mackerel egg survey. In 2021, however, the workshop had to be held online for the first time due to the continuing SARS-CoV2 pandemic. All egg identification and staging during the workshop were undertaken using images on the SmartDots WebApp, as opposed to real samples under microscopes. In advance of the workshop eggs were, however, sent to participants to be identified and staged under the microscope.

The majority of the time at the workshop was spent completing 2 rounds of identifying and staging mackerel, horse mackerel, hake and similar looking eggs. The results promoted discussion and highlighted specific problem areas. These discussions enabled further development of standard protocols, and enhancements to the species and stage descriptions. The results were reassuring and improved from the first to the second round of the exercises. However, and particularly in horse mackerel, bias in correctly identifying stage 1 eggs was higher than in previous workshops for both, experts and non-experts. These results can almost exclusively be explained by the change in workshop methodology that saw a move from a live view of the fish eggs to images.

As the mackerel and horse mackerel egg surveys are carried out once every three years, the workshop functions as a refresher for expert survey participants and as an introduction for new participants in egg analyses. It should however be realised that one week of workshop for egg identification and staging, particularly if carried out online and based on images, is not sufficient to train new participants. Institutes should ensure newcomers receive a thorough training while also allowing more experienced participants to refresh their knowledge ahead of the survey.

Again, as all previous workshops, the meeting demonstrated the importance of conducting the workshop a few months ahead of the mackerel and horse mackerel egg survey. For several valuable fish stocks in the Northeast Atlantic, the survey delivers the only fishery-independent SSB indices based on correctly identified and staged fish eggs. Ongoing discussion and training for consistency is, therefore, imperative. While many participants had problems working with images only, the use of image-based systems for (egg) analysis will become a central part of future workshops.

ii Expert group information

Expert group name	Workshop on Mackerel, Horse Mackerel and Hake Egg Identification and Staging (WKMACHIS)
Expert group cycle	Annual
Year cycle started	2021
Reporting year in cycle	1/1
Chair	Matthias Kloppmann, Germany
Meeting venue and date	11-15 October 2021, Bremerhaven (planned), Germany, but held online. (38, participants).

1 Introduction

In preparation for the 2022 international ICES coordinated mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) egg survey (MEGS), a workshop was held to standardise and calibrate the identification and staging of eggs of the survey's target species: mackerel, horse mackerel and hake (*Merluccius merluccius*).

The workshop was planned to be held at TI-SF, Bremerhaven, Germany, for the plankton analysts who will be involved in the 2022 survey. The aims of the workshop were to standardise procedures and produce definitive criteria for the identification and staging of mackerel, horse mackerel and hake eggs. The workshop also investigated the reasons for individual differences in the identification and staging of mackerel and horse mackerel eggs and attempted to harmonise these. It was also planned to evaluate the use of the 'spray' technique, for removing fish eggs from plankton samples but also to separate hake eggs from other eggs.

Due to the Covid19 pandemic and the associated restrictions for travelling as well as holding physical meetings, the workshop was required to be held online. While it was possible to carry out egg identification and staging trials using the ICES SmartDots web application, evaluation of the spray technique had to be cancelled.

To enable 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 1A and 1B, and to distinguish these from eggs in later stages of development but also from other species of the same stages. It is therefore vital that the analysts involved with sorting, identification and staging of mackerel, horse mackerel and hake eggs from the triennial egg surveys are able to accurately identify and stage the eggs of each of the target species (ICES, 2018). These workshops (previously WKMHMES and 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 challenges encountered while doing so. Previous workshops (ICES, 2001, 2004, 2006, 2009, 2012, 2015, 2018) were successful in developing a comprehensive set of criteria for both mackerel and horse mackerel egg identification and staging and these were reviewed during the 2021 workshop. With the exception of grey gurnard, no additions or changes were considered necessary for the identification criteria of both egg stage and species.

As usual for this workshop, inexperienced analysts were involved for their first time, and it was critical that they became fully aware of the procedures and criteria in advance of the 2022 surveys in the Northeast Atlantic.

2 Materials and Methods

2.1 Egg identification and staging – general remarks

The eggs and larvae of most of the species found in the MEGS area are well described by Russell, 1976. His book is well known and used by all the participants of the ICES triennial surveys. It is generally regarded as the definitive work on the subject in the area. Descriptions of the eggs of mackerel, horse mackerel and species with similarly sized eggs can also be found in Munk and Nielsen (2005), Rodriguez, *et al.* (2017) and Ré and Meneses (2009).

Some difficulties do occur, particularly with the identification of recently spawned eggs from species that do not show great differences in their morphological features. In some instances, it is even difficult to recognize differences between mackerel and horse mackerel eggs when the segmentation of the yolk is not distinct in the latter.

Some difficulties can occur with the identification of hake eggs, which are similar in size and appearance to several other species including mackerel, ling and megrim. The 'surface adhesion test' (SAT) described by Porebski (1975) and Coombs (1994) does help to separate hake eggs from those of other species, although it does not always produce consistent results.

Spraying of the samples also gives an indication of the species composition of the sample. Hake eggs, and eggs such as pearlside (*Maurollicus muelleri*), with its corrugated chorion, attract and retain microbubbles of air and are subsequently lifted upwards during the spraying procedure, tending to float at the surface. This is in contrast to mackerel and horse mackerel eggs, which drop downwards and can be drained.

Within WGMEGS the eggs of mackerel are classified into one of six morphological stages (1a, 1b, 2, 3, 4 and 5; Lockwood *et al.*, 1981), following the development criteria described for plaice (Simpson, 1959). For horse mackerel, and hake the description of stages is the same with the exception of stage 5, which does not exist for these species. Horse mackerel and hake larvae hatch at the end of egg stage 4 (Pipe and Walker, 1987; Coombs and Mitchell, 1982).

2.2 Egg sorting trials (ToR a)

The evaluation of the spray technique (Eltink, 2007) for sorting eggs from plankton samples involves practical work, which cannot be conducted during an online event. Therefore, this term of reference couldn't be considered during this workshop. Participants were instead advised to practise the method in their home institute prior to the 2022 surveys.

2.3 Pre-workshop exercise

About 2 months ahead of the workshop, 11 samples, each containing the same mixture of 24 eggs at different developmental stages of 4 different fish species (mackerel, horse mackerel, hake and grey gurnard, Table 2.1), were prepared and sent to 11 participating institutes. Each participant from the different institutes was asked to take a picture of the sample, and to stage and identify all eggs in the sample. The results had to be submitted one week ahead of the scheduled workshop to the organizing Thünen Institute.

Table 2.1: The composition of the egg samples sent to each of the participating institute. HOM = horse mackerel, MAC = mackerel, GGU = grey gurnard, HAK = hake.

egg no	species	stage
1	HOM	1b
2	HOM	1b
3	HOM	3
4	HOM	3
5	MAC	1a
6	MAC	1a
7	MAC	1a
8	MAC	1a
9	MAC	1b
10	MAC	1b
11	MAC	1b
12	MAC	1b
13	MAC	2
14	MAC	2
15	MAC	2
16	MAC	3
17	MAC	3
18	MAC	3
19	MAC	3
20	GGU	1b
21	GGU	1b
22	GGU	1b
23	HAK	2
24	HAK	2

2.4 Egg staging (ToRs b, c and d)

2.4.1 Egg staging trials

In lieu of the normal workshop circumstances where egg samples are staged and identified using microscopes, the online workshop necessitated the use of the SmartDots Web-application for egg identification and staging trials. To facilitate this, two SmartDots events were created prior to the workshop.

Images of a total of 600 eggs of mackerel, horse mackerel, hake (*Merluccius merluccius*), megrim (*Lepidorhombus whiffiagonis*), ling (*Molva molva*), grey gurnard (*Eutrigla gurnardus*), and other species, which can be found in egg survey samples, were uploaded in 2 separate identification and staging events to the SmartDots web-server. A scale was added to each image, converting distances in pixel to mm, enabling participants to measure egg and oil globule diameters.

During both rounds 300 eggs had to be staged and identified by each participant. All eggs were validated for species and stage. The eggs were mainly those of mackerel (110 eggs), horse mackerel (90), hake (40), ling (20), and grey gurnard (20) which are morphologically similar to those of the two target-species. A mixture of 20 eggs of other species was added to sum up to 300 eggs. It was hoped that these definitive eggs of known parentage, would enable participants' species identification to be judged more consistently. The egg images 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 TAEP and SSB are based. All participants were asked to stage all eggs, irrespective of species. The mackerel eggs on each image were staged to 1A, 1B, 2, 3, 4, 5 and the horse mackerel and hake eggs were staged to 1A, 1B, 2, 3, 4, as horse mackerel and hake larvae hatch before the eggs reach stage 5. Due to the fact that computers can only calculate with numeric values, stage 1A was changed to 0 and stage 1B to 1 in the results tables.

Each event was open for 24 hours, during which participants were permitted to view and annotate the egg images. Each participant logged in to the SmartDots Web-App and browsed through the images one by one. After selecting an image, participants were requested to annotate the image with at least the species name and stage and, if possible, also to measure both, egg and oil globule diameter.

Once each participant had staged and identified as many of the 300 eggs as possible during the 24 hours when the event was open, the downloaded results retrieved from the SmartDots site were entered into the standard Excel evaluation sheets. From here 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 species and/or to one developmental stage. These eggs were then viewed on the SmartDots site. 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, 2019) were reviewed (see section 2.4.2 below).

The second round, which was set up prior to the workshop, provided the same mix of the target species though at a slightly different composition of stages. Consequently, the lessons learned during the first round of analysis and subsequent discussions should be reflected in the second-round results

2.4.2 Egg staging criteria

2.4.2.1 Egg staging criteria for mackerel, horse mackerel (Western stock), and hake

On account of discussions following the first and second round of egg staging, the participants reviewed the description of the developmental stages for mackerel, horse mackerel, hake, ling, megrim and grey gurnard. The primary characteristics are based on those presented in Lockwood *et al.* (1977) for mackerel (Figures 2.1 and 2.2), but also include some other (secondary) characteristics, which the participants of the previous workshops thought were crucial in determining egg stage. At this workshop it was decided that the descriptions don't need a further update. Figures 2.3 and 2.4 show the development stages for horse mackerel and figure 2.5 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 1A

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 1b.

Stage 1B

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 2

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 3

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 4

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 5

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.

2.4.2.2 Egg staging criteria for the southern stock of horse mackerel

Contrary to the Western horse mackerel Stock AEPM analyses, where a development scale with 5 stages is used, the DEPM approach in the southern stock uses an 11-developmental-stage scale. This egg development scale was first developed by Cunha *et al.* (2008) but is not subject of the exercises during WKMACHIS. A revised version of the 11-stage scale now used by IPMA during their DEPM surveys for horse mackerel. The details are described in annex 4 of this report, and are exemplified in comparison to the 5-stage scale in figure 2.3.

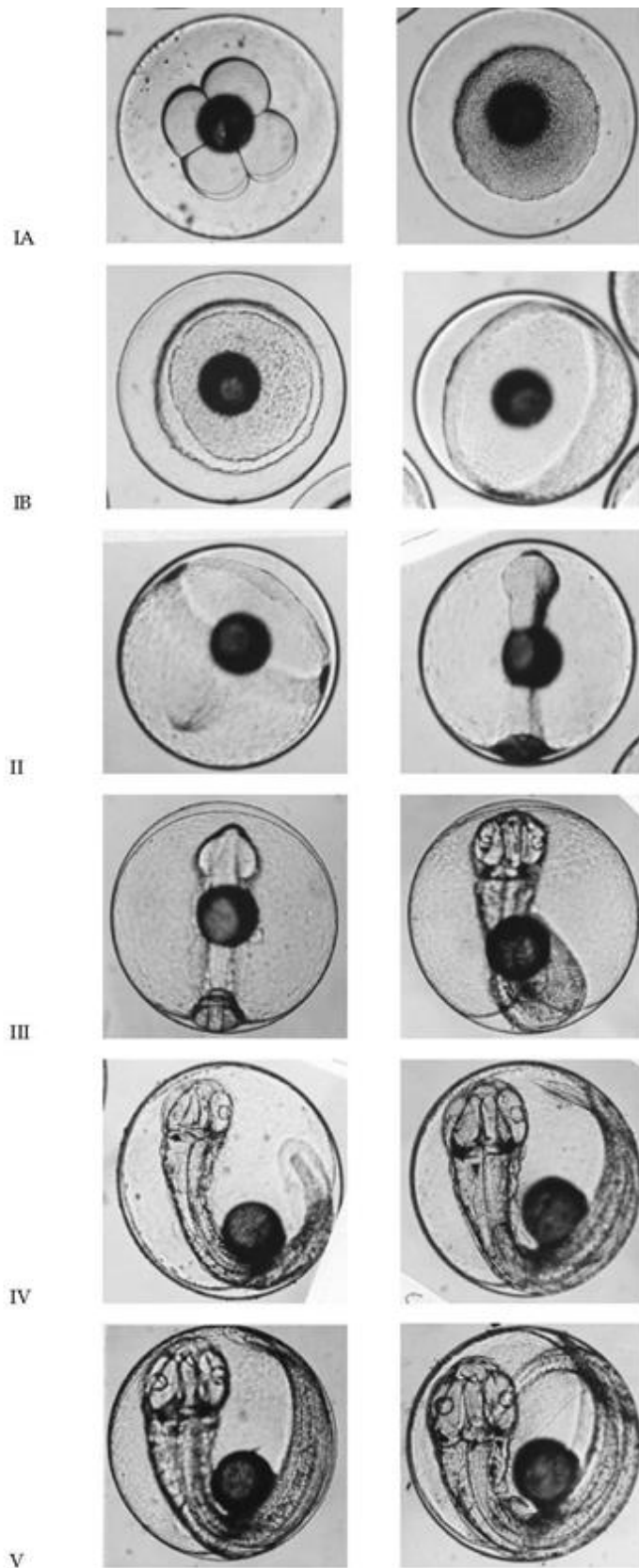


Figure 2.1: Mackerel eggs at the beginning and end of the six development stages.

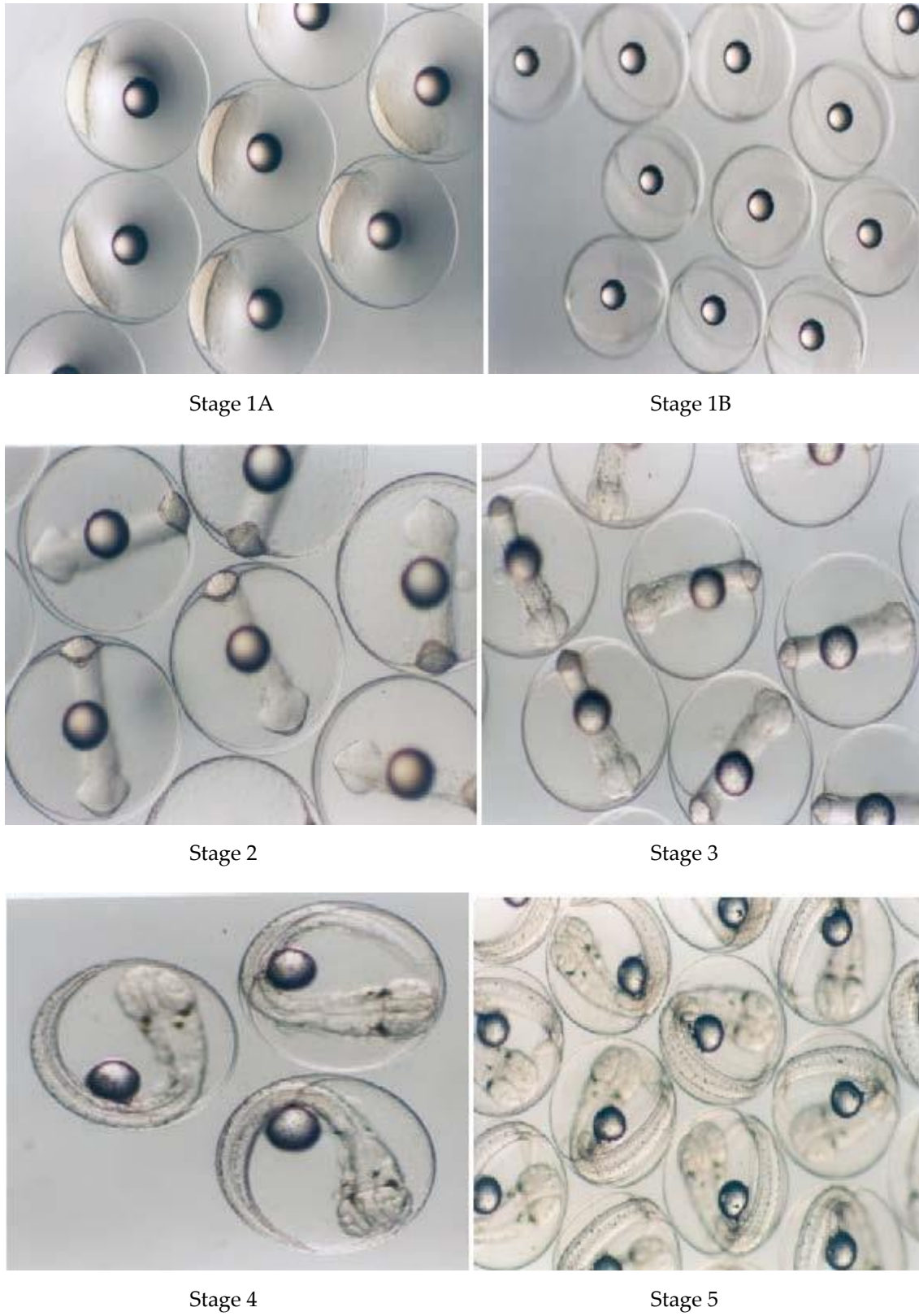


Figure 2.2. Development stages of mackerel from fertilization experiments.

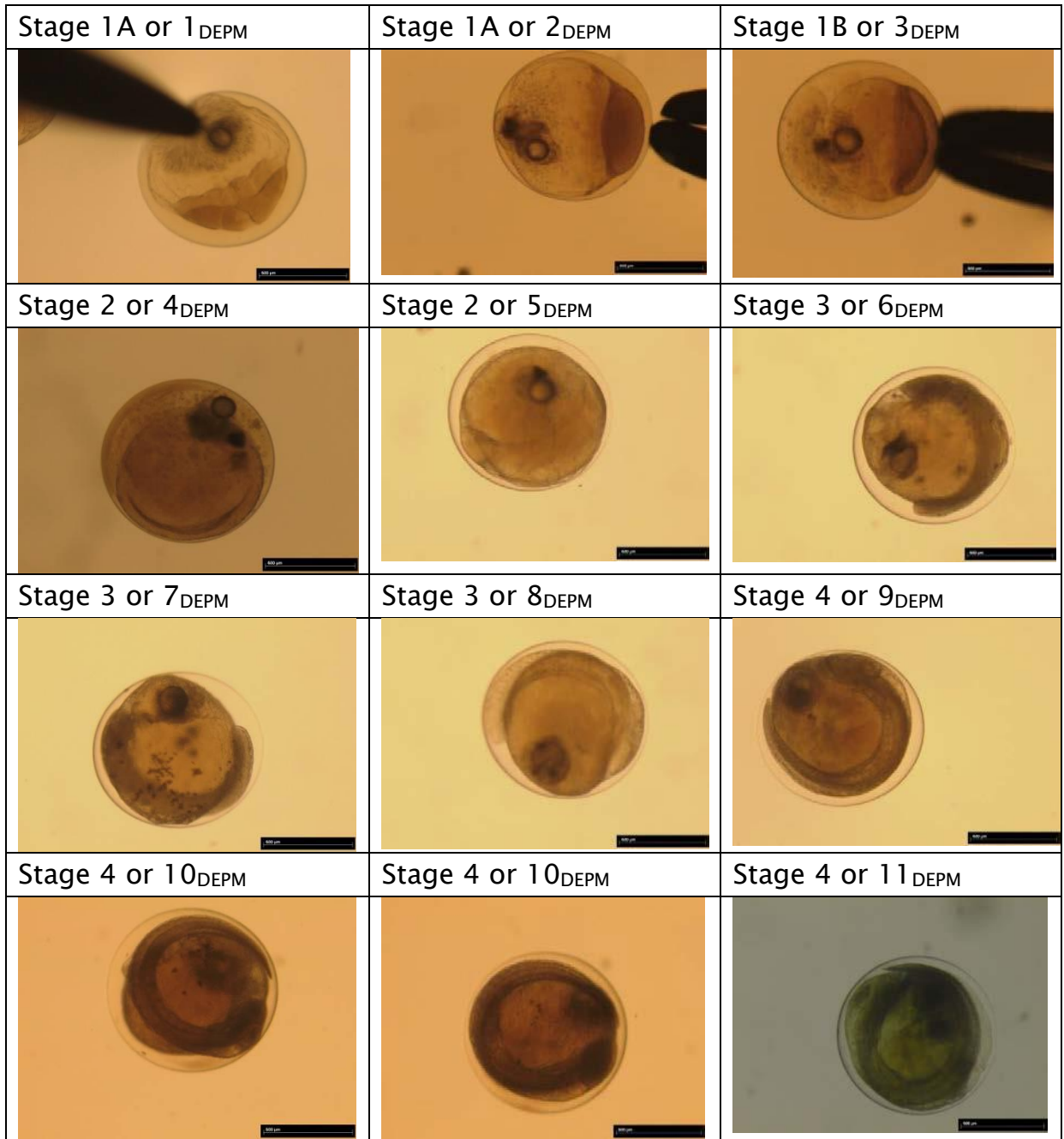
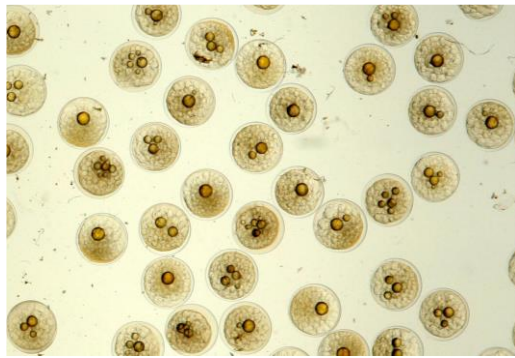
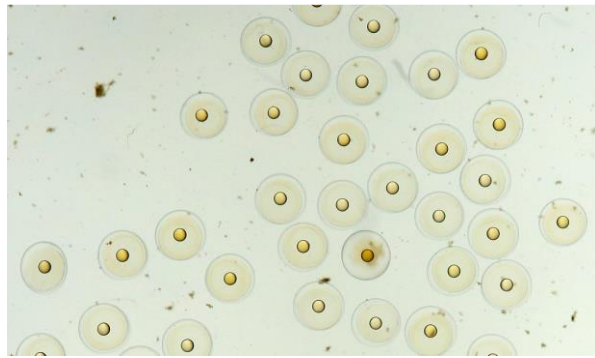


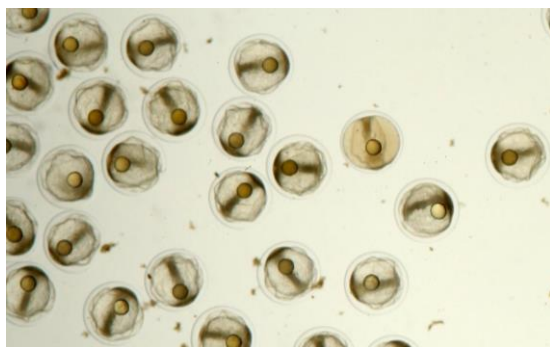
Figure 2.3. Development stages of horse mackerel from fertilization experiments. First stage number is the stage development used for the Western stock, second number is the stage development used for the DEPM in the Southern stock.



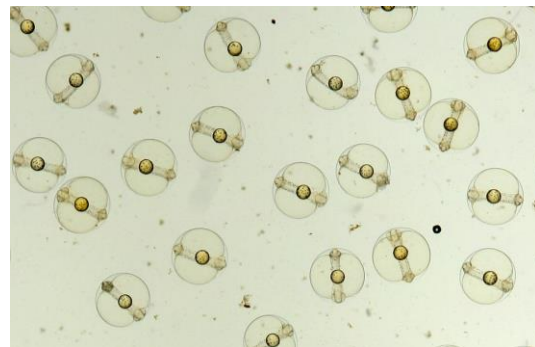
Stage IA



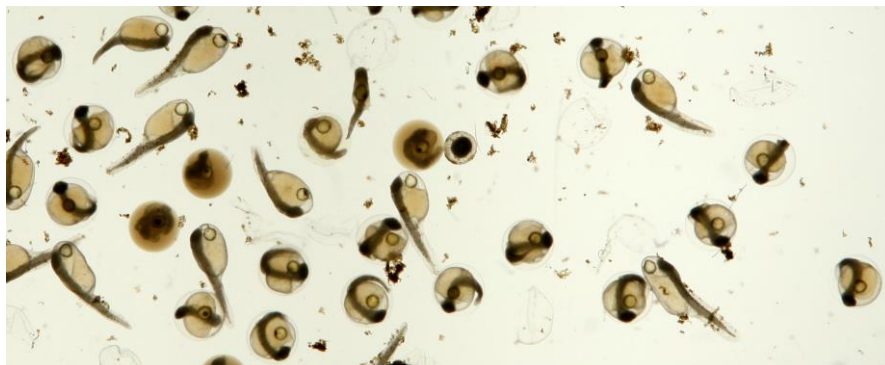
Stage IB



Late stage II

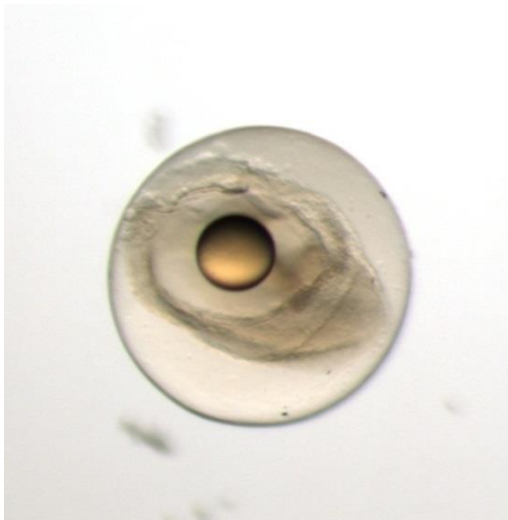


Early stage III

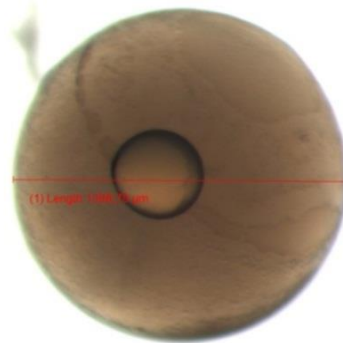


Late stage IV and hatching

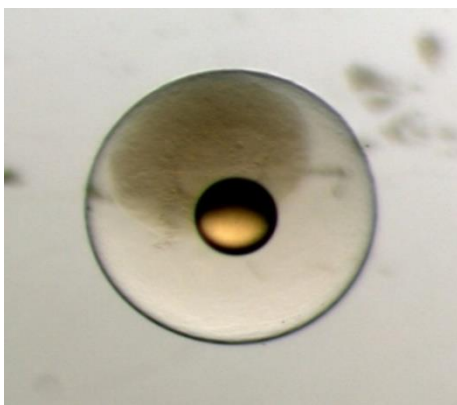
Figure 2.4 Development stages of horse mackerel from fertilisation experiments.



Stage 1A



Stage 1A



Stage 1B



Stage 2



Stage 3



Stage 4

Figure 2.5. Developmental stages of hake eggs from fertilization experiments.

2.5 Egg identification (ToRs b, c and d)

2.5.1 Egg identification trials

The same images of fish eggs (described in section 2.4 above) were also used for the egg identification exercise. As each participant moved from image to image on his or her screen, 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 2018 workshop report (ICES, 2018) was available to participants prior to the first staging round.

The results of the first round of egg identifications were downloaded from the SmartDots sites, collated and entered 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 selected from the SmartDots site and displayed (as described in section 2.4 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 prior to commencing the second round of analysis a review of the egg identification criteria produced by previous WKMHMES and WKFATHOM participants was undertaken. It was decided that the descriptions of the target and of most other very similar species did not require updating within the survey manual. There was however a lengthier discussion on the potential for confusing eggs of mackerel with those of grey gurnard, particularly in the North Sea survey area.

2.5.2 Egg identification criteria

Egg and oil globule size are the primary criteria used in identification of 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 2.2 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 also 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, therefore, not 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 egg identification and staging workshops carried out in the year prior to the survey year.

Table 2.2: Comparison of the Characteristics of Mackerel, Horse Mackerel, Blue Jack Mackerel, Megrim, Hake, Snipefish, Grey Gurnard and Ling 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) Egg Oil Globule		Reference	Area	Diagnostic Features
Mackerel (<i>Scomber scombrus</i>) (See Lockwood <i>et al.</i> , 1977)	1.0- 1.38	0.28- 0.35	Russell, 1976	North Sea, English Channel	<ul style="list-style-type: none"> • Unsegmented/ Homogenous yolk • Perivitelline space approx. 0.05 mm • Oil globule often orientated to the top of the egg • Yolk pigmented before hatching: a spot per side appears just posterior to the head. • Not typically found where water temperature at 20 m is less than 8.5 °C
	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 <i>et al.</i> , 2006	Biscay	
	0.97- 1.38	0.22- 0.38	Fritzsche, 1978	Mid-Atlantic Bight	
	1.0- 1.38			North Atlantic	
	0.97- 1.38	?	Johnstone <i>et al.</i> , 1934	Isle of Man	
	1.21- 1.33	~0.32	Holt, 1893	West of Ireland	
0.99- 1.39	0.20- 0.40	IPMA, 2019 survey	S and W Atlantic Iberia		
Horse Mackerel (<i>Trachurus trachurus</i>) (See Pipe and Walker, 1987)	0.81- 1.04	0.19- 0.28	Russell, 1976	North Sea, English Channel	<ul style="list-style-type: none"> • 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 mackerel. However, the pigmentation is not as strong as in hake. <ul style="list-style-type: none"> • Oil globule easily broken into several smaller pieces.
	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
Horse Mackerel (<i>Trachurus trachurus</i>)	0.72- 1.15	0.14- 0.35	IPMA, 2013, 2019 surveys	S an W Atlantic Iberia	
	0.90- 1.00	0.18- 0.28	Cunha <i>et al.</i> , 2008; Gonçalves <i>et al.</i> , 2013	Atlantic Iberian waters	
	max. 0.84	0.24- 0.26	Holt, 1893	English Channel	
Blue Jack Mackerel (<i>Trachurus picturatus</i>)	0.98- 1.10	0.19- 0.31	IPMA, fertilization experiment 2010 (Gonçalves <i>et al.</i> , 2013)	W Portugal	<ul style="list-style-type: none"> ● Segmented yolk ● Small perivitelline space ● Single yellow oil globule located towards the posterior portion of the yolk ● Two rows of spots appear along the dorsal body contours
Grey gurnard (<i>Eutrigla gurnardus</i>)	1.27- 1.55	0.25- 0.33	Russell, 1976	North Sea	<ul style="list-style-type: none"> ● Wrinkled chorion, resembling that of megrim egg, but striation lines more curved
Megrim (<i>Lepidorhombus whiffiagonis</i>)	1.02- 1.22	0.25- 0.30	Russell, 1976	North Sea, Irish Sea	<ul style="list-style-type: none"> ● Striated appearance of egg membrane*. (See below and Figure 2.6) ● 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
	1.07- 1.22	0.25- 0.30	Ehrenbaum, 1905-09	North Sea	
	1.07- 1.13	0.30	Holt, 1893	West of Ireland	
Megrim (<i>Lepidorhombus whiffiagonis</i>)	1.08- 1.30	0.29- 0.34	CEFAS unpublished data	Celtic Sea	
Hake (<i>Merluccius merluccius</i>)	0.94- 1.03	0.25- 0.28	Russell, 1976	North Sea, English Channel, Mediterranean	<ul style="list-style-type: none"> ● Positive surface adhesion test (SAT) is used to identify hake eggs (Porebski, 1975) and (Coombs, 1994).

*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.

Species	Diameter (mm) Egg Oil Globule		Reference	Area	Diagnostic Features
Hake (<i>Merluccius merluccius</i>) (See Coombs and Mitchell, 1982)	0.94- 1.03	~0.27	Ehrenbaum, 1905-09	North Sea, English Channel, Mediterranean	<ul style="list-style-type: none"> • 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, 1931-33	Mediterranean	
	1.06	0.26	Guevara-Fletcher <i>et al.</i> , 2015	Galicia waters	
	1.10- 1.16	0.27- 0.35	Shaw, 2003	Celtic Sea	
Longspine Snipefish (<i>Macrorhamphosus scolopax</i>)	1.00	0.2	Fritzsche, 1978	Europe	<ul style="list-style-type: none"> • 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 (<i>Molva spp.</i>)	0.97 – 1.13	0.28 – 0.31	Russell, 1976	North Sea	<ul style="list-style-type: none"> • Unsegmented yolk • Pigmented oil globule • Pigmentation in later stage embryo is concentrated into 2 distinct lines that run all the way along the back. • Most likely to occur in temperatures < 8.5 °C
Lings (<i>Molva spp.</i>)					

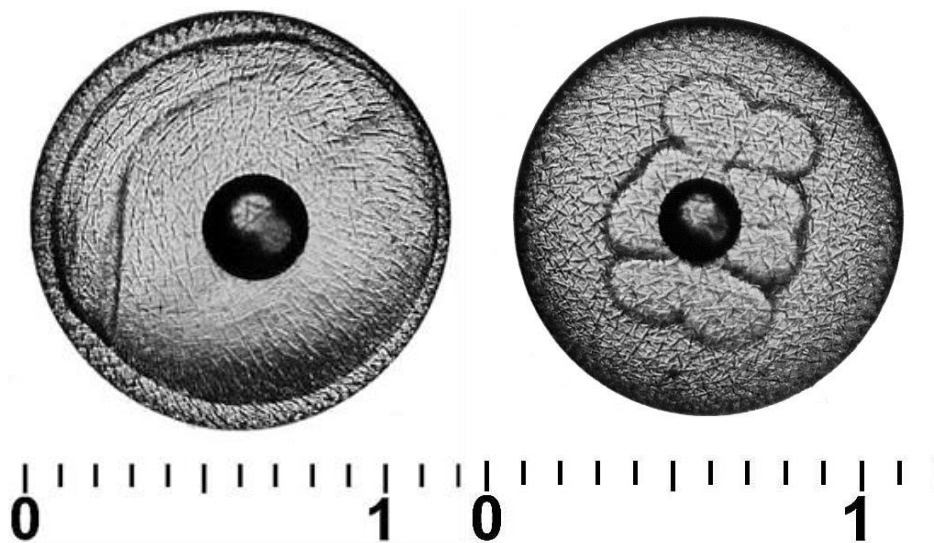


Figure 2.6: Eggs of megrim, showing the striations on the membrane.

Misclassification of mackerel and horse mackerel eggs in the southern survey areas (ICES sub-devisions 27.8.a,b,c and 27.9.a)

In the southern part of the area of the triennial mackerel and horse mackerel egg survey several 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: 0.71 mm – 1.04 mm (Demir, 1961; Padoa, 1956)
- Oil globule: 0.24 mm (Padoa, 1956)
- Description: Pelagic eggs, spherical, transparent. No perivitelline space. Oil globule colourless. Fine striated membrane (Padoa, 1956).
- Eggs are similar to *Trachurus trachurus*
- Distribution of adults appears in the reports of ICES-WGACEGG (e.g. ICES, 2021).

Trachurus picturatus

Description and measurements based on eggs from a single artificial fertilization experiment carried out in 2010 at IPMA (Figure 2.7).

- 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 (Gonçalves et al., 2013)
- Oil globule: 0.19 – 0.31 mm (Gonçalves et al., 2013)
- The species distribution is patchy and not regular each year. Report on adult distribution appears in the reports of ICES-WGACEGG (e. g. ICES, 2021)

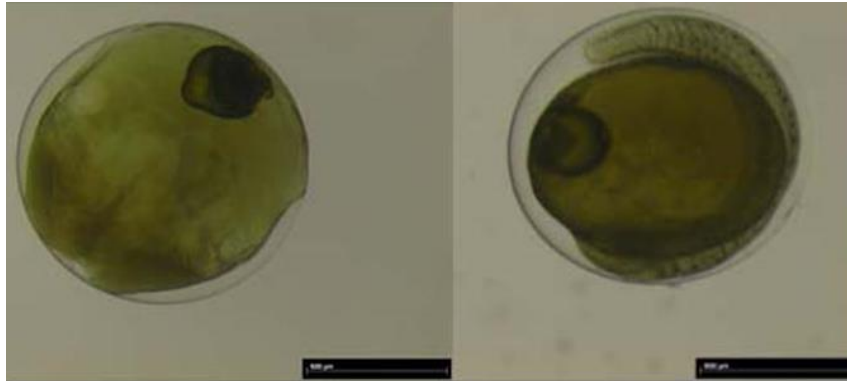


Figure 2.7. Eggs of *Trachurus picturatus* from a fertilization experiment at IPMA in 2010.

Scomber colias

- The eggs are spherical, on average ranging in diameter from 1.04 – 1.36 mm (Rodriguez et al., 2017). Similar description was offered by Ré and Meneses (2009), their given diameter range, however, with a considerably smaller minimum value (0.8 mm) is based on a description of eggs of *S. japonicus* from the Pacific (Ambrose, 1996) and probably not valid for *S. colias*.
- Oil globule 0.22-0.31 mm in diameter in (Ré and Meneses, 2009) the North Atlantic. According Rodriguez et al. (2017), 0.22-0.27 mm in the Mediterranean.
- 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 (Fahay, 1983), while in *S. scombrus* eggs the yolk is pigmented just before hatching, when a spot per side appears just posterior to the head (Fahay, 1983).
- The perivitelline space is narrow.
- Distribution of adults appears in the reports of ICES-WGACEGG (e.g. ICES, 2021).

Macroramphosus scolopax

- Egg diameter: 1.0 mm (Fritzsche, 1978)
- Oil globule: 0.20 mm (Fritzsche, 1978)
- 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 the species' distributions see for example Marques *et al.* (2005).

Boops boops

- Egg diameter: 0.93 mm (based on eggs from artificial fertilization at IPMA in 2008, see Figure 2.8).
- Oil globule: 0.18 mm (based on eggs from artificial fertilization at IPMA in 2008).
- Description: Pelagic eggs, spherical. Single oil globule with melanophores (Gaetani, 1937).
- Fish distribution is mapped in the reports of ICES-WGACEGG (e.g. ICES, 2021).

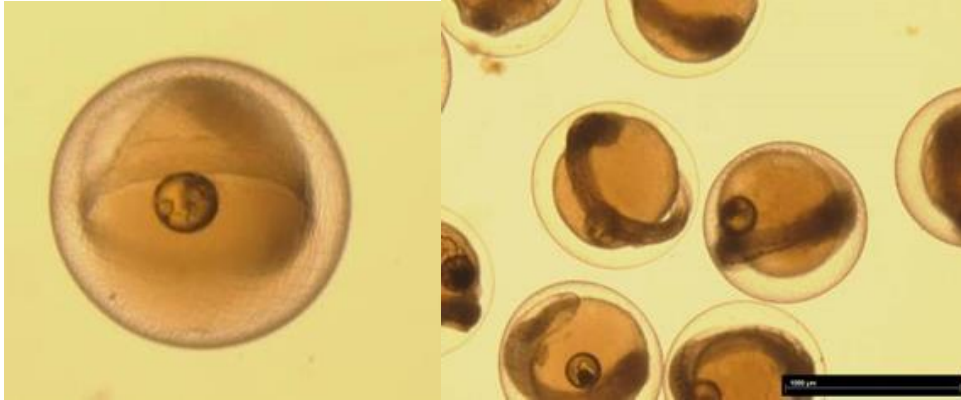


Figure 2.8. Eggs of *Boops boops* from fertilization experiments (IPMA).

3 Results

3.1 Results of the pre-workshop exercise

In total, 25 members of 9 institutes participated in the pre-workshop exercise. The summary of the exercise and the original setup of the samples is illustrated in figure 3.1. All participants correctly staged all stage 1 eggs (stage 1a and 1b combined), the stage that is used to calculate daily and total annual egg production in mackerel and in the western component of horse mackerel. However, discrimination between stages 1a and 1b failed in a majority of all participants: while the original setup contained 17 % 1a and 37 % 1b eggs, participants staged the eggs as 29 and 25 % 1a and 1b eggs. Also, the correct identification of later stages was less precise: In the original setup, only stages 2 and 3 were present with a proportion of 21 and 25 %, respectively. Participants identified stages 2, 3, 4 and 5 at proportions of 22, 21, 3 and < 1 %, respectively.

The amount of all survey target species was underestimated by the participants. While the original sample consisted of 63 % mackerel, 17 % horse mackerel and 8 % hake eggs, participants assigned on average 60 % of the eggs to mackerel, 15 % to horse mackerel and 6 % to hake. This resulted in a mean underestimation of 5, 12 and 26 % for mackerel, horse mackerel and hake, respectively.

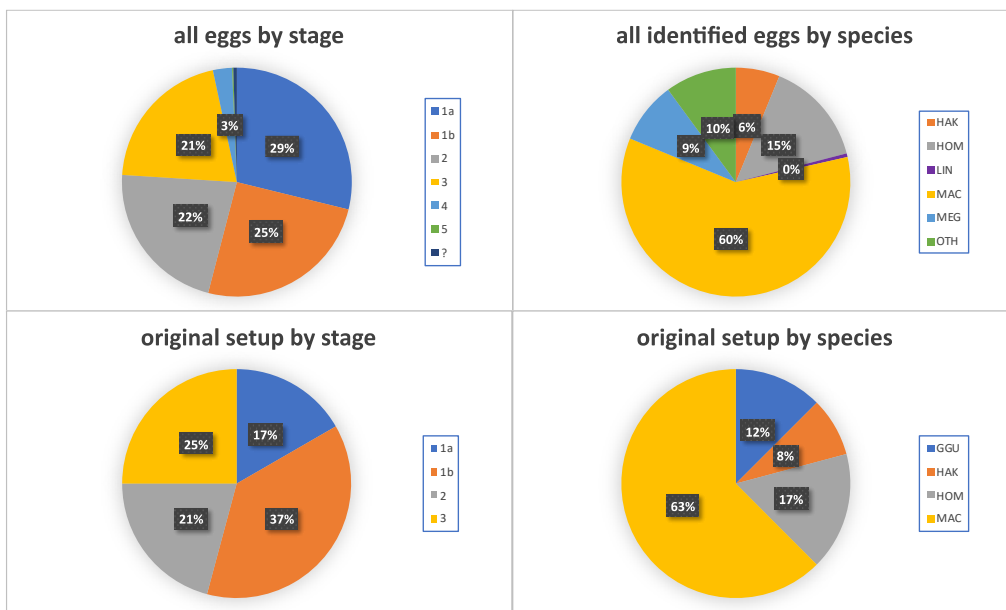


Figure 3.1 The results of the pre-workshop exercise. The two bottom panels illustrate the original setup of the samples sent to the participating institutes by stage (left) and by species right. The two top panels show the results by stage (left) and species (right). All panels show the relative proportion (%) of stage or species composition. MAC – mackerel, HOM – horse mackerel, HAK – hake, GGU – grey gurnard, LIN – ling, MEG – megrim, OTH – other.

3.2 Results of egg sorting exercise

No egg sorting was carried out during the online workshop.

3.3 Results of egg staging exercises

The results of the egg staging exercises are given in Tables 3.1 to 3.12.

Tables 3.1 to 3.6 presents the results for each participant for the first round of analysis for eggs of all species (Table 3.1), for mackerel eggs (Table 3.3) and for horse mackerel eggs (Table 3.5). About half of the participants at the workshop were inexperienced; hence results of only the expert readers are presented separately (Table 3.2, 3.4 and 3.6). Tables 3.7 to 3.12 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.

Tables 3.1 to 3.12 summarise 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 / under estimation 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 validated stage 1a+1b.

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

By studying the results presented in Tables 3.1 to 3.12, some encouraging improvements in the consistency of egg staging between participants can be observed from between the first and second round of analysis.

The overall agreement in egg stage for all species of eggs, in all stages of development was 59.9 % in the first round (Table 3.1). This increased to 68.3 % agreement in the second round of analysis (Table 3.7). The agreement between the expert readers was higher compared to overall and increased from 62.6 % to 73.7 % (Table 3.2 and 3.8). The overall agreement for all egg stages, for mackerel, increased from 52.3 % (Table 3.3) to 65.8 % (Table 3.9), for horse mackerel however, the score decreased from 36.5 % (Table 3.5) to 27.8 % (Table 3.11). For the experts, agreement for all egg stages, for mackerel, increased from 57.3 % (Table 3.4) to 71.4 % (Table 3.10), and for horse mackerel it decreased from 37.9 % to 30.3 % (Table 3.6 and 3.12).

The overall agreement for stage 1 eggs (1a+1b combined), the critical stage for the calculation of the annual egg production in both target species, showed improvements with an overall greater level of agreement, from 93 % in the first round to 95 % in the second round. (Tables 3.1 and 3.7). Agreement between the experts increased from 94 to 96 % (Tables 3.2 and 3.8). The overall agreement of stage 1 eggs, for mackerel, decreased from 97 % (Table 3.3) to 95 % (Table 3.9), and increased for horse mackerel from 84% (Table 3.5) to 86% (Tables 3.11). For experts' agreement of stage 1 eggs, for mackerel, was 97% for both rounds (Table 3.4 and Table 3.10), and for horse mackerel it increased from 81% to 88 % (Tables 3.6 and 3.12).

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 in the first round of analysis, some individuals showed very high levels of bias. In the first round of analysis there was no overall bias with a mean over- or underestimation of 11% for eggs of all species but individual bias ranged from an underestimate of -11% to an overestimate of 46% (Table.3.1). In the second round there was a slight overall overestimation of 3 %, but the range of individual bias reduced to between -19% to 20% (Table 3.7). For the experts the overall bias was an overestimate of 10% for eggs of all species in the first round and 1% in the second round. Individual bias ranged from an underestimate of -11% to an overestimate of 34% (Table 3.2) in the first round. In the second round the range of individual bias was reduced to between -12% and 12% (Table 3.8).

The mean over- or underestimation for stage 1 mackerel eggs (Tables 3.3 and 3.9) was 63 % in the first round and 18% in the second round of analysis. However, the bias of individual participants was much greater, ranging from 10% to 132% in the first round, but improving to between -6% to 80% in the second round of analysis. For experts the overall bias for mackerel stage 1 was 51% in the first round and 14% in the second (Tables 3.4 and 3.10). Individual bias ranged from 10% to 113% and narrowed to 2% to 52% in the second round. The overall bias for stage 1 horse mackerel eggs (Tables 3.5 and 3.11) was 74% in the first round and increased to 121% in the second round of analysis. However, the bias of individual participants was again much greater, ranging from -100% to 325% in the first round, but changed to between 59% and 400% in the second round of analysis. For experts the overall bias for horse mackerel stage 1 was 85% and 111% in the first and second round, respectively (Tables 3.6 and 3.12). Individual bias for horse mackerel in the first round ranged from -31% to 325% and deteriorated from 59% to 336%.

Table 3.1 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 validated egg stage by each participant.
- (E) The percentage agreement by validated stage 1a and 1b combined, by each participant.
- (F) The bias is indicated by the percentage over or under estimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

All EGGS first staging Egg Staging Workshop online, October 2021

A NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE																																
validated		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	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	TOTAL
Stage 1a =>	0	84	96	94	29	67	96	96	65	58	90	57	96	96	23	41	67	95	71	96	56	96	65	33	59	34	96	58	58	71	72	2115
Stage 1b =>	1	35	40	40	17	27	40	40	27	25	37	32	40	40	11	17	28	40	28	40	25	40	26	15	25	16	40	25	25	29	26	896
Stage 2 =>	2	36	42	42	9	27	42	42	26	23	39	20	42	42	9	15	26	42	29	42	22	42	25	11	23	12	42	23	23	28	24	870
Stage 3 =>	3	83	90	83	25	61	90	90	60	51	88	46	90	90	23	36	60	88	65	90	51	90	61	30	55	31	90	49	50	66	62	1944
Stage 4 =>	4	22	27	25	7	15	27	27	15	10	26	15	27	26	4	8	15	26	17	27	10	27	14	7	11	8	27	10	10	16	20	526
Stage 5 =>	5	4	5	5	2	3	5	5	3	3	5	4	5	5	1	3	3	5	3	5	3	5	3	2	3	2	5	3	3	3	3	109
Total	0-5	264	300	289	89	200	300	300	196	170	285	174	300	299	71	120	199	296	213	300	167	300	194	98	176	103	300	168	169	213	207	6460

B EGG STAGE COMPOSITION																																
validated		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	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	TOTAL
Stage 1a =>	0	92	85	146	45	47	145	65	84	50	91	52	107	106	18	45	49	88	78	60	53	109	73	26	58	16	93	53	48	59	100	2141
Stage 1b =>	1	23	36	6	10	79	13	89	10	35	38	29	47	49	16	15	43	52	67	122	29	56	31	24	38	37	49	33	39	64	11	1190
Stage 2 =>	2	60	71	65	22	34	54	84	49	32	53	27	81	46	30	25	50	57	30	67	29	61	55	32	46	30	43	32	30	52	51	1398
Stage 3 =>	3	65	82	51	6	27	62	46	36	30	62	52	42	60	1	26	35	69	16	31	34	53	24	10	21	14	86	29	31	25	29	1155
Stage 4 =>	4	18	19	14	6	8	22	10	16	15	15	29	10	18	33	2	5	19	24	15	12	16	19	9	5	7	4	26	15	15	8	432
Stage 5 =>	5	6	7	7	-	5	4	6	1	8	2	4	5	4	4	4	3	6	7	8	6	2	2	1	6	2	3	6	6	5	3	144
Total	0-5	264	300	289	89	200	300	300	196	170	285	174	300	299	71	120	199	296	213	300	167	300	194	98	176	103	300	168	169	213	207	6460

C OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)																																
validated		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	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	ALL
1a+1b		-3%	-11%	13%	20%	34%	16%	13%	2%	2%	2%	-9%	13%	14%	0%	3%	-3%	4%	46%	34%	1%	21%	14%	4%	14%	6%	4%	4%	5%	23%	13%	11%

D PERCENTAGE AGREEMENT BY EGG STAGE																																
validated		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	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	ALL
Stage 1a =>	0	80%	80%	93%	72%	48%	94%	40%	92%	71%	76%	81%	75%	79%	52%	73%	63%	84%	73%	35%	79%	74%	86%	42%	56%	24%	76%	76%	72%	49%	96%	72%
Stage 1b =>	1	17%	60%	10%	6%	74%	20%	58%	30%	76%	62%	63%	58%	70%	36%	41%	64%	88%	46%	93%	76%	78%	65%	47%	56%	44%	55%	76%	88%	79%	35%	57%
Stage 2 =>	2	72%	98%	62%	44%	26%	62%	45%	85%	83%	62%	60%	62%	60%	44%	73%	65%	83%	21%	33%	77%	52%	68%	64%	74%	42%	43%	87%	83%	43%	63%	61%
Stage 3 =>	3	65%	82%	51%	16%	36%	61%	36%	55%	55%	59%	85%	39%	60%	4%	58%	43%	70%	23%	28%	59%	44%	31%	17%	38%	26%	72%	55%	58%	27%	31%	49%
Stage 4 =>	4	59%	63%	48%	43%	33%	67%	19%	73%	50%	38%	47%	52%	77%	25%	38%	67%	69%	47%	33%	70%	55%	50%	57%	55%	25%	63%	60%	70%	44%	45%	52%
Stage 5 =>	5	100%	100%	100%	0%	67%	60%	60%	33%	100%	80%	75%	60%	100%	100%	67%	100%	80%	67%	80%	100%	40%	67%	50%	100%	50%	60%	67%	100%	100%	33%	74%
Weighted mean	0-5	64.4%	79.3%	60.9%	37.1%	44.0%	66.7%	40.0%	68.9%	67.6%	63.5%	73.0%	57.7%	69.9%	32.4%	61.7%	58.3%	79.1%	45.1%	41.0%	71.9%	60.3%	60.8%	38.8%	53.4%	30.1%	66.0%	70.2%	72.2%	46.0%	58.9%	59.9%
RANKING		12	1	15	28	24	10	26	8	9	13	3	20	7	29	14	19	2	23	25	5	17	16	27	21	30	11	6	4	22	18	6

E PERCENTAGE AGREEMENT STAGE 1A and 1B combined																																
validated		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	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	ALL
1a+1b		88%	88%	96%	98%	96%	97%	89%	93%	95%	91%	89%	97%	96%	71%	91%	88%	97%	95%	96%	96%	98%	97%	88%	90%	80%	91%	98%	98%	94%	98%	93%
RANKING		26	26	14	2	13	6	23	18	15	20	24	6	10	30	19	25	8	16	10	12	3	9	28	22	29	21	4	4	17	1	1

F BIAS																																
validated		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	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	ALL
Stage 1a =>	0	0.25	0.30	0.15	0.31	0.55	0.11	0.72	0.15	0.36	0.37	0.26	0.29	0.26	0.65	0.39	0.46	0.20	0.30	0.72	0.27	0.30	0.15	0.70	0.56	0.97	0.32	0.28	0.31	0.55	0.06	0.35
Stage 1b =>	1	-0.20	0.00	-0.85	-0.94	-0.11	-0.80	-0.13	-0.56	-0.24	-0.16	0.09	-0.38	-0.15	0.45	-0.47	0.11	-0.08	-0.32	-0.03	-0.24	-0.23	-0.19	-0.20	-0.36	-0.06	-0.13	-0.24	-0.12	0.10	-0.54	-0.24
Stage 2 =>	2	-0.17	-0.02	-0.62	-1.00	-0.93	-0.69	-0.64	-0.27	-0.09	-0.28	0.15	-0.69	-0.52	-0.78	-0.53	-0.12	-0.12	-1.14	-1.02	0.09	-0.69	-0.48	-0.27	-0.39	-0.75	-0.12	-0.13	-0.09	-0.82	-0.50	-0.45
Stage 3 =>	3	-0.28	-0.13	-0.66	-1.12	-0.80	-0.52	-0.79	-0.45	-0.16	-0.16	-0.07	-0.66	-0.33	-1.09	-0.44	-0.32	-0.22	-0.94	-0.99	-0.16	-0.80	-0.80	-1.17	-1.02	-0.90	-0.18	-0.16	-0.20	-1.06	-0.82	-0.54
Stage 4 =>	4	-0.41	-0.30	-0.68	-0.86	-0.73	-0.48	-0.96	-0.53	0.50	-0.12	-0.53	-0.67	-0.46	0.00	-0.13	-0.53	-0.35	-0.47	-0.85	0.30	-0.74	-0.79	-0.43	-0.27	-0.50	-0.59	0.40	0.30	-0.69	-0.60	-0.48
Stage 5 =>	5	0.00	0.00	0.00	-1.00	-1.00	-0.40	-0.40	-0.67	0.00	-0.20	-0.25	-0.40	0.00	0.00	-0.67	0.00	-0.20	-1.33	-0.60	0.00	-0.60	-1.33	-0.50	0.00	-0.50	-0.80	-0.67	0.00	0.00	-1.00	-0.39

Table 3.2 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 validated egg stage by each participant.
- (E) The percentage agreement by validated stage 1a and 1b combined, by each participant.
- (F) The bias is indicated by the percentage over or under estimation of each egg stage, as estimated by each participant, in relation to the validated stage. For each table the combined result is also given.

ALL EGGS first staging Egg Staging Workshop online, October 2021

A

NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	TOTAL
Stage 1a ==> 0	84	96	94	96	96	65	96	96	95	96	96	96	1106
Stage 1b ==> 1	35	40	40	40	40	27	40	40	40	40	40	40	462
Stage 2 ==> 2	36	42	42	42	42	26	42	42	42	42	42	42	482
Stage 3 ==> 3	83	90	83	90	90	60	90	90	88	90	90	90	1034
Stage 4 ==> 4	22	27	25	27	27	15	27	26	26	27	27	27	303
Stage 5 ==> 5	4	5	5	5	5	3	5	5	5	5	5	5	57
Total	0-5	264	300	289	300	300	196	300	299	296	300	300	3444

B

EGG STAGE COMPOSITION													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	TOTAL
Stage 1a ==> 0	92	85	146	145	65	84	107	106	88	60	109	93	1180
Stage 1b ==> 1	23	36	6	13	89	10	47	49	52	122	56	49	552
Stage 2 ==> 2	60	71	65	54	84	49	81	46	57	67	61	43	738
Stage 3 ==> 3	65	82	51	62	46	36	42	60	69	31	53	86	683
Stage 4 ==> 4	18	19	14	22	10	16	18	33	24	12	19	26	231
Stage 5 ==> 5	6	7	7	4	6	1	5	5	6	8	2	3	60
Total	0-5	264	300	289	300	300	196	300	299	296	300	300	3444

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
1a+1b	-3%	-11%	13%	16%	13%	2%	13%	14%	4%	34%	21%	4%	10%

D

PERCENTAGE AGREEMENT BY EGG STAGE													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
Stage 1a ==> 0	80%	80%	93%	94%	40%	92%	75%	79%	84%	35%	74%	76%	75%
Stage 1b ==> 1	17%	60%	10%	20%	58%	30%	58%	70%	88%	93%	78%	55%	54%
Stage 2 ==> 2	72%	98%	62%	62%	45%	85%	62%	62%	83%	33%	52%	43%	62%
Stage 3 ==> 3	65%	82%	51%	61%	36%	55%	39%	60%	70%	28%	44%	72%	55%
Stage 4 ==> 4	59%	63%	48%	67%	19%	73%	52%	77%	69%	33%	56%	63%	56%
Stage 5 ==> 5	100%	100%	100%	60%	60%	33%	60%	100%	80%	40%	40%	60%	74%
Weighted mean	64.4%	79.3%	60.9%	66.7%	40.0%	68.9%	57.7%	69.9%	79.1%	41.0%	60.3%	66.0%	62.6%
RANKING	7	1	8	5	12	4	10	3	2	11	9	6	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
1a+1b	88%	88%	96%	97%	89%	93%	97%	96%	97%	96%	98%	91%	94%
RANKING	11	11	7	2	10	8	2	5	4	5	1	9	

F

BIAS													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
Stage 1a ==> 0	0.25	0.30	0.15	0.11	0.72	0.15	0.29	0.26	0.20	0.72	0.30	0.32	0.32
Stage 1b ==> 1	-0.20	0.00	-0.85	-0.80	-0.13	-0.56	-0.38	-0.15	-0.08	-0.03	-0.23	-0.13	-0.29
Stage 2 ==> 2	-0.17	-0.02	-0.62	-0.69	-0.64	-0.27	-0.69	-0.52	-0.12	-1.02	-0.69	-0.12	-0.48
Stage 3 ==> 3	-0.28	-0.13	-0.66	-0.52	-0.79	-0.45	-0.66	-0.33	-0.22	-0.99	-0.80	-0.18	-0.50
Stage 4 ==> 4	-0.41	-0.30	-0.68	-0.48	-0.96	-0.53	-0.67	-0.46	-0.35	-0.85	-0.74	-0.59	-0.59
Stage 5 ==> 5	0.00	0.00	0.00	-0.40	-0.40	-0.67	-0.40	0.00	-0.20	-0.60	-0.60	-0.80	-0.33

Table 3.4 Mackerel eggs first staging, expert readers only.

(A) The numbers of eggs at each validated 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 validated egg stage by each participant.

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

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

For each table the combined result is also given.

MAC EGGS first staging Egg Staging Workshop online, October 2021

A NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	TOTAL
Stage 1a ==> 0	31	37	36	34	37	23	34	35	37	36	37	35	412
Stage 1b ==> 1	8	11	11	10	9	5	10	10	11	10	11	10	116
Stage 2 ==> 2	19	21	21	20	21	13	20	20	22	21	20	20	238
Stage 3 ==> 3	16	18	18	15	15	8	14	18	19	18	19	19	197
Stage 4 ==> 4	8	10	10	11	11	6	10	11	11	10	10	11	119
Stage 5 ==> 5	3	4	4	4	4	2	4	4	4	3	4	4	44
Total	0-5	118	120	119	104	134	67	102	124	111	150	107	1420

B EGG STAGE COMPOSITION													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	TOTAL
Stage 1a ==> 0	48	46	65	47	40	31	36	41	38	30	40	61	523
Stage 1b ==> 1	18	15	4	7	39	4	24	26	15	68	20	32	272
Stage 2 ==> 2	26	26	20	20	23	17	22	24	25	26	16	17	262
Stage 3 ==> 3	15	21	20	17	20	7	10	14	17	14	17	35	207
Stage 4 ==> 4	5	5	4	9	6	7	5	14	10	6	12	16	99
Stage 5 ==> 5	6	7	6	4	6	1	5	5	6	6	2	3	57
Total	0-5	118	120	119	104	134	67	102	124	111	150	107	1420

C OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
1a+1b	69%	27%	47%	23%	72%	25%	36%	49%	10%	113%	25%	107%	51%

D PERCENTAGE AGREEMENT BY EGG STAGE													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
Stage 1a ==> 0	74%	92%	94%	91%	51%	87%	65%	74%	95%	31%	76%	89%	76%
Stage 1b ==> 1	25%	82%	18%	10%	56%	60%	90%	100%	100%	100%	82%	90%	69%
Stage 2 ==> 2	79%	100%	76%	80%	48%	92%	65%	90%	100%	38%	55%	40%	71%
Stage 3 ==> 3	69%	94%	89%	80%	60%	88%	50%	67%	84%	56%	74%	84%	75%
Stage 4 ==> 4	50%	50%	40%	64%	9%	100%	40%	91%	73%	30%	80%	64%	56%
Stage 5 ==> 5	100%	100%	100%	75%	75%	50%	75%	100%	100%	67%	50%	75%	82%
Weighted mean	49.2%	75.0%	63.9%	67.3%	35.1%	73.1%	56.9%	64.5%	86.5%	29.3%	67.3%	45.1%	57.3%
RANKING	9	2	7	4	11	3	8	6	1	12	5	10	

E PERCENTAGE AGREEMENT STAGE 1A and 1B combined													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
1a+1b	95%	96%	98%	98%	96%	93%	98%	98%	98%	98%	98%	98%	97%
RANKING	11	9	3	7	10	12	7	5	1	4	1	5	

F BIAS													
validated stage	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
0	0.29	0.14	0.08	0.12	0.54	0.22	0.38	0.29	0.08	0.72	0.27	0.14	0.27
1	-0.50	-0.18	-0.82	-0.90	-0.44	-0.40	-0.10	0.00	0.00	0.00	-0.18	-0.10	-0.29
2	-0.32	0.00	-0.48	-0.25	-0.62	-0.15	-0.65	-0.20	0.00	-0.90	-0.65	-0.20	-0.37
3	-0.19	-0.06	-0.11	-0.07	-0.40	-0.13	-0.50	0.00	0.05	-0.50	-0.16	0.16	-0.15
4	-0.38	-0.10	-0.20	-0.18	-1.00	0.00	-0.30	-0.09	0.09	-0.70	-0.20	-0.36	-0.29
5	0.00	0.00	0.00	-0.25	-0.25	-0.50	-0.25	0.00	0.00	-1.00	-0.50	-0.50	-0.25

Table 3.7 All eggs second staging.

- (A) The numbers of eggs at each validated 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 validated egg stage by each participant.
 - (E) The percentage agreement by validated stage 1a and 1b combined, by each participant.
 - (F) The bias is indicated by the percentage over or under estimation of each egg stage, as estimated by each participant, in relation to the validated stage.
- For each table the combined result is also given.

ALL EGGS second staging Egg Staging Workshop online, October 2021

NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	TOTAL	
Stage 1a =>	0	70	108	100	61	93	85	107	70	81	78	71	107	69	62	70	76	66	74	108	69	108	82	108	14	88	76	77	69	56	81	108	2492
Stage 1b =>	1	41	62	61	37	59	52	62	41	55	47	41	62	41	37	41	42	40	42	62	41	62	52	62	10	46	45	46	40	33	55	62	1479
Stage 2 =>	2	26	43	43	22	36	32	42	25	26	31	28	43	26	24	25	27	24	27	43	25	43	32	42	6	34	27	29	25	20	26	43	945
Stage 3 =>	3	47	60	56	46	58	55	60	46	52	36	60	47	40	46	49	35	48	60	46	60	54	59	10	49	51	52	45	33	46	60	1512	
Stage 4 =>	4	18	24	24	15	21	20	24	18	18	20	19	24	18	16	17	20	15	17	24	18	24	20	23	2	22	20	20	18	11	18	24	592
Stage 5 =>	5	1	3	3	-	2	2	3	1	1	1	1	3	1	1	1	1	1	1	3	1	3	2	3	1	2	1	1	1	1	3	50	
Total	0-5	203	300	287	181	269	246	298	201	227	229	196	299	202	180	200	215	181	209	300	300	242	297	43	241	220	225	198	154	227	300	7070	

EGG STAGE COMPOSITION																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	TOTAL	
Stage 1a =>	0	86	105	133	69	37	103	32	84	67	88	63	126	62	33	102	105	74	73	106	63	109	83	67	11	47	100	68	62	27	64	126	2375
Stage 1b =>	1	32	61	30	40	137	37	116	26	72	43	41	50	50	47	26	14	35	66	84	49	59	60	111	14	82	19	55	51	62	74	69	1712
Stage 2 =>	2	26	55	53	15	31	31	71	33	24	35	35	46	27	70	30	28	20	10	42	22	43	41	65	6	44	49	26	18	23	23	29	1071
Stage 3 =>	3	41	56	54	52	42	53	46	40	39	31	51	44	20	29	47	33	32	50	34	66	32	43	9	41	42	64	48	23	43	55	1300	
Stage 4 =>	4	16	16	14	5	20	19	29	18	20	21	21	16	5	11	20	17	21	17	28	20	24	11	2	22	8	11	16	18	18	18	523	
Stage 5 =>	5	2	7	3	-	2	3	4	-	3	4	5	3	5	2	1	2	7	1	4	3	2	-	1	5	2	1	3	1	5	3	89	
Total	0-5	203	300	287	181	269	246	298	201	227	229	196	299	202	180	200	215	181	209	300	200	300	242	297	43	241	220	225	198	154	227	300	7070

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)																																
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL
1a+1b	6%	-2%	1%	11%	14%	2%	-12%	-1%	2%	5%	-7%	4%	2%	-19%	15%	1%	3%	20%	12%	2%	-1%	7%	5%	4%	-4%	-2%	0%	4%	0%	1%	15%	3%

PERCENTAGE AGREEMENT BY EGG STAGE																																		
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL		
Stage 1a =>	0	91%	88%	93%	87%	31%	93%	21%	90%	73%	79%	82%	91%	83%	37%	97%	95%	97%	61%	71%	70%	81%	83%	55%	71%	35%	82%	79%	80%	36%	72%	80%	73%	
Stage 1b =>	1	54%	79%	46%	68%	86%	62%	60%	51%	89%	53%	76%	63%	90%	62%	54%	31%	78%	55%	69%	63%	61%	79%	85%	90%	54%	18%	80%	88%	76%	91%	65%	67%	
Stage 2 =>	2	62%	91%	77%	36%	42%	78%	69%	92%	73%	65%	79%	79%	85%	88%	64%	67%	75%	22%	60%	64%	72%	78%	76%	83%	56%	67%	66%	60%	50%	69%	64%		
Stage 3 =>	3	77%	82%	64%	76%	41%	78%	57%	83%	74%	48%	75%	75%	81%	30%	54%	67%	77%	50%	65%	54%	82%	54%	46%	80%	53%	59%	81%	82%	42%	78%	60%	65%	
Stage 4 =>	4	78%	63%	42%	20%	43%	60%	79%	94%	72%	60%	79%	71%	72%	19%	59%	65%	80%	59%	58%	67%	63%	85%	30%	50%	59%	40%	35%	67%	64%	72%	42%	60%	
Stage 5 =>	5	100%	100%	100%	-	50%	100%	100%	0%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	33%	100%	67%	100%	0%	100%	100%	100%	100%	100%	100%	100%	100%	33%	78%
Weighted mean	0-5	75.4%	83.3%	70.7%	68.5%	48.0%	78.0%	48.7%	80.6%	77.1%	63.3%	78.6%	83.2%	46.1%	71.0%	69.8%	84.5%	52.2%	66.7%	64.0%	74.0%	75.2%	59.9%	79.1%	48.1%	57.7%	74.2%	78.3%	50.0%	77.5%	64.0%	68.3%		
RANKING		12	2	17	19	30	9	28	4	11	23	7	6	3	31	16	18	1	26	20	21	15	13	24	5	29	25	14	8	27	10	21		

PERCENTAGE AGREEMENT STAGE 1A and 1B combined																																
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL
1a+1b	97%	96%	96%	99%	97%	99%	83%	95%	99%	98%	90%	98%	99%	72%	99%	97%	98%	97%	98%	97%	96%	96%	98%	100%	87%	89%	97%	99%	88%	98%	98%	95%
RANKING		16	22	23	5	19	6	30	24	7	8	26	11	3	32	2	20	12	15	14	17	25	21	9	1	29	27	18	4	28	13	9

BIAS																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL	
Stage 1a =>	0	0.10	0.18	0.14	0.13	0.75	0.11	0.90	0.16	0.30	0.24	0.28	0.13	0.22	0.97	0.03	0.09	0.06	0.43	0.33	0.33	0.26	0.22	0.49	0.29	0.74	0.28	0.25	0.22	0.84	0.36	0.24	0.33
Stage 1b =>	1	-0.37	-0.08	-0.48	-0.27	-0.10	-0.29	0.21	-0.39	-0.11	-0.38	0.05	-0.34	-0.10	0.03	-0.41	-0.64	-0.18	-0.40	-0.27	-0.32	-0.26	-0.10	-0.10	0.00	-0.51	-0.09	-0.13	-0.12	-0.09	-0.32	-0.21	
Stage 2 =>	2	-0.23	-0.02	-0.12	-0.14	-0.11	-0.06	0.10	-0.12	-0.04	0.13	0.07	-0.30	0.08	-0.13	-0.68	0.11	-0.08	-0.52	-0.44	0.08	0.12	-0.28	-0.19	-0.17	0.03	-0.26	0.21	0.08	-0.10	0.04	-0.37	-0.11
Stage 3 =>	3	-0.23	-0.20	-0.39	-0.26	-0.53	-0.11	-0.23	-0.26	0.00	-0.42	-0.03	-0.15	-0.17	-0.68	-0.78	-0.16	0.00	-0.23	-0.06	0.20	-0.17	-0.46	-0.58	0.00	-0.31	-0.55	-0.08	-0.04	-0.09	-0.04	-0.28	-0.38
Stage 4 =>	4	-0.28	-0.08	-0.63	-1.07	-0.57	-0.35	-0.17	-0.06	0.06	-0.30	0.11	-0.25	-0.06	-0.81	-0.41	-0.35	-0.07	-0.12	-0.46	0.00	-0.29	-0.15	-0.78	-0.50	-0.18	-0.75	-0.70	-0.11	-0.45	0.06	-0.58	-0.32
Stage 5 =>	5	0.00	0.00	0.00	-	-0.50	-0.50	0.00	-1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.67	0.00	-1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.67	-0.22

Table 3.8 All eggs second staging, expert readers only.

- (A) The numbers of eggs at each validated 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 validated egg stage by each participant.
- (E) The percentage agreement by validated stage 1a and 1b combined, by each participant.
- (F) The bias is indicated by the percentage over or under estimation of each egg stage, as estimated by each participant, in relation to the validated stage.

For each table the combined result is also given.

ALL EGGS second staging Egg Staging Workshop online, October 2021

A

NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE																		
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	TOTAL	
Stage 1a ==> 0	70	108	100	107	70	81	78	69	62	69	108	82	108	69	56	108	1345	
Stage 1b ==> 1	41	62	61	62	41	55	47	41	37	41	62	52	62	40	33	62	799	
Stage 2 ==> 2	26	43	43	42	25	26	31	26	24	25	43	32	42	25	20	43	516	
Stage 3 ==> 3	47	60	56	60	46	46	52	47	40	46	60	54	59	45	33	60	811	
Stage 4 ==> 4	18	24	24	24	18	18	20	18	16	18	24	20	23	18	11	24	318	
Stage 5 ==> 5	1	3	3	3	1	1	1	1	1	1	3	2	3	1	1	3	29	
Total	0-5	203	300	287	298	201	227	229	202	180	200	300	242	297	198	154	300	3818

B

EGG STAGE COMPOSITION																		
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	TOTAL	
Stage 1a ==> 0	86	105	133	32	84	67	88	62	33	63	109	83	67	62	27	126	1227	
Stage 1b ==> 1	32	61	30	116	26	72	43	50	47	49	59	60	111	51	62	69	938	
Stage 2 ==> 2	26	55	53	71	33	24	35	27	70	22	43	41	65	18	23	29	635	
Stage 3 ==> 3	41	56	54	46	40	40	39	44	20	34	66	32	43	48	23	55	681	
Stage 4 ==> 4	16	16	14	29	18	20	21	16	5	28	20	24	11	16	18	18	290	
Stage 5 ==> 5	2	7	3	4	-	4	3	3	5	4	3	2	-	3	1	3	47	
Total	0-5	203	300	287	298	201	227	229	202	180	200	300	242	297	198	154	300	3818

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
1a+1b	6%	-2%	1%	-12%	-1%	2%	5%	2%	-19%	2%	-1%	7%	5%	4%	0%	15%	1%

D

PERCENTAGE AGREEMENT BY EGG STAGE																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
Stage 1a ==> 0	91%	88%	93%	21%	90%	73%	79%	83%	37%	70%	81%	83%	55%	80%	36%	80%	72%
Stage 1b ==> 1	54%	79%	46%	60%	51%	89%	53%	90%	62%	63%	61%	79%	85%	88%	76%	65%	69%
Stage 2 ==> 2	62%	91%	77%	69%	92%	73%	65%	85%	88%	64%	72%	78%	76%	60%	50%	44%	72%
Stage 3 ==> 3	77%	82%	64%	57%	83%	74%	48%	30%	54%	82%	54%	46%	82%	42%	42%	60%	64%
Stage 4 ==> 4	78%	63%	42%	79%	94%	60%	72%	72%	19%	67%	63%	85%	30%	64%	64%	42%	62%
Stage 5 ==> 5	100%	100%	100%	100%	0%	100%	100%	100%	100%	100%	67%	100%	0%	100%	100%	33%	76%
Weighted mean	75.4%	83.3%	70.7%	48.7%	80.6%	77.1%	63.3%	83.2%	46.1%	64.0%	74.0%	75.2%	59.9%	78.3%	50.0%	64.0%	68.6%
RANKING	6	1	9	15	3	5	12	2	16	10	8	7	13	4	14	10	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
1a+1b	97%	96%	96%	83%	95%	99%	98%	99%	72%	97%	95%	96%	98%	99%	88%	98%	94%
RANKING	7	10	11	15	12	3	4	1	16	8	13	9	5	2	14	5	

F

BIAS																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
Stage 1a ==> 0	0.10	0.18	0.14	0.90	0.16	0.30	0.24	0.22	0.97	0.33	0.26	0.22	0.49	0.22	0.84	0.24	0.35
Stage 1b ==> 1	-0.37	-0.08	-0.48	0.21	-0.39	-0.11	-0.38	-0.10	0.03	-0.32	-0.26	-0.10	-0.10	-0.13	-0.12	-0.32	-0.19
Stage 2 ==> 2	-0.23	-0.02	-0.12	0.10	-0.12	-0.04	0.13	0.08	-0.13	0.08	0.12	-0.28	-0.19	0.08	-0.10	-0.37	-0.07
Stage 3 ==> 3	-0.23	-0.20	-0.39	-0.23	-0.42	-0.00	-0.42	-0.17	-0.26	0.00	-0.17	-0.46	-0.58	-0.04	-0.09	-0.57	-0.28
Stage 4 ==> 4	-0.28	-0.08	-0.63	-0.17	-0.06	0.06	-0.20	-0.06	-0.81	0.00	-0.29	-0.15	-0.78	-0.11	-0.45	-0.58	-0.29
Stage 5 ==> 5	0.00	0.00	0.00	0.00	-1.00	0.00	0.00	0.00	0.00	0.00	-1.00	0.00	-1.00	0.00	0.00	-0.67	-0.24

Table 3.9 Mackerel eggs second staging.

- (A) The numbers of eggs at each validated 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 validated egg stage by each participant.
 - (E) The percentage agreement by validated stage 1a and 1b combined, by each participant.
 - (F) The bias is indicated by the percentage over or under estimation of each egg stage, as estimated by each participant, in relation to the validated stage.
- For each table the combined result is also given.

MAC EGGS second staging Egg Staging Workshop online, October 2021

A

NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	TOTAL	
Stage 1a ==>	0	18	28	27	10	20	22	26	19	23	6	20	25	19	10	19	21	19	17	28	19	21	21	27	2	21	20	20	19	16	23	28	614
Stage 1b ==>	1	27	42	40	22	38	33	37	24	41	4	32	39	27	14	27	29	27	28	39	27	41	34	40	5	27	29	30	26	22	41	41	
Stage 2 ==>	2	14	21	21	3	19	18	21	13	15	6	16	20	14	8	14	15	15	11	20	14	22	17	19	4	14	14	16	13	13	15	21	
Stage 3 ==>	3	6	6	7	1	5	7	6	5	6	2	3	5	5	3	5	5	4	3	5	6	7	5	7	1	5	5	6	3	6	7		
Stage 4 ==>	4	5	8	8	-	4	3	5	5	4	-	1	5	5	-	3	3	4	2	6	4	8	2	5	-	4	5	5	4	2	4		
Stage 5 ==>	5	1	3	3	-	1	2	3	1	-	-	1	3	1	-	1	1	1	1	-	2	2	2	1	2	1	1	-	-	-	2		
Total	0-5	84	121	116	44	116	89	117	73	101	26	75	99	74	43	78	79	72	96	131	82	110	86	110	18	88	93	85	80	66	100	150	2702

B

EGG STAGE COMPOSITION																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	TOTAL	
Stage 1a ==>	0	32	41	55	16	7	30	12	28	28	11	19	27	16	10	33	43	24	36	48	29	32	26	27	5	25	46	20	23	16	26	50	841
Stage 1b ==>	1	27	39	20	21	28	54	20	44	7	30	38	32	18	22	11	24	33	54	24	37	33	52	7	33	10	34	30	29	44	51	977	
Stage 2 ==>	2	14	25	21	4	15	17	32	13	17	5	20	21	15	13	14	13	14	2	19	15	20	21	22	4	13	20	14	13	16	19	484	
Stage 3 ==>	3	7	5	11	2	16	7	10	6	8	2	3	6	2	4	7	4	16	5	10	12	3	7	-	10	12	14	11	5	9	19	239	
Stage 4 ==>	4	3	4	6	1	6	4	5	6	2	-	1	2	3	-	4	4	4	6	5	2	6	1	2	1	3	3	2	2	8	101		
Stage 5 ==>	5	1	7	3	-	1	3	4	-	2	1	2	5	2	-	1	1	2	3	-	2	3	2	-	1	4	2	1	1	-	3	60	
Total	0-5	84	121	116	44	116	89	117	73	101	26	75	99	74	43	78	79	72	96	131	82	110	86	110	18	88	93	85	80	66	100	150	2702

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)																																
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL
1a+1b	31%	14%	12%	16%	34%	5%	5%	12%	13%	80%	-6%	2%	4%	17%	20%	8%	4%	53%	52%	15%	11%	7%	18%	71%	21%	14%	8%	18%	18%	9%	46%	18%

D

PERCENTAGE AGREEMENT BY EGG STAGE																																		
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL		
Stage 1a ==>	0	83%	96%	100%	80%	20%	91%	31%	95%	83%	67%	85%	84%	79%	60%	100%	95%	95%	76%	82%	84%	90%	81%	74%	100%	62%	90%	80%	84%	63%	78%	75%	79%	
Stage 1b ==>	1	67%	81%	50%	77%	97%	76%	57%	71%	90%	75%	84%	87%	96%	79%	38%	85%	61%	85%	62%	67%	80%	76%	93%	80%	56%	21%	90%	96%	77%	90%	83%	76%	
Stage 2 ==>	2	79%	100%	81%	67%	47%	89%	81%	92%	100%	83%	94%	95%	100%	100%	79%	73%	93%	9%	75%	79%	86%	88%	79%	100%	57%	86%	75%	85%	62%	93%	67%	81%	
Stage 3 ==>	3	83%	83%	57%	0%	60%	71%	100%	100%	83%	100%	67%	80%	80%	33%	40%	80%	100%	67%	40%	83%	85%	85%	40%	25%	0%	80%	60%	100%	83%	33%	83%	57%	70%
Stage 4 ==>	4	60%	50%	63%	-	50%	67%	80%	100%	50%	60%	60%	60%	-	67%	100%	75%	50%	63%	50%	0%	-	50%	20%	40%	50%	100%	50%	50%	50%	50%	50%	55%	
Stage 5 ==>	5	100%	100%	100%	-	100%	50%	100%	0%	-	100%	100%	100%	-	100%	100%	100%	100%	0%	-	100%	100%	0%	100%	100%	100%	100%	-	-	-	50%	84%	84%	
Weighted mean	0-5	63.1%	77.7%	65.5%	61.4%	48.3%	77.5%	50.4%	78.1%	77.2%	53.8%	82.7%	83.8%	85.1%	60.5%	69.2%	63.3%	87.5%	36.5%	58.8%	63.4%	76.4%	73.3%	67.3%	61.1%	50.0%	44.1%	74.1%	73.8%	57.6%	76.0%	51.3%	65.8%	
RANKING		19	6	16	20	29	7	27	5	8	25	4	3	2	22	14	18	1	31	23	17	9	13	15	21	28	30	11	12	24	10	26		

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL	
1a+1b	97%	96%	96%	99%	97%	99%	83%	95%	99%	98%	90%	98%	93%	72%	99%	97%	98%	97%	98%	97%	95%	96%	98%	100%	87%	89%	97%	99%	88%	98%	98%	95%	
RANKING		16	22	23	5	19	6	30	24	7	8	26	11	3	31	2	20	12	15	14	17	25	21	9	1	29	27	18	4	28	13	9	

F

BIAS																																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32	ALL	
Stage 1a ==>	0	0.17	0.04	0.00	0.20	0.90	0.09	0.73	0.05	0.17	0.33	0.15	0.16	0.21	0.40	0.00	0.10	0.05	0.24	0.18	0.16	0.10	0.19	0.26	0.00	0.38	0.10	0.20	0.16	0.50	0.39	0.29	0.22
Stage 1b ==>	1	-0.19	-0.10	-0.50	-0.23	-0.03	-0.09	0.27	-0.29	-0.10	-0.25	0.03	-0.13	-0.04	-0.21	-0.30	-0.62	-0.15	-0.39	-0.15	-0.33	-0.20	-0.06	-0.08	-0.20	-0.04	-0.72	-0.03	-0.04	-0.23	-0.10	-0.17	-0.17
Stage 2 ==>	2	-0.21	0.00	0.05	-0.67	0.11	-0.06	0.19	-0.15	0.00	-0.33	0.06	-0.10	0.00	0.00	-0.36	0.07	-0.13	-0.45	-0.15	0.07	0.00	-0.12	-0.21	0.00	0.29	-0.21	0.13	0.15	0.08	0.07	-0.14	-0.04
Stage 3 ==>	3	-0.17	-0.17	-0.43	1.00	0.00	0.00	0.00	0.00	-0.17	0.00	0.33	-0.20	-0.20	-0.67	-0.60	0.20	0.00	-1.00	-0.80	-0.17	0.14	-1.00	-0.71	1.00	0.20	-0.40	0.00	-0.17	0.00	-0.17	-0.14	-0.20
Stage 4 ==>	4	-1.00	0.50	-0.38	-	-0.75	0.33	0.20	0.00	0.50	-	1.00	-0.40	0.00	-	-0.33	0.00	0.25	-0.50	-0.33	0.50	-0.13	-0.50	-1.40	-	0.50	-0.40	-0.60	0.00	0.00	0.50	-0.50	-0.15
Stage 5 ==>	5	0.00	0.00	0.00	-	0.00	-0.50	0.00	-1.00	-	-	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00	-0.00	-	0.00	0.00	-1.00	0.00	0.00	0.00	-	-	-	-0.50	-0.16	

Table 3.10 Mackerel eggs second staging, expert readers only.

- (A) The numbers of eggs at each validated 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 validated egg stage by each participant.
- (E) The percentage agreement by validated stage 1a and 1b combined, by each participant.
- (F) The bias is indicated by the percentage over or under estimation of each egg stage, as estimated by each participant, in relation to the validated stage.

For each table the combined result is also given.

MAC EGGS second staging Egg Staging Workshop online, October 2021

A

NUMBER OF EGG STAGE READINGS BY VALIDATED EGG STAGE																		
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	TOTAL	
Stage 1a ==> 0	18	28	27	22	26	19	23	25	19	28	19	21	21	20	19	23	358	
Stage 1b ==> 1	27	42	40	33	37	24	41	39	27	39	27	41	34	30	26	41	548	
Stage 2 ==> 2	14	21	21	18	21	13	15	20	14	20	14	22	17	16	13	15	274	
Stage 3 ==> 3	6	6	7	7	6	5	6	5	5	6	6	7	5	5	6	6	93	
Stage 4 ==> 4	5	8	8	3	5	5	4	5	5	4	4	8	2	5	4	4	81	
Stage 5 ==> 5	1	3	3	2	3	1	-	3	1	1	-	2	2	1	-	-	23	
Total	0-5	84	121	116	89	117	73	101	99	74	131	82	110	86	85	80	100	1548

B

EGG STAGE COMPOSITION																		
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	TOTAL	
Stage 1a ==> 0	32	41	55	30	12	28	28	27	16	48	29	32	26	20	23	26	473	
Stage 1b ==> 1	27	39	20	28	54	20	44	38	32	54	24	37	33	34	30	44	558	
Stage 2 ==> 2	14	25	21	17	32	13	17	21	15	19	15	20	21	14	13	16	293	
Stage 3 ==> 3	7	5	11	7	10	6	8	6	6	5	10	12	3	14	11	9	130	
Stage 4 ==> 4	3	4	6	4	5	6	2	2	3	5	2	6	1	2	2	2	55	
Stage 5 ==> 5	1	7	3	3	4	-	2	5	2	-	2	3	2	1	1	3	39	
Total	0-5	84	121	116	89	117	73	101	99	74	131	82	110	86	85	80	100	1548

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
1a+1b	31%	14%	12%	5%	5%	12%	13%	2%	4%	52%	15%	11%	7%	8%	18%	9%	14%

D

PERCENTAGE AGREEMENT BY EGG STAGE																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
Stage 1a ==> 0	83%	96%	100%	91%	31%	95%	83%	84%	79%	82%	84%	90%	81%	80%	84%	78%	82%
Stage 1b ==> 1	67%	81%	50%	76%	57%	71%	90%	87%	96%	85%	67%	80%	76%	90%	96%	90%	79%
Stage 2 ==> 2	79%	100%	81%	89%	81%	92%	100%	95%	81%	75%	79%	86%	88%	75%	85%	93%	87%
Stage 3 ==> 3	83%	83%	57%	71%	100%	100%	83%	80%	80%	40%	83%	85%	40%	100%	83%	83%	78%
Stage 4 ==> 4	50%	63%	67%	67%	80%	100%	50%	40%	60%	67%	50%	63%	50%	40%	50%	50%	59%
Stage 5 ==> 5	100%	100%	100%	50%	100%	0%	-	100%	100%	0%	-	100%	100%	100%	-	-	87%
Weighted mean	63.1%	77.7%	65.5%	77.5%	50.4%	78.1%	77.2%	83.8%	85.1%	58.8%	63.4%	76.4%	73.3%	74.1%	73.8%	76.0%	71.4%
RANKING	14	4	12	5	16	3	6	2	1	15	13	7	11	9	10	8	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
1a+1b	96%	97%	100%	98%	78%	100%	100%	100%	100%	100%	100%	100%	95%	98%	100%	98%	97%
RANKING	14	13	1	11	16	1	1	1	1	1	1	1	15	12	1	10	

F

BIAS																	
validated stage	Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31	ALL
Stage 1a ==> 0	0.17	0.04	0.00	0.09	0.73	0.05	0.17	0.16	-0.21	0.18	0.16	0.10	0.19	0.20	0.16	0.39	0.19
Stage 1b ==> 1	-0.19	-0.10	-0.50	-0.09	0.27	-0.29	-0.10	-0.13	-0.04	-0.15	-0.33	-0.20	-0.06	-0.03	-0.04	-0.10	-0.13
Stage 2 ==> 2	-0.21	0.00	0.05	-0.06	0.19	-0.15	0.00	-0.10	0.00	-0.15	0.07	0.00	-0.12	0.13	0.15	0.07	-0.01
Stage 3 ==> 3	-0.17	-0.17	-0.43	0.00	0.00	0.00	-0.17	-0.20	-0.00	-0.20	-0.17	0.14	-1.00	0.00	-0.17	-0.17	-0.20
Stage 4 ==> 4	-1.00	0.50	-0.38	0.33	0.20	0.00	0.50	-0.40	0.00	-0.33	0.50	-0.13	-0.50	-0.60	0.00	0.50	-0.06
Stage 5 ==> 5	0.00	0.00	0.00	-0.50	0.00	-1.00	-	0.00	0.00	-1.00	-	0.00	0.00	0.00	-	-	-0.13

3.4 Results of the egg identification exercises

The same images of eggs, which were used for egg staging, were also used for the egg identification exercises. Most of the eggs used were from artificial fertilisations and so the species of those eggs was definitely known. Those eggs originating from field sampling were double checked for correct species determination by the organizers of the workshop and also considered as validated. 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 3.13 to 3.16. About half of the participants at the workshop were inexperienced; hence results of the expert readers 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, validated species determination.

Sub-tables A show the number of eggs at each actual species that were assessed by each participant. The numbers at each validated 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 over-estimation 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 species.

The results 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, particularly in the environment of this workshop, where eggs could only be identified on images instead of real ones. Though some facts on the origin (ICES area and sampling date) of the egg was available with the egg images, more detailed information that would be helpful for identification, e.g. on specific location and the environmental conditions of the eggs, was not given. Also, participants were not able to perform the SAT test on individual eggs in the trays. After the first round of analysis there was some discussion on the features which aid fish egg identification, and some references and criteria were reviewed (see section 2.5) to help with the identification of eggs which are similar to those of mackerel and horse mackerel. This, in turn, helped improving identification results of the second round (Tables 3.15 – 3.16). For mackerel eggs, the percentage agreement increased from 86% to 89% with the actual species and for expert readers from 90% to 94%. For horse mackerel the agreement increased from 79% to 81% for actual species and for experts from 83% to 86%. For hake the agreement increased from 35% to 68%, experts from 51% to 80%.

Table 3.13. Species identification with validated species, first identification.
(A) The numbers of eggs at each validated species read by each participant.
(B) The numbers of eggs allocated to each species by each participant.
(C) The over / underestimation by each participant.
(D) The percentage agreement by validated species by each participant.

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Species composition using validated species																																
validated species	Reader 1 to Reader 30																														TOTAL	
	Mackerel	94	109	109	44	68	109	68	109	68	109	109	26	43	69	109	74	109	60	109	67	34	60	35	109	60	60	70	77	2336		
Horse Mackerel	81	92	92	35	61	92	92	61	54	92	58	92	23	40	59	92	65	92	54	92	60	32	57	34	92	54	54	64	71	2029		
Hake	34	41	41	11	27	41	41	27	21	41	18	41	11	16	29	41	26	41	21	41	27	13	23	14	41	21	21	30	30	871		
Ling	17	18	18	6	13	18	18	13	11	18	16	18	5	6	13	18	14	18	11	18	13	5	12	5	18	11	11	15	17	412		
Other Species	39	40	40	16	31	40	40	31	25	40	28	40	40	6	15	32	40	35	40	25	40	30	15	24	15	40	25	34	38	930		
Total	1-5	265	300	300	112	200	300	300	200	171	300	188	300	300	71	120	202	300	214	300	171	300	197	99	176	103	300	171	172	213	233	6578

Species composition as estimated per participant and whole group																																	
validated species	Reader 1 to Reader 30																														TOTAL		
	Mackerel	118	120	119	44	98	104	134	67	77	137	36	102	124	19	71	65	111	95	150	77	107	91	29	50	43	164	75	77	92		97	2693
Horse Mackerel	80	83	90	43	66	67	81	57	60	85	43	91	101	7	42	52	87	43	123	59	99	71	15	43	24	89	60	59	60	60	1940		
Hake	21	32	34	6	3	37	19	19	1	9	20	26	21	4	-	38	43	16	17	1	39	5	9	22	7	13	2	2	15	33	514		
Ling	-	-	21	3	8	17	19	18	11	9	-	24	13	3	2	3	19	11	-	9	19	1	-	11	4	-	9	10	18	8	270		
Other Species	46	65	36	16	25	75	47	39	22	60	89	57	41	38	5	44	40	49	10	25	36	29	46	50	25	34	25	24	28	35	1161		
Total	1-5	0-5	264	300	289	89	200	300	300	196	170	285	174	300	299	71	120	199	296	213	300	167	300	194	98	176	103	300	168	169	213	207	6460

Percentage overestimation/underestimation																															
validated species	Reader 1 to Reader 30																														ALL
	Mackerel	26%	10%	9%	0%	44%	-5%	23%	-1%	28%	26%	-47%	-6%	14%	-27%	65%	-6%	2%	28%	38%	28%	-2%	36%	-15%	-17%	23%	50%	25%	28%	31%	
Horse Mackerel	-1%	-10%	-2%	23%	8%	-27%	-12%	-7%	11%	-8%	-26%	-1%	10%	-70%	5%	-12%	-5%	-34%	34%	9%	8%	18%	-53%	-25%	-29%	-3%	11%	9%	-6%	-15%	-4%
Hake	-38%	-22%	-17%	-43%	-89%	-10%	-54%	-30%	-95%	-78%	11%	-37%	-49%	-64%	-	31%	5%	-38%	-59%	-95%	-5%	-81%	-31%	-4%	-50%	-68%	-90%	-90%	-50%	10%	-41%
Ling	-	-	17%	-50%	-38%	-6%	6%	38%	0%	-50%	-	33%	-28%	-40%	-67%	-77%	6%	-21%	-	-18%	6%	-92%	-	-8%	-20%	-	-18%	-9%	20%	-53%	-34%
Other Species	18%	63%	-10%	-19%	88%	18%	26%	-12%	50%	218%	43%	3%	533%	-67%	38%	0%	40%	-75%	0%	-10%	-3%	207%	108%	67%	-15%	0%	-8%	-18%	-8%	25%	

Percentage agreement in species identification per species																																
validated species	Reader 1 to Reader 30																														ALL	
	Mackerel	90%	92%	91%	70%	93%	87%	89%	84%	93%	73%	49%	84%	90%	58%	91%	87%	94%	82%	90%	93%	91%	87%	68%	65%	91%	92%	93%	84%	83%		86%
Horse Mackerel	84%	87%	91%	94%	92%	55%	72%	70%	98%	68%	71%	78%	92%	22%	85%	73%	90%	52%	88%	98%	96%	97%	34%	58%	53%	83%	100%	98%	80%	52%	79%	
Hake	41%	73%	80%	27%	0%	63%	12%	59%	5%	2%	50%	59%	73%	0%	0%	62%	80%	0%	20%	5%	83%	19%	23%	35%	7%	10%	5%	30%	7%	35%		
Ling	0%	0%	100%	50%	54%	89%	89%	85%	73%	50%	0%	100%	72%	20%	33%	23%	100%	79%	0%	73%	89%	0%	0%	92%	80%	0%	73%	73%	93%	47%	56%	
Other Species	51%	70%	48%	44%	68%	80%	50%	74%	84%	55%	96%	85%	80%	83%	27%	75%	85%	74%	23%	88%	78%	77%	93%	50%	47%	73%	88%	62%	58%	68%		
Weighted mean	1-5	70.6%	79.3%	84.3%	68.8%	73.5%	73.3%	68.0%	75.0%	81.3%	58.3%	58.5%	80.0%	81.0%	36.6%	65.8%	73.3%	90.3%	61.7%	65.3%	81.9%	89.3%	73.1%	51.5%	58.5%	60.2%	69.3%	82.5%	82.0%	72.3%	57.1%	72.7%
RANKING		17	10	3	19	12	13	20	11	7	27	26	9	8	30	21	14	1	23	22	6	2	15	29	25	24	18	4	5	16	28	

Table 3.14. Species identification with validated species, first identification, expert readers only.

- (A) The numbers of eggs at each validated species read by each participant.
- (B) The numbers of eggs allocated to each species by each participant.
- (C) The over / underestimation by each participant.
- (D) The percentage agreement by validated egg stage by each participant.

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Species composition using validated species													
validated species	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	TOTAL
Mackerel 1	94	109	109	109	109	68	109	109	109	109	109	109	1252
Mackerel 2	81	91	91	91	91	61	91	91	91	91	91	91	1052
Horse Mackerel 3	34	42	42	42	42	27	42	42	42	42	42	42	481
Ling 4	17	18	18	18	18	13	18	18	18	18	18	18	210
Other Species 5	39	40	40	40	40	31	40	40	40	40	40	40	470
Total 1-5	265	300	300	300	300	200	300	300	300	300	300	300	3465

Species composition as estimated per participant and whole group													
validated species	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	TOTAL
Mackerel 1	118	120	119	104	134	67	102	124	111	150	107	164	1420
Mackerel 2	80	83	90	67	81	57	91	101	87	123	99	89	1048
Horse Mackerel 3	21	32	34	37	19	19	26	21	43	17	39	13	321
Ling 4	-	-	21	17	19	18	24	13	19	-	19	-	150
Other Species 5	46	65	36	75	47	39	57	41	40	10	36	34	526
Total 1-5	265	300	300	300	300	200	300	300	300	300	300	300	3465

Percentage overestimation/underestimation													
validated species	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
Mackerel 1	26%	10%	9%	-5%	23%	-1%	-6%	14%	2%	38%	-2%	-50%	13%
Mackerel 2	-1%	-9%	-1%	-26%	-11%	-7%	0%	11%	-4%	35%	9%	-2%	0%
Horse Mackerel 3	-38%	-24%	-19%	-12%	-55%	-30%	-38%	-50%	2%	-60%	-7%	-69%	-33%
Ling 4	-	-	17%	-6%	6%	38%	33%	-28%	6%	-	6%	-	-29%
Other Species 5	18%	63%	-10%	88%	18%	26%	43%	3%	0%	-75%	-10%	-15%	12%

Percentage agreement in species identification per species													
validated species	Reader 1	Reader 2	Reader 3	Reader 6	Reader 7	Reader 8	Reader 12	Reader 13	Reader 17	Reader 19	Reader 21	Reader 26	ALL
Mackerel 1	90%	92%	91%	87%	89%	84%	84%	90%	94%	90%	91%	92%	90%
Mackerel 2	84%	88%	92%	56%	71%	70%	78%	93%	91%	88%	96%	84%	83%
Horse Mackerel 3	41%	74%	79%	62%	12%	59%	57%	38%	81%	19%	81%	10%	51%
Ling 4	0%	0%	100%	89%	89%	85%	100%	72%	100%	0%	89%	0%	60%
Other Species 5	51%	70%	48%	80%	50%	74%	85%	80%	85%	23%	78%	73%	66%
Weighted mean	70.6%	79.7%	84.3%	73.3%	67.7%	75.0%	79.7%	81.3%	90.7%	65.0%	89.0%	69.7%	77.3%
RANKING	9	5	3	8	11	7	5	4	1	12	2	10	

Table 3.15. Species identification with validated species, second identification.

- (A) The numbers of eggs at each validated species read by each participant.
- (B) The numbers of eggs allocated to each species by each participant.
- (C) The over / underestimation by each participant.
- (D) The percentage agreement by validated species by each participant.

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		Species composition using validated species																																TOTAL
		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32		
Mackerel	1	72	110	110	66	100	90	109	72	91	82	78	110	72	68	72	78	71	76	110	72	110	90	110	19	86	79	80	72	59	91	110		
	2	60	90	90	58	83	73	89	60	59	73	60	90	60	54	59	67	47	63	90	59	90	73	90	10	71	69	72	59	41	59	90		
	3	31	40	40	30	36	36	40	31	31	33	16	40	31	30	31	31	29	30	40	31	40	35	40	5	35	31	32	31	22	31	40		
	4	13	20	20	12	16	15	20	13	16	15	15	20	13	13	13	14	14	14	20	13	20	15	20	1	18	14	14	13	10	16	20		
	5	27	40	39	27	35	32	40	25	30	27	27	40	26	20	26	26	20	27	40	25	40	29	40	8	31	27	27	25	22	30	40		
Total		1-5	203	300	299	193	270	246	298	201	227	230	196	300	202	185	201	216	181	210	300	200	300	242	300	43	241	220	225	200	154	227	300	7110

		Species composition as estimated per participant and whole group																																TOTAL
		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32		
Mackerel	1	84	121	116	46	116	89	117	73	101	26	75	99	74	43	78	79	72	96	131	82	110	86	110	18	88	93	85	81	66	100	150		
	2	56	78	85	44	82	67	68	64	51	62	47	95	59	21	64	55	45	42	89	51	97	74	70	13	58	67	58	52	33	53	83		
	3	36	54	41	37	24	27	43	27	35	28	24	32	32	26	24	44	28	13	57	32	44	39	25	1	30	14	46	34	26	33	26		
	4	13	12	23	10	10	21	20	11	15	14	-	21	14	16	13	13	16	13	14	12	16	13	-	-	17	15	15	12	8	15	16	408	
	5	14	35	34	56	38	42	50	26	25	100	50	53	23	79	22	25	20	46	9	23	33	30	95	11	48	31	21	21	21	26	25	1132	
Total		0-5	203	300	299	193	270	246	298	201	227	230	196	300	202	185	201	216	181	210	300	200	300	242	300	43	241	220	225	200	154	227	300	7110

		Percentage overestimation/underestimation																																ALL
		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32		
Mackerel	1	17%	10%	5%	-30%	16%	-1%	7%	1%	11%	-6%	-4%	-10%	3%	-37%	8%	1%	1%	26%	19%	14%	0%	-4%	0%	-5%	2%	18%	6%	13%	12%	10%	36%	3%	
	2	-7%	-13%	-6%	-24%	-1%	-8%	-24%	7%	-14%	-15%	-22%	6%	-2%	-61%	8%	-18%	-4%	-33%	-1%	-14%	8%	1%	-22%	30%	-18%	-3%	-19%	-12%	-20%	-10%	-8%	-11%	
	3	16%	35%	3%	23%	-33%	-25%	8%	-13%	13%	-15%	50%	-20%	3%	-13%	-23%	42%	-3%	-57%	43%	3%	10%	11%	-38%	-80%	-14%	-55%	44%	10%	18%	6%	-35%	-2%	
	4	0%	-40%	15%	-17%	-38%	40%	0%	-15%	-6%	-7%	-	5%	8%	23%	0%	-7%	14%	-7%	-30%	-8%	-20%	-13%	-	-	-6%	7%	7%	-8%	-20%	-6%	-20%	-13%	
	5	-48%	-13%	-13%	107%	9%	31%	25%	4%	-17%	270%	85%	33%	-12%	295%	-15%	-4%	0%	70%	-78%	-8%	-18%	3%	138%	38%	55%	15%	-22%	-16%	-5%	-13%	-38%	23%	

		Percentag agreement in species identification per species																																ALL
		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	Reader 25	Reader 26	Reader 27	Reader 28	Reader 29	Reader 30	Reader 31	Reader 32		
Mackerel	1	99%	98%	96%	56%	87%	94%	90%	93%	98%	22%	94%	88%	99%	51%	96%	95%	99%	82%	90%	97%	92%	90%	91%	68%	85%	94%	96%	96%	96%	95%	98%	95%	89%
	2	88%	83%	93%	64%	81%	86%	73%	95%	85%	71%	78%	94%	93%	30%	97%	78%	94%	51%	67%	83%	93%	92%	77%	90%	72%	87%	78%	85%	71%	85%	87%	81%	81%
	3	90%	90%	88%	60%	36%	67%	70%	77%	84%	58%	81%	75%	87%	37%	71%	87%	93%	17%	58%	81%	85%	83%	48%	0%	60%	23%	88%	84%	73%	81%	45%	68%	
	4	92%	50%	95%	83%	63%	100%	85%	77%	94%	87%	0%	100%	100%	100%	92%	86%	100%	86%	55%	92%	75%	80%	0%	0%	83%	93%	93%	92%	70%	94%	80%	78%	
	5	44%	68%	74%	85%	60%	81%	58%	88%	73%	56%	93%	88%	85%	70%	73%	73%	95%	70%	18%	72%	63%	66%	85%	75%	48%	74%	63%	68%	73%	77%	53%	69%	
Weighted mean		1-5	86.7%	85.3%	91.3%	64.8%	73.3%	86.6%	77.5%	89.6%	89.0%	50.9%	80.6%	89.0%	93.6%	48.1%	89.1%	85.2%	96.1%	61.9%	66.7%	87.0%	86.3%	86.0%	74.0%	65.1%	72.6%	79.1%	84.9%	87.0%	80.5%	89.0%	79.3%	80.3%
RANKING			11	15	3	28	24	12	22	4	7	30	18	6	2	31	5	16	1	29	26	9	13	14	23	27	25	21	17	9	19	7	20	

Table 3.16. Species identification with validated species, first identification, expert readers only.

- (A) The numbers of eggs at each validated species read by each participant.
- (B) The numbers of eggs allocated to each species by each participant.
- (C) The over / underestimation by each participant.
- (D) The percentage agreement by validated egg stage by each participant.

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		Species composition using validated species																	TOTAL
		Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31		
Mackerel	1	72	110	110	90	109	72	91	110	72	110	72	110	90	80	72	91	1461	
Horse Mackerel	2	60	90	90	73	89	60	59	90	60	90	59	73	72	59	59	59	1173	
Hake	3	31	40	40	36	40	31	31	40	31	40	31	40	35	32	31	31	560	
Ling	4	13	20	20	15	20	13	16	20	13	20	13	20	15	14	13	16	261	
Other Species	5	27	40	39	32	40	25	30	40	26	40	25	40	29	27	25	30	515	
Total	1-5	203	300	299	246	298	201	227	300	202	300	200	300	242	225	200	227	3970	

		Species composition as estimated per participant and whole group																	TOTAL
		Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31		
Mackerel	1	84	121	116	89	117	73	101	99	74	131	82	110	86	85	81	100	1549	
Horse Mackerel	2	56	78	85	67	68	64	51	95	59	89	51	97	74	58	52	53	1097	
Hake	3	36	54	41	27	43	27	35	32	32	57	32	44	39	46	34	33	612	
Ling	4	13	12	23	21	20	11	15	21	14	14	12	16	13	15	12	15	247	
Other Species	5	14	35	34	42	50	26	25	53	23	9	23	33	30	21	21	26	465	
Total	1-5	203	300	299	246	298	201	227	300	202	300	200	300	242	225	200	227	3970	

		Percentage overestimation/underestimation																	ALL
		Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31		
Mackerel	1	17%	10%	5%	-1%	7%	1%	11%	-10%	3%	19%	-14%	0%	-4%	6%	13%	10%	6%	
Horse Mackerel	2	-7%	-13%	-6%	-8%	-24%	7%	-14%	6%	-2%	-14%	-14%	8%	1%	-19%	-12%	-10%	-6%	
Hake	3	16%	35%	3%	-25%	8%	-13%	13%	-20%	3%	43%	3%	10%	11%	44%	10%	6%	9%	
Ling	4	0%	-40%	15%	40%	0%	-15%	-6%	5%	8%	-30%	-8%	-20%	-13%	7%	8%	-6%	-5%	
Other Species	5	-8%	-13%	-13%	31%	25%	4%	-17%	33%	-12%	-78%	-8%	-18%	3%	-22%	-16%	-13%	-10%	

		Percentage agreement in species identification per species																	ALL
		Reader 1	Reader 2	Reader 3	Reader 7	Reader 8	Reader 9	Reader 10	Reader 13	Reader 14	Reader 20	Reader 21	Reader 22	Reader 23	Reader 28	Reader 29	Reader 31		
Mackerel	1	99%	98%	96%	94%	90%	93%	98%	88%	99%	94%	97%	92%	90%	96%	96%	98%	94%	
Horse Mackerel	2	88%	83%	93%	86%	73%	95%	85%	94%	93%	67%	83%	93%	92%	78%	85%	85%	86%	
Hake	3	90%	90%	88%	67%	70%	77%	84%	75%	87%	58%	81%	85%	83%	88%	84%	81%	80%	
Ling	4	92%	50%	95%	100%	85%	77%	94%	100%	100%	55%	92%	75%	80%	93%	92%	94%	85%	
Other Species	5	46%	68%	74%	81%	58%	88%	73%	88%	85%	18%	72%	63%	63%	68%	68%	77%	67%	
Weighted mean	0.5	86.7%	85.3%	91.3%	86.6%	77.5%	89.6%	89.0%	89.0%	89.6%	66.7%	87.0%	86.3%	86.0%	84.9%	87.0%	89.0%	85.5%	
RANKING		9	13	2	10	15	3	5	4	1	16	7	11	12	14	7	5		

3.5 Species identification and staging error matrix (ToR c)

Uncertainty in fish egg identification and staging can be quantified by an error matrix (EM). The elements of an EM are the probabilities that a sampled egg of a validated species/stage *a* is assigned to one of the observed species/stages. For the majority of the eggs in this workshop the validated species came from fertilization experiments. For the remainder the validated species was the visual identification from the individual providing the egg (or egg image) for this workshop. Before adding the egg to the exercise, the species was checked by the organisers of the workshop. 'True species' can be gained from fertilization experiments, but these are time and cost consuming. Also, it can be difficult to fertilize eggs and keep them alive until larvae hatch.

For the staging error matrices, the validated stage was the visual staging from the individual providing the egg (or egg image) for this workshop. Before adding the egg to the exercise, the stage was checked by the organisers of the workshop.

For the mackerel and horse mackerel staging error matrices, all readings of either mackerel or horse mackerel were used. Thus, if a reader has misidentified an egg as mackerel, the stage of this egg was included in the mackerel staging error matrix.

3.5.1 Data on egg identification uncertainty

During this workshop, 300 images of eggs were available for both identification rounds. In both rounds 110 mackerel, 90 horse mackerel and 40 hake eggs were available (Table 3.17). For various reasons, not all readers were able to identify all eggs (see section 4).

Table 3.17. Number of images per validated species for each identification exercise.

Species	Development stage	N images 1 st round	N images 2 nd round
Mackerel	1A	40	28
	1B	11	42
	2	22	22
	3	21	7
	4	12	8
	5	4	3
Horse mackerel	1A	4	21
	1B	14	7
	2	13	15
	3	53	36
	4	6	11
Hake	1A	16	13
	1B	4	7

Species	Development stage	N images 1 st round	N images 2 nd round
Hake	2	4	5
	3	6	10
	4	9	5
Ling	1A	17	19
	1B	3	1
Other	1A	19	28
	1B	8	4
	2	3	1
	3	10	7

Not all of the participants in the workshop provide egg data for the survey. The participants were divided in a group of experts, that provided data in the most recent surveys or will provide data for the 2022 survey and have experience with egg identification, and other participants.

3.5.2 The Error Matrices

For the construction of the error matrices only experts' readings were included, where the experts identified and stage 200 or more eggs. The resulting error matrices for species identification, staging of all, mackerel and horse mackerel eggs of the first round are given in tables 3.18 – 3.24, and of the second round in tables 3.25 – 3.31.

Table 3.18. Species identification error matrix based on the first identification exercise.

Actual species	Observed species				
	Mackerel	Horse mackerel	Hake	Ling	Other
Mackerel	0.89	0.06	0.01	0.00	0.04
Horse mackerel	0.01	0.83	0.04	0.01	0.11
Hake	0.33	0.09	0.51	0.03	0.04
Ling	0.15	0.05	0.10	0.57	0.13
Other	0.20	0.12	0.02	0.00	0.66

Table 3.22. All species staging error matrix based on the first identification exercise, stages 1A and 1B combined to stage 1.

		Observed stage				
Actual stage	1	2	3	4	5	
1	0.94	0.05	0.01	0.00	0.00	
2	0.32	0.62	0.05	0.00	0.00	
3	0.08	0.33	0.55	0.04	0.00	
4	0.07	0.06	0.25	0.56	0.06	
5	0.00	0.02	0.02	0.13	0.82	

Table 3.23. Mackerel staging error matrix based on the first identification exercise, stages 1A and 1B combined to stage 1.

		Observed stage				
Actual stage	1	2	3	4	5	
1	0.96	0.03	0.00	0.00	0.00	
2	0.25	0.71	0.03	0.01	0.00	
3	0.03	0.21	0.70	0.05	0.00	
4	0.02	0.06	0.26	0.54	0.12	
5	0.00	0.02	0.04	0.14	0.80	

Table 3.24. Horse mackerel staging error matrix based on the first identification exercise, stages 1A and 1B combined to stage 1.

		Observed stage				
Actual stage	1	2	3	4	5	
1	0.87	0.11	0.02	0.00	0.00	
2	0.40	0.53	0.08	0.00	0.00	
3	0.09	0.39	0.49	0.04	0.00	
4	0.06	0.09	0.41	0.43	0.01	
5	0.00	0.00	0.00	0.00	1.00	

Table 3.25. Species identification error matrix based on the second identification exercise.

		Observed species				
Actual species	Mackerel	Horse mackerel	Hake	Ling	Other	
Mackerel	0.94	0.01	0.00	0.00	0.04	
Horse mackerel	0.02	0.86	0.10	0.00	0.02	
Hake	0.08	0.05	0.80	0.02	0.06	
Ling	0.00	0.04	0.08	0.85	0.03	
Other	0.20	0.08	0.04	0.02	0.67	

Table 3.26. All species staging error matrix based on the second identification exercise.

		Observed stage					
Actual stage	1A	1B	2	3	4	5	
1A	0.77	0.19	0.02	0.00	0.01	0.00	
1B	0.25	0.69	0.05	0.00	0.00	0.00	
2	0.05	0.06	0.75	0.13	0.00	0.00	
3	0.03	0.03	0.13	0.73	0.08	0.00	
4	0.00	0.00	0.02	0.24	0.67	0.07	
5	0.00	0.00	0.00	0.00	0.17	0.83	

Table 3.27. Mackerel staging error matrix based on the second identification exercise.

		Observed stage					
Actual stage	1A	1B	2	3	4	5	
1A	0.79	0.20	0.00	0.00	0.00	0.00	
1B	0.18	0.77	0.04	0.00	0.00	0.00	
2	0.02	0.04	0.87	0.07	0.00	0.00	
3	0.02	0.02	0.14	0.78	0.03	0.01	
4	0.01	0.00	0.01	0.19	0.59	0.20	
5	0.00	0.00	0.00	0.00	0.13	0.87	

Table 3.28. Horse mackerel staging error matrix based on the second identification exercise.

		Observed stage				
Actual stage	1A	1B	2	3	4	5
1A	0.46	0.17	0.10	0.13	0.13	0.00
1B	0.16	0.13	0.12	0.49	0.11	0.00
2	0.17	0.08	0.34	0.32	0.08	0.00
3	0.12	0.06	0.17	0.52	0.13	0.00
4	0.01	0.00	0.03	0.72	0.24	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00

Table 3.29. All species staging error matrix based on the second identification exercise, stages 1A and 1B combined to stage 1.

		Observed stage				
Actual stage	1	2	3	4	5	
1	0.96	0.03	0.00	0.00	0.00	
2	0.11	0.75	0.13	0.00	0.00	
3	0.06	0.13	0.73	0.08	0.00	
4	0.00	0.02	0.24	0.67	0.07	
5	0.00	0.00	0.00	0.17	0.83	

Table 3.30. Mackerel staging error matrix based on the second identification exercise, stages 1A and 1B combined to stage 1.

		Observed stage				
Actual stage	1	2	3	4	5	
1	0.97	0.03	0.00	0.00	0.00	
2	0.06	0.87	0.07	0.00	0.00	
3	0.03	0.14	0.78	0.03	0.01	
4	0.01	0.01	0.19	0.59	0.20	
5	0.00	0.00	0.00	0.13	0.87	

Table 3.31. Horse mackerel staging error matrix based on the second identification exercise, stages 1A and 1B combined to stage 1.

		Observed stage				
Actual stage	1	2	3	4	5	
1	0.53	0.11	0.24	0.12	0.00	
2	0.25	0.34	0.32	0.08	0.00	
3	0.17	0.17	0.52	0.13	0.00	
4	0.01	0.03	0.72	0.24	0.00	
5	0.00	0.00	0.00	0.00	0.00	

4 Discussion

During this workshop and for the first time all egg identification and staging trials were run solely using images instead of real eggs viewed under a microscope. This posed several challenges when compared to previous staging workshops. In particular no possibility to directly analyse the eggs through either viewing from different sides or through varying magnification or focus. Though the total number of 300 eggs was well within the range of what participants had encountered during recent workshops, the process of analysing egg images turned out to be far more time consuming than dealing with samples of real eggs with the result that the majority of participants were unable to complete the trial and fell short of the 300 images set aside for each trial.

In a plenary session it was discussed what the results of the workshop represent and if results, particularly those from the error matrices, could be used in the assessment of the total egg productions. The goal of WKMACHIS is to refresh the analysts participating in the mackerel and horse mackerel egg surveys. The surveys are carried out triennially and for most survey participants egg identification and 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 skills. For this, ring tests should be carried out during or after the survey to assess the performance of survey participants.

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

4.1 Egg sorting exercise and SAT test

No egg sorting and surface adhesion tests were carried out during the online workshop.

4.2 Egg staging and identification exercises

For the first time during egg identification and staging workshops, participants were only presented with images of eggs, which they had to identify and stage on their home screens, instead of real eggs viewed under microscopes. Instead of being able to handle each egg with tweezers and view it utilizing different lighting, magnifications and focus, participants were confronted with a single image per egg, taken at a fixed magnification (mostly 4 x), lighting (mostly dark field) and focus. Though extensive care was taken that all necessary diagnostic features enabling correct identification and staging of the egg were visible in the images, the inability to manipulate an egg during the identification and staging process was felt by most participants to be at the very least, concerning. This might shake the confidence of some readers in their own judgement, which in turn may ultimately impact their identification and staging results.

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, 2015, 2018), and further consultations at this workshop, these egg staging criteria have been

reviewed (section 2.4). These characteristics are the result of many years of personal experience (from various participants) in staging preserved fish eggs from plankton samples.

After the first round of the egg staging and identifying exercise the main discussion was with the stages 1A and 1B. To clarify the characteristics that separate these from the other stages, several 1A and 1B images were viewed on the screen and discussed. However, correct discrimination between those two stages did not improve considerably during the second round. While for calculation of the annual egg production only eggs of stage 1 (i.e. 1A and 1B combined) are used, the apparent inability to correctly discriminate between those two stages will not negatively influence the results of the egg survey at the current design. Any move, however, towards utilizing and implementing a finer staging system should be considered with care and should certainly involve thorough training of participants in correct egg staging.

At only 86 %, the agreement in correctly identifying horse mackerel eggs was low even after the second identification and staging trial and most likely assigning them to hake. Particularly for the crucial stage 1 eggs, the uncertainty matrix revealed a high error potential in correct assignment of horse mackerel eggs of that stage. Two factors, which result from the image-based trials, may be responsible for this unsatisfactory result. Hake and horse mackerel have a highly overlapping range in egg diameter. Though the segmented yolk, which only exists in horse mackerel, is a characteristic, the feature may not be visible in the sampled egg at all or was not distinctly displayed in the image. The surface adhesion test, which would clearly separate hake egg from other eggs cannot be applied on images. On the other hand, the additional structure, which is introduced through that segmentation, might be misjudged as some embryonic development, particularly when readers are not enabled to move a specimen, trying to judge its view from another angle.

Image quality may have been a major source of error in both species and stage identification. For mackerel, where over the most recent surveys rearing experiments have been carried out, a high number of good quality egg samples of all stages was available. This resulted in higher choice of good quality images of mackerel eggs, possibly contributing to the better results obtained for this species. The choice on good quality horse mackerel eggs was, in contrast, much lower. A high number of images were from eggs, which originated from rearing experiments carried out in 2013. Hence, the quality of the eggs had already suffered from a long period of storage in formaldehyde.

To be able to properly train the participants during these workshops it is imperative to have samples and images of good quality eggs of the main species: mackerel, horse mackerel, hake, megrim, grey gurnard and ling. All participants of the WGMEGGS 2022 are therefore requested to collect eggs of these species, preferable validated from artificial fertilization experiments and, if possible, take high resolution images. It would also be good if information on the origin of the eggs would be given to aid the identification. For samples collected during the surveys, these data are always available, providing assurance regarding their provenance.

5 Other items discussed at the workshop

5.1 Standardization of sample processing and data reporting within MEGS

At the beginning of the workshop, all participants were asked to fill in a table on sample processing methods. Participants were requested to provide information on the following subjects:

Subjects and instructions for filling in the table

Country: name of country of the survey participant

Institute: name or acronym of participating institute

Survey: name and/or acronym of the survey

ICES area: area code

Target species: the name(s) of the target species of the survey

Non-target species: name(s) of any species for which data are generated and which is/are not target of the survey

Survey purpose: the purpose of the survey w.r.t. the target species

Assessment group; relevant survey output: The ICES assessment group and the provided survey output (index) for assessment, usually WGWIDE or WGACEGG for area 27.9.a

Gear: the acronym of the gear used for catching the eggs and larvae. Preferably using ICES vocabulary

Gear deployment: mode of deployment of gear (e.g. vertical, horizontal, double-oblique) Preferably using ICES vocabulary

Mesh (μm): The mesh width of the net used for the catches in μm

Codend mesh (μm): mesh width of the codend if different from the latter

Location of fish egg sorting and identification: where samples are sorted and larvae identified – on board or in the lab

Spray method applied and number of applications per sample: yes or no, and if yes, the number or application per sample

Fish egg sorting and processing (fresh/preserved): is sorting and processing of larval sample done on fresh or preserved samples

Subsampling (y/n) and method: is subsampling regularly applied and which method is chosen (e.g. Folsom splitter, other type of splitter, subsampling by weight, numbers). Some free text is allowed here

Identification of eggs: visual or molecular/genetic

Identification method: visual methods: microscopic on the real sample or on an image of the sample maybe aided by image analysis. Genetic: barcoding, metabarcoding,

Measurements: Counts, diameters of egg, oil globule

Smallest units: 0.01 mm, 0.1 mm...

Method of measurement: microscopic aided by eyepiece graticule, image analysis....

Egg samples kept (y/n): Are larvae kept/stored (y) or discarded (n) after analysis

Preservation of eggs: preservation fluid and concentration (%)

Buffer for eggs: name of the buffering agent if applicable

Other fish eggs kept (y/n): Are larvae kept/stored (y) or discarded (n) after analysis

Preservation of other fish eggs: preservation fluid and concentration (%)

Buffer for other fish eggs: name of the buffering agent if applicable

Remainder of plankton sample kept (y/n): Is remainder kept/stored (y) or discarded (n) after analysis

Preservation of remainder: preservation fluid and concentration (%)

Buffer for remainder: name of the buffering agent if applicable

Comments, suggestions for future methods: your thoughts and comments

From the table entries (the overview table is presented in annex 5) it became apparent that while sampling procedures appear to be well standardized through the MEGS survey manual (ICES 2019), work up of samples is done differently among the different institutes and/or nations. Major differences include whether samples are processed fresh or preserved on either ship or land, and the utilization of image-based systems for egg identification, staging and measuring. Some participants use sub-sampling in their sample analysis. However, while the minimum amount of counted and measured individuals per target species are defined in the survey manual (e.g. ICES 2019), methods on how these numbers shall be achieved are neither described in manuals, nor documented in survey protocols.

Formaldehyde at 4 % concentrations still appears to be the major preservation fluid to be used, while some institutes and/or nations have switched to ethanol not only for safety reasons. Ethanol also allows for utilization of genetic methods in egg identification. Staging, however, is not possible in ethanol preserved eggs, as they become opaque, and characteristics necessary for correct stage assignment are no longer visible. In particular where fresh and preserved sample work-up as well as the differing preservation methods – prior to sample analysis and measurement of eggs – are used, measures should be taken to assure data comparability. For buffering of formaldehyde, only one chemical, sodium acetate, is used.

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Annex 1: List of participants

Name	Institute	Country (of institute)	Email
Matthias Kloppmann, chair	TISF	Germany	matthias.kloppmann@thuener.de
Linford Mann	CEFAS	UK-England	linford.mann@cefasc.co.uk
James Pettigrew	CEFAS	UK-England	james.pettigrew@cefasc.co.uk
Rob van Ree	CEFAS	UK-England	robert.vanree@cefasc.co.uk
Valeska Borges	TISF	Germany	valeska.borges@thuener.de
Sakis Kroupis	TISF	Germany	sakis.kroupis@thuener.de
Birgit Suer	TISF	Germany	birgit.suer@thuener.de
Karin Krüger	TISF	Germany	k.krueger@thuener.de
Jens Ulleweit	TISF	Germany	jens.ulleweit@thuener.de
Bastian Huwer	DTU-Aqua	Denmark	bhu@aquadtu.dk
Anne-Mette Kroner	DTU-Aqua	Denmark	amkro@aquadtu.dk
Mette Kjellerup Schiønning	DTU-Aqua	Denmark	mekjs@aquadtu.dk
Brendan O'Hea	MI	Ireland	brendan.ohea@marine.ie
Grainne NiChonchuir	MI	Ireland	grainne.nichonchuir@marine.ie
Dave Tully	MI	Ireland	david.tully@marine.ie
Isabel Riveiro	IEO	Spain	isabel.riveiro@ieo.es
Gersom Costas	IEO	Spain	gersom.costas@ieo.es
Luisa Iglesias	IEO	Spain	luisa.iglesias@ieo.es
Dolores García	IEO	Spain	mariadolores.garcia@ieo.es
Javier Valtierra	IEO	Spain	javier.valtierra@ieo.es
Maria Manuel Angélico	IPMA	Portugal	mmangelico@ipma.pt
Elisabete Henriques	IPMA	Portugal	ehenriques@ipma.pt
Finlay Burns	MSS	UK-Scotland	burnsf@marlab.ac.uk
Hannah Holah	MSS	UK-Scotland	Hannah.Holah@gov.scot
Jim Drewery	MSS	UK-Scotland	J.Drewery@MARLAB.AC.UK
Sólva Káradóttir Eliassen	FMRI	Faroese	Solvae@hav.fo
Durita Sørensen	FMRI	Faroese	duritas@hav.fo
Cindy van Damme	WUR	The Netherlands	cindy.vandamme@wur.nl
Ewout Blom	WUR	The Netherlands	ewout.blom@wur.nl
Erika Koelemij	WUR	The Netherlands	erika.koelemij@wur.nl
Paula Alvarez	AZTI	Spain	palvarez@azti.es
Beatriz Beldarrain	AZTI	Spain	bbeldarrain@azti.es
Merete Fonn	HI	Norway	merete.fonn@hi.no
Grethe Thorsheim	HI	Norway	grethe.thorsheim@hi.no
Thassya dos Santos Schmidt	HI	Norway	thassya.dos.santos.schmidt@hi.no
Bahar Mozfar	HI	Norway	bahar.mozfar@hi.no
Frøydis Tousgaard Rist	HI	Norway	froydis.rist@hi.no
Anders Thorsen	HI	Norway	anders.thorsen@hi.no

Annex 2: Resolutions

The **Workshop on Mackerel, Horse Mackerel and Hake Eggs Identification and Staging (WKMACHIS)** chaired by Matthias Kloppmann*, Germany, will meet online, 11-15 October 2021 to:

- a) Carry out internationally comparative plankton sorting trials on typical MEGS survey samples to evaluate and standardize the effectiveness of plankton sampling procedures. This should follow the pattern of trial – analysis– identification of problem areas – retrieval; **ICES Science plan [3.1](#)**
- b) Carry out comparative egg identification and staging trials for mackerel, horse mackerel and hake eggs following the methodology used in the previous egg staging workshops in order to quality assure the egg production estimates for the target species; **ICES Science plan [3.1](#)**
- c) Discuss sources of misidentification and -staging of fish eggs and prepare an uncertainty matrix of mackerel, horse mackerel and hake egg identification and staging; **ICES Science plan [3.1](#)**
- d) Review available documentation on species identification and staging of fish eggs, define **standard protocols and updated relevant descriptions and pictures in the survey manual**; **ICES Science plan [3.1](#)**

WKMACHIS will report by 19 November 2021 for the attention of EOSG, WGMEGS and WGBIOP

Supporting Information

Priority	High priority to ensure the quality of data provided to WGWISE for the production of advice.
Scientific justification	<p>Sorting fish eggs from plankton samples, their staging and identification to species remains one of the key proficiencies in the execution of the mackerel and horse mackerel egg surveys. As this 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. WGMEGS strongly feels that this is best done through the mechanism of a regular workshop to compare results between survey participants. In the context of the triennial egg surveys, it proved 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 since 2000, and were very successful in achieving these aims. It is recommended that experiences gathered during these be used for setting up the procedures for the proposed workshop in 2022. 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.</p> <p>The workshop will also be tasked to update the descriptions and photographs given in the MEGS manual to assist in the plankton sample handling procedure.</p>
Resource requirements	None

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

Annex 3: Agenda

Agenda for the WKMACHIS online meeting, 11 –15 October 2021.

Monday 11 October

10:00

- Start of meeting – Welcome and general announcements
- Presentation on workshop history and Introduction to fish egg identification and staging
- Introduction into use of SmartDots for fish egg identification and staging events

12:00 Lunch break and opening of 1st egg identification and staging round

- 1st individual egg identification and staging trial using SmartDots at <https://smartdots.ices.dk/manage/ViewLarvaeEvent?tblEventID=366>

Tuesday 12 October

09:00

- Continue 1st individual egg identification and staging trial

13:00 Closing of 1st round

- Introduction into the objectJ tool for egg sample analysis – presentation by **Cindy van Damme**. Each participant is then asked to analyse 2 egg sample images using ObjectJ during the course of the next 2 days until Thursday, 12 October, lunch time.
- Plenary – Planning for the 2022 survey (presentation and discussion led by **Brendan O’Hea**)
- Break out in sub-groups to review available information on fish egg identification and staging of MEGS target species, updates on descriptions from 2018 report and MEGS manual, while WKMACHIS chair analyses the results of the first round.

16:00 End of the day

Wednesday 13 October

10:00

- Review available information species identification and staging of fish eggs (presentations of sub-groups)
- Presentation and discussion of results of 1st identification round

12:00 Lunch and opening of 2nd egg identification and staging round

- 2nd individual egg identification and staging trial at <https://smartdots.ices.dk/manage/ViewLarvaeEvent?tblEventID=368>

17:30 End of the day

Thursday 14 October

09:00

- Continue 2nd individual larvae identification trial

13:00 Closing of 2nd round,

- Plenary and break out in sub-groups to update of MEGS manual, in particular sample processing procedures while WKMACHIS chair analyses the results of the second round.

15:00 Break

15:15

- Presentation of MEGS manual updates, 2022 survey plan updates
- Presentation and discussion of results of 2nd identification round and of ObjectJ trials

17:30 End of the day

Friday 15 October

10:00

- Presentation and discussion of uncertainty matrix of mackerel, horse mackerel and hake egg identification and staging – **Cindy van Damme**
- Presentation on transect design (the double-0 rule) during surveys – **Gersom Costas**
- Compile and discuss overview of methods of fish egg sampling and sample processing, preservation used

11:15 Report writing: discussion, conclusions, recommendations and future, e.g. creating an image database on ELH stages of marine fish

12:00 Final discussions – using SmartDots for egg identification and staging workshops

12:30 End of the workshop

Annex 4: 11-stage scale as used for DEPM in southern horse mackerel

Stage 1

The egg shows, in the animal pole, an initial superficial thickening coming from the cell division, which, under dim reflected light, is easily visible. This stage lasts until individual cells are easily distinguishable from each other, and counting is possible (until 64 cells).

Equivalent to stages 1A of Pipe and Walker (1987) and 1 of King et al. (1977). Unfertilized eggs are included in this stage (however, they are difficult to distinguish).

Stage 2

In the animal pole, the cells continue to divide and it is impossible to count them. The cleavage proceeds until a blastodermal cap – blastodisc - is formed. Eggs are sometimes pear-shaped being the animal pole more evident (in a hood-shaped).

Equivalent to stages 1A of Pipe and Walker (1987) and 1 of King et al. (1977).

Stage 3

The formation of the blastodisc occurs. Its edge is thicker and it is visible, as a ring, from both sides: from the vegetative pole and when viewed from the lateral.

Equivalent to stages 1B of Pipe and Walker (1987) and 1 of King et al. (1977).

Stage 4

The outline of the embryo is clearly defined in the median line of the embryonic shield although its head and tail cannot be distinguished. On the lateral side, eggs have a contour that resembles “underwear”. The blastopore is still large.

Equivalent to stages 2 of Pipe and Walker (1987) and 2 of King et al. (1977). The blastopore is still large.

Stage 5

The head and the tail of the embryo become visible. The cephalic region become 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. In this stage, it is possible to see the somites, although not so clearly, and pigmentation may begin to appear.

Equivalent to stages 2 of Pipe and Walker (1987) and 2 of King et al. (1977).

Stage 6

In this stage the embryo has thickened out and becomes bulbous. However the angle formed by the tail and yolk is $\geq 90^\circ$. The closure of the blastopore takes place. The optical vesicles are visible. The somites are more or less clear.

Equivalent to stages 2 of Pipe and Walker (1987) and 2 and 3 of King et al. (1977).

Stage 7

The embryo tail begins to separate from the yolk mass. The angle formed by the tail and the yolk is $< 90^\circ$ 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 3 of Pipe and Walker (1987) and 3 and 4 of King et al. (1977).

Stage 8

This stage starts when the length of the free tail is greater than the length of the head and ends when the embryo reaches $\frac{3}{4}$ of the circumference of the egg (when viewed from the lateral side).

Equivalent to stages 3 of Pipe and Walker (1987) and 4 of King et al. (1977).

Stage 9

This stage begins with the embryo occupying $\frac{3}{4}$ of the circumference of the egg and runs until it reaches $\frac{7}{8}$.

Equivalent to stages 3 of Pipe and Walker (1987) and 4 of King et al. (1977).

Stage 10

This stage begins with the embryo occupying $\frac{7}{8}$ of the circumference of the egg and ends when the tail reaches the head but without touching it.

Equivalent to stages 4 of Pipe and Walker (1987) and 5 of King et al. (1977).

Stage 11

The tail touches the head and may grow beyond it. At the end of this stage, the embryo hatches.

Equivalent to stages 4 of Pipe and Walker (1987) and 5 of King et al. (1977).

Annex 5: Overview Table Sampling and Sample Processing Methods

Country	Institute	Survey	ICES area	target species	non-target fish eggs	survey purpose	assessment group relevant survey output	gear	gear deployment	mesh (µm)	location of fish egg sorting and identification (ship/lab)	spray method applied and number of applications per sample	fish egg sorting and processing (fresh/preserved)	subsampling (y/n) and method	identification of eggs (visual/genetic)	identification method	measurements	smallest unit	method of measurement	egg samples kept (y/n)	preservation of eggs	buffer eggs	other fish eggs samples kept (y/n)	preservation of other fish eggs	buffer for other fish eggs	remainder of plankton sample kept (y/n)	preservation of remainder	buffer for remainder	comments, suggestion for future methods
Germany	TSP	MEGS	27.6.a 27.7.b,c,g,h,j,k 27.8.a	mackerel, horse mackerel	hake	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Nackthai	dO	280	ship and lab	yes; 3	preserved	y; counting eggs until desired number of target species is achieved	visual	microscopic, sample	counts, egg and oil globule diameter	0.1 mm below	microscope eyepiece graticule; image analysis	y	4 % formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	image processing, metabarcoding
Spain	IEO	MEGS	27.8.a-d 27.9.a	mackerel, horse mackerel	hake	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Bongo	dO	250	ship and lab	yes; 3	preserved	y; counting eggs until desired number of target species and stages is achieved in	visual	microscopic, sample	counts, egg and oil globule diameter	0.1 mm below	microscope eyepiece graticule; image analysis	y	4 % formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	
Netherlands	WMR	MEGS	27.7.k, 27.7.l, 27.7.g, 27.7.h, 27.8.d.2, 27.8.a	mackerel, horse mackerel	hake on ling eggs are measured and staged. The eggs of all the other species are counted	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Gulf VII	dO	280	ship and lab	yes; continue to spray until few eggs are in sample, than sort remainder by hand.	preserved	y; 1) for large samples folsom splitter first; 2) after folsom splitter and for small samples staging eggs until desired number of target species is achieved, count remainder of target species in the tub/sample	visual	Image analyzing (Object)	counts, egg and oil globule diameter	0.1 mm below	Image analyzing	y	4 % formaldehyde buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y for 5 years after the survey	4% formaldehyde, buffered	sodium acetate trihydrate	
Netherlands	WMR	NSMEGS up to 2021	27.4.a-c	mackerel	The eggs of all the other species are counted	TAP up to 2017, in 2021 DEPM of mackerel, fecundity of mackerel	WGOWIDE	Gulf VII	dO	500	ship and lab	yes; continue to spray until few eggs are in sample, than sort remainder by hand.	preserved	y; 1) for large samples folsom splitter first; 2) after folsom splitter and for small samples staging eggs until desired number of target species is achieved, count remainder of target species in the tub/sample	visual	Image analyzing (Object)	counts, egg and oil globule diameter	0.1 mm below	Image analyzing	y	4 % formaldehyde buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y for 5 years after the survey	4% formaldehyde, buffered	sodium acetate trihydrate	
Spain	AZTI	MEGS	27.8.a-d-27.	mackerel, horse mackerel	hake	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Bongo	dO	250	ship and lab	yes; 3	preserved	y; counting eggs until desired number of target species and stages is achieved in	visual	microscopic, sample	counts, measurements to help in the identification, but are not robust	if are required 0.1 mm below	microscope eyepiece graticule; image analysis	y	4 % formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	
Norway	IMR	MEGS	27.4.a, 27.5.b, 1a-b, 27.2.a.2	mackerel	horse mackerel, hake	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Gulf VII	dO	280	ship	no	fresh	y; counting eggs until desired number of target species and stages is achieved in	visual	microscopic, sample	counts, egg and oil globule diameter	0.1 mm below	image analysis	y	95% ethanol	sodium acetate	y	95% ethanol	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	larvae were preserved in 4% buffered sodium-phosphate formaldehyde
Denmark	DTU Aqua	NSMEGS	27.4.a-c	mackerel		TAP up to 2017, in 2021 DEPM of mackerel, fecundity of mackerel	WGOWIDE	Nackthai	dO	500	ship and lab	yes; at least 3 or until no more eggs are found	preserved	y; counting eggs until desired number of target species is achieved	visual	microscopic, sample	counts, egg and oil globule diameter	0.1 mm below	microscope eyepiece graticule; image analysis	y	4 % formaldehyde, buffered, afterwards in Steedman sorting fluid	sodium acetate	y	4 % formaldehyde, buffered, afterwards in Steedman sorting fluid	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	image processing
Faroe Islands	FAMRI	MEGS	27.2.a 27.4.a 27.5b 27.6a	mackerel, horse mackerel	ling, hake, gurnard	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Gulf VII	dO	500	Ship	yes; continue to spray until few eggs are in sample, than sort remainder by hand.	preserved	y - when necessary. Counting eggs until desired number of target species is achieved.	visual	microscopic, sample	counts, egg and oil globule diameter	0.1 mm below	Image analyzing	y	4 % formaldehyde buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	
Portugal	IPMA	DEPM_FL	27.9.a	sardine	anchovy, horse mackerel	DEPM	WGACEG	CalVET_25c	V	150	lab	n	preserved	n	visual	microscopic, sample	counts, lengths	1L, 1 mm below	on photos taken on microscope	y	4% formalin	sodium acetate	y	4% formalin	sodium acetate	y	4% formalin	sodium acetate	
Portugal	IPMA	DEPM_HOM	27.9.a	horse-mackerel	anchovy, sardine	DEPM	WGMEGGS	CalVET_40c	V	150	lab	n	preserved	n	visual	microscopic, sample	counts, lengths	1L, 1 mm below	on photos taken on microscope	y	96 % ethanol; 4% formalin	none; sodium acetate	y	96 % ethanol; 4% formalin	none; sodium acetate	y	96 % ethanol; 4% formalin	none; sodium acetate	photos kept for all HDM and MAC eggs
Portugal	IPMA	Acoustics surveys	27.9.a	pelagic sps	pelagic sps	Acoustics	WGACEG	BONGO	dO	200, 500	lab	n	preserved	n	visual	microscopic, sample	counts, lengths	1L, 1 mm below	on photos taken on microscope	y	4% formalin	sodium acetate	y	4% formalin	sodium acetate	y	4% formalin	sodium acetate	
Scotland	MSS	MEGS	27.4.a 27.6a-b 27.7b,c,d,g,h, 27.8a	mackerel and horse mackerel	hake and ling from 2002 maouriculis, boarfish and anchovy will be counted	TAP of mackerel and horse mackerel, fecundity of mackerel	WGOWIDE	Gulf VII	dO	250	ship	Opportunities, when it is used, 3 times	preserved	n	visual	microscopic, sample	counts, egg and oil globule diameter	0.1 mm below	microscope eyepiece graticule; image analysis	y	4 % formaldehyde buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	