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Report of the

Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES)

20–25 October 2003
CEFAS, Lowestoft, England

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1 SUMMARY

In preparation for the 2004 international ICES coordinated mackerel and horse mackerel egg survey, a workshop was held at CEFAS, Lowestoft (20-25 October 2003) for all the plankton analysts who would be involved with the 2004 survey. The aim of the workshop was to investigate the reasons for individual differences in the identification and staging of mackerel and horse mackerel eggs and to eventually harmonise these. In addition, a new technique for removing fish eggs from plankton samples was evaluated.

To enable the calculation of the numbers of spawning female fish in a stock by using the Annual Egg Production Method (AEPM, Lockwood *et al*, 1981, Armstrong *et al*, 2001) it is essential to correctly identify (both in terms of species and age) the number of freshly spawned eggs, *i.e.*, the eggs of the stages Ia and Ib, and to distinguish these from eggs of the stage II. Prior to the workshop, a plankton sample exchange was initiated in 2001 to test the precision in egg sorting, identification and staging of the individual laboratories. The results from this sample exchange (see section 7.1) showed significant differences between the participants in the numbers of eggs retrieved from the plankton samples, the identification of those eggs to species and the staging (ageing) of the eggs. Unfortunately it was impossible to separate the three components of the plankton analysis in this exercise, which made interpretation of the results extremely difficult. These differences caused real concern for members of WGMEGS. Consequently, the Working Group recommended (ICES, 2002) that a further egg workshop be held at CEFAS (following the successful workshop held in 2000, ICES, 2001), but this time to evaluate egg sorting and identification as well as egg staging.

At the beginning of the workshop the possible causes of the differences in the results of the plankton exchange were discussed. It became clear that the procedures employed for the sorting and staging of mackerel and horse mackerel eggs differed considerably between participants (see section 3.1). In addition, there was some variation by individuals when allocating eggs to the various developmental stages, despite clear guidelines provided by the results of the previous workshop (ICES, 2001).

However, from the statistical analysis of the first round of staging during the 2003 workshop, it became apparent that the agreement for stages Ia and Ib combined was 96% for eggs of all species. This was a surprisingly high level of agreement given the results of the sample exchange. In the second round of staging the level of agreement improved still further reaching 97%. (At the 2000 workshop the level of agreement reached 96% for mackerel and 94% for horse mackerel). The agreement for all stages of eggs combined improved from 80% in the first round to 87% in the second round. This was again better than the results obtained in 2000, where the agreement for horse mackerel increased from 74% in the first reading to 85% in the second, and mackerel increased from 71% to 82%.

The initial over-estimation of stage I eggs (Stages Ia+Ib combined) was 4% in the 2003 workshop. In the second round this had decreased to just 1% over-estimation. (In 2000, there was an under-estimate of 2.5% for mackerel and an over-estimate of 1.5% for horse mackerel). Again, this was a very pleasing result which, if applied to the 2004 survey would only produce a very small over-estimate of egg production. The individual levels of over or under-estimation of stage I eggs was however much larger, varying between an under-estimate of 10% to an over-estimate of 27% in the first round. However, this also improved during the second round of analysis to between -10% and +10%. Even though the individual deviations seem to be high, the exercise has clearly shown that on average the estimate of stage I eggs was reasonably good, given that it is the results from all the participants combined which produce the stage I egg abundance estimate from a tri-ennial survey.

Whilst the egg workshop was being conducted some histology training was undertaken by various participants under the instruction of Mr Peter Witthames (CEFAS). The aims were:

- a) To provide training in the application of image analysis to the assessment of fecundity in both mackerel and horse mackerel.
- b) To intercalibrate mackerel fecundity determined by the stereometric and gravimetric methods
- c) To produce and distribute a fecundity manual for all participants involved with the assessment of fecundity during the 2004 tri-ennial surveys.

The training benefited all concerned and the production of a manual will help standardise procedures during the 2004 survey.

1.1 Acknowledgements

All participants wish to express their thanks and gratitude to their respective institutes for providing the necessary funding to enable them to take part in the egg workshop and/or fecundity training. The chairman would also like to thank Dr Guus Eltink of RIVO, Netherlands who provided so much valuable support and advice, as well as the all-important spreadsheets in which to record and analyse the results.

2 INTRODUCTION

2.1 Background

The annual analytical assessments of the northeast Atlantic mackerel and horse mackerel stocks rely on commercial catch data, which are usually insufficient for a fully qualified analytical assessment. For mackerel and horse mackerel the only available fishery independent biomass indices are derived from the international triennial egg survey. These surveys have been conducted every three years (since 1977), with increasing international participation and correspondingly greater coverage of the spawning area. This demonstrates the importance placed upon these surveys, by all participating nations and ICES, for providing reliable estimates of spawning stock biomass (SSB). At present nine European partners conduct the survey (Portugal, Spain, Spain-Basque Country, England, Ireland, Scotland, Norway, The Netherlands and Germany). Each nation conducts at least one survey, whilst some nations conduct two or more. Due to the great effort invested in the survey programme and the tremendous costs involved, it can only be conducted every third year, the next being in 2004. During a survey year sampling starts when spawning commences in Portuguese waters in January. The coverage of the spawning area increases and shifts slowly northwards as the season progresses and the waters of the Continental Shelf warm. By the summer, spawning has finished in the southern area but sampling continues in the area north of Ireland and west of Scotland until mid July.

The plankton samples taken during the surveys are sorted and the fish eggs allocated to species and individual stages in each of the participating laboratories. The data are eventually combined and result in the production of biomass estimates (SSB) for both mackerel and horse mackerel.

However, results from a recent exchange of plankton samples (section 7.1) have indicated unexpected large differences between participants in the identification of eggs and of the allocation of those eggs to development stages. If neglected this could have a direct impact on the estimate of SSB produced from the 2004 survey. These problems cannot be addressed by circulating instructions or samples from lab to lab. For these reasons the respective ICES working group (Mackerel and Horse Mackerel Egg Survey Working Group, WGMEGS) recommended that a workshop be organised to help resolve these differences (ICES 2002). It is the results of this workshop, held at CEFAS, Lowestoft, England in October 2003, which are presented here.

2.2 Terms of reference

- a) To review results of the plankton sorting exchange programme in 2001/02 and present conclusions identifying main areas of uncertainty.
- b) To review available documentation on identifying eggs to species and define standard protocols.
- c) To carry out comparative sorting trials on typical survey samples. This should follow the pattern of trial - analysis - retrieval - identification of problem areas.
- d) To review any information available on other egg ID procedures - particularly DNA probes.
- e) To carry out a comparative egg staging trial following the pattern used in the 2000 egg staging workshop.
- f) To produce a set of standard pictures and descriptions for species ID and egg staging.

2.3 Scientific justification

Identification of eggs to species and the staging of those eggs remain two of the key areas in the execution of the mackerel and horse mackerel egg surveys. As this process is carried out by a number of different analysts in many different countries, and then the data combined, it is vital that the process be standardised. WGMHMSA and WGMEGS feel strongly that this is best done through the mechanism of sample exchange programmes and regular

workshops to compare results. In the context of the triennial egg surveys it would seem appropriate to hold a workshop prior to every survey to standardise approaches and methodologies in the run-up to the surveys. This will have the advantage of training new participants as well as harmonising the approach of experienced analysts. An egg-staging workshop was held in 2000 and was very successful in achieving some of these aims. It is proposed that this be used as a model for the workshop to be held in 2003. However, a small-scale exchange programme carried out in 2001/02 showed that there may also be some problems in the identification of eggs to species. Therefore, it is proposed to extend the scope of the workshop to address this aspect of sample handling as well. It is expected that the workshop will use the proven method of carrying out a set of sorting and staging trials, analysing the results and identifying problems, then repeating the trials on the basis of the new understanding.

The workshop will also be tasked to produce a standard manual of descriptions and photographs to assist in the plankton sample handling procedure. Such material is available but not currently assembled into a single, agreed and standard manual.

Currently identification to species depends on visual examination. A number of other approaches to egg identification are available, notably the use of DNA probes. The workshop is asked to examine the state-of-the-art in fish egg species identification and to make appropriate recommendations.

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2.5 Timetable

- 19 October 2003. Travel of participants to Lowestoft, England.
- 20 October 2003. Welcome and introduction. Presentation of the results of the 2001-02 egg exchange. Begin 1st round of egg identification and staging. Deliver results to the statistical co-ordinator. Explanation and use of the 'spray technique' to remove eggs from plankton samples.
- 21 October 2003. Continuation of the analysis of the samples. Each participant, using 15 different microscopes, read a total of 375 eggs in the first round. Presentation of working documents on genetic identification of eggs and the use of image analysis. Use of 'Spray technique' continued.
- 22 October 2003. Presentation of the results from the first round of analysis. Discussion of the results and the criteria for egg identification and egg staging. Discussion of those eggs with low agreement (either in species identification or staging) in the first round analysis by projecting microscope images on a large screen. Use of 'Spray technique' continued.
- 23 October 2003. Explanation of bias plots from the first round. Begin second round of egg analysis (375 eggs at 15 microscopes). Description and discussion of very early larvae using slides from artificial fertilisations. 'Spray technique' continued. Report text passed around for comments.
- 24 October 2003. Second round of analysis finished. Use of 'Spray technique' finished. Results from the second round of analysis compiled and presented. Discussion and analysis of the results of the second reading. Drafting sections of the workshop report. Compiling a list of 'recommendations' and items for discussion in the report.
- 25 October 2003. Travel home.

2.6 Workshop Agenda

1. Introduction and welcome. S. Milligan (CEFAS).
2. Brief introduction to the histology workshop. P. Withames (CEFAS).
3. Presentation of the results of the sample exchange experiment 2001/02. S. Milligan (CEFAS). Discussion and evaluation. TOR a).
4. Presentation of the work of MARINEGGS in genetically identifying species of fish eggs. P. Alvarez (AZTI). TOR d).
5. Presentation of other working documents.
6. Discussion on egg identification and staging to be used during the workshop.
7. Discussion on plankton sorting techniques, including the 'spray technique' used by RIVO and AZTI.
8. Visual identification and staging of prepared samples of mackerel, horse mackerel, and morphologically similar fish eggs.
9. Applying the 'spray technique' to remove eggs from prepared plankton samples.
10. Statistical analysis of the first round of results from the comparative egg identification and staging exercise.
11. Discussion and evaluation of results including group analysis of those egg stages which are highlighted as causing the greatest difficulties.
12. Repeat of the egg identification and staging exercise.
13. Statistical analysis and interpretation of the second set of results.

14. Discussion and evaluation of the performance of individual participants.
15. Discussion and evaluation of the 'spray technique'.
16. Production of the report on the statistical evaluation of individual performance.
17. Discussion on the interpretation of egg stages and species recognition.
18. Production of a technical manual to include sorting, identification and staging of fish eggs for the use of all participants in the WGMEGS 2004 surveys.
19. Recommendations.
20. Any other business.

3 MATERIALS AND METHODS

3.1 Egg sorting exercise (Addressing TOR c)

The plankton samples collected as part of the 2001 triennial surveys were sorted and analysed according to the procedures described in Annex A of section 7.1 (the results of the plankton sample exchange 2001/02). Different procedures were employed by the various institutes and the effectiveness of each procedure was difficult to quantify. In an attempt to standardise the procedure and to make the task less time-consuming and less prone to human error, a 'new' mechanical method for effectively removing fish eggs from plankton samples was devised by Dr A Eltink of RIVO-DLO, Netherlands.

This sorting procedure, using a fine spray of water (described below), has been refined by personnel at AZTI, Basque Country, Spain and is now in routine use both at RIVO and AZTI for the removal of fish eggs from plankton samples.

3.1.1 The 'Spray Technique'

In an attempt to evaluate the effectiveness of the new sorting technique two plankton samples (typical plankton from the 2001 survey) were prepared, each containing a total of 500 mackerel and horse mackerel eggs. As many participants as possible were asked to undertake the following procedure to remove and count the eggs from the prepared samples.

The formaldehyde was rinsed from the sample in a 270µm mesh sieve. The plankton was then washed into a glass beaker with a little seawater. A normal garden spray pump was used to 3/4's fill the beaker with pressurised water. The spray jet was rotated around the sides of the beaker to limit damage to the plankton. The pressure and the spray caused aeration of the sample with many fine bubbles, which gave the sample a cloudy appearance. The sample was then left to stand for one to two minutes whilst air bubbles became trapped in the parts of the plankton that had projections (legs, antennae etc). The aerated plankton floated to the surface and all smooth structures including the fish eggs sank to the bottom. The floating plankton and surface liquid were then carefully decanted from the top of the sample, leaving the fish eggs on the bottom of the beaker. The beaker was held at an angle of approximately 45° over a black background and the eggs rolled slowly to one side enabling them to be easily removed using a pipette. This process was repeated until very few eggs were found at the bottom of the beaker (a maximum of 4-5 times). It is recommended that the waiting time is increased for each subsequent spraying to allow the more buoyant eggs time to settle out from the rest of the plankton. The sample was then fully sorted using a binocular microscope, to remove any remaining eggs from the plankton.

The numbers of eggs removed after each spraying and those eggs remaining in the plankton were counted, and the results recorded in a spreadsheet (see section 4.1).

3.2 Egg staging (Addressing TOR's e & f)

3.2.1 Egg staging exercise

A total of 375 mackerel, horse mackerel and megrim (*Lepidorhombus whiffiagonis*, Walbaum) eggs were placed in 15 small, Perspex trays. Each tray contained 50 small wells but only the first 25 wells were used to hold one egg each. Each tray was numbered and placed on a microscope stage. The rows and columns of each tray were labelled so that the position of each individual egg could be identified.

The fish eggs used in the analysis were mainly mackerel and horse mackerel from the English survey which took place in the Celtic Sea area during April / May 2001. In addition, a few validated eggs (from artificial fertilisations) of

mackerel, horse mackerel and megrim were placed at random in the trays to provide definitive eggs of known parentage, against which participants' species identification could be judged (see section 3.3 below). The eggs were selected at random with the intention of providing the full range of egg stages, but with greater emphasis on stage I eggs on which the estimates of SSB are based. The mackerel and megrim eggs in each tray were staged to Ia, Ib, II, III, IV, V and the horse mackerel were staged to Ia, Ib, II, III, IV, as horse mackerel larvae hatch before the eggs reach stage V. Due to the fact that computers can only calculate with numeric values, stage Ia was changed to 0 and stage Ib to 1 in the result tables.

Each participant moved from one microscope to another in order to complete the staging of all 375 eggs. In this way, the results of the egg stage readers were not affected by differences in the quality of the microscopes. Unlike the workshop held in 2000, most of the microscopes were modern and provided good optical quality. There were, however, limitations to the amount of transmitted light provided by some microscopes and only a few were fitted with eyepiece graticules.

Once each participant had staged each of the eggs and the results had been entered into a result spreadsheet, a full discussion on egg staging 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 amongst participants indicated problems in allocating an egg consistently to one developmental stage. These eggs were then placed under a microscope equipped with a digital camera and displayed on a large screen. Discussions then took place on the diagnostic features visible in the egg, which generally led to an agreement on the most likely developmental stage. In this way, the egg staging criteria (ICES, 2001) were revised (see section 3.2.1 below) before being used for the second round of analysis.

3.2.2 Egg stage criteria

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

3.2.2.1 Stage Ia

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

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

3.2.2.2 Stage Ib

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

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

3.2.2.3 Stage II

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

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

3.2.2.4 Stage III

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

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

3.2.2.5 Stage IV

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

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

3.2.2.6 Stage V

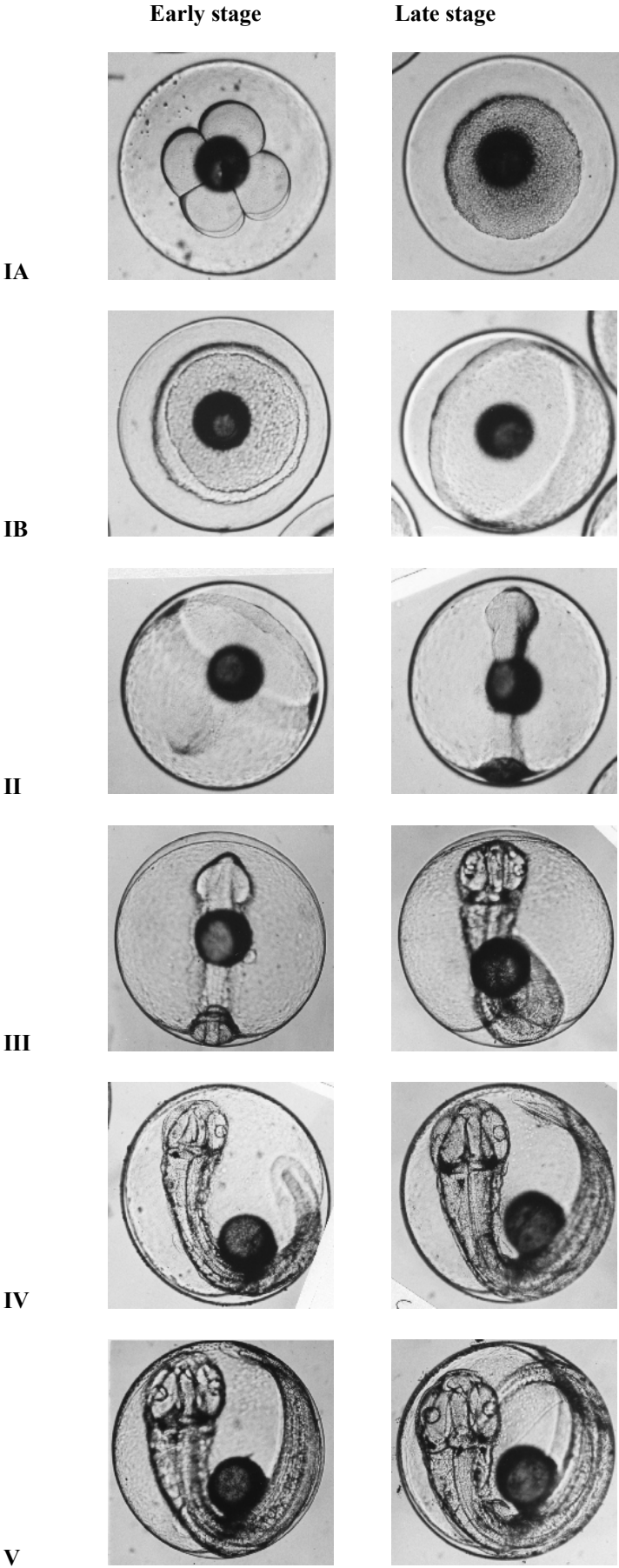
Primary characteristics : Growth of the embryo until the tail has reached the nose.

Secondary characteristics : Pigmentation develops in the eye.

NB

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

Figure 3.2-1. Mackerel eggs at the begining and end of the six development stages.



3.3 Egg identification (Addressing TOR's b & f)

3.3.1 Egg identification exercise

The same trays of fish eggs (described in section 3.2 above) were also used for the egg identification exercise. As each participant moved from microscope to microscope they were asked to provide a species identification for each egg, in addition to a development stage. A short presentation on the main features of mackerel and horse mackerel eggs was delivered before the first round of analysis. This provided some useful preliminary information on egg identification to less experienced participants and enabled the more experienced analysts to discuss the criteria they used to identify mackerel and horse mackerel eggs.

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

3.3.2 Egg identification criteria

The text table (see below) summarises published descriptions of mackerel, horse mackerel and other species of eggs with similar morphological features. It particularly concentrates on egg and oil globule sizes, which may vary through the spawning season and from area to area. A complete reference list is given at the end of this report.

In addition to the published descriptions given in the text table (below), various other criteria are used by participants to help with egg identification based their own knowledge and experience. These criteria can be regarded as secondary characteristics and are described for each species below. Photographs of known horse mackerel eggs from the southern area are shown in Figure 3.3-1 for comparison with mackerel eggs shown in Figure 3.2-1.

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

- Oil globule often orientated to the top of the egg during analysis with the embryo following the circumference of the egg.

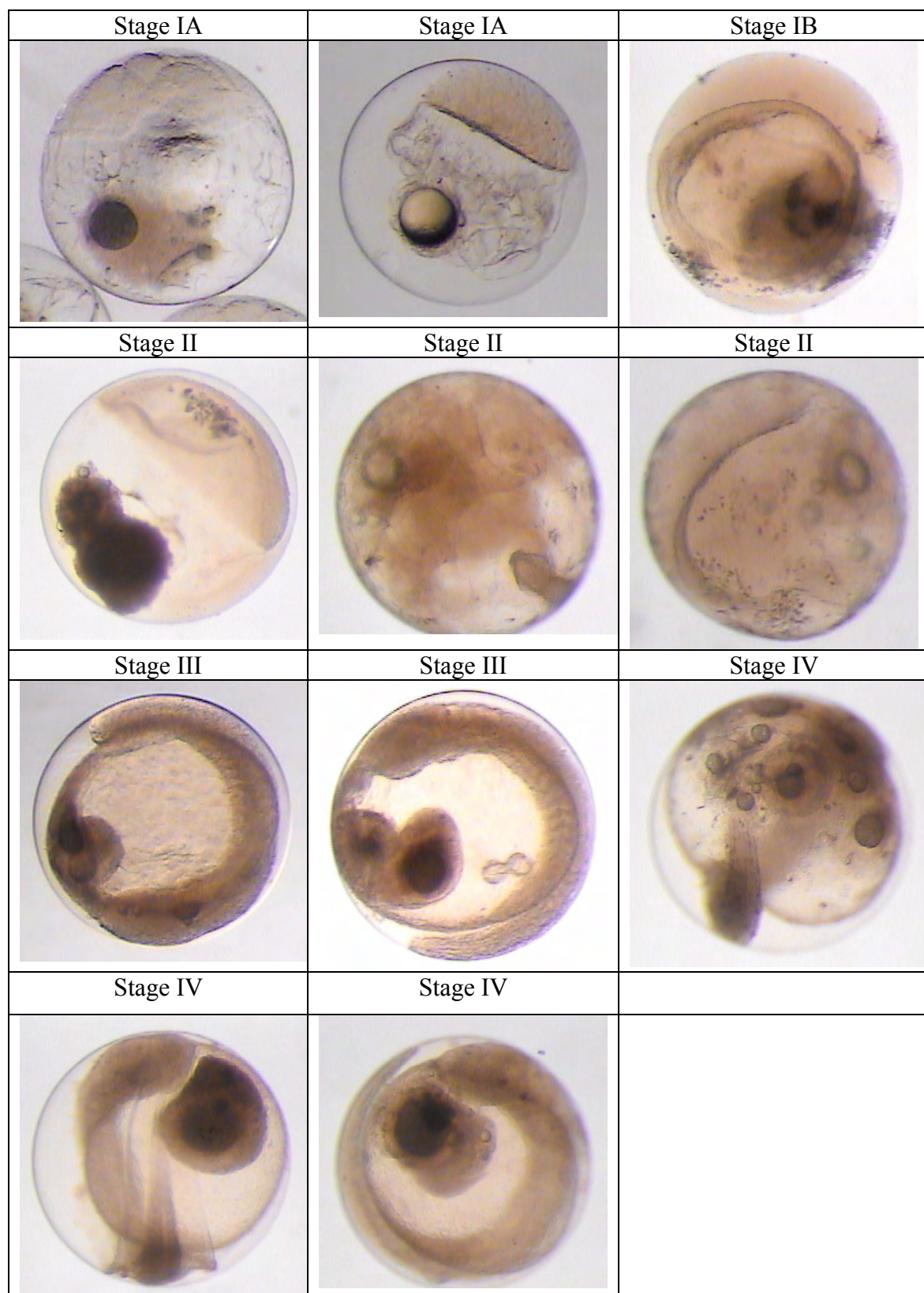
Horse Mackerel (*Trachurus trachurus*). (See Pipe and Walker, 1987)

- Oil globule easily broken into several smaller pieces. This seems to be more common in eggs found in the southern area, particularly in eggs from the Portuguese coast.
- Some early stage eggs from the southern area also lack colour in the yolk, which is unusual, as horse mackerel eggs normally have a darker yolk than mackerel.
- The oil globule migrates to the head of the embryo after stage 2.
- In stages 3 and 4 the embryo show very strong pigmentation.

Megrim (*Lepidorhombus whiffiagonis*)

- Striated punctuate appearance of egg membrane.
- Oil globule is closer to egg membrane than in mackerel.
- Embryo thinner than a mackerel embryo.
- Yolk unsegmented and the egg has a small perivitelline space.

Figure 3.3-1. Horse mackerel eggs in each of the five development stages.



Comparison of the Characteristics of Mackerel, Horse Mackerel, Megrim, Hake and Snipefish Eggs (Details of fixative and concentration unknown)

Species	Diameter (mm)		Other Features Noted	Area	Reference
	Egg	Oil Globule			
Mackerel (<i>Scomber scombrus</i>)	1.0-1.38	0.28-0.35	Unsegmented yolk	North Sea, English Channel	Russell, 1976
	1.09-1.36	0.26-0.37	Homogenous yolk	N.W. Atlantic	Fahay, 1983
	0.97-1.38	0.25-0.35		Irish Sea, North Sea	Ehrenbaum, 1905-09
	1.071-1.193	0.285-0.360		Mediterranean	D'Ancona et al., 1956
	0.97-1.38		Perivitelline space approx 0.05mm	Mid-Atlantic Bight	Development of Fishes of the Mid-Atlantic Bight, 1978
	1.0-1.38	0.22-0.38		North Atlantic	
	0.86-1.04			Mediterranean	
Horse Mackerel (<i>Trachurus trachurus</i>)	0.97-1.38	?		Isle of Man	Johnstone, Scott and Chadwick, 1934
	1.21-1.33	~0.32		West of Ireland	Holt, 1893
	0.9-1.4	?		NE Atlantic	Froese and Pauly, 2003
	0.81-1.04	0.19-0.28	Segmented yolk	North Sea, English Channel	Russell, 1976
	1.03-1.09	0.26-0.27	Segmented yolk	North Sea	Holt, 1898
	0.81-0.93	0.22-0.23		Plymouth	
	0.84-1.04	0.19-0.24	Totally segmented yolk	North Sea, English Channel	Ehrenbaum, 1905-09
Megrim (<i>Lepidorhombus whiffiagonis</i>)	0.81-1.04	0.19-0.24	Segmented yolk	North Sea, English Channel	D'Ancona et al., 1956
	Max. 0.84	0.24-0.26	Granular yolk	English Channel	Holt, 1893
	0.76-1.07	0.19-0.29	Segmented yolk	Europe	Froese and Pauly, 2003
	1.02-1.22	0.25-0.30	Striated membrane. Pigment on oil globule as larva develops	North Sea, Irish Sea	Russell, 1976
	1.07-1.22	0.25-0.30	Fine "meshwork" on inside of membrane. Pigment on oil globule as larva develops	North Sea	Ehrenbaum, 1905-09
	1.07-1.13	0.30	Striations on inside of membrane	West of Ireland	Holt, 1893
	1.08-1.30	0.29-0.34	Striated membrane	Celtic Sea	Milligan et al, In prep.
Hake (<i>Merluccius merluccius</i>)	1.02-1.22	0.25-0.3	Slight ridges on inside of membrane	Europe	Froese and Pauly, 2003
	0.94-1.03	0.25-0.28	Pigmented oil globule	North Sea, English Channel, Mediterranean	Russell, 1976
	0.94-1.03	~0.27	Black and yellow pigment on oil globule	North Sea, English Channel, Mediterranean	Ehrenbaum, 1905-09
	0.94-1.03	~0.27		?	D'Ancona et al., 1956
	1.10-1.16	0.27-0.35		Celtic Sea	Shaw, 2003
	0.94-1.03	0.25-0.28		Europe	Froese and Pauly, 2003
	1.00	0.2	Amber/rose single oil globule Membrane is light amber with grainy reflections	Europe	Development of Fishes of the Mid-Atlantic Bight, 1978

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

- Pigmented oil globule.
- Towards the end of its development the embryo begins to show the characteristic postanal pigmentation of three bars.
- Positive surface adhesion test (SAT) is also used to identify hake eggs (Porebski, 1976) and (Coombs, 1994).

Longspine snipefish (*Macrorhamphosus scolopax*)

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

4 RESULTS

4.1 Results of the egg sorting exercise

The results of the egg sorting exercise using the 'spray technique' are given in Table 4.1-1. Two plankton samples were prepared with 500 fish eggs (a mix of mackerel and horse mackerel eggs) present in each. The decrease in egg numbers was very apparent whilst using the first prepared sample. After six participants had used the spray technique, over 20% of the eggs had been lost and a second sample had to be used. A further ten participants were able to use the second sample to evaluate the technique with only a 10% loss of eggs.

Table 4.1-1 shows the numbers and percentage of eggs removed by each use of the spray technique, and the numbers of eggs remaining in the plankton sample. In the first sample, the percentage of the original egg numbers (500) removed by the spray technique was between 77% and 97%. After some improvements to the technique and increased care when spraying, this improved to between 88% and 98% of the eggs removed from the second sample. This improvement was also shown in the numbers of eggs remaining in the sample. In the first sample at least six eggs were left in the plankton sample by each participant after spraying a maximum of three times. In the second sample, only on one occasion were more than six eggs found in the plankton sample after spraying.

4.2 Results of the egg staging exercise

4.2.1 Egg staging results

The results of the egg staging exercise are given in Tables 4.2-1 to 4.2-8.

Tables 4.2-1 to 4.2-4 present the results for each participant for the first round of analysis for eggs of all species (Table 4.2-1), for mackerel eggs (Table 4.2-2), for horse mackerel eggs (Table 4.2-3) and for eggs of other species (Table 4.2-4). Tables 4.2-5 to 4.2-8 present the results for the second round of analysis in exactly the same way.

The original assessment of each egg, by each participant, for stage (and species), was input into a primary result table (not presented here). Once the results were available from every participant a modal stage could be calculated for each egg. This modal assessment of stage was presumed to be 'correct' although it does not necessarily mean that this was the true stage. In the case when two or more stages appeared at equal frequency, the stage used by the most experienced readers was used as the modal stage.

Tables 4.2-1 to 4.2-8 summarise the results into eight sub-tables labelled A-H, 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 allocation of egg stages by each participant against each modal stage.

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

Sub-tables D show the allocation of each modal egg stage against each participant's assessment of egg stage.

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

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

Sub-tables G show the bias (over or under estimation) of each participants assessment of egg stage 1a+1b compared to the modal stage 1a+1b.

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

By studying the results presented in Tables 4.2-1 to 4.2-8, some encouraging improvements in the consistency of egg staging between participants can be observed from the first to the second round of analysis.

The overall agreement in egg stage for all species of eggs, in all stages of development was 80% in the first round (Table 4.2-1). This increased to 87% agreement in the second round of analysis (Table 4.2-5). The overall agreement for all egg stages by species increased from 75% (Table 4.2-2) to 82% (Table 4.2-6) for mackerel, 82% (Table 4.2-3) to 88% (Table 4.2-7) for horse mackerel and 90% (Table 4.2-4) to 96% (Table 4.2-8) for eggs of other species.

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

The overall agreement in the assessment of stage 1 (1a+1b) eggs of all species was 96% in the first round (Table 4.2-1). This increased to 97% agreement in the second round of analysis (Table 4.2-5). The overall agreement of stage 1 eggs (by species) increased from 92% (Table 4.2-2) to 94% (Table 4.2-6) for mackerel, 96% (Table 4.2-3) to 97% (Table 4.2-7) for horse mackerel and remained at 99% (Tables 4.2-4 and 4.2-8) for eggs of other species.

The bias in allocating eggs to stage 1 (1a+1b) as a percentage over or underestimation, are given in sub-tables G. Although the overall bias was reasonable, particularly after the second round of analysis, some individuals showed surprisingly high levels of bias. In the first round of analysis the overall bias was 4% for eggs of all species but individual bias ranged from an underestimate of 10% to an overestimate of 27% (Table 4.2-1). In the second round this did improve to an overall bias of just 1%, with a range of individual bias from -10% to +10%.

The overall bias was greatest for stage 1 mackerel eggs (Tables 4.2-1 and 4.2-5). This was 15% in the first round reducing to 8% in the second round of analysis. However, the bias of individual participants was much greater, ranging from -23% to +89% in the first round, to between -9% to +33% in the second round of analysis. The overall estimates of bias for stage 1 eggs of horse mackerel and other eggs were, however, much better as they ranged between -1% and +1%.

Figures 4.2-1 to 4.2-8 show the egg stage bias plots in which the mean egg stage ± 2 standard deviations of each stage reader and all stage readers combined are plotted against the modal egg stage.

4.2.2 Results by microscope / tray number

Tables 4.2-9 to 4.2-16 show the egg staging results by each microscope and its associated tray of eggs. Within each table the top sub-tables show the number of eggs at each modal stage in each tray, the middle sub-tables show the percentage agreement at each modal stage by tray/microscope and the bottom sub-tables show the bias at each modal stage by tray/microscope. This analysis highlights any problems associated with a particular microscope or tray of eggs.

Most of the microscopes were modern, stereoscopic, zoom microscopes with good sources of transmitted light. Only one microscope (Number 7) was more than 20 years old and consequently it did not have the optical quality of the more modern microscopes. This is reflected in the results of percentage agreement, particularly for mackerel eggs (Tables 4.2-10 and 4.2-14). However, most of the differences between microscopes/trays can be accounted for by differences in egg quality, and the mix of species and stages in each of the trays.

4.3 Results of the egg species identification exercise

The same trays of eggs used for egg staging were used for the egg identification exercise. The procedures employed were almost identical except where the species was definitely known.

The original assessment of each egg, by each participant, for species identification, was input into a primary result table (not presented here). Once the results were available from every participant a modal species could be calculated for each egg. This modal assessment of species was presumed to be 'correct' except where the egg species was known (from artificial fertilisations). In these cases the 'validated' species was used as the correct assessment and participants could be judged against this directly.

Summaries of the results from the two rounds of egg species determination are presented in Tables 4.3-1 and 4.3-2. Each of these tables are divided into four sub-tables labelled A-D, where the performance of each participant is judged against the modal / actual species.

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

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

Sub-tables C show the percentage under or 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 modal / actual species.

The results do show some improvements in the allocation of eggs to the various species, from the first to the second round of analysis. However, they also highlight the difficulties in being able to positively identify eggs where there are few distinguishing features other than the size of egg and oil globule diameters. This is apparent when comparing the results in sub-tables C (Tables 4.3-1 and 4.3-2). The percentage over-estimation to mackerel eggs increased from 6% to 11%, from the first to second rounds, whilst there was a significant decrease in the numbers of eggs allocated to other species from +29% to -16%.

After the first round of analysis there was some discussion on the features which aid fish egg identification. Some references and criteria were produced (see section 3.3.2) to help with the identification of eggs which are similar to those of mackerel and horse mackerel. This helped with the identification of horse mackerel eggs during the second round of analysis where the percentage under-estimation decreased from -18% to -6%.

These discussions and criteria also helped to improve the mean percentage agreement between participants' identification of eggs to species and the modal species (Tables 4.3-1D and 4.3-2D). For mackerel eggs the percentage agreement increased from 84% to 95% and for horse mackerel the improvement rose from 70% to 88%. These results were very re-assuring particularly as most of the microscopes were not fitted with eyepiece graticules to enable measurement of egg or oil globule diameters.

Table 4.1-1

Evaluation of the 'Spray method' by estimating the percentage of eggs removed from the remaining eggs and by estimating the cumulative percentage of eggs removed by separation, by participant and for all participants combined.

NUMBER OF EGGS REMOVED BY EACH SEPARATION																				
Sample number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	TOTAL
Actual No. of eggs	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	8000
Egg sorter	Sco IG	Ire DL	Spa IM	Ire DOB	Eng SM	Nor EH	Ger MK	Ger JU	Net Cvd	Por CV	Sco FB	Spa BB	Spa ALL	Spa CF	Nor JdL	Ire DOB				
1st separation	476	480	396	388	426	384	444	430	461	457	448	408	437	440	434	427				
2nd separation	8	5	6	2	5	2	34	29	13	5	5	40	12	6	10	13				
3rd separation	1	2	2	0	0	2	13	9	3	2	3	6	0	3	2	2				
TOTAL (spray method)	485	485	404	390	431	386	491	468	477	464	456	454	449	449	444	442				
Eggs found by hand sorting	10	6		14	6	6	2	2	0	0	1	5	4	2	0	8				
TOTAL (spray and hand sorting)	495	491	404	404	437	392	493	470	477	464	457	459	453	451	444	450				
Eggs found (%) by spray and hand	99%	98%	81%	81%	87%	78%	99%	94%	95%	93%	91%	92%	91%	90%	89%	90%				
10% loss of eggs (50 eggs) after applying 29 separations by the spray method																				
0.3% loss of eggs per separation by the spray method																				

PERCENTAGE OF EGGS REMOVED BY EACH SEPARATION BY SPRAYING																				
Sample number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	Mean
Egg sorter	Sco IG	Ire DL	Spa IM	Ire DOB	Eng SM	Nor EH	Ger MK	Ger JU	Net Cvd	Por CV	Sco FB	Spa BB	Spa ALL	Spa CF	Nor JdL	Ire DOB				
1st separation	95%	96%	79%	78%	85%	77%	89%	86%	92%	91%	90%	82%	87%	88%	87%	85%				
2nd separation	33%	25%	6%	2%	7%	2%	61%	41%	33%	12%	10%	43%	19%	10%	15%	18%				
3rd separation	6%		2%	0%	0%		59%	22%	12%	5%	6%	12%	0%	6%	3%	10%				

CUMULATIVE PERCENTAGE OF EGGS REMOVED BY SPRAY METHOD																				
Sample number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	Mean
Egg sorter	Sco IG	Ire DL	Spa IM	Ire DOB	Eng SM	Nor EH	Ger MK	Ger JU	Net Cvd	Por CV	Sco FB	Spa BB	Spa ALL	Spa CF	Nor JdL	Ire DOB				
1st separation	95%	96%	79%	78%	85%	77%	89%	86%	92%	91%	90%	82%	87%	88%	87%	85%				
2nd separation	97%	97%	80%	78%	86%	77%	96%	92%	95%	92%	91%	90%	90%	89%	89%	88%				
3rd separation	97%		81%	78%	86%	77%	98%	94%	95%	93%	91%	91%	90%	90%	89%	88%				

All Eggs first stageing. Egg Stageing Workshop, Lowestoft, October 2003

Table 4.2-1

- (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 at each modal stage.
 (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage.
 (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant.
 (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage.
 (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

		NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																											
MODAL stage	Stage	Spa CF	Eng MS	Soi IG	Spa ALL	Ire DL	Spa IM	Eng CB	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL			
		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					
>	0	206	-	183	187	184	200	188	181	184	204	188	205	188	175	183	165	204	174	188	149	111	111	111	3647				
>	1	11	-	11	10	11	11	11	11	11	10	11	11	11	10	11	11	11	10	11	11	11	10	215					
>	2	81	-	77	76	77	81	77	81	77	80	77	81	77	75	74	71	81	72	75	76	70	70	1538					
>	3	47	-	44	44	44	46	46	48	44	47	45	47	46	46	44	45	48	42	46	45	43	43	907					
>	4	29	-	27	27	26	29	27	29	27	29	27	29	27	27	27	27	29	25	27	27	26	26	548					
>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
11	0-5	374	0	342	344	342	367	349	350	343	370	348	373	349	333	339	319	373	323	349	308	280	280	6855					

Modal egg stages divided by each readers egg stages (Table (A) divided by the readers egg stages)

Modal stage		Spa CF	Eng MS	Soi IG	Spa ALL	Ire DL	Spa IM	Eng CB	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Por CV	Spa PC	Reader 12	Not EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%
Reader	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	%	
Stage 1a	0	137	-	182	170	182	180	170	166	175	173	161	173	100	164	142	136	116	168	170	143	100	100	87%			
Stage 1a	0	1	-	1	10	2	12	17	8	1	12	17	24	85	7	35	25	82	1	5	5	1	10	10%			
Stage 1a	0	2	-	5	5	8	1	7	3	14	10	2	3	1	4	4	6	4	12	1	1	1	1	3%			
Stage 1a	0	3	-	2	2	-	-	-	3	4	-	-	-	-	3	2	-	-	1	-	-	-	-	0%			
Stage 1a	0	4	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	0%			
Stage 1a	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%			
Stage 1b	1	0	5	-	10	3	4	1	1	4	-	-	-	3	4	1	10	1	8	1	2	5	30%				
Stage 1b	1	1	-	-	4	7	6	10	7	6	3	7	11	6	5	10	1	8	1	3	9	4	4	50%			
Stage 1b	1	2	6	-	1	3	-	4	1	7	4	-	-	2	1	-	-	2	1	7	-	1	20%				
Stage 1b	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%			
Stage 1b	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%			
Stage 1b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%			
Stage 2	2	0	4	-	4	6	8	1	-	3	8	4	-	4	7	5	6	22	6	9	2	3	8	7%			
Stage 2	2	1	-	-	1	11	-	1	-	4	-	4	30	8	10	7	24	16	12	-	16	15	10%				
Stage 2	2	2	76	-	65	68	69	76	77	63	35	43	43	40	40	54	56	25	58	45	71	42	46	72%			
Stage 2	2	3	1	-	8	1	2	11	-	1	2	40	30	3	22	6	5	-	1	6	4	15	1	10%			
Stage 2	2	4	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	0%			
Stage 2	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%			
Stage 3	3	0	-	-	-	-	-	-	-	5	-	-	1	-	1	-	1	-	1	-	-	1	1	1%			
Stage 3	3	1	-	-	1	1	-	-	1	-	-	-	-	-	-	5	3	-	-	-	-	-	-	1%			
Stage 3	3	2	7	-	10	3	-	6	4	1	-	1	1	-	5	12	25	8	8	9	3	5	12%				
Stage 3	3	3	40	-	41	33	22	45	36	42	34	41	33	35	36	27	11	34	31	36	29	35	75%				
Stage 3	3	4	-	3	-	19	1	4	2	4	6	4	12	11	4	-	5	6	2	1	11	2	11%				
Stage 3	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	0%				
Stage 4	4	0	-	-	-	-	-	-	-	1	-	-	1	1	-	-	1	-	-	-	-	-	-	1%			
Stage 4	4	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	0%				
Stage 4	4	2	-	1	1	-	-	-	-	1	-	1	-	-	-	2	4	-	-	-	-	-	2%				
Stage 4	4	3	11	-	1	14	2	6	1	8	3	1	2	6	5	13	11	2	7	14	-	7	21%				
Stage 4	4	4	17	-	26	12	23	26	21	23	26	25	27	20	22	12	11	27	18	12	23	19	75%				
Stage 4	4	5	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	1	3	-	1%				
Stage 5	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Total	0-5	374	0	342	344	342	367	349	350	343	370	348	373	349	333	339	319	373	323	349	308	260	6855				

EGG STAGE COMPOSITION AS ESTIMATED BY READER

		Spa CF	Eng MS	Soa IG	Spa ALL	Ire DL	Spa IM	Eng CB	Reader 5	Reader 6	Reader 7	Reader 8	Soa FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU		
Stage	Reader	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	TOTAL		
1a ==>	0	206	-	196	179	194	182	171	170	183	177	161	185	111	174	149	170	123	186	173	148	114	114	3362			
1b ==>	1	1	-	1	16	20	18	28	15	11	15	28	66	99	22	57	53	106	14	8	31	29	29	638			
2 ==>	2	96	-	66	87	59	81	83	91	68	57	57	46	45	61	74	58	74	58	99	46	53	53	1359			
3 ==>	3	54	-	50	50	26	62	37	51	42	86	73	36	63	50	47	22	37	45	55	44	43	43	973			
4 ==>	4	17	-	29	12	42	24	30	23	29	34	29	40	31	26	12	16	33	20	13	34	21	21	515			
5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8			
Total	0-5	374	0	342	344	342	367	349	350	343	370	348	373	349	333	339	319	373	323	349	308	280	280	6855			

Table 4.2-1 Continued

All Eggs first stageing. Egg Stageing Workshop, Lowestoft, October 2003

D

Each readers egg stages divided by the modal egg stages (Table (C) divided by modal egg stage)

Modal Stage	Spa CF	Eng MS	Soo IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Soo FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net OxD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Reader 1	197	-	182	170	182	180	170	166	175	173	161	179	100	164	142	136	116	168	170	143	100	94%	
Stage 1a	0	1	5	-	10	3	4	1	4	-	-	-	3	4	1	10	1	8	1	2	5	2%	
Stage 1a	0	2	4	-	4	6	8	1	-	3	8	4	-	4	7	5	6	9	2	3	8	3%	
Stage 1a	0	3	-	-	-	-	-	-	-	5	-	-	-	1	-	1	-	1	-	-	1	0%	
Stage 1a	0	4	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	0%	
Stage 1a	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 1b	1	0	1	-	10	2	12	17	8	1	12	17	24	85	7	35	25	82	1	5	10	56%	
Stage 1b	1	1	-	-	4	7	6	10	7	6	3	7	11	6	5	10	1	8	1	3	9	4	17%
Stage 1b	1	2	-	-	1	11	-	-	-	4	-	30	8	10	7	24	16	12	-	16	15	25%	
Stage 1b	1	3	-	-	-	1	-	-	-	-	-	-	-	-	5	3	-	-	-	-	-	1%	
Stage 1b	1	4	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	0%	
Stage 1b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 2	2	0	6	-	1	8	1	7	3	14	10	2	3	1	4	4	6	4	12	1	1	7%	
Stage 2	2	1	6	-	1	3	-	4	1	7	4	-	2	1	-	2	1	7	-	1	3	3%	
Stage 2	2	2	76	-	65	68	69	76	77	63	35	43	40	54	56	25	58	45	71	42	46	82%	
Stage 2	2	3	7	-	10	3	-	6	4	1	-	-	1	5	12	25	8	3	9	3	5	8%	
Stage 2	2	4	1	-	-	1	-	-	-	1	-	-	-	-	2	4	-	-	-	-	-	1%	
Stage 2	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 3	3	0	2	-	-	2	-	-	3	4	-	-	-	3	2	-	-	1	-	-	-	2%	
Stage 3	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 3	3	2	1	-	8	1	2	11	-	2	40	30	3	22	6	5	-	1	6	4	15	16%	
Stage 3	3	3	40	-	41	33	22	45	36	42	34	41	33	35	36	27	11	34	31	36	29	70%	
Stage 3	3	4	11	-	1	14	2	6	1	8	3	1	2	-	6	5	13	11	2	7	14	12%	
Stage 3	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	0	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	1%	
Stage 4	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	2	-	-	-	-	19	1	4	2	4	6	4	12	11	4	5	6	2	1	11	2	19%
Stage 4	4	3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	4	17	-	26	12	23	23	26	21	23	26	25	27	20	22	12	11	27	18	23	19	80%
Stage 4	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25%	
Stage 5	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75%	
Stage 5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Total	0-5	374	0	342	344	342	367	349	350	343	370	348	373	349	333	339	319	373	323	349	308	260	6555

E

PERCENTAGE AGREEMENT BY EGG STAGE

MODAL stage	Spa CF	Eng MS	Soo IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Soo FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net OxD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Stage 1a	0	96%	-	99%	91%	99%	90%	92%	95%	85%	86%	87%	88%	94%	78%	82%	57%	97%	90%	96%	90%	87%	90%
Stage 1b	0	1	0%	0%	40%	64%	55%	91%	64%	55%	30%	64%	100%	55%	91%	9%	73%	10%	27%	82%	40%	50%	
Stage 2	0	2	94%	88%	73%	85%	99%	95%	82%	44%	56%	53%	52%	72%	76%	35%	72%	63%	92%	55%	66%	72%	
Stage 3	0	3	85%	79%	50%	98%	78%	88%	77%	87%	70%	70%	76%	78%	61%	24%	71%	74%	78%	64%	81%	76%	
Stage 4	0	4	59%	44%	88%	79%	96%	72%	85%	90%	93%	93%	74%	81%	44%	41%	93%	72%	44%	85%	73%	75%	
Stage 5	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Weighted mean	0-5	88.2%	-	91.8%	83.4%	88.0%	91.1%	89.4%	87.8%	75.1%	79.5%	78.6%	57.6%	84.4%	72.9%	57.7%	65.1%	81.4%	83.7%	79.5%	78.5%	80%	
RANKING	4	-	1	1	10	4	7	5	2	3	6	16	13	14	20	19	18	11	9	12	15	15	

F

PERCENTAGE AGREEMENT STAGE 1a and 1b combined

MODAL stage	Spa CF	Eng MS	Soo IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Soo FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net OxD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Reader 1	94%	-	99%	95%	100%	94%	99%	95%	95%	88%	93%	99%	97%	97%	97%	98%	96%	97%	90%	99%	98%	96%	96%
1a+1b	17	-	3	14	1	16	2	15	13	20	18	5	8	9	10	7	12	11	19	4	6	6	
RANKING	17	-	3	14	1	16	2	15	13	20	18	5	8	9	10	7	12	11	19	4	6	6	

G

BIAS = over- / underestimation of stage 1a+1b combined

MODAL stage	Spa CF	Eng MS	Soo IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Soo FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net OxD	Spa PA	Spa BB	Ger SC	Ger JU	%
Reader 1	-5%	-	-2%	-1%	-10%	-5%	0%	-4%	5%	-10%	-5%	18%	6%	6%	6%	27%	7%	9%	-9%	12%	18%	4%
1a+1b	5	-	3	2	15	8	1	4	6	16	7	18	9	10	11	20	12	13	14	17	19	19
RANKING	5	-	3	2	15	8	1	4	6	16	7	18	9	10	11	20	12	13	14	17	19	19

H

PRECISION = Coefficient of Variation (CV)

MODAL stage	Spa CF	Eng MS	Soo IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Soo FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net OxD	Spa PA	Spa BB	Ger SC	Ger JU	%
Reader 1	27%	-	7%	26%	0%	22%	7%	21%	38%	38%	24%	10%	15%	26%	24%	15%	18%	21%	29%	8%	13%	10%
Stage 1a+1b	1	27%	-	19%	16%	17%	6%	11%	22%	25%	25%	38%	13%	28%	26%	36%	27%	32%	14%	34%	30%	17%
Stage 2	2	13%	-	8%	18%	18%	5%	16%	12%	26%	11%	9%	8%	18%	28%	34%	18%	20%	15%	20%	17%	25%
Stage 3	3	16%	-	5%	17%	9%	11%	5%	12%	17%	12%	7%	19%	10%	19%	26%	7%	12%	16%	17%	12%	14%
Stage 4	4	21.5%	-	9.8%	22.4%	9.0%	17.9%	7.8%	31.2%	29.5%	20.9%	17.6%	19.2%	24.1%	24.2%	23.0%	19.3%	22.7%	23.1%	17.0%	17.9%	14%
Stage 5	5	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	5	5	14%
Weighted mean	1-5	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	5	5	14%
RANKING	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	16	5	5	14%
Weighted mean	1-5	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	5	5	14%
RANKING	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	16	5	5	14%
Weighted mean	1-5	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	5	5	14%
RANKING	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16	16	5	5	14%
Weighted mean	1-5	12	-	3	13	2	8	1	4	20	19	11	6	9	17	18	10	14	16			

Mackerel Eggs first staging.

Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-2

- (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 at each modal stage.
 (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage.
 (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant.
 (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage.
 (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

A

NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																							
Modal stage	Spa CF Reader 1	Eng MS Reader 2	Sco IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Nor Jdl Reader 8	Sco FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net Cvd Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL	
Stage 1a ==>	0	46	-	33	34	34	34	43	35	44	34	44	35	45	35	33	33	44	33	35	33	28	736
Stage 1b ==>	1	5	-	5	4	5	5	5	5	4	5	5	5	4	5	5	5	5	5	5	4	96	
Stage 2 ==>	2	53	-	49	48	49	53	49	53	49	52	49	53	49	46	47	53	46	49	48	43	986	
Stage 3 ==>	3	43	-	40	40	42	41	43	40	42	41	43	41	41	40	40	43	38	41	41	38	818	
Stage 4 ==>	4	26	-	24	24	23	26	24	26	24	26	24	26	24	24	24	26	22	24	24	23	488	
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total	0-5	173	0	151	150	151	169	154	171	152	168	154	172	154	150	149	171	144	154	151	136	3124	

B

Modal egg stages divided by each readers egg stages (Table A) divided by the readers egg stages)																						
Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JDL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%
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	All	
Stage 1a	0	43	-	33	24	34	30	23	36	32	29	33	13	28	23	30	22	28	24	32	23	76%
Stage 1a	0	1	-	7	-	9	12	5	-	6	8	10	22	3	10	2	17	1	4	1	4	16%
Stage 1a	0	2	1	-	3	-	4	-	3	2	9	6	2	-	1	1	5	3	6	-	1	6%
Stage 1a	0	3	2	-	-	-	-	-	-	-	-	-	-	2	1	1	-	1	1	-	-	1%
Stage 1a	0	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1a	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1b	1	0	3	-	2	1	-	-	-	-	-	-	1	1	1	5	1	4	1	1	2	23%
Stage 1b	1	1	-	1	4	3	5	4	4	2	4	5	3	2	4	-	3	1	2	4	2	55%
Stage 1b	1	2	2	-	1	2	-	1	1	2	1	-	1	1	-	-	1	-	2	-	-	16%
Stage 1b	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1b	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 2	2	0	1	-	3	6	-	-	1	5	4	-	3	3	5	16	3	4	-	2	4	6%
Stage 2	2	1	-	-	1	8	-	1	-	4	-	3	22	7	6	14	9	11	-	11	10	12%
Stage 2	2	2	52	-	44	43	51	48	51	39	26	28	31	36	30	17	40	29	48	31	29	74%
Stage 2	2	3	-	-	4	1	2	-	1	1	22	18	3	8	1	5	-	2	1	4	-	8%
Stage 2	2	4	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	0%
Stage 2	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 3	3	0	-	-	-	-	-	-	-	5	-	-	-	1	-	1	-	-	-	-	-	1%
Stage 3	3	1	-	-	1	-	-	-	3	-	-	-	-	-	5	3	-	-	-	-	-	1%
Stage 3	3	2	5	-	8	1	-	3	3	-	-	1	-	4	10	22	6	8	6	3	5	10%
Stage 3	3	3	38	-	38	31	20	41	35	38	31	37	38	30	31	32	25	10	33	26	32	77%
Stage 3	3	4	-	2	-	19	1	3	2	4	5	3	12	10	4	4	4	2	-	11	1	11%
Stage 3	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 4	4	0	-	-	-	-	-	-	-	1	-	-	1	-	-	1	-	-	-	-	-	1%
Stage 4	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 4	4	2	1	-	-	-	-	-	1	-	-	-	-	-	2	4	-	-	-	1	-	2%
Stage 4	4	3	11	-	13	2	5	1	7	3	1	-	6	4	11	9	2	5	12	-	7	21%
Stage 4	4	4	14	-	23	11	20	21	23	19	20	23	26	17	20	11	10	24	17	11	20	76%
Stage 4	4	5	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	1	3	-	1%
Stage 5	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	173	0	151	150	151	169	154	171	152	168	154	172	154	150	149	171	144	154	151	136	3124

C

EGG STAGE COMPOSITION AS ESTIMATED BY READER																						
Stage	Spa CF Reader 1	Eng MS Reader 2	Sco IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Nor JdL Reader 8	Sco FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net Cvd Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL
0	47	-	39	29	41	30	23	37	43	33	21	34	18	33	29	53	26	36	25	35	29	661
1	-	-	-	10	12	12	18	9	8	8	15	37	32	13	25	19	29	13	6	17	16	299
2	61	-	44	55	34	57	51	58	42	38	35	29	32	41	43	44	52	40	62	34	35	887
3	51	-	43	45	24	48	36	46	35	60	57	33	45	39	42	19	36	36	49	30	39	813
4	14	-	25	11	39	22	26	21	24	28	26	39	27	24	11	14	28	19	11	31	17	457
5	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	1	4	-	7
0-5	173	0	151	150	151	169	154	171	152	168	154	172	154	150	150	149	171	144	154	151	136	3124

Table 4.2-2 Continued

Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

D

Each readers egg stages divided by the modal egg stages (Table C) divided by modal egg stage)

Modal Stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Reader 19	Reader 20	Reader 21	Ger JU	%	
Stage 1a	0	43	-	33	24	34	30	23	36	32	29	21	33	13	28	23	30	22	28	24	32	23	23	85%	
Stage 1a	0	1	3	-	5	2	1	-	-	-	-	-	-	1	1	1	5	1	4	1	1	2	4	4%	
Stage 1a	0	2	1	-	1	3	6	-	1	5	4	-	1	3	3	5	16	3	4	-	2	4	4	9%	
Stage 1a	0	3	-	-	-	-	-	-	-	5	-	-	-	-	1	-	-	-	-	-	-	-	-	1%	
Stage 1a	0	4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	0%	
Stage 1a	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 1b	1	0	-	-	7	-	9	12	5	-	6	8	10	22	3	10	2	17	1	4	1	4	4	40%	
Stage 1b	1	1	-	-	1	4	3	5	4	4	2	4	4	3	2	4	-	3	1	2	4	2	2	18%	
Stage 1b	1	2	-	-	1	1	8	-	1	-	3	22	7	8	6	14	9	11	-	-	11	10	10	38%	
Stage 1b	1	3	-	-	-	1	-	-	-	-	-	-	-	-	5	3	-	-	-	-	-	-	-	3%	
Stage 1b	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 1b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 2	2	0	1	-	-	3	-	4	-	3	2	9	6	2	-	1	1	5	3	6	-	1	1	5%	
Stage 2	2	1	2	-	-	1	2	-	1	1	2	1	-	1	1	-	1	-	2	-	2	-	2	2%	
Stage 2	2	2	52	-	44	43	33	51	48	51	39	26	28	28	31	36	30	17	40	29	48	31	29	83%	
Stage 2	2	3	5	-	8	1	-	3	-	3	-	-	-	1	-	4	10	22	6	8	6	3	5	10%	
Stage 2	2	4	1	-	-	-	-	-	-	-	1	-	-	-	2	4	-	-	-	-	-	-	-	1%	
Stage 2	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 3	3	0	2	-	-	-	-	-	-	-	-	-	-	-	2	1	-	1	-	1	-	-	-	1%	
Stage 3	3	1	-	-	-	2	-	-	-	-	-	18	3	8	1	5	-	-	-	-	-	-	-	0%	
Stage 3	3	2	-	4	1	2	2	-	1	1	22	18	3	8	1	5	-	1	2	1	4	-	-	-	0%
Stage 3	3	3	38	-	38	31	20	41	35	38	31	37	38	30	31	32	25	10	33	28	35	26	32	77%	
Stage 3	3	4	11	-	1	13	2	5	1	7	3	1	1	-	6	4	11	9	2	5	12	-	7	12%	
Stage 3	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	3	-	-	-	19	1	3	2	4	5	3	12	10	4	4	4	2	-	-	11	-	1	19%	
Stage 4	4	4	14	-	23	11	20	21	23	19	20	23	26	17	20	11	10	24	17	11	20	16	16	81%	
Stage 4	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Total	0-5	173	0	151	150	151	169	154	171	152	168	154	172	154	150	150	149	171	144	154	151	136	136	3124	

E

PERCENTAGE AGREEMENT BY EGG STAGE

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Reader 19	Reader 20	Reader 21	Ger JU	%
Stage 1a ==>	0	93%	-	71%	100%	70%	66%	82%	94%	66%	60%	73%	37%	85%	66%	91%	50%	85%	69%	97%	82%	82%	82%	ALL
Stage 1b ==>	1	0%	-	25%	80%	60%	100%	80%	80%	50%	80%	100%	60%	50%	80%	0%	60%	20%	40%	80%	50%	50%	50%	95%
Stage 2 ==>	2	98%	-	90%	90%	67%	98%	96%	80%	50%	57%	48%	63%	75%	65%	36%	75%	63%	98%	65%	67%	67%	74%	
Stage 3 ==>	3	88%	-	95%	78%	50%	98%	85%	88%	78%	88%	93%	70%	76%	78%	63%	25%	77%	74%	85%	63%	64%	77%	
Stage 4 ==>	4	54%	-	96%	46%	87%	81%	96%	73%	83%	88%	96%	100%	71%	83%	46%	42%	92%	77%	46%	83%	70%	76%	
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Weighted mean	0-5	85.0%	-	91.4%	73.3%	73.5%	86.4%	87.0%	86.5%	82.9%	69.6%	74.9%	69.8%	61.7%	78.7%	62.0%	45.0%	71.3%	71.5%	77.9%	74.8%	75.0%	75%	
RANKING	5	-	1	13	12	4	2	3	6	17	11	16	19	7	18	20	15	14	8	10	9	9	9	

F

PERCENTAGE AGREEMENT STAGE 1a and 1b combined

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Reader 19	Reader 20	Reader 21	Ger JU	%
Stage 1a ==>	0	90%	-	100%	89%	100%	88%	100%	92%	92%	77%	83%	96%	98%	92%	95%	97%	88%	89%	78%	100%	97%	97%	ALL
Stage 1b ==>	1	13	-	1	14	1	17	1	12	10	20	18	8	5	11	9	6	16	14	19	1	7	7	92%
RANKING	13	-	1	14	1	17	1	12	10	20	18	8	5	11	9	6	16	14	14	19	1	7	7	92%

G

BIAS = over- / underestimation of stage 1a+1b combined

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Reader 19	Reader 20	Reader 21	Ger JU	%
Stage 1a+1b ==>	0	-8%	-	3%	3%	35%	-13%	3%	-6%	31%	-15%	-10%	42%	25%	24%	35%	89%	12%	28%	-23%	37%	41%	41%	ALL
Stage 1b ==>	1	5	-	2	2	16	8	1	4	14	9	6	19	12	11	15	20	7	13	10	17	18	18	15%
RANKING	5	-	2	2	16	8	1	4	14	9	6	19	12	11	15	20	7	13	10	17	18	18	18	15%

H

PRECISION = Coefficient of Variation (CV)

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Reader 19	Reader 20	Reader 21	Ger JU	%
Stage 1a+1b ==>	0	39%	-	0%	28%	0%	30%	0%	26%	25%	35%	33%	19%	15%	42%	33%	16%	30%	37%	39%	0%	17%	17%	ALL
Stage 2 ==>	2	7%	-	15%	17%	30%	7%	10%	23%	27%	27%	25%	41%	31%	26%	31%	36%	25%	32%	7%	23%	28%	28%	15%
Stage 3 ==>	3	11%	-	7%	18%	16%	5%	13%	12%	27%	11%	9%	13%	13%	18%	29%	34%	16%	17%	13%	19%	13%	24%	
Stage 4 ==>	4	17%	-	5%	15%	9%	11%	5%	12%	18%	12%	5%	0%	20%	10%	19%	27%	7%	11%	17%	18%	13%	16%	
Stage 5 ==>	5	19.0%	-	7.7%	19.6%	15.3%	14.3%	6.5%	15.1%	23.9%	22.6%	19.7%	21.9%	20.6%	25.3%	28.9%	21.5%	26.1%	18.4%	18.1%	18.9%	18%	18%	
Weighted mean	1-5	19.0%	-	7.7%	19.6%	15.3%	14.3%	6.5%	15.1%	23.9%	22.6%	19.7%	21.9%	20.6%	25.3%	28.9%	21.5%	26.1%	18.4%	18.1%	18.9%	18%	18%	
RANKING	9	-	2	10	5	3	1	4	16	15	11	14	12	17	20	19	13	18	7	6	8	8	8	

I

Table 4.2-3

Horse Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

- (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 at each modal stage.
 (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage.
 (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant.
 (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage.
 (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

A

NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																								
MODAL stage	Spa CF Reader 1	Eng MS Reader 1	Scot IG Reader 1	Spa ALL Reader 1	Ire DL Reader 1	Spa IM Reader 1	Eng CB Reader 1	Nor J/L Reader 1	Scot FB Reader 1	Por CV Reader 1	Por FV Reader 1	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net CVD Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL		
Stage 1a ==>	0	107	-	101	103	105	103	97	101	107	103	107	103	96	99	92	107	96	103	94	79	2004		
Stage 1b ==>	1	6	-	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	6	6	119		
Stage 2 ==>	2	25	-	25	25	25	25	25	25	25	25	25	25	24	25	21	25	23	25	25	24	492		
Stage 3 ==>	3	2	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	40		
Stage 4 ==>	4	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	60		
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2715		
Total	0-5	143	0	137	139	141	139	133	137	143	139	143	139	131	135	124	143	129	139	130	114	2715		

Table 4.2-3 Continued

Horse Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

D

Each readers egg stages divided by the modal egg stages (Table C) divided by modal egg stage)

Model Stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Stage 1a =>	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	ALL
Stage 1a	0	102	-	100	100	100	99	100	92	94	98	94	90	74	71	70	95	97	89	73	95%	
Stage 1a	0	1	2	-	5	1	3	1	1	4	-	-	2	3	-	5	-	4	-	1	3	2%
Stage 1a	0	2	3	-	3	3	2	1	2	3	-	-	3	4	2	1	6	3	5	2	1	4
Stage 1a	0	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	0%
Stage 1a	0	4	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	0%
Stage 1b	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1b	1	0	-	1	1	2	2	2	1	3	5	9	56	4	22	19	36	-	-	4	6	64%
Stage 1b	1	0	-	-	3	3	3	5	3	2	1	3	6	3	6	1	5	-	1	5	2	20%
Stage 1b	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15%
Stage 1b	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1b	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 1b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 2	2	0	5	-	2	-	4	1	3	1	3	4	-	1	2	2	1	1	6	1	-	9%
Stage 2	2	1	4	-	1	2	-	2	-	5	3	-	1	-	-	-	1	1	5	-	1	7%
Stage 2	2	2	21	-	18	22	20	15	25	23	21	9	13	14	8	15	23	8	15	16	20	81%
Stage 2	2	3	1	-	1	1	1	1	1	1	1	2	1	2	2	1	1	-	-	-	-	2%
Stage 2	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 2	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 3	3	0	-	-	-	-	-	-	-	3	2	-	-	1	-	-	-	-	-	-	-	6%
Stage 3	3	1	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 3	3	2	1	-	4	-	9	-	2	1	15	11	12	5	-	-	-	1	3	9	1	65%
Stage 3	3	3	1	-	1	1	1	2	1	2	1	1	2	2	-	-	-	-	-	-	-	17%
Stage 3	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	12%
Stage 3	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 4	4	0	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	5%
Stage 4	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 4	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2%
Stage 4	4	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13%
Stage 4	4	4	3	-	3	1	3	2	3	2	3	2	1	3	2	1	1	3	1	1	3	80%
Stage 4	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 5	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 5	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 5	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 5	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
Stage 5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Stage 5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Total		0-5	143	0	137	139	137	141	139	133	137	143	139	131	135	124	143	129	139	130	114	2715
Weighted mean		0-5	83.8%	-	89.1%	91.4%	92.7%	85.8%	95.7%	91.0%	89.1%	78.3%	81.3%	83.9%	44.6%	65.3%	65.0%	87.6%	85.6%	83.1%	81.6%	82%
RANKING		7	-	5	3	2	2	9	1	4	5	16	15	12	20	11	17	8	10	13	14	-

E

PERCENTAGE AGREEMENT BY EGG STAGE

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	
Stage 1a =>	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
Stage 1a	0	95%	-	99%	97%	99%	94%	97%	95%	93%	92%	91%	45%	94%	75%	77%	65%	99%	94%	95%	92%
Stage 1a	1	0%	-	0%	50%	50%	50%	83%	50%	33%	17%	50%	50%	50%	100%	17%	83%	0%	17%	83%	33%
Stage 2 =>	2	84%	-	72%	88%	80%	60%	100%	92%	84%	36%	52%	32%	63%	92%	38%	60%	70%	80%	40%	58%
Stage 3 =>	3	50%	-	50%	50%	50%	100%	0%	50%	100%	50%	50%	100%	100%	0%	0%	50%	0%	50%	50%	50%
Stage 4 =>	4	100%	-	100%	33%	100%	67%	100%	67%	100%	100%	67%	100%	67%	33%	33%	100%	33%	33%	100%	100%
Weighted mean		0-5	83.8%	-	89.1%	91.4%	92.7%	85.8%	95.7%	91.0%	89.1%	78.3%	81.3%	83.9%	44.6%	65.3%	65.0%	87.6%	85.6%	83.1%	81.6%
RANKING		7	-	5	3	2	2	9	1	4	5	16	15	12	20	11	17	8	10	13	14

F

PERCENTAGE AGREEMENT STAGE 1a and 1b combined

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	
Stage 1a+1b =>	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
1a+1b	92%	-	99%	96%	100%	95%	99%	95%	94%	90%	100%	98%	98%	97%	98%	98%	98%	90%	99%	99%	99%
RANKING		18	-	4	13	1	15	3	14	16	19	17	8	9	12	11	7	10	20	5	6

G

BIAS = over- / underestimation of stage 1a+1b combined

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	
Stage 1a+1b =>	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
1a+1b	-5%	-	2%	-1%	5%	-5%	-1%	-3%	-3%	-10%	-6%	3%	2%	-1%	11%	7%	5%	-8%	5%	11%	1%
RANKING		13	-	4	1	10	9	1	8	7	17	14	6	5	3	19	15	11	16	12	18

H

PRECISION = Coefficient of Variation (CV)

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	
Stage 1a+1b =>	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
1a+1b	25%	-	10%	18%	49%	22%	9%	21%	21%	40%	23%	13%	21%	23%	14%	13%	14%	27%	10%	11%	9%
Stage 2 =>	2	21%	-	26%	18%	28%	24%	0%	14%	21%	24%	32%	31%	14%	36%	31%	29%	37%	34%	34%	27%
Stage 3 =>	3	28%	-	20%	28%	28%	0%	47%	28%	0%	20%	71%	0%	0%	47%	47%	71%	47%	37%	34%	28%
Stage 4 =>	4	0%	-	0%	33%	0%	16%	0%	16%	0%	0%	8%	0%	16%	17%	0%	17%	17%	0%	0%	18%
Stage 5 =>	5	0%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13%
Weighted mean		1-5	23.9%	-	12.6%	18.6%	4.6%	21.6%	8.1%	19.4%	40.5%	35.5%	23.1%	8.5%	16.6%	20.8%	18.3%	16.4%	17.4%	26.6%	15.2%
RANKING		17	-	4	11	1	14	2	12	20	19	16	3	8	15	10	7	9	18	5	6

OTHER Eggs first stageing. **Egg Stageing Workshop, Lowestoft, October 2003**

Table 4.2-4

- (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 at each modal stage. (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage. (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant. (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage. (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

A

NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																							
MODAL stage	Spa CF Reader 1	Eng MS Reader 2	Sco IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Nor JdL Reader 8	Sco FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net CVD Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL	
Stage 1a ==>>>	0	53	-	49	50	49	52	50	40	49	53	50	53	50	46	49	40	53	45	50	22	4	907
Stage 1b ==>>>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>>>	2	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	60
Stage 3 ==>>>	3	2	-	2	2	2	3	3	2	3	2	2	3	2	3	2	3	3	2	3	2	3	49
Stage 4 ==>>>	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5 ==>>>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	58	0	54	55	54	57	56	46	54	59	55	58	56	52	54	46	59	50	56	27	10	1016

[illegible]

EGG STAGE COMPOSITION AS ESTIMATED BY READER																						
Stage	Spa CF Reader 1	Eng MS Reader 2	Sco IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Nor JDL Reader 8	Sco FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net CVD Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL
0	52	-	49	46	48	51	47	38	49	46	46	48	41	46	45	35	24	45	49	22	4	831
1	1	-	-	2	1	1	3	1	-	3	4	5	7	-	3	7	29	-	1	-	-	68
2	4	-	3	4	4	3	5	4	4	2	3	3	4	4	4	3	4	-	5	1	3	65
3	1	-	2	3	1	2	1	3	1	8	3	2	4	2	2	1	1	5	1	4	2	49
4	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	1	3
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Total	58	0	54	55	54	57	56	46	54	59	55	58	56	52	54	46	59	50	56	27	10	1016

Table 4.2-4 Continued

OTHER Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

D			Each readers egg stages divided by the modal egg stages (Table (C) divided by modal egg stage)																							
Stage estimated by reader	Modal Stage	Spa CF Reader 1	Eng MS Reader 2	Sco IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Nor Jdl Reader 8	Sco FB Reader 9	Por CV Reader 10	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net CVD Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	% All				
Stage 1a 0	0	52	-	49	46	48	51	47	38	49	46	48	41	46	45	35	24	45	49	22	4	100%				
Stage 1a 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1a 2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1a 3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1a 4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1a 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1b 0	0	1	-	2	1	1	3	1	1	3	4	5	7	-	3	4	29	-	1	-	-	96%				
Stage 1b 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1b 2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1b 3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4%				
Stage 1b 4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 1b 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 2 0	0	-	-	-	-	-	-	-	1	-	2	-	2	-	1	-	-	-	-	-	-	11%				
Stage 2 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 2 2	2	3	-	3	3	3	3	3	3	3	2	3	1	3	3	3	3	3	1	3	3	71%				
Stage 2 3	3	1	-	1	1	1	2	2	1	1	-	-	1	1	-	2	1	-	2	-	-	18%				
Stage 2 4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 2 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 3 0	0	-	-	-	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	8%				
Stage 3 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 3 2	2	-	-	-	-	-	-	-	-	-	3	1	2	2	2	1	-	3	-	2	-	22%				
Stage 3 3	3	3	1	2	1	1	2	1	3	1	3	2	2	2	2	1	1	2	1	2	2	69%				
Stage 3 4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 3 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 4 0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 4 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 4 2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 4 3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 4 4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 4 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%				
Stage 5 0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5 1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5 2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5 3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5 4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Stage 5 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Weighted mean	0-5	58	0	54	55	54	57	56	46	54	59	58	56	52	54	46	59	50	56	27	10	1016				
Total																										
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19	20	9	8	10	16					
RANKING		5	14	6	2	13	7	13	7	3	17	14	18	4	10	19										

All Eggs second stageing. Egg Stageing Workshop, Lowestoft, October 2003

Table 4.2-5

- (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 at each modal stage. (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage. (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant. (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage. (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

A

NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																					
MODAL stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net Cvd	Spa PA	Spa BB	Ger SC	Ger JU
	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
0	207	-	206	207	199	207	206	203	203	205	207	206	207	207	195	184	205	207	206	158	190
Stage 1a =>>>																					
Stage 1b =>>>	1	7	-	7	6	7	7	84	7	7	7	7	7	7	7	7	7	7	7	7	7
Stage 2 =>>>	2	84	-	84	82	84	84	83	84	81	84	84	84	84	82	84	84	84	84	84	82
Stage 3 =>>>	3	52	-	52	52	52	52	52	52	51	52	51	52	52	52	52	52	52	52	51	52
Stage 4 =>>>	4	25	-	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Stage 5 =>>>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	375	0	374	375	364	375	374	370	371	369	375	373	375	375	361	352	373	375	374	325	7361

[illegible]

EGG STAGE COMPOSITION AS ESTIMATED BY READER																						
Stage	Spa CF	Eng MS	Sci IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sci FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL
0	200	-	203	195	193	187	195	204	212	190	193	201	217	198	170	63	219	207	192	172	189	3800
Stage 1a =>																						
Stage 1b =>	1	-	8	13	22	17	26	8	4	15	11	29	18	22	36	108	9	14	9	8	17	401
Stage 2 =>	2	99	-	73	71	85	84	84	77	76	82	58	53	82	84	115	70	86	92	73	68	1691
Stage 3 =>	3	49	-	62	66	63	49	48	58	50	56	54	62	48	43	51	43	51	68	59	60	1072
Stage 4 =>	4	20	-	28	22	46	23	30	26	38	31	31	25	25	28	15	32	17	13	13	12	497
Stage 5 =>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	375	0	374	375	375	374	370	371	369	375	373	375	375	361	352	373	375	374	325	366	7361

Table 4.2-5 Continued

All Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

D

Each readers egg stages divided by the modal egg stages (Table C) divided by modal egg stage)

Modal Stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Reader 1	195	-	201	189	188	187	184	195	201	186	193	196	195	197	161	56	200	197	191	157	185	96%	
Stage 1a =>	0	1	2	1	1	2	3	6	1	3	2	4	4	4	2	4	4	4	1	5	3	1%	
Stage 1a	0	2	3	1	2	3	1	4	4	3	1	1	18	1	4	6	15	5	1	10	11	2%	
Stage 1a	0	3	-	-	3	-	-	1	1	-	-	-	-	-	2	-	-	-	-	-	-	0%	
Stage 1a	0	4	-	-	-	-	-	1	1	-	-	-	-	-	1	-	-	-	-	-	-	0%	
Stage 1a	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 1b	1	0	3	-	2	10	12	19	3	2	12	6	9	10	10	24	97	5	4	1	5	62%	
Stage 1b	1	1	3	-	5	3	4	6	4	1	3	5	3	3	7	4	1	3	2	3	3	17%	
Stage 1b	1	2	1	-	1	-	1	1	1	-	-	17	5	5	7	10	1	7	2	6	9	20%	
Stage 1b	1	3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	0%	
Stage 1b	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 1b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 2	2	0	9	-	2	5	1	8	3	4	-	1	-	-	4	-	31	-	5	11	-	6%	
Stage 2	2	1	2	-	1	3	-	2	1	-	3	2	-	-	1	5	-	1	3	1	-	2%	
Stage 2	2	2	78	-	69	68	75	78	78	75	67	72	56	49	77	69	62	66	72	75	67	60	87%
Stage 2	2	3	10	-	1	2	2	2	2	2	-	1	3	5	10	16	4	8	2	4	8	5%	
Stage 2	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	0%	
Stage 2	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 3	3	0	-	1	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1%	
Stage 3	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 3	3	2	-	13	11	3	9	4	-	4	11	12	10	12	1	1	6	2	7	1	2	10%	
Stage 3	3	3	42	-	47	46	29	51	45	46	50	38	44	44	47	34	33	41	43	50	45	43	80%
Stage 3	3	4	5	-	1	7	-	3	-	2	4	-	-	3	3	4	12	-	8	11	13	14	8%
Stage 3	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	0	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1%	
Stage 4	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4	4	2	-	-	2	-	1	5	3	-	13	8	6	2	3	5	3	7	-	-	2	1	17%
Stage 4	4	3	-	4	1	21	1	5	3	-	-	-	-	-	-	-	-	-	-	-	-	81%	
Stage 4	4	4	20	-	24	18	25	22	25	22	20	25	25	22	22	20	12	25	17	13	11	11	8%
Stage 4	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 5	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	375	0	374	375	364	375	374	370	371	369	375	373	375	361	352	373	375	374	325	366	7361	

E

PERCENTAGE AGREEMENT BY EGG STAGE

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Stage 1a =>	0	94%	-	98%	91%	94%	90%	89%	96%	99%	91%	93%	95%	94%	95%	83%	98%	95%	93%	99%	97%	91%	ALL
Stage 1a	0	1	43%	71%	67%	71%	66%	57%	67%	14%	43%	43%	43%	100%	57%	14%	43%	29%	43%	14%	43%	43%	90%
Stage 2	2	93%	-	82%	83%	89%	93%	84%	89%	83%	86%	67%	67%	98%	84%	74%	79%	86%	89%	80%	73%	83%	
Stage 3	3	81%	-	90%	88%	96%	98%	87%	88%	96%	75%	86%	90%	95%	85%	63%	79%	83%	86%	88%	83%	83%	
Stage 4	4	80%	-	96%	72%	100%	88%	100%	88%	80%	100%	100%	100%	88%	88%	48%	100%	68%	52%	44%	44%	81%	
Stage 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	81%	
Weighted mean	0-5	90.1%	-	92.5%	86.7%	86.3%	90.7%	90.4%	93.2%	93.5%	86.4%	90.4%	86.9%	84.3%	79.8%	46.6%	88.8%	88.3%	88.8%	86.5%	84.8%	87%	
RANKING	0-5	8	-	4	13	16	5	7	2	1	15	6	12	18	3	19	9	11	10	14	17	17%	

F

PERCENTAGE AGREEMENT STAGE 1a and 1b combined

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Reader 1	195	-	201	189	188	187	184	195	201	186	193	196	195	197	161	56	200	197	191	157	185	96%	
Stage 1a+1b	1a+1b	95%	-	98%	95%	100%	95%	98%	98%	100%	95%	95%	100%	99%	100%	95%	100%	97%	93%	99%	99%	97%	ALL
RANKING	1a+1b	16	-	9	16	5	13	9	11	1	15	13	4	8	1	18	20	1	12	19	7	6	97%
ALL	ALL	97%	-	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%

G

BIAS = over- / underestimation of stage 1a+1b combined

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Reader 1	195	-	201	189	188	187	184	195	201	186	193	196	195	197	161	56	200	197	191	157	185	96%	
Stage 1a+1b	1a+1b	-3%	-	-1%	-3%	5%	-5%	-4%	1%	3%	-3%	-5%	8%	10%	3%	-2%	-10%	8%	3%	-6%	9%	10%	ALL
RANKING	1a+1b	8	-	1	5	13	11	1	3	7	10	11	16	19	5	4	20	15	8	14	17	18	1%
ALL	ALL	10%	-	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%

H

PRECISION = Coefficient of Variation (CV)

Modal stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%	
Reader 1	195	-	201	189	188	187	184	195	201	186	193	196	195	197	161	56	200	197	191	157	185	96%	
Stage 1a+1b	1a+1b	21%	-	18%	31%	7%	20%	13%	24%	0%	23%	20%	7%	21%	0%	33%	0%	16%	23%	8%	14%	9%	ALL
Stage 2	2	14%	-	19%	22%	21%	15%	13%	12%	17%	19%	16%	30%	34%	15%	23%	24%	19%	16%	23%	17%	17%	
Stage 3	3	14%	-	10%	18%	17%	5%	12%	14%	7%	14%	12%	12%	10%	13%	20%	15%	16%	7%	12%	14%	12%	
Stage 4	4	11%	-	5%	12%	0%	9%	0%	17%	18%	0%	0%	0%	9%	9%	17%	0%	13%	17%	17%	15%	11%	
Stage 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Weighted mean	1-5	17.7%	-	16.0%	26.2%	11.1%	16.0%	12.2%	19.5%	5.9%	19.4%	16.8%	12.3%	21.7%	5.7%	32.0%	28.4%	7.4%	16.5%	18.8%	13.0%	12%	
RANKING	1-5	13	-	8	18	4	9	5	16	2	15	11	6	17	1	20	19	3	10	14	7	12%	
ALL	ALL	12%	-	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	12%	

Mackerel Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-6

- (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 at each modal stage.
 (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage.
 (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant.
 (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage.
 (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

A

NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																													
MODAL	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	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	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL
0	39	-	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	776
Stage 1a ==>	4	-	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	79
Stage 2 ==>	53	-	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	1052
Stage 3 ==>	48	-	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	968
Stage 4 ==>	22	-	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	440
Stage 5 ==>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	166	0	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	3305
Total	166	0	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	166	3305

B

Modal egg stages divided by each readers egg stages (Table A) divided by the readers egg stages																								
Reader	Spa CF	Erg MS	Spa IG	Spa ALL	Ire DL	Spa IM	Erg CB	Nor JDL	Spa FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%		
Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	All		
Modal stage																								
Stage 1a	0	33	-	37	31	31	26	24	34	39	29	32	33	32	33	19	5	35	35	31	39	35	79%	
Stage 1b	0	1	1	-	1	6	7	10	14	3	8	5	5	7	6	14	23	4	4	4	-	3	16%	
Stage 2a	0	3	-	1	1	-	3	1	1	-	2	1	1	-	-	11	-	-	4	-	-	-	4%	
Stage 2b	0	3	-	1	1	-	3	1	1	-	2	1	1	-	-	11	-	-	4	-	-	-	1%	
Stage 3a	0	4	-	-	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	0%	
Stage 3b	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 4a	0	1	-	1	1	-	-	-	2	4	1	-	3	3	-	1	2	3	1	3	2	3	36%	
Stage 4b	1	1	-	1	1	-	4	4	2	-	4	1	1	4	2	1	2	-	2	-	1	47%		
Stage 5a	1	2	-	1	1	-	2	1	-	1	-	-	-	-	1	2	-	1	1	-	-	14%		
Stage 5b	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1%		
Stage 6a	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%		
Stage 6b	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%		
Stage 7a	2	0	1	-	1	-	1	2	2	-	-	-	10	-	-	5	9	1	-	6	6	5%		
Stage 7b	2	1	-	1	-	4	-	1	1	-	-	12	4	5	6	6	-	6	2	6	7	6%		
Stage 8a	2	2	-	47	48	44	51	48	49	50	42	45	38	37	48	44	38	43	46	50	41	38	85%	
Stage 8b	2	3	-	4	2	2	3	-	-	8	8	3	3	2	-	1	4	1	-	1	-	-	4%	
Stage 9a	2	4	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 9b	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 10a	3	0	-	-	3	-	-	-	1	-	-	-	-	-	2	-	-	1	-	-	-	-	1%	
Stage 10b	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 11a	3	2	7	1	1	1	-	1	-	1	-	1	3	4	8	16	1	7	1	4	5	-	6%	
Stage 11b	3	3	41	43	43	26	47	42	47	34	40	40	43	41	32	29	40	40	47	42	42	84%		
Stage 12a	3	4	-	4	1	21	1	5	3	13	8	6	2	3	5	3	7	-	2	1	-	9%		
Stage 12b	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 13a	4	0	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 13b	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 14a	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 14b	4	2	-	1	6	-	3	-	2	3	-	-	-	3	4	10	-	8	10	10	11	18%		
Stage 15a	4	4	18	21	16	22	19	22	19	18	22	22	22	19	19	11	22	14	11	11	11	81%		
Stage 15b	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%		
Stage 16a	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 16b	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 17a	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 17b	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 18a	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Stage 18b	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%	
Total	166	0	166	166	166	166	166	164	166	161	166	165	165	166	165	166	166	166	166	166	163	3305		

C

EGG STAGE COMPOSITION AS ESTIMATED BY READER																								
Stage	Spa CF	Eng M5	Spa IG	Spa ALL	Eng DL	Spa IM	Eng GB	Nor Jdl	Spa FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Eng DOB	Net M5	Net CV	Spa PA	Spa BB	Ger SC	Ger JU			
0	35	-	39	36	34	26	25	40	46	30	32	36	45	33	23	11	46	40	32	48	43			
1	4	-	4	7	13	14	19	6	1	10	9	18	12	15	23	30	6	10	8	6	11			
2	64	-	50	52	54	54	50	50	51	43	47	40	40	52	54	68	44	54	57	47	43			
3	45	-	48	51	28	52	45	46	50	43	48	43	46	44	40	43	41	48	58	52	54			
4	18	-	25	20	43	20	27	18	18	35	30	28	21	22	25	14	29	14	11	13	12			
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
0-5	166	0	166	166	163	166	166	164	166	161	166	165	166	166	165	166	166	166	166	166	163			
TOTAL																								

Horse Mackerel Eggs second stageing. **Egg Stageing Workshop, Lowestoft, October 2003**

Table 4.2-7

- (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 at each modal stage. (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage. (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant. (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage. (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

A

NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																						
MODAL stage	Spa CF Reader 1	Eng MS Reader 2	Sco IG Reader 3	Sco ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Noi Jdl Reader 8	Sco FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net Cvd Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL
Stage 1a ==>>>	0	118	-	118	111	118	117	117	114	117	118	117	118	118	109	114	116	118	117	117	116	2325
Stage 1b ==>>>	1	2	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	40	
Stage 2 ==>>>	2	28	-	28	27	28	28	28	28	28	28	28	28	28	27	28	28	28	28	28	588	
Stage 3 ==>>>	3	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20	
Stage 4 ==>>>	4	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	60	
Stage 5 ==>>>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total	152	0	151	152	144	152	151	151	148	151	152	151	152	152	142	148	150	152	151	151	3003	

[illegible]

C

EGG STAGE COMPOSITION AS ESTIMATED BY READER																							
Stage	Spa CF Reader 1	Eng MS Reader 2	Sci IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Nor JdL Reader 8	Sci FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net CVD Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL	
Stage 1a =>>>	0	117	-	115	109	108	111	111	110	111	114	122	116	103	33	123	116	110	121	120	2202		
Stage 1b =>>>	1	1	3	5	9	2	5	1	3	4	1	11	5	5	10	72	2	-	2	5	190		
Stage 2 =>>>	2	29	-	20	23	23	28	31	30	32	15	10	27	27	37	21	28	32	23	20	509		
Stage 3 =>>>	3	3	-	10	13	1	8	1	5	4	8	11	1	1	-	5	1	7	5	5	94		
Stage 4 =>>>	4	2	-	3	2	3	3	3	2	3	3	4	3	3	2	1	3	3	2	-	48		
Stage 5 =>>>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3003		
Total	0-5	152	0	151	152	144	151	151	148	151	152	151	152	152	142	148	150	152	151	151	150	3003	

OTHER Eggs second stageing. **Egg Stageing Workshop, Lowestoft, October 2003**

Table 4.2-8

- (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 at each modal stage. (C) The numbers of eggs allocated to each stage by each participant. (D) The numbers of eggs allocated to each modal stage by each participant's estimation of stage. (E) The percentage agreement by modal egg stage by each participant. (F) The percentage agreement by modal stage 1a and 1b combined, by each participant. (G) The bias is indicated by the percentage over or under estimation of stages 1a and 1b eggs combined, as estimated by each participant, in relation to the modal stage. (H) The precision of each participant in allocating eggs to each modal stage is given by the Coefficient of Variation. For each table the combined result is also given.

MODAL stage	NUMBER OF READERS EGG STAGE READINGS BY MODAL EGG STAGE																				TOTAL
	Spa CF Reader 1	Eng MS Reader 2	Sci IG Reader 3	Sci ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	No JdL Reader 8	Sci FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Nor EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net CVD Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21
0	50	-	50	50	50	50	50	48	50	50	50	50	50	50	47	31	50	50	50	2	36
Stage 1a =>>>																					
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Stage 1b =>>>																					
2	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	20
Stage 2 =>>>																					
3	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	60
Stage 3 =>>>																					
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	59
Stage 4 =>>>																					
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Stage 5 =>>>																					
Total	57	0	57	57	57	57	57	55	57	57	57	57	57	57	54	38	57	57	57	8	1053

[illegible]

C

EGG STAGE COMPOSITION AS ESTIMATED BY READER																						
Stage	Spa CF Reader 1	Eng MS Reader 2	Spa IG Reader 3	Spa ALL Reader 4	Ire DL Reader 5	Spa IM Reader 6	Eng CB Reader 7	Not Jdl Reader 8	Spa FB Reader 9	Por CV Reader 10	Por FV Reader 11	Spa PC Reader 12	Not EH Reader 13	Ger MK Reader 14	Ire DOB Reader 15	Net MS Reader 16	Net Cvd Reader 17	Spa PA Reader 18	Spa BB Reader 19	Ger SC Reader 20	Ger JU Reader 21	TOTAL
Stage 1a ==>	0	48	-	49	50	51	49	47	51	50	50	51	50	49	44	19	50	51	50	3	36	898
Stage 1b ==>	1	2	-	1	1	2	1	4	3	1	3	3	3	2	3	6	1	-	1	-	1	25
Stage 2 ==>	2	6	-	3	4	3	3	4	3	3	3	3	3	3	3	10	5	4	3	3	5	77
Stage 3 ==>	3	1	-	4	2	3	3	2	3	3	3	-	-	3	3	3	1	2	3	2	1	51
Stage 4 ==>	4	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	2
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Total	0-5	57	0	57	57	57	57	55	57	57	57	57	57	57	54	38	57	57	57	8	43	1053

Table 4.2-8 Continued

OTHER Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

D	Each readers egg stages divided by the modal egg stages (Table C) divided by modal egg stage)																																			
	Modal Stage	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor Jdl	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	%													
	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	ALL														
Stage 1a =>	0	48	-	49	50	50	47	49	-	50	50	50	50	50	49	42	19	50	50	2	36	99%														
	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1%														
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	0%														
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
Stage 1b =>	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	1	0	1	-	1	-	1	1	-	1	1	1	1	1	1	2	6	-	-	-	-	44%														
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	56%														
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
Stage 2 =>	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	6	-	-	-	-	12%														
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1%														
	2	2	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	2	3	3	71%														
	3	2	-	-	1	-	1	-	-	-	-	-	-	-	-	1	-	2	1	1	2	16%														
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
Stage 3 =>	0	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	4%														
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	3	1	-	3	2	3	2	2	3	3	3	3	3	3	3	2	3	1	2	2	1	92%														
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
Stage 4 =>	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50%														
Stage 5 =>	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	50%														
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%														
Total		57	0	57	57	57	57	57	55	57	57	57	57	57	57	54	38	57	57	57	8	43	1053													

PERCENTAGE AGREEMENT BY EGG STAGE	
Modal Stage	Reader 1
Stage 1a =>	96%
Stage 1b =>	100%
Stage 2 =>	100%
Stage 3 =>	33%
Stage 4 =>	-
Stage 5 =>	-
Weighted mean	93.0%
RANKING	17

PERCENTAGE AGREEMENT STAGE 1a and 1b combined	
Modal Stage	Reader 1
Stage 1a+1b =>	98%
Stage 2 =>	100%
Stage 3 =>	100%
Stage 4 =>	100%
Stage 5 =>	100%
Weighted mean	98.2%
RANKING	17

BIAS = over- / underestimation of stage 1a+1b combined	
Modal Stage	Reader 1
Stage 1a+1b =>	-2%
Stage 2 =>	-2%
Stage 3 =>	-2%
Stage 4 =>	-2%
Stage 5 =>	-2%
Weighted mean	-2%
RANKING	16

PRECISION = Coefficient of Variation (CV)	
Modal Stage	Reader 1
Stage 1a+1b =>	14%
Stage 2 =>	0%
Stage 3 =>	25%
Stage 4 =>	4%
Stage 5 =>	15%
Weighted mean	13.6%
RANKING	16

All Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-9 Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED																
MODAL stage	SAMPLING STRATA															No. of eggs
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0	17	17	17	14	17	14	15	7	13	8	11	14	14	15	13	206
1	-	-	-	1	1	1	-	1	2	1	1	1	-	1	1	11
2	3	1	4	5	2	5	5	10	4	10	10	6	7	5	4	81
3	4	5	2	3	4	3	1	3	4	3	3	2	4	3	4	48
4	1	2	2	2	1	2	4	4	2	3	-	2	-	1	3	29
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	375

PERCENTAGE AGREEMENT																
MODAL stage	SAMPLING STRATA															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Agreement
0	90%	87%	81%	90%	84%	88%	82%	92%	87%	82%	89%	94%	84%	83%	91%	87.0%
1	-	-	-	47%	42%	45%	-	65%	55%	65%	50%	45%	-	47%	35%	50.2%
2	62%	80%	58%	83%	70%	78%	71%	78%	74%	70%	69%	64%	76%	62%	79%	72.0%
3	88%	62%	67%	93%	71%	83%	80%	78%	78%	77%	58%	73%	75%	67%	78%	75.2%
4	55%	90%	50%	78%	70%	60%	70%	88%	80%	88%	-	60%	-	47%	82%	75.4%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	85.1%	81.6%	73.9%	86.5%	78.3%	81.2%	77.6%	83.0%	80.3%	76.5%	75.5%	80.5%	80.2%	74.4%	83.4%	80.3%
Weighted																

BIAS																
MODAL stage	SAMPLING STRATA															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean bias
0	0.10	0.16	0.37	0.10	0.26	0.17	0.26	0.08	0.14	0.24	0.11	0.06	0.19	0.21	0.13	0.17
1	-	-	-	0.11	-0.05	-0.05	-	0.15	-0.10	-0.25	-0.40	-0.25	-	-0.06	-0.05	-0.10
2	-0.33	0.10	-0.46	-0.01	-0.22	-0.05	-0.14	-0.09	-0.15	0.01	-0.13	-0.42	-0.13	-0.26	-0.18	-0.14
3	-0.03	-0.01	0.17	0.07	-0.35	0.13	-0.20	-0.15	-0.02	-0.05	-0.47	0.08	-0.08	0.04	0.13	-0.06
4	-0.45	-0.20	-0.50	-0.22	-0.55	-0.55	-0.31	-0.10	-0.20	-0.05	-	-0.43	-	-0.63	-0.18	-0.27
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	0.01	0.10	0.15	0.05	0.08	0.06	0.07	-0.04	0.02	0.06	-0.07	-0.10	0.06	0.05	0.04	0.03
Weighted																

Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-10

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED																	No. of eggs
MODAL stage	SAMPLING STRATA															No. of eggs	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	4	5	10	2	4	2	4	1	1	3	1	2	1	2	4	46	
1	-	-	-	-	1	-	-	1	1	-	1	-	-	1	-	5	
2	3	1	4	3	2	2	4	3	3	4	6	4	7	4	3	53	
3	4	1	2	3	4	3	1	3	4	3	2	2	4	3	4	43	
4	1	1	2	2	1	1	3	4	2	3	-	2	-	1	3	26	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
TOTAL	12	8	18	10	12	8	12	12	11	13	10	10	12	11	14	173	

PERCENTAGE AGREEMENT																	Agreement
MODAL stage	SAMPLING STRATA															Agreement	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	86%	82%	78%	64%	51%	95%	60%	90%	80%	71%	90%	93%	70%	72%	83%	76.2%	
1	-	-	-	-	42%	-	-	65%	70%	-	50%	-	-	47%	-	55.2%	
2	62%	80%	58%	86%	70%	79%	72%	80%	75%	80%	74%	71%	76%	57%	92%	74.2%	
3	88%	60%	67%	93%	71%	83%	80%	78%	78%	77%	62%	73%	75%	67%	78%	76.9%	
4	55%	85%	50%	78%	70%	55%	73%	88%	80%	88%	-	60%	-	47%	82%	75.6%	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mean Weighted	78.0%	79.6%	69.2%	82.1%	61.7%	81.6%	69.0%	81.7%	76.8%	79.0%	70.5%	73.5%	75.0%	60.7%	82.9%	75.1%	

BIAS																	Mean bias
MODAL stage	SAMPLING STRATA															Mean bias	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	0.14	0.22	0.34	0.36	0.71	0.13	0.61	0.10	0.20	0.44	0.10	0.08	0.30	0.28	0.28	0.32	
1	-	-	-	-	-0.05	-	-	0.15	-0.30	-	-0.40	-	-	-0.06	-	-0.14	
2	-0.33	0.10	-0.46	-0.05	-0.22	-0.34	-0.23	-0.07	-0.27	-0.05	0.02	-0.39	-0.13	-0.32	0.08	-0.16	
3	-0.03	0.30	0.17	0.07	-0.35	0.13	-0.20	-0.15	-0.02	-0.05	-0.41	0.08	-0.08	0.04	0.13	-0.04	
4	-0.45	-0.20	-0.50	-0.22	-0.55	-0.60	-0.28	-0.10	-0.20	-0.05	-	-0.43	-	-0.63	-0.18	-0.26	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mean Weighted	-0.08	0.16	0.05	0.03	0.04	-0.08	0.04	-0.07	-0.13	0.06	-0.10	-0.21	-0.08	-0.12	0.09	-0.02	

Horse Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-11

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED																	No. of eggs
MODAL stage		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0	10	8	4	7	10	8	3	7	2	5	7	10	9	10	9	9	107
1	-	-	1	1	-	1	-	1	1	-	1	-	1	-	-	1	6
2	-	-	2	-	-	-	1	7	1	6	2	4	2	-	1	1	25
3	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	2
4	-	1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	3
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	10	10	4	10	10	10	10	10	10	9	9	10	10	10	10	11	143

PERCENTAGE AGREEMENT																	Agree-ment
MODAL stage		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0	91%	85%	88%	92%	91%	86%	89%	92%	88%	88%	85%	87%	96%	82%	87%	94%	88.9%
1	-	-	-	47%	-	45%	-	-	40%	65%	65%	-	45%	-	-	35%	46.2%
2	-	-	-	79%	-	-	65%	77%	70%	63%	63%	61%	50%	-	84%	40%	67.1%
3	-	45%	-	-	-	-	-	-	-	-	-	50%	-	-	-	-	47.5%
4	-	95%	-	-	-	65%	60%	-	-	-	-	-	-	-	-	-	73.3%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	91.5%	81.8%	87.5%	84.7%	91.3%	79.6%	83.5%	81.4%	80.6%	68.3%	73.0%	81.5%	82.2%	86.8%	84.1%	-	82.4%

Weighted

BIAS																	Mean bias
MODAL stage		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0	0.09	0.18	0.42	0.09	0.16	0.19	0.15	0.08	0.14	0.14	0.15	0.13	0.05	0.22	0.17	0.07	0.14
1	-	-	-	0.11	-	-0.05	-	-	0.10	0.10	-0.25	-	-0.25	-	-	-0.05	-0.07
2	-	-	-	0.05	-	-	0.20	-0.10	0.20	0.04	0.04	-0.34	-0.48	-	0.00	-0.95	-0.13
3	-	0.10	-	-	-	-	-	-	-	-	-	-0.60	-	-	-	-	-0.25
4	-	-0.20	-	-	-	-0.50	-0.40	-	-	-	-	-	-	-	-	-	-0.37
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	0.09	0.14	0.42	0.08	0.16	0.10	0.10	-0.04	0.14	0.03	-0.13	-0.09	0.22	0.15	-0.04	-	0.07

Weighted

OTHER Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-12

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED															
MODAL stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	No. of eggs
	3	4	3	5	3	4	3	3	5	3	5	5	3	4	15
0															53
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
2	-	-	-	-	-	3	-	-	-	-	-	-	-	-	3
3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	3
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	3	7	3	5	3	7	3	3	5	3	5	5	3	4	59

PERCENTAGE AGREEMENT															
MODAL stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Agree-ment
	92%	96%	83%	99%	96%	88%	94%	94%	87%	91%	91%	92%	96%	81%	91.6%
0															-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	76.7%
2	-	69%	-	-	-	77%	-	-	-	-	-	-	-	-	69.4%
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	92.2%	84.5%	83.3%	98.9%	96.0%	83.0%	94.1%	93.9%	87.4%	90.9%	91.0%	92.2%	96.1%	80.6%	89.7%
Weighted															

BIAS															
MODAL stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean bias
	0.10	0.04	0.39	0.01	0.06	0.15	0.06	0.06	0.13	0.09	0.10	0.08	0.04	0.28	0.10
0															-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	0.13	-	-	-	-	-	-	-	-	0.13
3	-	-0.18	-	-	-	-	-	-	-	-	-	-	-	-	-0.18
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	0.10	-0.06	0.39	0.01	0.06	0.14	0.06	0.06	0.13	0.09	0.10	0.08	0.04	0.28	0.09
Weighted															

All Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-13

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED																	No. of eggs
MODAL stage	SAMPLING STRATA																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	17	18	14	14	16	14	14	7	15	9	12	14	14	15	14	207	
1	-	-	-	1	1	1	1	-	-	-	1	1	-	1	-	7	
2	4	1	6	5	2	5	5	11	4	10	9	6	7	5	4	84	
3	4	3	4	3	5	4	2	3	4	3	3	2	4	4	4	52	
4	-	3	1	2	1	1	3	4	2	3	-	2	-	-	3	25	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
TOTAL	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	375	

PERCENTAGE AGREEMENT																	Agree- ment
MODAL stage	SAMPLING STRATA																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	91%	89%	93%	94%	90%	91%	87%	96%	90%	88%	96%	96%	90%	88%	88%	91.0%	
1	-	-	-	50%	53%	55%	35%	-	-	-	50%	35%	-	70%	-	49.6%	
2	71%	90%	83%	93%	76%	94%	72%	84%	84%	83%	76%	73%	93%	89%	86%	82.8%	
3	76%	78%	93%	90%	88%	78%	70%	88%	86%	87%	68%	78%	89%	81%	85%	83.1%	
4	-	85%	95%	88%	75%	75%	73%	81%	78%	90%	-	60%	-	-	85%	80.8%	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mean	85.3%	87.6%	90.6%	91.3%	86.6%	87.4%	78.9%	87.3%	87.4%	85.7%	83.6%	83.3%	90.4%	86.6%	87.0%	86.6%	

Weighted

BIAS																	Mean bias
MODAL stage	SAMPLING STRATA																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	0.17	0.12	0.09	0.09	0.12	0.14	0.17	0.05	0.12	0.14	0.06	0.06	0.13	0.16	0.16	0.12	
1	-	-	-	-0.20	-0.16	0.15	0.00	-	-	-	-0.50	0.05	-	-0.20	-	-0.12	
2	-0.06	0.10	-0.18	0.04	-0.21	-0.02	-0.08	-0.12	-0.11	0.06	-0.02	-0.40	-0.11	-0.11	-0.14	-0.09	
3	-0.10	-0.08	0.02	0.03	-0.04	0.04	0.25	-0.08	-0.06	-0.03	-0.32	0.23	-0.06	0.06	0.03	-0.02	
4	-	-0.15	-0.05	-0.13	-0.25	-0.40	-0.37	-0.20	-0.23	-0.10	-	-0.43	-	-	-0.17	-0.22	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mean	0.09	0.06	0.01	0.04	0.04	0.07	0.06	-0.08	0.03	0.06	-0.03	-0.08	0.03	0.08	0.05	0.03	

Weighted

Mackerel Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-14

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED																	No. of eggs
MODAL stage	SAMPLING STRATA																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	6	5	2	1	3	2	2	1	2	4	1	2	1	3	4	39	
1	-	-	-	1	1	-	1	-	-	-	1	-	-	-	-	4	
2	3	1	3	3	2	2	4	4	3	4	6	4	7	4	3	53	
3	4	1	4	3	5	4	2	3	4	3	2	2	4	3	4	48	
4	-	1	1	2	1	-	3	4	2	3	-	2	-	-	3	22	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
TOTAL	13	8	10	10	12	8	12	12	11	14	10	10	12	10	14	166	

PERCENTAGE AGREEMENT																	Agree- ment
MODAL stage	SAMPLING STRATA																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	85%	84%	80%	90%	62%	90%	56%	95%	73%	76%	90%	95%	60%	71%	81%	79.0%	
1	-	-	-	50%	53%	-	35%	-	-	-	50%	-	-	-	-	46.8%	
2	78%	90%	93%	90%	76%	98%	75%	85%	87%	88%	82%	71%	93%	87%	93%	85.4%	
3	76%	80%	93%	90%	88%	78%	70%	88%	86%	87%	73%	78%	89%	80%	85%	83.8%	
4	-	95%	95%	88%	75%	-	73%	81%	78%	90%	-	60%	-	-	85%	81.1%	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mean	80.8%	85.6%	90.5%	85.5%	75.4%	86.1%	67.2%	85.4%	82.3%	84.6%	77.4%	75.0%	88.8%	80.3%	85.5%	81.9%	

Weighted

BIAS																	Mean bias
MODAL stage	SAMPLING STRATA																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	0.28	0.18	0.23	0.15	0.50	0.21	0.49	0.05	0.30	0.25	0.10	0.05	0.40	0.41	0.28	0.27	
1	-	-	-	-0.20	-0.16	-	0.00	-	-	-	-0.50	-	-	-	-	-0.22	
2	-0.20	0.10	0.03	0.05	-0.21	-0.05	-0.20	-0.14	-0.13	-0.09	0.03	-0.44	-0.11	-0.15	0.02	-0.11	
3	-0.10	0.20	0.02	0.03	-0.04	0.04	0.25	-0.08	-0.06	-0.03	-0.28	0.23	-0.06	0.13	0.03	0.00	
4	-	-0.05	-0.05	-0.13	-0.25	-	-0.37	-0.20	-0.23	-0.10	-	-0.43	-	-	-0.17	-0.21	
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mean	0.05	0.14	0.06	-0.01	0.04	0.06	-0.04	-0.13	-0.05	0.02	-0.07	-0.21	-0.05	0.10	0.05	-0.00	

Weighted

Horse Mackerel Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-15

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED															
MODAL stage	SAMPLING STRATA														
	1	2	3	4	5	6	7	8	9	10	8	10	10	10	No. of eggs
0	8	10	9	8	10	9	9	3	8	2	2	7	10	9	118
1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	2
2	1	-	3	2	-	-	1	7	1	6	6	2	-	1	28
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
4	-	2	-	-	-	1	-	-	-	-	-	-	-	-	3
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	9	12	12	10	10	11	10	10	9	8	10	10	10	11	152

PERCENTAGE AGREEMENT															
MODAL stage	SAMPLING STRATA														
	1	2	3	4	5	6	7	8	9	10	10	11	11	12	Agree-ment
0	93%	90%	94%	92%	97%	90%	91%	97%	91%	95%	95%	91%	94%	96%	92.5%
1	-	-	-	-	-	55%	-	-	-	-	-	-	-	35%	45.0%
2	50%	-	72%	98%	-	-	60%	83%	75%	79%	79%	-	64%	75%	76.9%
3	-	-	-	-	-	-	-	-	-	-	-	-	60%	-	60.0%
4	-	80%	-	-	-	75%	-	-	-	-	-	-	-	-	78.3%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	88.2%	88.2%	88.8%	92.8%	97.0%	85.5%	88.1%	86.9%	89.3%	83.1%	83.1%	90.5%	81.8%	85.5%	88.5%
Weighted															

BIAS															
MODAL stage	SAMPLING STRATA														
	1	2	3	4	5	6	7	8	9	10	10	11	11	12	Mean bias
0	0.12	0.12	0.08	0.13	0.03	0.13	0.13	0.03	0.12	0.08	0.08	0.11	0.11	0.05	0.10
1	-	-	-	-	-	0.15	-	-	-	-	-	-	-	0.05	0.10
2	0.35	-	-0.40	0.02	-	-	0.40	-0.11	-0.05	0.17	0.17	-0.12	-0.40	-0.33	-0.06
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.40
4	-	-0.20	-	-	-	-0.40	-	-	-	-	-	-	-	-	-0.27
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	0.15	0.07	-0.04	0.11	0.03	0.09	0.16	-0.07	0.10	0.14	0.14	-0.01	0.11	-0.02	0.06
Weighted															

OTHER Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Table 4.2-16

Eggs staged, percentage agreement and bias by stratum and modal egg stage.
The strata correspond to egg trays and their associated microscopes.

NUMBER OF EGGS STAGED															
MODAL stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	No. of eggs
	3	3	3	5	3	3	3	3	5	3	5	5	3	3	15
0															50
1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
2	-	-	-	-	-	3	-	-	-	-	-	-	-	-	3
3	-	2	-	-	-	-	-	-	-	-	-	-	-	1	3
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	3	5	3	5	3	6	3	3	5	3	5	5	3	5	57

PERCENTAGE AGREEMENT															
MODAL stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Agree-ment
	96%	98%	98%	100%	98%	95%	96%	96%	96%	98%	100%	95%	98%	98%	
0															97.5%
1	-	-	-	-	-	-	-	-	-	-	-	-	-	70%	70.0%
2	-	-	-	-	-	92%	-	-	-	-	-	-	-	-	91.7%
3	-	77%	-	-	-	-	-	-	-	-	-	-	-	85%	79.7%
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	96.4%	89.6%	98.2%	100.0%	98.1%	93.1%	96.2%	96.5%	95.7%	98.2%	100.0%	95.5%	98.1%	89.9%	95.8%
Weighted															

BIAS															
MODAL stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean bias
	0.07	0.02	0.02	0.00	0.04	0.13	0.09	0.07	0.04	0.02	0.00	0.07	0.06	0.02	
0															0.04
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.20	-0.20
2	-	-	-	-	-	0.00	-	-	-	-	-	-	-	-	0.00
3	-	-0.23	-	-	-	-	-	-	-	-	-	-	-	-0.15	-0.20
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	0.07	-0.08	0.02	0.00	0.04	0.06	0.09	0.07	0.04	0.02	0.00	0.07	0.06	-0.06	0.02
Weighted															

SPECIES IDENTIFICATION first determination Egg Stageing Workshop, Lowestoft, October 2003

Table 4.3-1

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

A

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

Modal or actual species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DO'B	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL
	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	
Mackerel	1	173	-	151	150	169	154	171	152	168	154	172	154	154	151	151	171	143	154	152	143	3137
Horse Mackerel	2	143	-	137	138	137	141	139	142	143	139	143	139	138	134	136	143	129	139	139	125	2761
Other species	3	58	-	54	55	54	57	59	54	59	55	58	56	56	53	55	59	50	56	54	52	1110
Total	1-3	374	0	342	343	341	367	349	343	370	348	373	349	348	338	342	373	322	349	345	320	7008

B

Species compositions as estimated per participant and whole group

Species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DO'B	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL
	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	
Mackerel	1	185	-	161	157	176	163	112	157	218	198	214	202	182	122	144	184	140	168	110	170	3323
Horse Mackerel	2	130	-	100	108	98	131	55	117	148	137	106	53	77	114	108	115	135	136	163	93	2254
Other species	3	59	-	81	75	86	56	205	69	4	13	53	94	89	102	90	74	47	45	72	57	1431
Total	1-3	374	0	342	343	341	367	349	343	370	348	373	349	348	338	342	373	322	349	345	320	7008

C

Percentage overestimation / underestimation

Modal or actual species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DO'B	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	ALL
	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	
Mackerel	1	7%	-	7%	5%	4%	6%	-35%	3%	30%	29%	24%	31%	18%	-19%	-5%	8%	-2%	9%	-28%	19%	6%
Horse Mackerel	2	-9%	-	-27%	-22%	-7%	-6%	-61%	-15%	3%	-1%	-26%	-62%	-44%	-15%	-21%	-20%	5%	-2%	17%	-26%	-18%
Other species	3	2%	-	50%	36%	5%	0%	247%	28%	-93%	-76%	-9%	68%	59%	92%	64%	25%	-6%	-20%	33%	10%	29%

D

Percentage agreement in species identification per species

Modal or actual species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DO'B	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	ALL
	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	
Mackerel	1	98%	-	96%	94%	94%	94%	39%	88%	90%	96%	96%	83%	84%	66%	54%	71%	87%	95%	66%	94%	84%
Horse Mackerel	2	85%	-	72%	67%	85%	88%	35%	77%	88%	91%	71%	36%	49%	64%	34%	54%	91%	91%	85%	71%	70%
Other species	3	83%	-	89%	83%	84%	77%	86%	81%	7%	24%	55%	66%	84%	79%	56%	63%	84%	77%	89%	83%	71%
Weighted mean	1-3	89.6%	85.1%	86.3%	81.5%	89.1%	88.5%	45.2%	82.8%	76.2%	82.8%	80.2%	61.8%	70.4%	67.5%	48.2%	63.3%	87.9%	90.5%	77.4%	83.4%	76.7%
RANKING	2	7	6	11	3	4	4	20	9	14	10	12	18	15	16	19	17	5	1	13	8	

SPECIES IDENTIFICATION SECOND determination Egg Stageing Workshop, Lowestoft, October 2003

Table 4.3-2

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

A

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

Model or actual species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL
	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	
Mackerel	1	166	-	166	166	166	166	164	166	161	166	165	166	166	165	166	166	166	166	166	166	3311
Horse Mackerel	2	152	-	151	150	152	152	149	148	151	152	151	152	150	141	149	149	151	151	152	152	3007
Other species	3	57	-	57	57	57	57	55	57	57	57	57	57	57	54	57	57	57	57	57	57	1135
Total	1-3	375	0	374	375	375	375	368	371	369	375	373	375	373	360	372	372	374	374	375	375	7453

B

Species compositions as estimated per participant and whole group

Species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	TOTAL
	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	
Mackerel	1	176	-	175	169	177	175	251	175	167	176	172	237	206	163	187	180	189	174	172	173	3669
Horse Mackerel	2	148	-	143	153	141	150	106	146	151	149	146	130	113	148	136	142	135	144	152	149	2828
Other species	3	51	-	56	54	51	57	11	50	51	50	55	8	54	49	49	50	50	56	51	53	956
Total	1-3	375	0	374	375	373	375	368	371	369	375	373	375	373	360	372	372	374	374	375	375	7453

C

Percentage overestimation / underestimation

Model or actual species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	ALL
	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	
Mackerel	1	6%	-	5%	2%	7%	5%	53%	5%	4%	6%	4%	43%	24%	-1%	13%	8%	14%	5%	4%	4%	11%
Horse Mackerel	2	-3%	-	-5%	2%	-7%	-1%	-29%	-1%	0%	-2%	-3%	-14%	-25%	5%	-9%	-5%	-11%	-5%	0%	-2%	-6%
Other species	3	-11%	-	-2%	-11%	0%	-12%	-80%	-12%	-11%	-12%	-4%	-86%	-5%	-9%	-14%	-12%	-12%	-2%	-11%	-7%	-16%

D

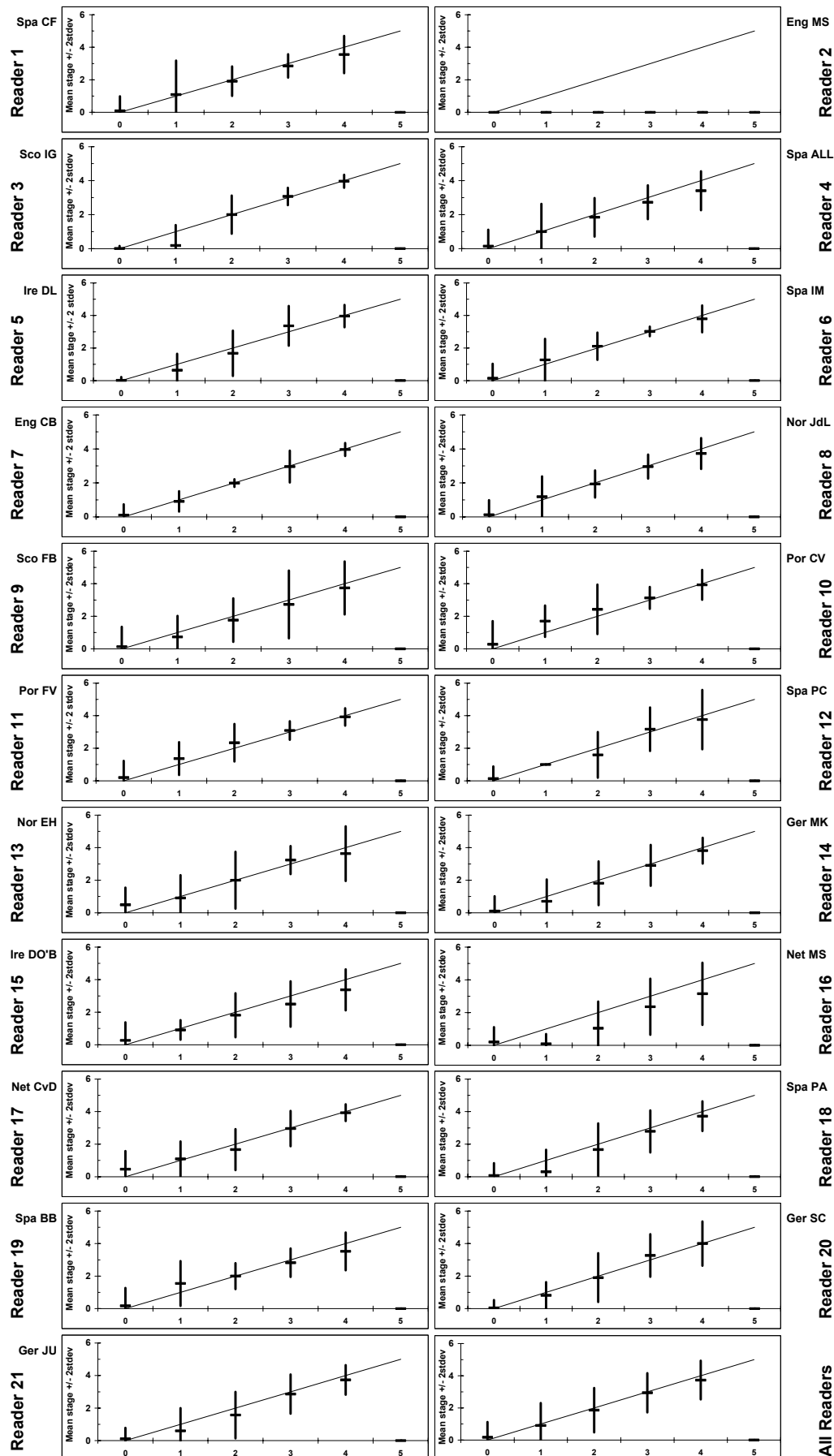
Percentage agreement in species identification per species

Model or actual species	Spa CF	Eng MS	Sco IG	Spa ALL	Ire DL	Spa IM	Eng CB	Nor JdL	Sco FB	Por CV	Por FV	Spa PC	Nor EH	Ger MK	Ire DOB	Net MS	Net CVD	Spa PA	Spa BB	Ger SC	Ger JU	ALL
	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	
Mackerel	1	95%	-	97%	95%	97%	96%	94%	96%	96%	97%	93%	96%	95%	88%	90%	96%	98%	97%	95%	95%	95%
Horse Mackerel	2	92%	-	93%	88%	89%	94%	65%	94%	95%	95%	89%	82%	69%	92%	82%	91%	85%	93%	94%	91%	88%
Other species	3	84%	-	86%	82%	86%	86%	15%	86%	88%	86%	88%	7%	88%	80%	75%	86%	84%	88%	88%	89%	78%
Weighted mean	1-3	92.3%	93.6%	89.3%	93.8%	92.3%	93.6%	70.4%	93.5%	94.3%	94.4%	90.6%	76.8%	83.6%	85.6%	84.7%	92.5%	90.1%	94.1%	93.3%	92.3%	89.7%
RANKING	10	6	15	4	10	5	20	7	2	1	13	19	18	16	17	9	14	3	8	10		

All Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Figure 4.2-1

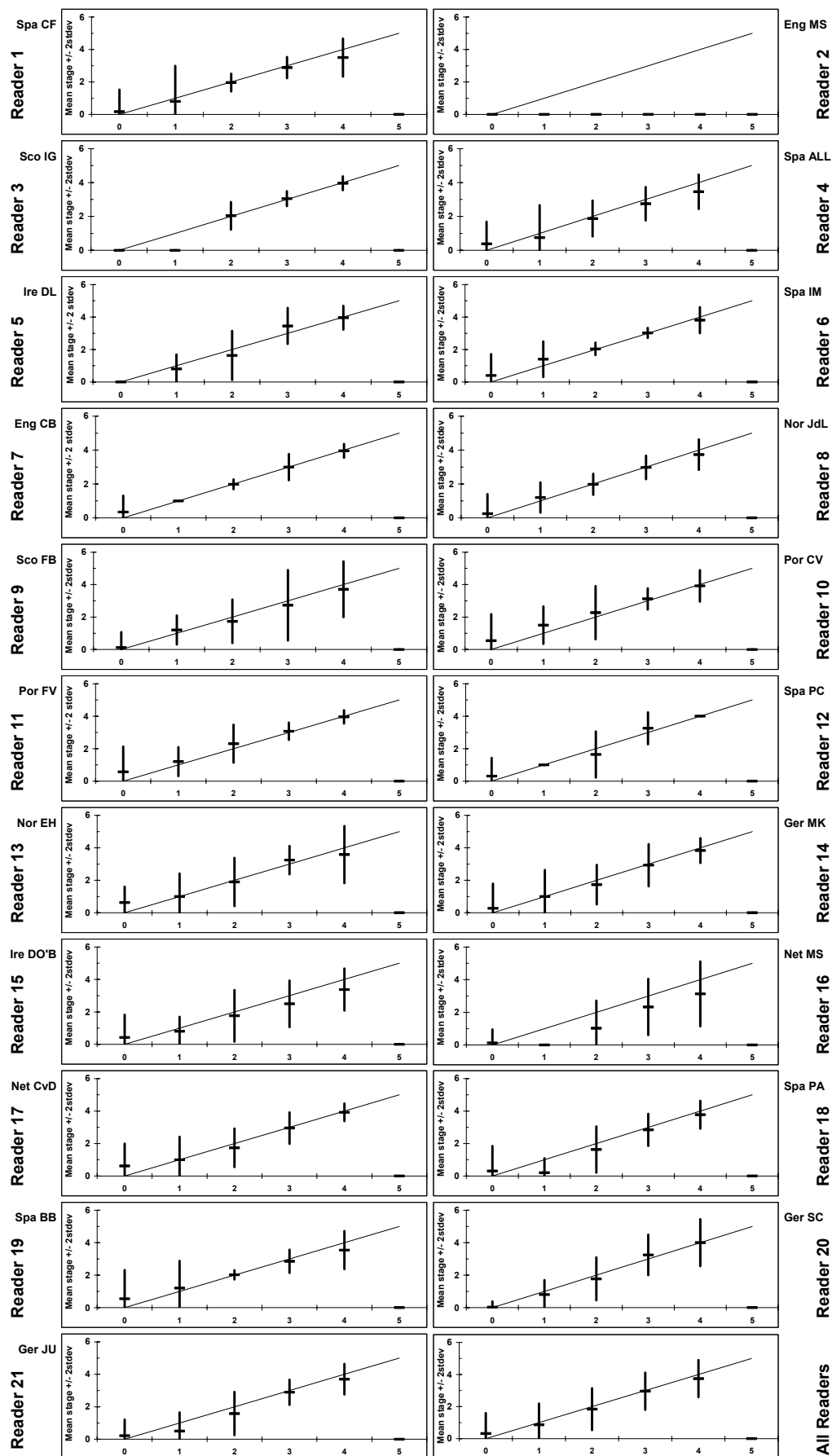
In the egg stage bias plots below the mean egg stage recorded \pm 2stddev of each stage reader and all stage readers combined are plotted against the MODAL egg stage. The estimated mean egg stage corresponds to MODAL egg stage, if the estimated mean egg stage is on the 1:1 equilibrium line (solid line).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



Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Figure 4.2-2

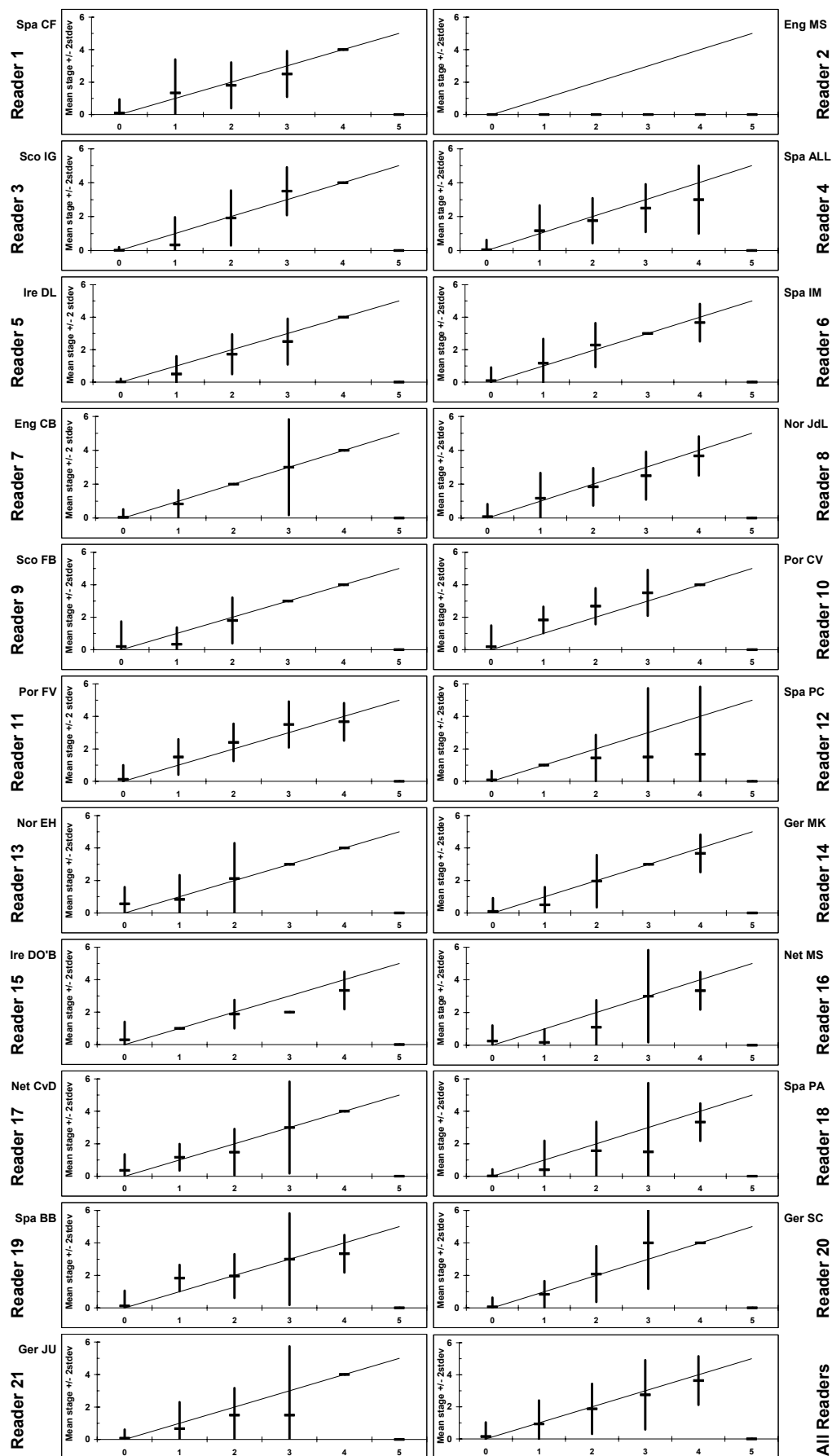
In the egg stage bias plots below the mean egg stage recorded \pm 2stdev of each stage reader and all stage readers combined are plotted against the MODAL egg stage. The estimated mean egg stage corresponds to MODAL egg stage, if the estimated mean egg stage is on the 1:1 equilibrium line (solid line).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



Horse Mackerel Eggs first staging. Egg Staging Workshop, Lowestoft, October 2003

Figure 4.2-3

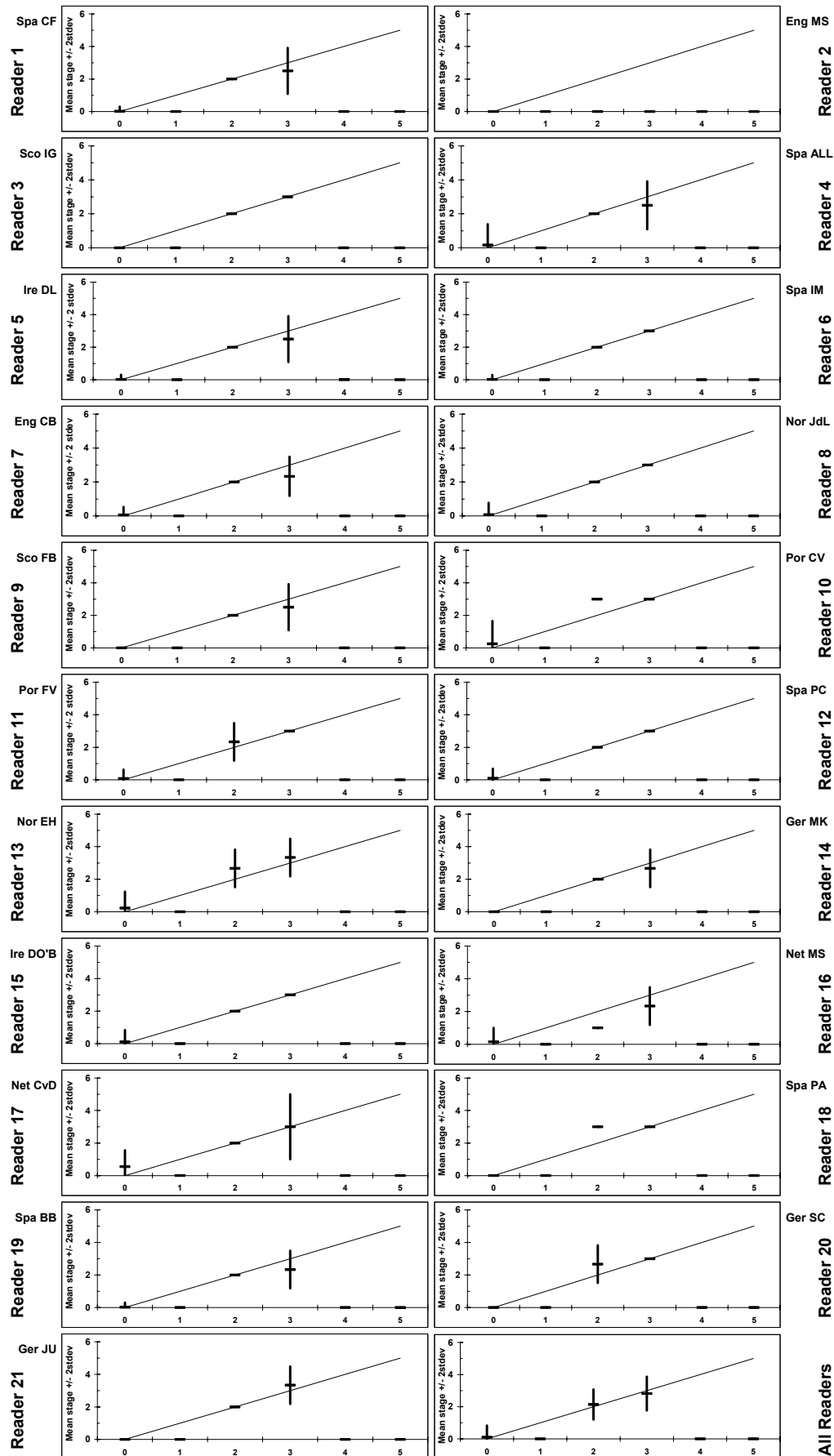
In the egg stage bias plots below the mean egg stage recorded \pm 2stdev of each stage reader and all stage readers combined are plotted against the MODAL egg stage. The estimated mean egg stage corresponds to MODAL egg stage, if the estimated mean egg stage is on the 1:1 equilibrium line (solid line).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



OTHER Eggs first staging. Egg Stageing Workshop, Lowestoft, October 2003

Figure 4.2-4

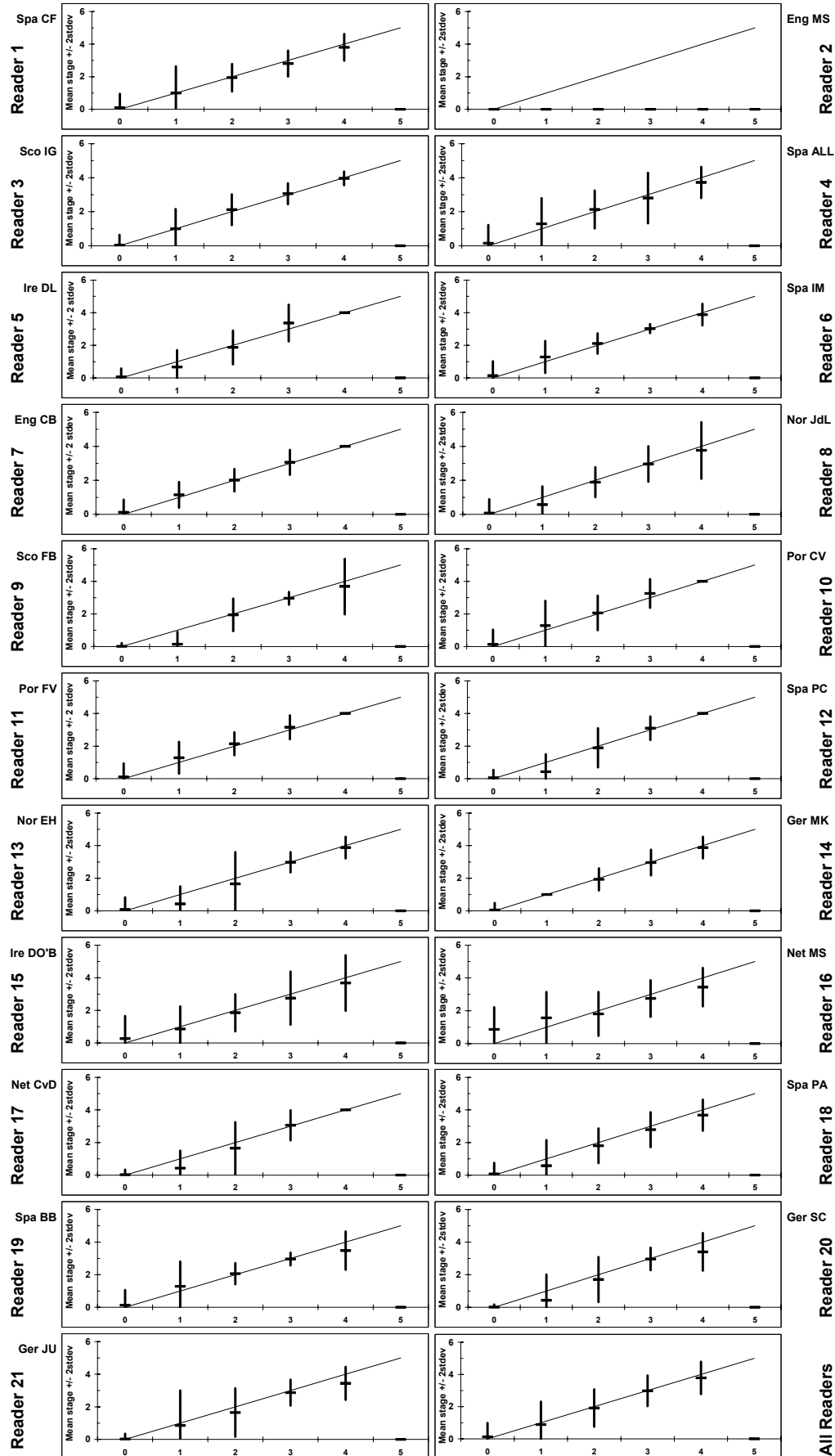
In the egg stage bias plots below the mean egg stage recorded ± 2 stdev of each stage reader and all stage readers combined are plotted against the MODAL egg stage. The estimated mean egg stage corresponds to MODAL egg stage, if the estimated mean egg stage is on the 1:1 equilibrium line (solid line).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



All Eggs second stageing. Egg Stageing Workshop, Lowestoft, October 2003

Figure 4.2-5

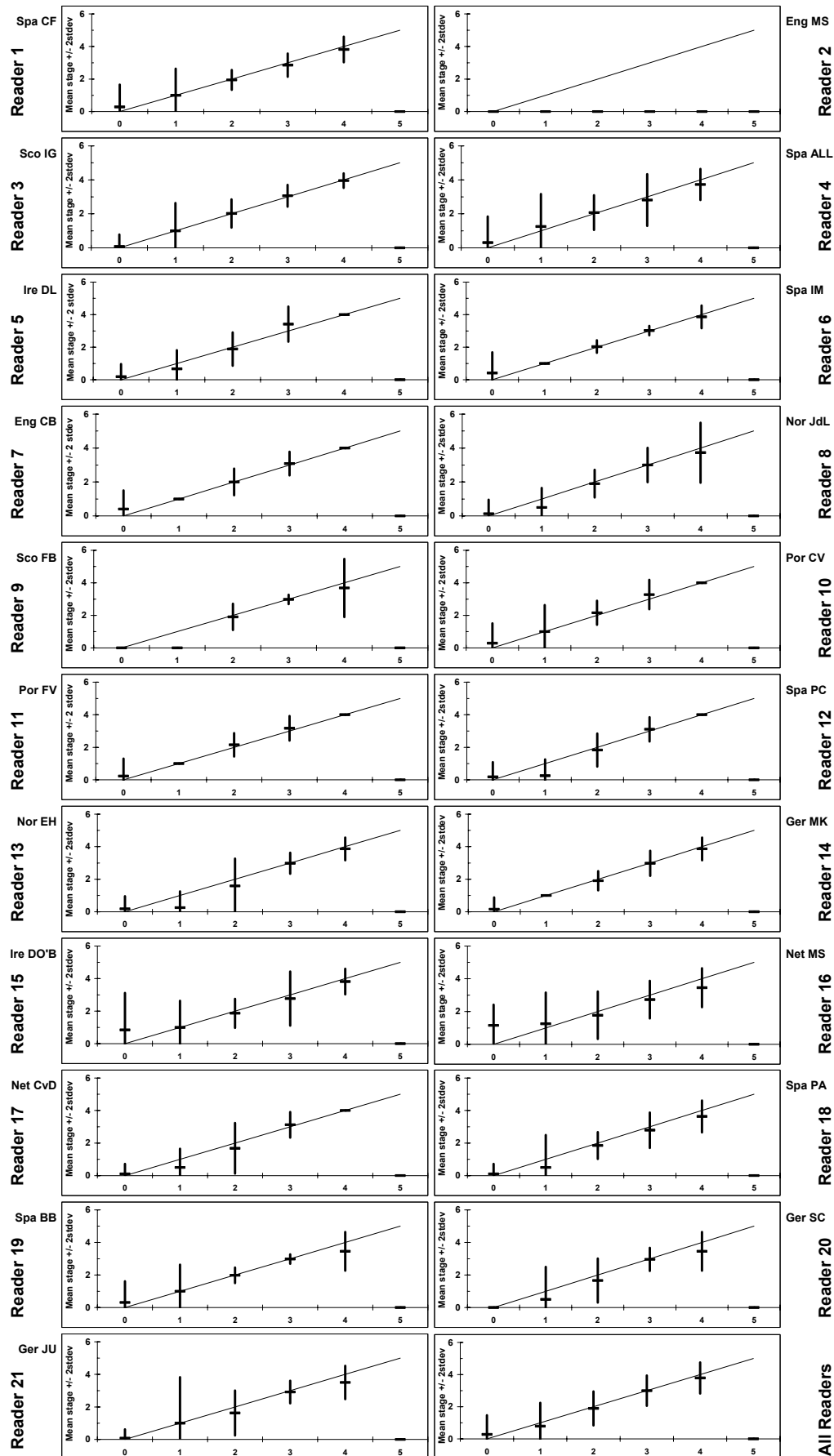
In the egg stage bias plots below the mean egg stage recorded ± 2 stdev of each stage reader and all stage readers combined are plotted against the MODAL egg stage. The estimated mean egg stage corresponds to MODAL egg stage, if the estimated mean egg stage is on the 1:1 equilibrium line (solid line).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



Mackerel Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Figure 4.2-6

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

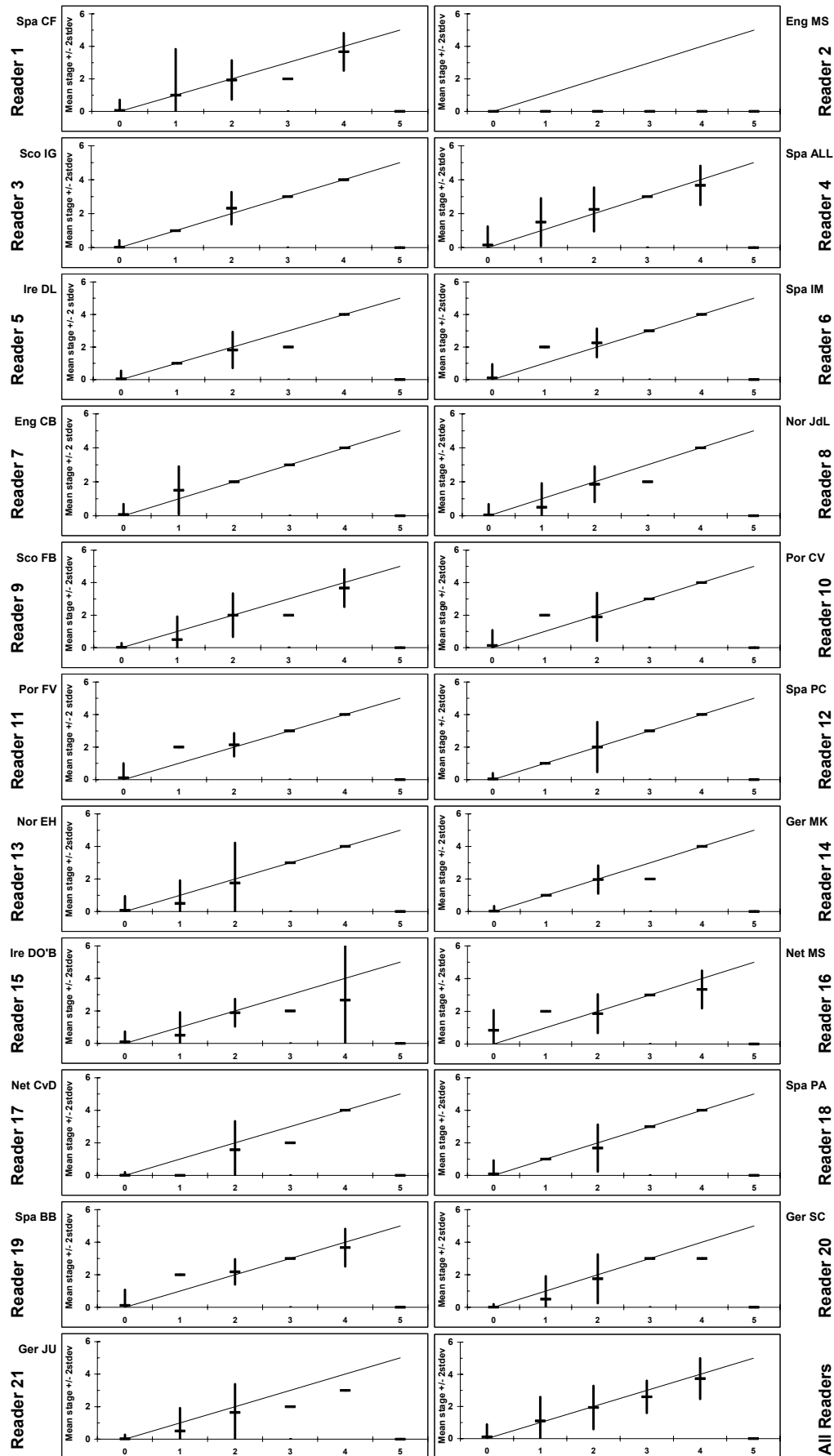


Horse Mackerel Eggs second staging.

Egg Staging Workshop, Lowestoft, October 2003

Figure 4.2-7

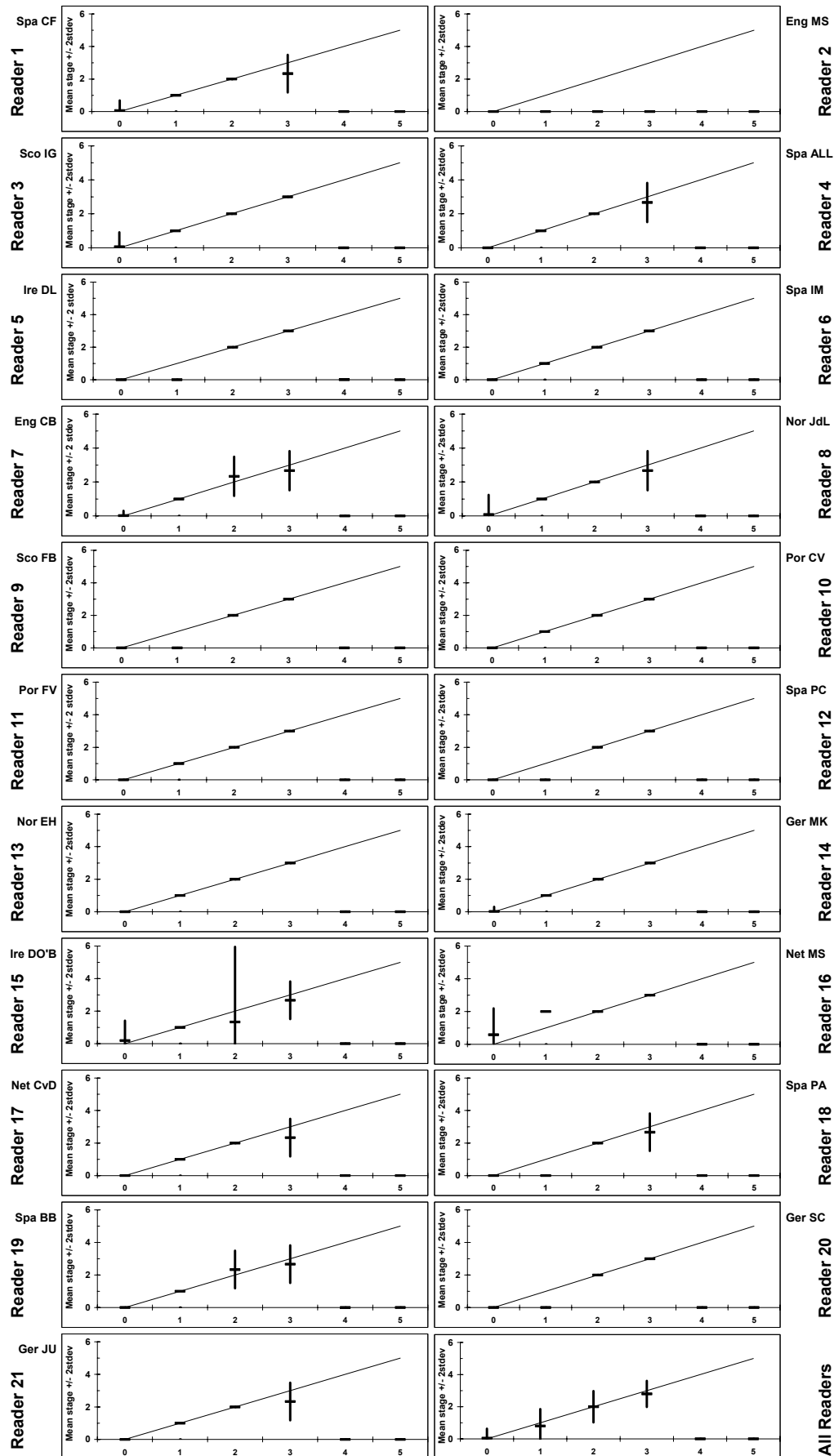
In the egg stage bias plots below the mean egg stage recorded ± 2 stdev of each stage reader and all stage readers combined are plotted against the MODAL egg stage. The estimated mean egg stage corresponds to MODAL egg stage, if the estimated mean egg stage is on the 1:1 equilibrium line (solid line).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



OTHER Eggs second staging. Egg Staging Workshop, Lowestoft, October 2003

Figure 4.2-8

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



5 DISCUSSION

5.1 Discussion of the egg sorting exercise

The evaluation of the 'Spray technique' for the removal of fish eggs from plankton samples proved to be very valuable. The results (Table 4.1-1) indicate that on average 87% of the eggs were removed during the first spraying and that after three sprayings 90% of the eggs had been removed. However, these are probably conservative estimates as each individual result assumes that there were 500 eggs available in the sample to be removed. This was probably only the case for the first participant to use each sample, as eggs were lost during the spraying and in the reconstitution of the samples. The results for the first participants to use each sample are probably more realistic, and indicate that between 97% and 98% of the eggs will be removed after three sprayings of a sample.

The problems experienced during this evaluation of the 'Spray technique' were mainly generated by the fact that only a few samples were prepared with exactly 500 eggs in them. This meant that each sample had to be used on several occasions and that each time they were used, eggs were lost. This would not happen when using this technique on actual survey samples.

However, the problem of egg loss did highlight the fact that great care should be taken at all times when using the spray and all participants were made aware of this before the second sample was used. This resulted in a decrease in the numbers of eggs lost, from 1.4% per separation in the first sample to 0.3% loss per separation in the second sample.

These results are very encouraging and it is recommended that each institute use the spray technique as the primary method for removing fish eggs from the plankton samples collected during the 2004 surveys.

5.2 Discussion of the egg staging exercise

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

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

One of the main problems encountered during the workshop was the distinction between the stages 1a and 1b, although this appeared to be less contentious than at the 2000 workshop. The eggs used were mainly from the English tri-ennial survey conducted during April/May 2001. They had therefore been preserved for more than two years and had been sorted at least once before. They were often quite dark, making the development difficult to see and there was sometimes distortion of the cluster of cells or embryo, making stage determination difficult. This was particularly true of eggs in the earlier stages of development. However, following discussions after the first round of analysis, further clarification of egg stage criteria was produced (section 3.2.2). This led to improvements in the percentage agreement of stage 1 (1a+1b) eggs of both target species, with agreement for mackerel increasing from 92% to 94% and agreement for horse mackerel increasing from 96% to 97%. These results are very pleasing particularly given the extremely disappointing results from the 2001 sample exchange (section 7.1).

Unfortunately, there were not many late stage eggs (stages 4 & 5) available for the analysis whilst there were large numbers of stage 1a eggs. This could have affected the results, as a participant who arbitrarily assigned all the eggs to stage 1a would have achieved a high level of agreement for that stage. It is important for future workshops that all egg stages are fully represented perhaps in the proportions expected from an average survey sample.

These results (Tables 4.2-1 to 4.2-8) certainly highlight the need to conduct regular quality assurance workshops and the very valuable benefit, which can be gained by bringing practitioners together to discuss problems and clarify procedures.

5.3 Discussion of the egg identification exercise

The problem of egg species identification was not addressed during the 2000 workshop. However, it was identified as a potential source of error, which should be addressed by this workshop, following the 2001 sample exchange (section 7.1). The eggs used for this task were the same as those used for the egg staging exercise and included 'validated' eggs (of known species) from artificial fertilisations.

The exercise proved to be extremely valuable, not least in the production of some egg identification criteria (section 3.3.2) from both published sources and from the experience gained by several participants over many years. The benefits are highlighted by the increase in the mean percentage agreement in the identification of each species (Table 4.3-1 and 4.3-2). For mackerel, the percentage agreement for species identification increased from 84% to 95%. For horse mackerel the agreement increased from 70% to 88% and for other eggs the agreement increased from 71% to 78%. This is very encouraging, given the results of the 2001 sample exchange and taking into account that the eggs were old and in some cases damaged (see 5.2 above).

The mean agreement for horse mackerel eggs improved by 18% mainly because the validated horse mackerel eggs were unusual in their appearance. Many of the participants, particularly those **not** familiar with samples from the southern area, found these eggs extremely difficult to identify in the first round of analysis. The oil globules were very fragmented and there was little sign of segmentation in the yolk. As a result of discussions, participants were made aware of these features, which seem to be more common for horse mackerel eggs from the southern area (particularly the Portuguese coast). They were then able to correctly identify these eggs in the second round of analysis.

5.4 Problems experienced

5.4.1 Problems encountered during the workshop

- The levels of agreement seen in these results (both for stage and species) are probably lower than in the analysis of real survey samples. Not only were the eggs old and some validated eggs were unusual, but also eggs were occasionally, accidentally moved from one well to another. This would obviously lead to low levels of agreement if different participants were analysing different eggs in the same wells. The eggs also became more and more damaged during the course of the two rounds of analysis as twenty participants manipulated each egg to look for the salient features.
- The stage clip on microscope/tray number 3 slipped during the first round of analysis and all the eggs were disturbed. Only six participants had looked at this tray before this happened. There were consequently several hundred fewer assessments (both of stage and species) of eggs during the first round of analysis (Tables 4.2-1 to 4.2-8).
- Discussion amongst participants was difficult to prevent whilst the eggs were being analysed. Independent assessment of the eggs is critical to prevent the introduction of bias or incorrect assignment of modal stages/species. All discussions should be reserved for the plenary sessions to enable every participant to comment fully on the features observed.
- The participants were unfamiliar with the microscopes used for the analysis. This did lead to some problems at the beginning of the analysis where the lighting on some microscopes was not adjusted to its optimum settings.

5.4.2 Problems encountered with survey samples

- Some very dark and badly preserved eggs from Portugal were available for discussion. It was concluded that the bad preservation was due to 40% formaldehyde being added directly to the plankton sample at sea. It is recommended that the standard procedure should be used (Annex 1), where 4% formaldehyde, buffered with sodium acetate trihydrate, is used to wash the plankton sample from the cod-end of the plankton sampler into the sample jar.

- Eggs can die in the process of being caught by the plankton samplers and are sometimes very dark or damaged. This is particularly the case on long deployments of the sampler (>30mins), i.e. on the majority of the tri-ennial survey tows. The eggs die in the net and osmoregulation ceases, causing discolouration of the yolk and distortion of the developing embryo. Higher than ambient temperatures also adversely affect the condition of fish eggs. The procedures described in Annex 1 are designed to alleviate (as far as practicable) any adverse effects on egg development caused by capture or preservation of the plankton samples. See papers by Cameron, Westernhagen and Dethlefsen (References, Section 8) which suggest several causes of malformations in fish eggs.
- Parasites can also cause abnormal development in fish eggs. Mackerel eggs are particularly prone to *Ichthyodinium chabaudi* infections in the southern area (Meneses *et al*, 2003). Once infected the embryo usually becomes distorted and dies.

6 RECOMMENDATIONS

- a) It is almost impossible to organise and run workshops such as this without some financial assistance. Standardisation of procedures and techniques is a requirement of all ICES working groups and is recognised as being vitally important. However, without access to central financial resources, each participant is wholly reliant on funding from their own institute for travel and subsistence. It is recommended that each institute include workshops such as this in their bid for EU Data Regulations funding.
- b) It is recommended that all microscopes at the next workshop are fitted with eyepiece graticules. These graticules should be calibrated to the same standard i.e. that one eyepiece unit (epu) should be equivalent to the same number of millimeters, regardless of microscope used.
- c) A full range of egg stages of all species, should be available for analysis during the next workshop (scheduled for 2006).
- d) The Spray technique should be used as the primary method for sorting eggs from the rest of the plankton during the 2004 tri-ennial surveys.
- e) All participants are reminded that the procedures described at Annex 1 should be followed during the 2004 surveys. Particularly that 4% formaldehyde, buffered with sodium acetate tri-hydrate, is the standard survey fixative and that plankton samples should never come into contact with formaldehyde of a concentration greater than 4%.
- f) It is recommended that all participants carry out artificial fertilisations of any species, which have eggs similar to those of mackerel and horse mackerel. It would be useful if egg and oil globule diameters are measured and that photographs are taken of as many stages as possible. It would also be beneficial if the eggs are preserved at various stages of development and any morphological changes noted.
- g) All participants are requested to make measurements of egg and oil globule diameters from as many mackerel and horse mackerel eggs as possible from their standard survey samples in 2004. These data would provide both temporal and spatial resolution to egg sizes, for the first time.
- h) Based on the experiences at the workshop a recommended binocular microscope should have the following features:

Options for a black or white stage plate for use with incident (top) light
 A transparent stage plate for transmitted (bottom) light
 Dark field illumination for contrast
 Adjustable brightness
 Magnification with click stops.
 A choice of 10x and 20x eyepieces
 Adjustable binocular head and ergonomic design to allow flexibility of movement.
 Adjustable focus on all eyepieces
 Calibrated eyepiece graticules
 Double (fibre optic) cold light source, with adjustable focus, to avoid shadows
 Mechanical stages to position samples easily in the field of view and to hold the samples firmly.
 Filters and polarisation

Note: The numbering of figures and tables begin at 1 for each individual working document.

7.1 Results of a comparative plankton sample sorting, egg identification and staging exercise, 2002

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Introduction

The results of an EU funded mackerel and horse mackerel egg staging workshop (ICES, 2001) showed good agreement between WGMEGS participants in the allocation of mackerel and horse mackerel eggs to the various development stages. A recommendation of this workshop was for a sample exchange to be conducted following the 2001 tri-ennial surveys. The aims were to help maintain standards in egg staging and also to address the potential problems of sorting and identification of fish eggs.

Method

Three plankton samples were selected from the English (CEFAS) survey, Cirolana 4/01 (Apr-May 2001) which contained large numbers of mackerel and horse mackerel eggs in all stages of development. The samples were relabelled (to prevent participants gaining access to the original results) and passed around each institute in turn. Standard institute protocols were employed, to sort all fish eggs from the samples (or sub-samples). These protocols varied considerably and are presented at Annex A.

CEFAS were the only participant to split the samples (using a Folsom splitter) to quarters to reduce sorting time. Most participants preferred to limit the time taken for staging the eggs by either:

a) Sub-sampling the eggs sorted, until at least 100 eggs of both target species had been identified and staged.

Or more usually:

b) Identifying the eggs, before staging sub-samples of mackerel and horse mackerel eggs.

The total numbers of fish eggs were recorded from each sample and mackerel and horse mackerel eggs were identified and counted. A minimum of 100 eggs of each species were taken at random and allocated to development stages. All the eggs sorted and staged were then returned to the samples before they were passed on to the next institute. The results were sent to CEFAS, in a standard spreadsheet format, where the data were collated and summarised.

Following completion of the comparative exercise it was decided that it might be useful for CEFAS to re-examine the samples. The purpose of this re-analysis was to discover whether significant numbers of eggs were lost during transit around Europe and to assess the physical condition of the eggs due to the frequent handling caused by this exercise. Standard CEFAS procedures were employed with each sample being re-examined by a different analyst who had NO access to any previous results. Each sample was again split and all fish eggs were removed from a quarter of the sample. The eggs were identified and staged as normal (Annex A) before being returned to the samples.

During the WGMEGS meeting in Dublin (April, 2002) there was some discussion about a quick technique for separating pelagic fish eggs from plankton samples. This involves the use of a spray to generate bubbles, which raise the majority of the plankton to the surface leaving the eggs near the bottom of the sample (see AZTI's protocol at Annex A). This technique was also employed by CEFAS, as a re-analysis on a different quarter of each sample, as a comparison between the 'standard' method and this newer, quicker sorting method.

Results

International comparisons

A brief presentation of the incomplete results was given at the WGMEGS meeting, held in Dublin in April 2002 (ICES, 2002). These preliminary results showed large differences between institutes in the total numbers of fish eggs present in each sample and in egg species identification. There were also differences in the allocation of the eggs to each of the development stages. These discrepancies prompted much discussion and were of some concern.

The sample exchange was completed during October 2002, and the full results are presented below.

Tables 1 - 3 show the basic results, with the numbers of mackerel and horse mackerel eggs at each development stage and the total numbers of other fish eggs found in each of the three samples. The participants conducted sub-sampling in various ways in order to reduce the time taken to process the samples (see Annex A). The tables show the numbers of eggs staged by each participant from each sub-sample. The sub-sampling factors are given to enable the total numbers of eggs at each stage for each sample, to be calculated. In figures 1-8, the egg numbers have been raised to numbers per sample. In addition, egg stages 1a and 1b have been combined to be consistent with the standard estimate of egg production from the tri-ennial surveys, where the abundance of total stage 1 eggs is used.

Figure 1 shows the total numbers of mackerel, horse mackerel and other fish eggs found in each of the samples by each participant. The x-axis also shows the order in which the samples were analysed by country (institute), beginning with England (CEFAS) and ending with Spain, Basque country (AZTI). Figure 1 shows some large differences between countries which are not always easy to explain, such as:

- a) The higher numbers of both mackerel and horse mackerel eggs found by Germany in sample 1.
- b) The larger numbers of horse mackerel eggs found by Germany in samples 2 and 3.
- c) The low numbers of horse mackerel eggs and the extremely high numbers of other eggs found by Scotland in sample 3.

The (raised) numbers of mackerel eggs allocated to development stage by each country are shown in Figures 2 and 4. There are some large differences in the allocation of mackerel eggs to stage, partly due to the difference in total numbers of mackerel eggs found by each country. However, some of the biggest differences occur when one or two countries allocate stages to eggs in totally different proportions to the majority of the other countries. For instance, in all three samples the Netherlands appear to be overestimating early stage mackerel eggs (stages 1 & 2) and underestimating the numbers of eggs in the later stages of development. These differences can be seen quite clearly in Figure 4, where the mean numbers at stage and the standard deviations are plotted on the charts.

Figures 3 and 5 show the numbers of horse mackerel eggs allocated to development stage by each country. Germany found the greatest numbers of horse mackerel eggs in all three samples and this is reflected in the numbers allocated to each stage. Scotland found very few horse mackerel eggs in sample 3 and consequently allocated very few eggs to stages 1 and 2 in this sample. Again, in every sample, one or two countries allocated eggs to stages in different proportions to the rest of the participants. For example, Portugal found lower than average numbers of stage 2 eggs in samples 1 and 2 and consequently higher numbers of stage 3 eggs. In sample 3, Portugal found large numbers of stage 1 eggs and correspondingly lower numbers of stage 2.

It must be noted that the means and standard deviations presented in Figures 4 and 5 are calculated from ALL the results. It may be better to exclude some of the more obvious out-liers before calculating the means and standard deviations of each data set.

CEFAS comparisons

Once each participant had examined the samples, they were returned to CEFAS where they were re-analysed, using two different procedures a) using standard CEFAS protocols and b) using a spray to separate the eggs from the plankton (see AZTI's protocol at Annex A). The results of these re-analyses and the comparison with the original CEFAS counts are shown in Figures 6 - 8.

Once again, it is difficult to see any consistent pattern in the results. These inconsistencies are apparent even in the overall numbers of eggs found during each sorting. It could be expected that small numbers of eggs would be lost

during transit and handling and this is clearly shown in the results for sample 1 (Figure 6). Unfortunately, this does not appear to be the case for sample 2 where more eggs of each target species were found during both re-analyses. However, the results for sample 3 appear to show a significant loss (over 40%) of eggs during the sample exchange.

The subsequent staging of mackerel and horse mackerel eggs from the re-analyses and the comparisons with the original CEFAS staging are shown in Figures 7 and 8. Again, no clear pattern emerges. This could be partially due to the fact that each analysis was carried out on different sub-samples (quarters) of each sample.

Discussion

The results of this sample exchange are quite disturbing, particularly following the successful egg staging workshop held at Lowestoft in December 2000 (ICES, 2001). Large variations in all aspects of sample sorting, egg identification and staging occur between participants in the analysis of these three samples. Some of this variation can be attributed to differences in analytical procedures between institutes (Annex A), but others are more difficult to explain.

There are only a few observations, which are common across all three samples. For example, Germany found the largest number of horse mackerel eggs in all three samples, and the Netherlands consistently found larger numbers of early stage mackerel eggs compared to every other participant.

Apart from the examples given above, there does not appear to be any obvious pattern in the differences seen in the results of these analyses. This makes interpretation of the results very difficult and observation of where the most serious problems lie, almost impossible. It is also very difficult to separate the various components (sorting, identification and staging of fish eggs) of this sample analysis, in these results.

Fish eggs were generally sorted from the whole sample (all participants except CEFAS) but differences in egg identification, in sub-sampling procedures and in the criteria used for egg staging have all affected the numbers of eggs found at each development stage by each participant.

It must be noted that part of sample 1 was spilt whilst at RIVO. Although every effort was made to recover the eggs, this could have affected the results obtained from RIVO, and those of all the subsequent participants.

Inevitably, as the samples were passed from institute to institute, some eggs were damaged or lost. The numbers of eggs lost is difficult to quantify as even total fish egg numbers varied from one participant to the next. When the samples were re-analysed at CEFAS, it was noted that the sample condition had deteriorated to the point where it was very difficult to be certain of species identification and even more difficult to assign development stages to many of the eggs with any degree of certainty.

Recommendations

The results presented above for this limited sample sorting comparison has shown that substantial differences occur between WGMEGS participants, in the analysis of plankton samples. This is obviously of considerable concern and could have a significant impact on the variance in the estimate of SSB of both mackerel and horse mackerel if similar differences occur in the standard survey samples.

As no definitive, 'correct' answer is available, each participant can only be judged against the group 'mean' results. There is, of course, no guarantee that these 'mean' results are any closer to the 'correct' answer than the results from any participant. Unfortunately many of the differences observed are not consistent from sample to sample and recommendations for improvements in procedures to individual participants are therefore difficult to make. Some general recommendations may, however, help to improve the current situation before the start of the 2004 surveys.

1. One of the major differences between participants was the way in which the samples were treated during sorting, egg identification and staging (Annex A). It would be useful if these procedures were standardised across all participants, particularly the way in which sub-sampling is conducted. This would, of course, need to address a number of issues, including total volume of sample, total numbers of fish eggs and numbers of mackerel and horse mackerel eggs present in the sample.
2. ICES have suggested that data quality checks be conducted periodically for all aspects of ICES co-ordinated surveys. The last meeting of WGMEGS recommended that another egg workshop, this time to include the separate aspects of sample sorting, egg species identification and egg staging, be conducted prior to the 2004 surveys. It has since been agreed that this workshop will take place at CEFAS during October 2003.

Although the previous egg workshop (ICES, 2001) showed good agreement between participants in the staging of eggs, it is obvious from the results above that sample sorting and egg identification problems also exist and should be addressed urgently.

3. Following the second egg workshop it is crucial that further Quality Assurance exercises be conducted during the sampling season in 2004 to maintain consistency of standards between all the participants. Recommendations as to the form of these exercises should be an important part of the output from this second workshop.

Although the results of the 2002 plankton sample exchange are disappointing, it is hoped that much has been learnt and many of the potential problems can be addressed prior to the next mackerel and horse mackerel egg survey in 2004.

Table 1.

a) The numbers of mackerel eggs counted and staged by each participant in sample 1, and the total number of 'other' eggs found.

Participant	Numbers of Mackerel Eggs by stage (unraised)					Raising Factors			Total number of non-MAC or HOM eggs
	Stage 1a	Stage 1b	Total	Stage 2	Stage 3	Stage 4	Stage 5	Total Number Staged	Total Number in Sample
England & Germany (I)	3	0	3	23	43	32	1	102	408
Norway (IM)	6	3	9	38	94	30	4	155	652
Scotland (R)	7		7	11	74	52	11	175	499
Ireland (MI)	22	0	22	28	262	153	13	155	436
Netherlands (RIVO) 1			21	35	36	19	2	478	478
Netherlands (RIVO) 2			24	32	38	17	1	113	495
Portugal (IPIMAR)			22	12	228	122	33	112	496
Spain (IEO)	14	1	15	62	213	87	20	417	417
Spain BC (I)	3	0	3	18	50	27	2	397	397
								100	421
									83

b) The numbers of horse mackerel eggs counted and staged by each participant in sample 1.

Participant	Numbers of Horse Mackerel Eggs by stage (unraised)						Raising factors			Total Number in Sample
	Stage 1a	Stage 1b	Total	Stage 2	Stage 3	Stage 4	Total Number Staged	Sorting Raising Factor	Staging Raising Factor	
England & Germany (I)	11	25	36	25	40	13	114	4	1	456
Norway (IM)	29	27	56	30	64	15	165	1	4.2069	606
Scotland (R)	43		43	29	78	28	178	1	2.854	472
Ireland (MI)	152	6	158	81	216	74	529	1	2.8105	501
Netherlands (RIVO) 1			29	41	27	5	102	1	1	529
Netherlands (RIVO) 2			35	34	25	6	100	1	4.24	432
Portugal (IPIMAR)			161	37	240	67	505	1	4.17	417
Spain (IEO)	66	20	86	103	170	36	395	1	1	505
Spain BC (I)	24	7	31	24	35	10	100	1	1	395
									4.67	467

Table 2.

a) The numbers of mackerel eggs counted and staged by each participant in sample 2, and the total number of 'other' eggs found.

Participant	Numbers of Mackerel Eggs by stage (unraised)							Raising Factors			Total	Number in Sample	Number of non-HOM or MAC eggs
	Stage 1a	Stage 1b	Total	Stage 2	Stage 3	Stage 4	Stage 5	Total Number Staged	Sorting Raising Factor	Staging Raising Factor			
England & Germany (I)	8	3	11	14	11	14	7	57	4	1	228	56	
Norway (IM)	0	24	24	10	23	17	17	91	1	3.2943	300	128	
Scotland (F)	35	5	40	10	28	17	13	108	1	2.689	290	5	
Ireland (MI)	38		38	14	17	35	14	118	1	1.9154	227	207	
Netherlands (RIVO) 2	58	21	79	30	41	62	22	234	1	1	234	15	
Portugal (IPIMAR)			48	19	22	7	1	97	1	2.48	241	15	
Spain (IEO)	58	2	60	12	49	50	35	219	1	1	219	1	
Spain BC (I)	30	3	33	41	38	41	34	214	1	1	214	64	
				15	21	16	15	100	1	2.26	226	0	

b) The numbers of horse mackerel eggs counted and staged by each participant in sample 2.

Participant	Numbers of Horse Mackerel Eggs by stage (unraised)						Raising Factors			Total Number in Sample
	Stage 1a	Stage 1b	Total	Stage 2	Stage 3	Stage 4	Total Number Staged	Sorting Raising Factor	Staging Raising Factor	
			Stage 1							
England & Germany (I)	11	8	19	39	41	13	112	4	1	448
Norway (IM)	3	29	32	81	30	26	169	1	3.2943	557
Scotland (F)	21	2	23	68	46	17	154	1	2.689	414
Ireland (MI)	24		24	92	71	48	235	1	1.9154	450
Netherlands (RIVO) 2	55	6	61	169	125	101	456	1	1	456
Portugal (IPIMAR)			80	44	28	13	165	1	2.36	389
Spain (IEO)	34	4	38	114	173	83	429	1	1	429
Spain BC (I)	10	1	11	155	104	86	383	1	1	383
				41	34	14	100	1	4.35	435

Table 3.

a) The numbers of mackerel eggs counted and staged by each participant in sample 3, and the total number of 'other' eggs found.

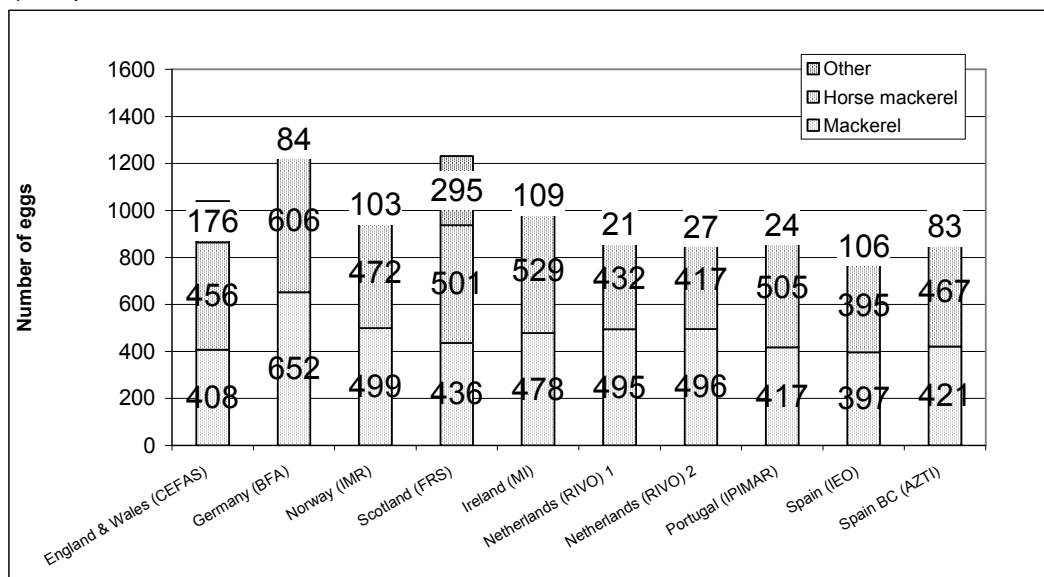
Participant	Numbers of Mackerel Eggs by stage (unraised)						Raising Factors			Total Number in Sample	Number of non-HOM or MAC eggs
	Stage 1a	Stage 1b	Total	Stage 2	Stage 3	Stage 4	Stage 5	Total Number Staged	Sorting Raising Factor	Staging Raising Factor	
			Stage 1								
England & Wales	11	1	12	5	28	16	16	77	4	1	308
Germany (IPIMAR)	1	10	11	4	17	6	5	43	1	5.9202	255
Norway (ILM)	20	3	23	10	48	18	23	122	1	2.526	309
Scotland (RIVM)	3		3	1	20	15	20	59	1	4.7425	280
Ireland (MII)	63	6	69	15	109	78	39	310	1	1	310
Netherlands (RIVO) 1			35	40	28	10	5	118	1	2.53	298
Portugal (IPIMAR)			62	31	80	64	54	291	1	1	291
Spain (IEO)	34	11	45	24	118	42	53	282	1	1	282
Spain BC (IEO)	16	3	19	5	42	15	19	100	1	2.84	284
											21

b) The numbers of horse mackerel eggs counted and staged by each participant in sample 3.

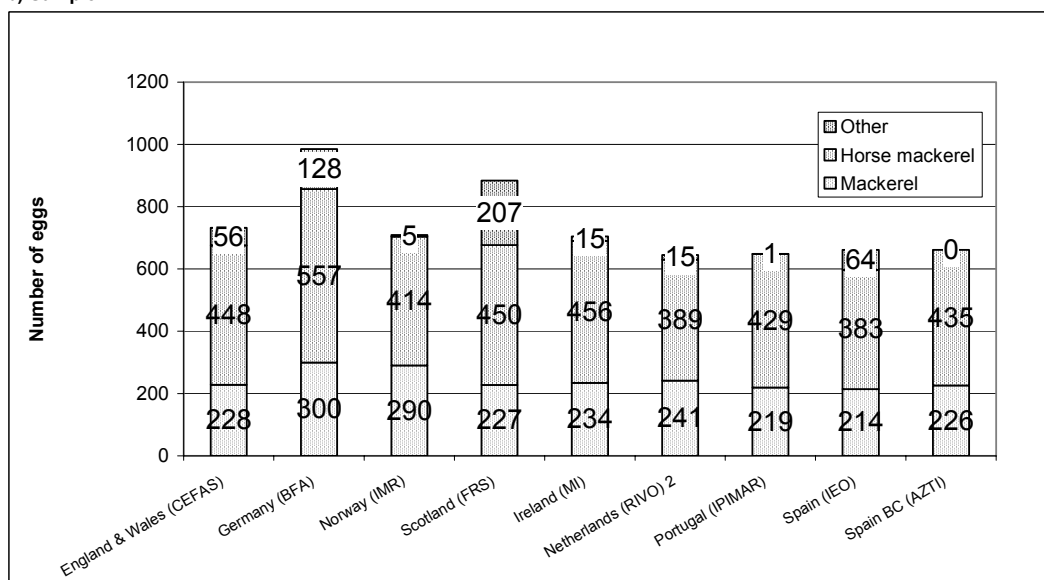
Participant	Numbers of Horse Mackerel Eggs by stage (unraised)						Raising Factors			Total Number in Sample
	Stage 1a	Stage 1b	Total	Stage 2	Stage 3	Stage 4	Total Number Staged	Sorting Raising Factor	Staging Raising Factor	
			Stage 1							
England & Wales	43	2	45	42	7	6	100	4	1.56	624
Germany (IPIMAR)	27	48	75	35	24	5	139	1	5.9202	823
Norway (ILM)	93		93	82	38	19	232	1	2.526	586
Scotland (RIVM)	3		3	7	11	8	29	1	4.7425	137
Ireland (MII)	268	14	282	168	100	70	620	1	1	620
Netherlands (RIVO) 1			80	53	18	8	159	1	3.39	539
Portugal (IPIMAR)			364	83	73	63	583	1	1	583
Spain (IEO)	177	5	182	153	91	43	469	1	1	469
Spain BC (IEO)	35	2	37	35	21	7	100	1	5.38	538

Figure 1. Numbers of mackerel, horse mackerel and other eggs counted by each participant.

a) Sample 1.



b) Sample 2.



c) Sample 3.

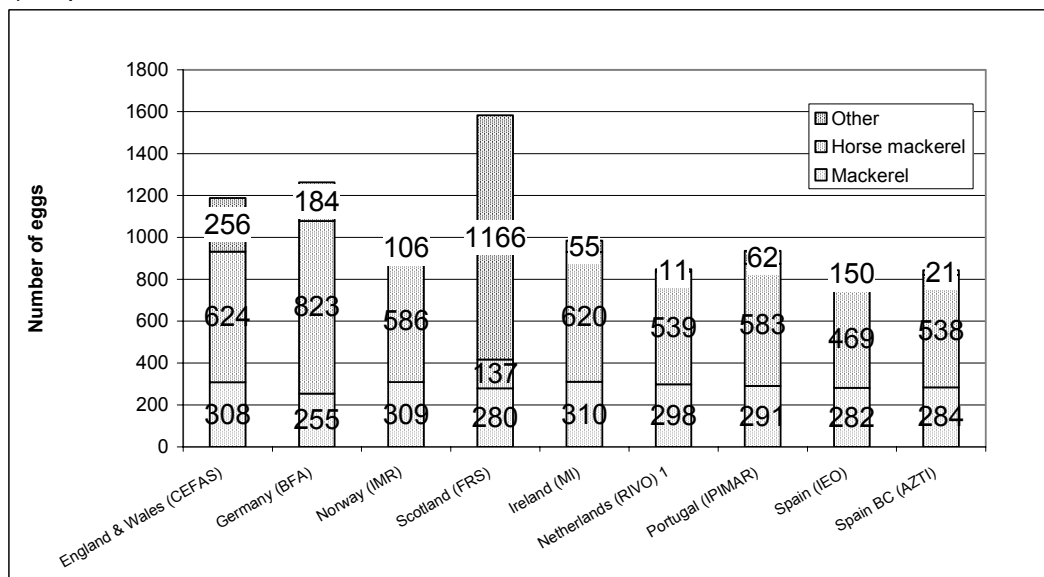
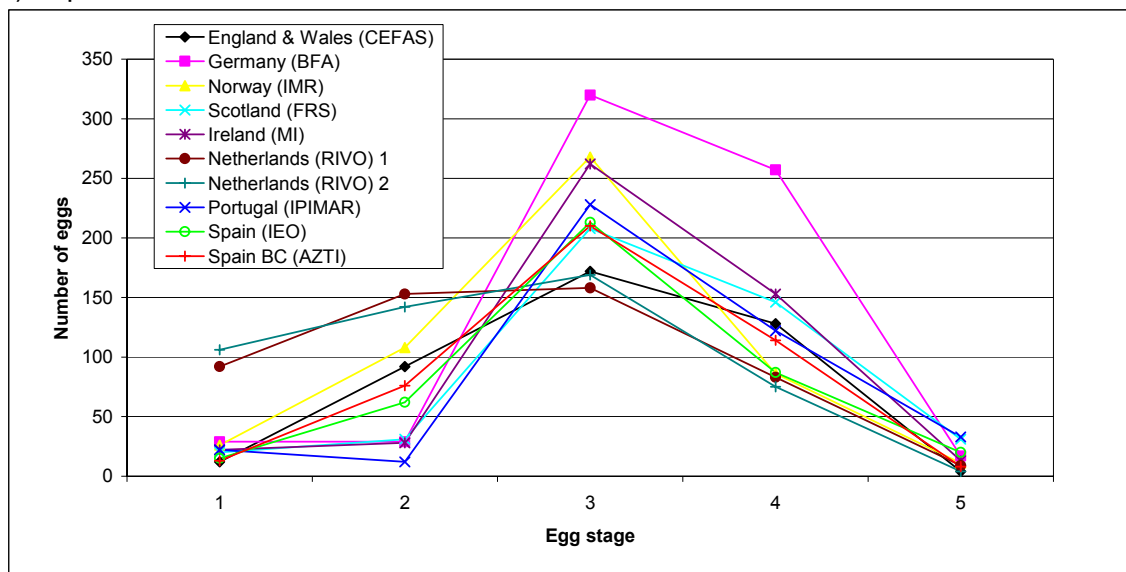
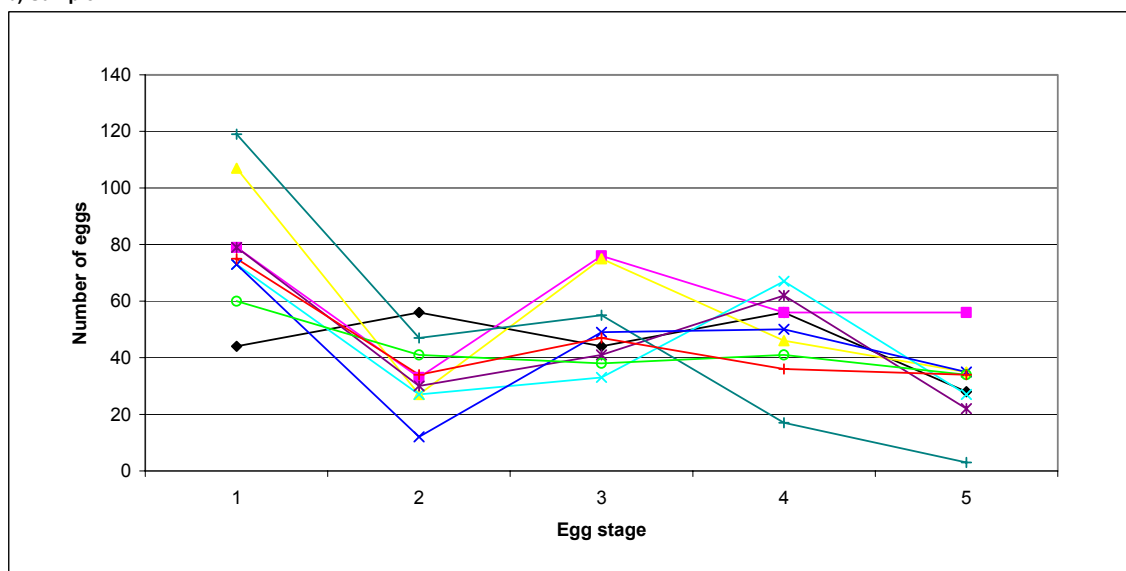


Figure 2. Numbers of mackerel eggs allocated to stage by each participant.

a) Sample 1.



b) Sample 2.



c) Sample 3.

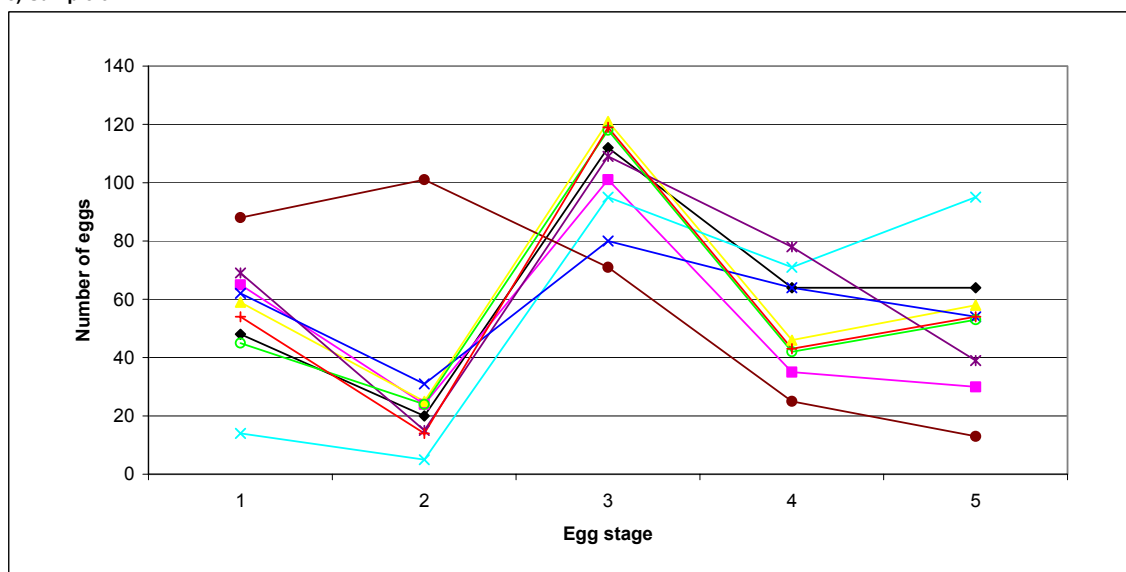
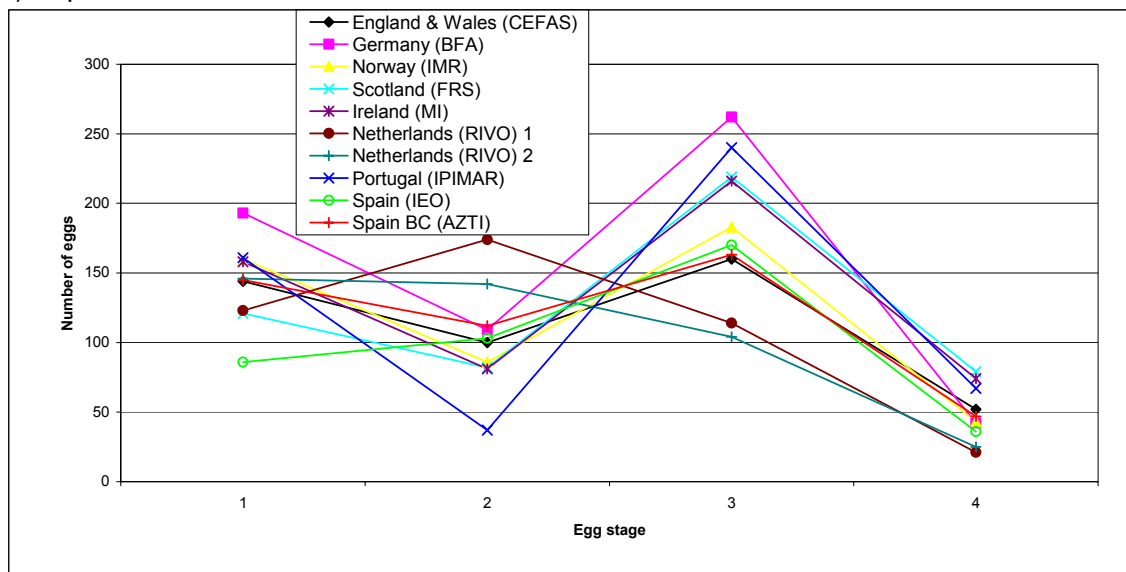
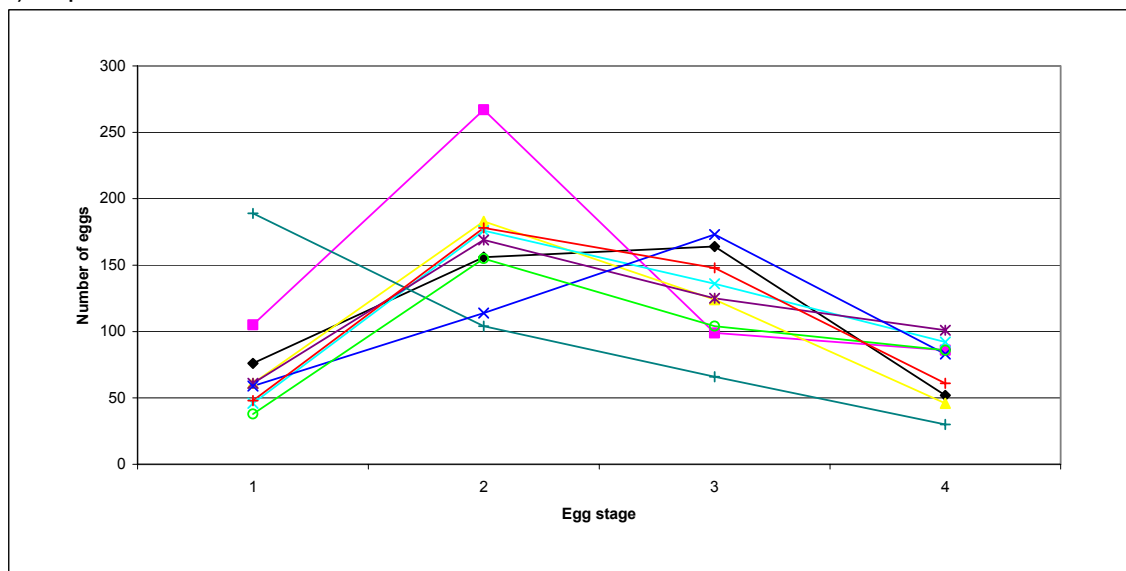


Figure 3. Numbers of horse mackerel eggs allocated to stage by each participant.

a) Sample 1.



b) Sample 2.



c) Sample 3.

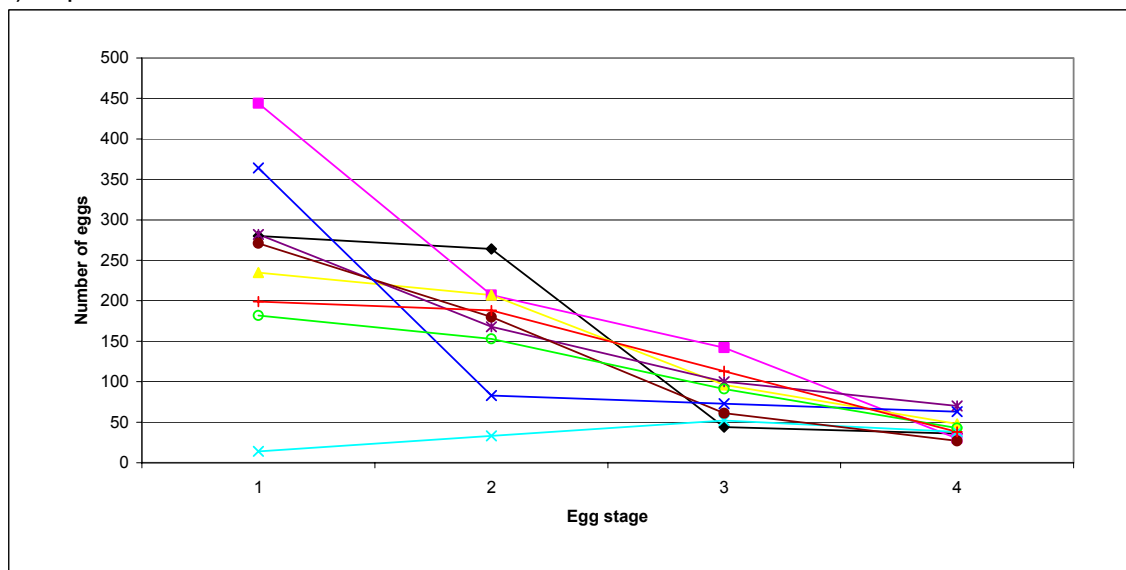
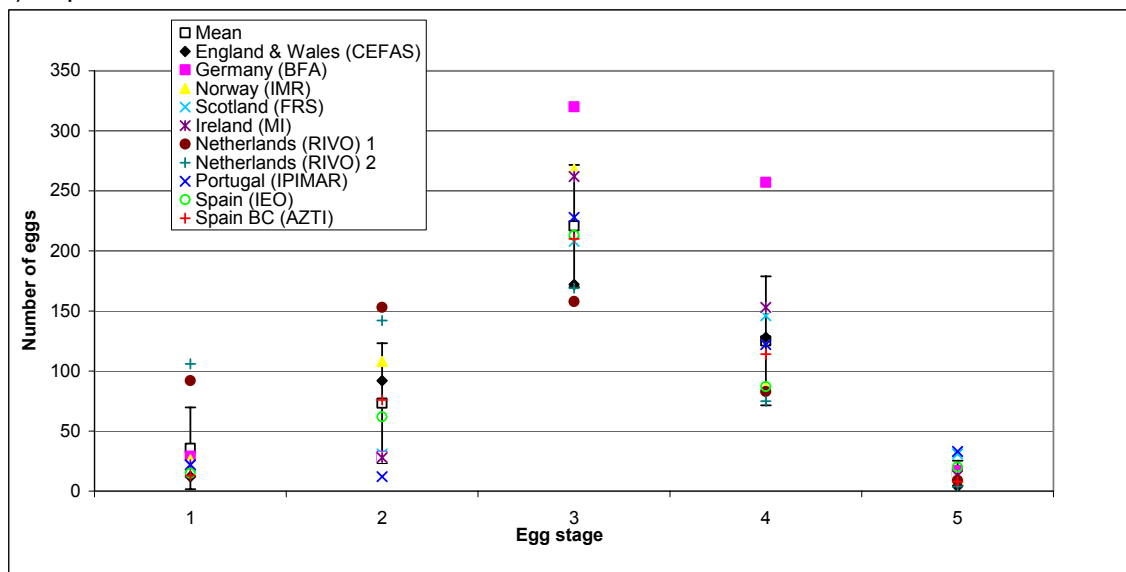
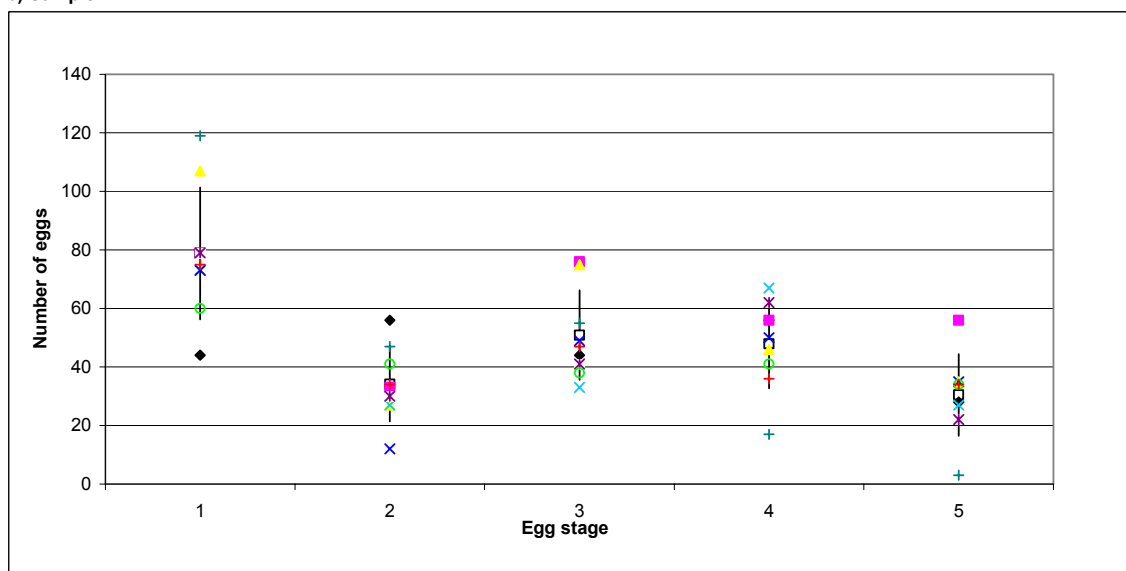


Figure 4. Numbers of mackerel eggs allocated to stage by each participant, compared to the mean and standard deviation.

a) Sample 1.



b) Sample 2.



c) Sample 3.

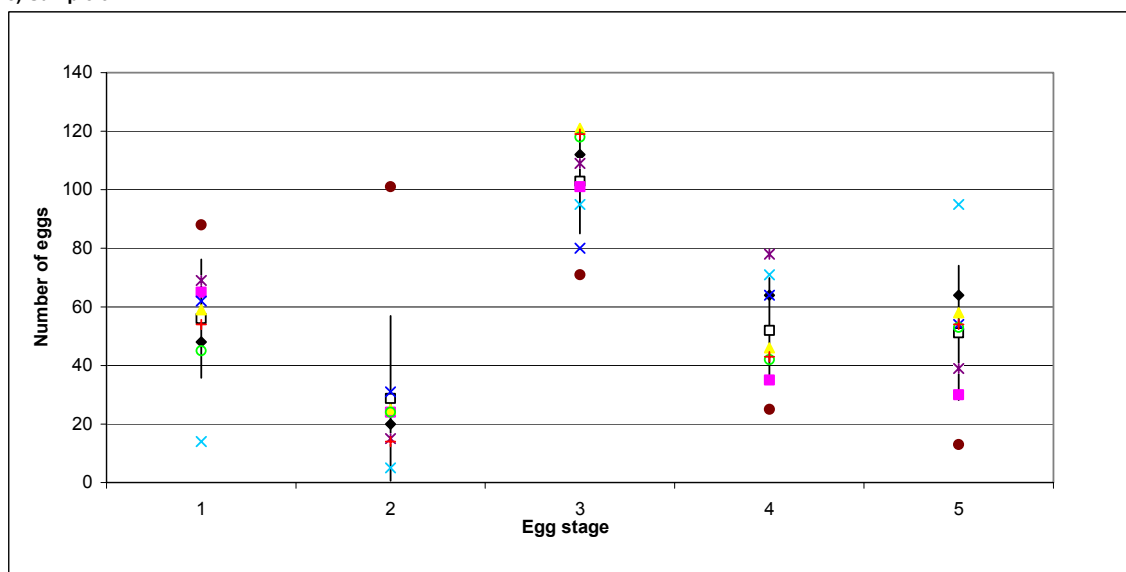
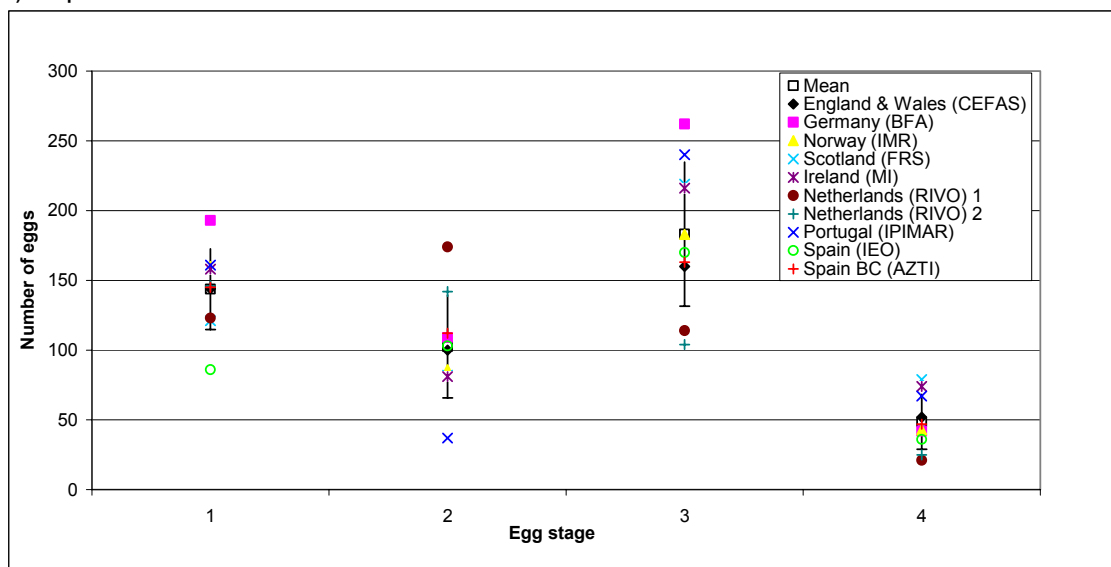
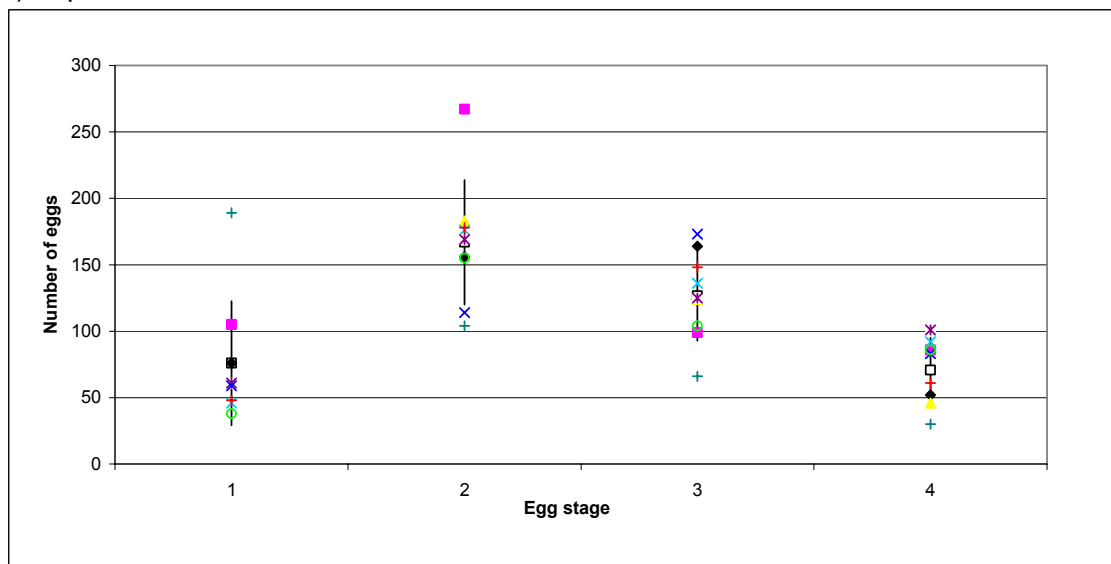


Figure 5. Numbers of horse mackerel eggs allocated to stage by each participant, with the mean and standard deviation.

a) Sample 1.



b) Sample 2.



c) Sample 3.

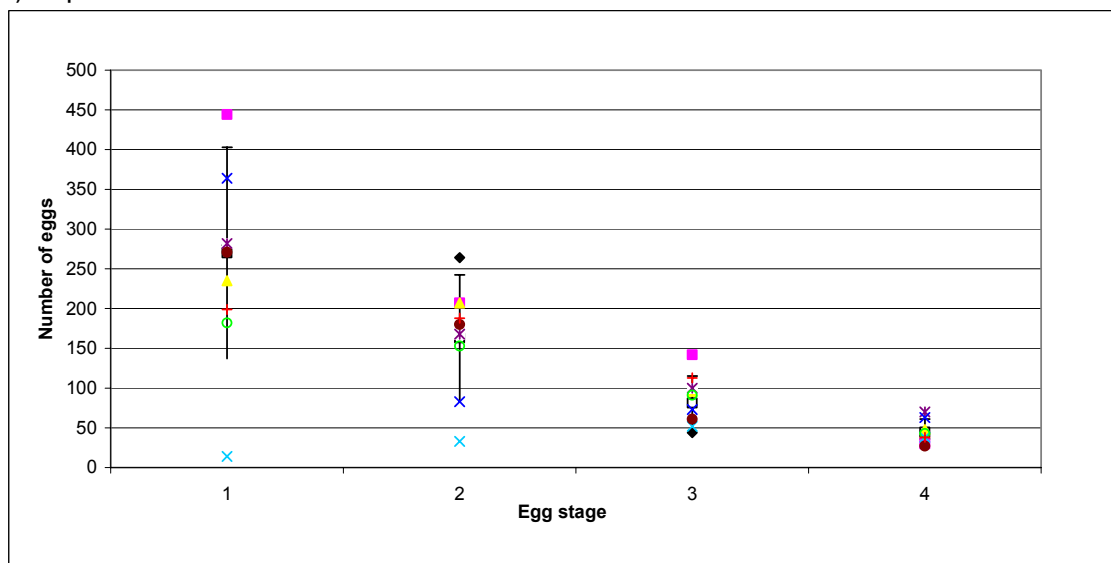
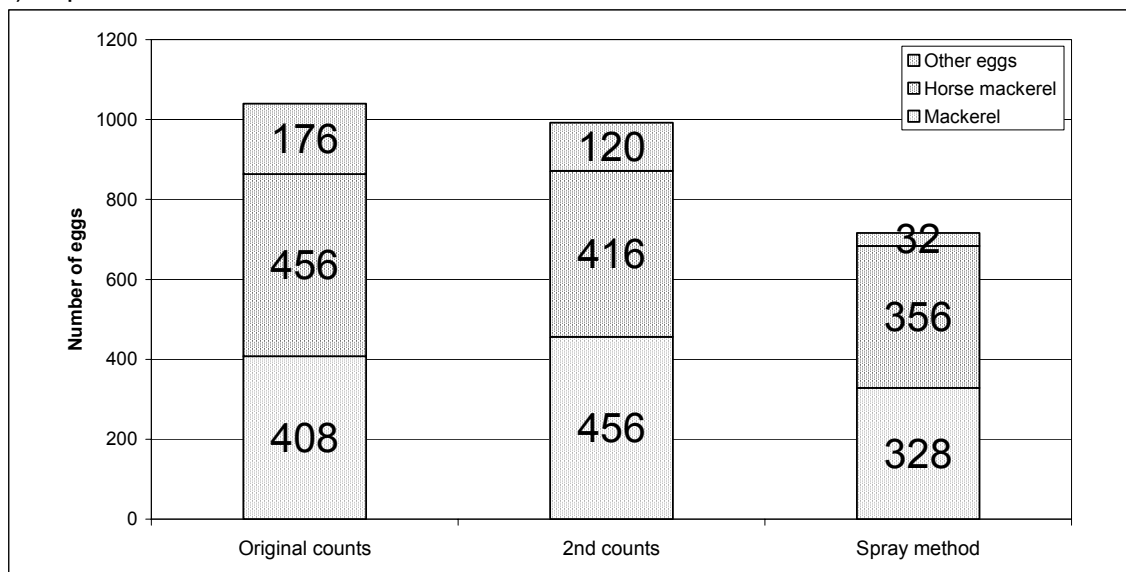
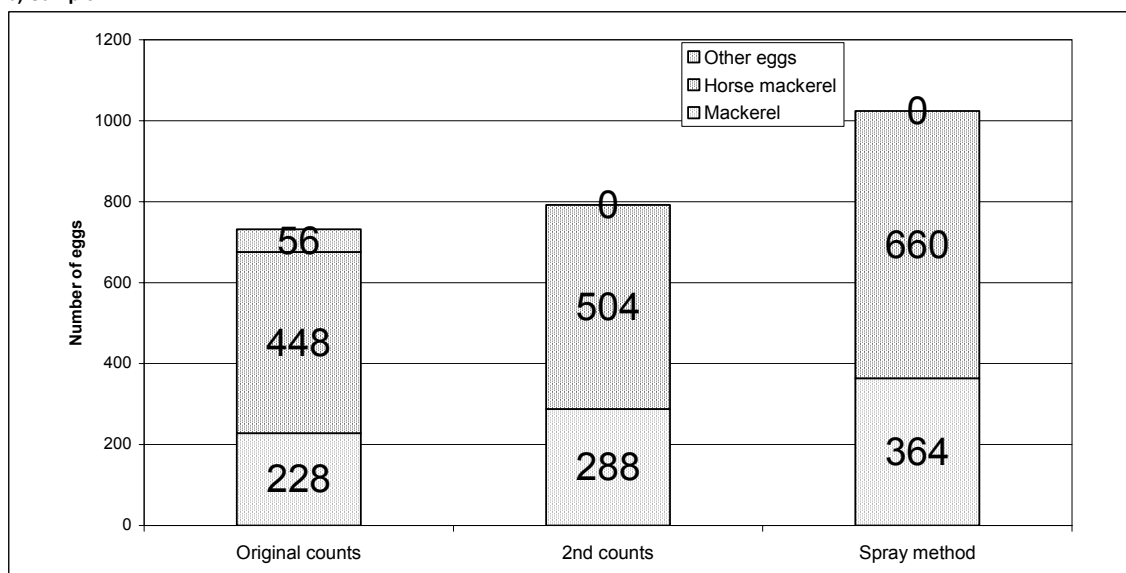


Figure 6. Number of mackerel, horse mackerel and other eggs counted by CEFAS before and after the 2001 sample exchange.

a) Sample 1.



b) Sample 2.



c) Sample 3.

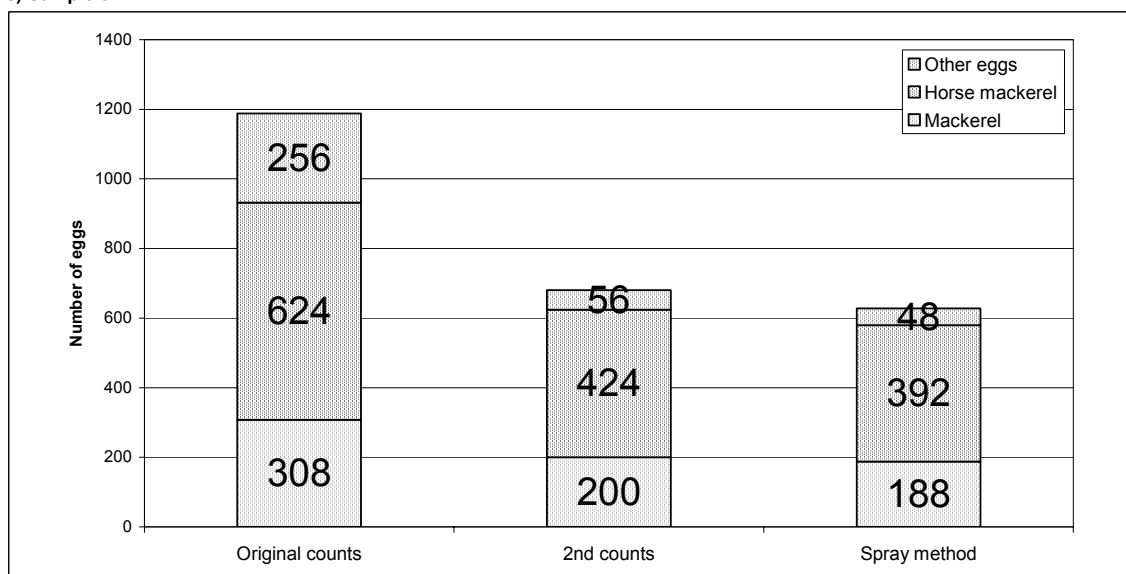
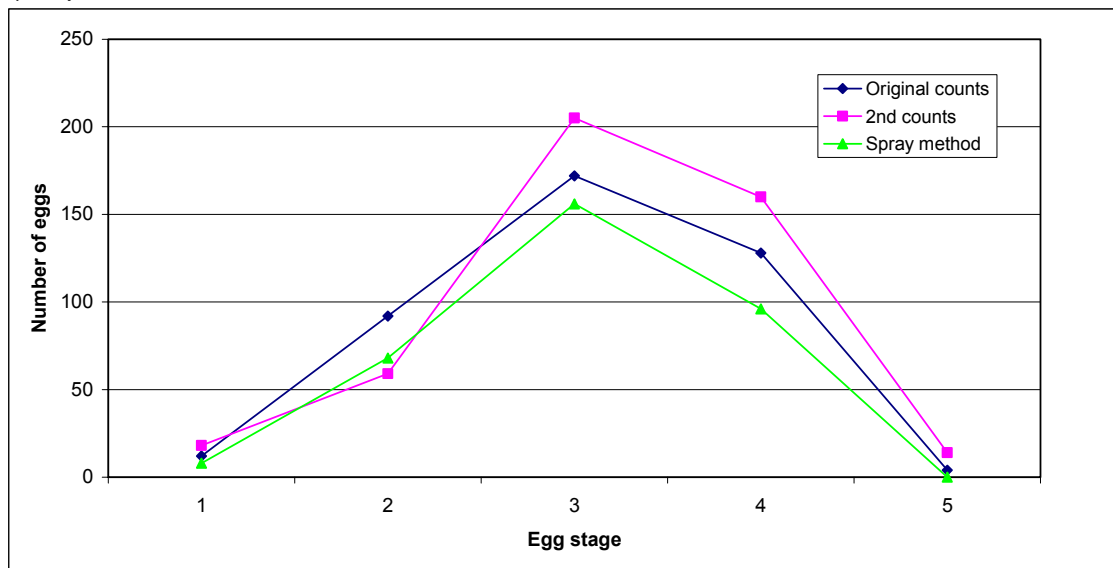
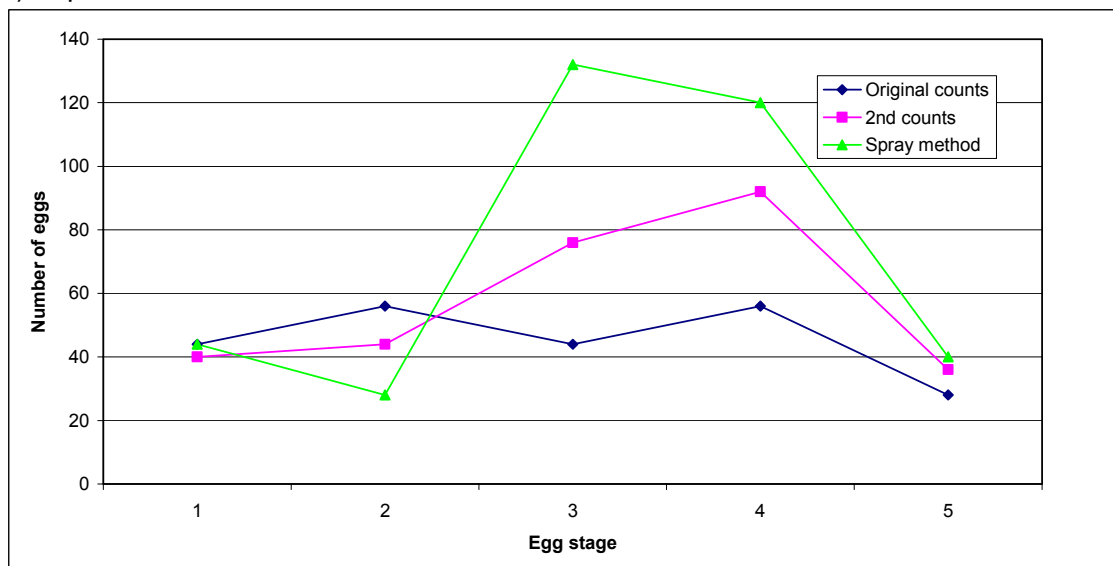


Figure 7. Numbers of mackerel eggs allocated to stage by CEFAS before and after the 2001 sample exchange.

a) Sample 1.



b) Sample 2.



c) Sample 3.

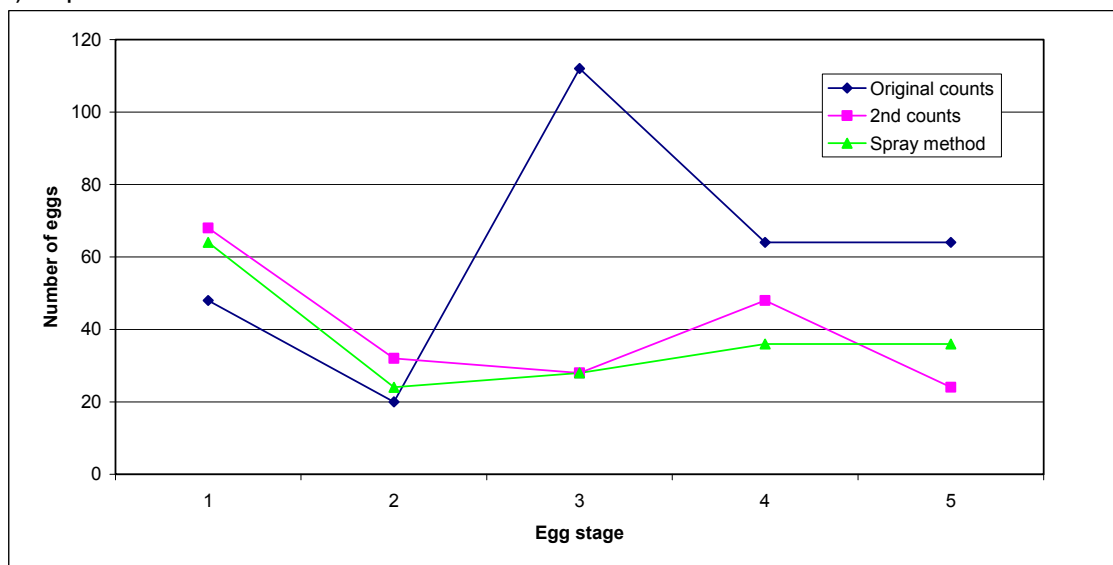
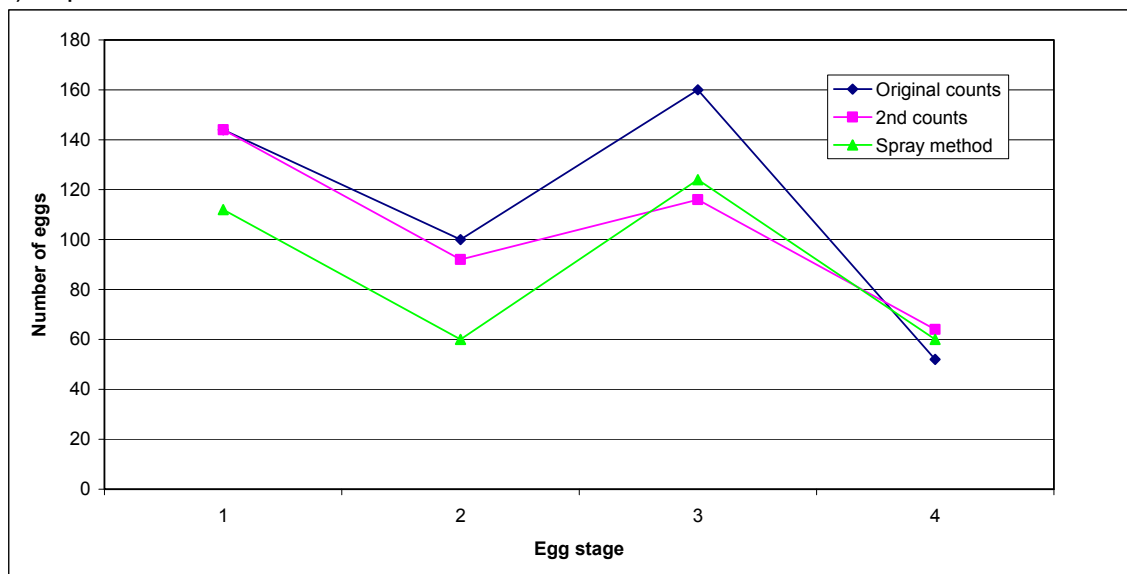
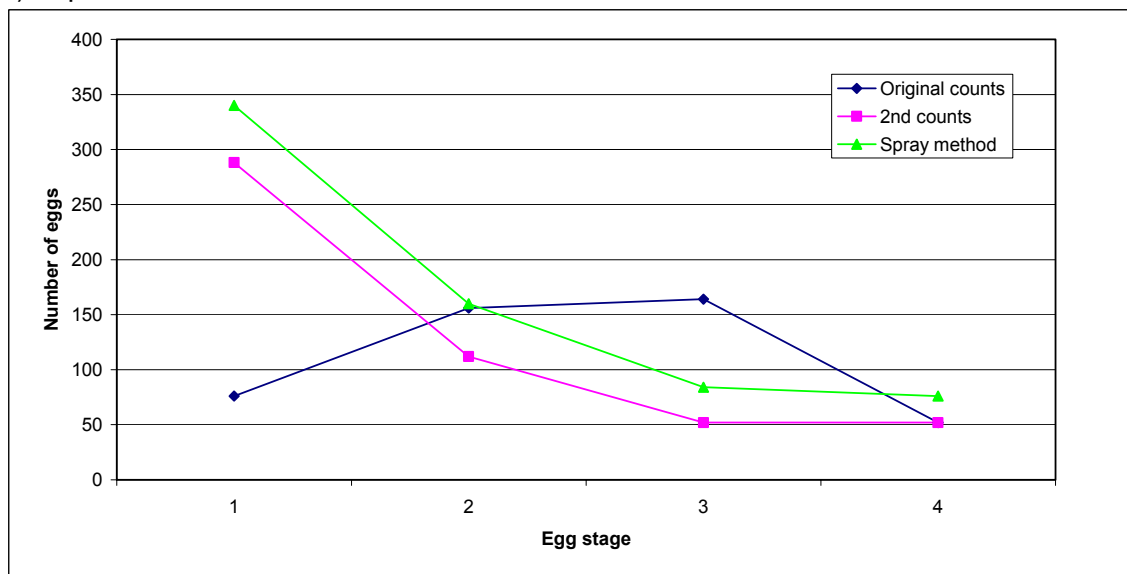


Figure 8. Numbers of horse mackerel eggs allocated to stage by CEFAS before and after the 2001 sample exchange.

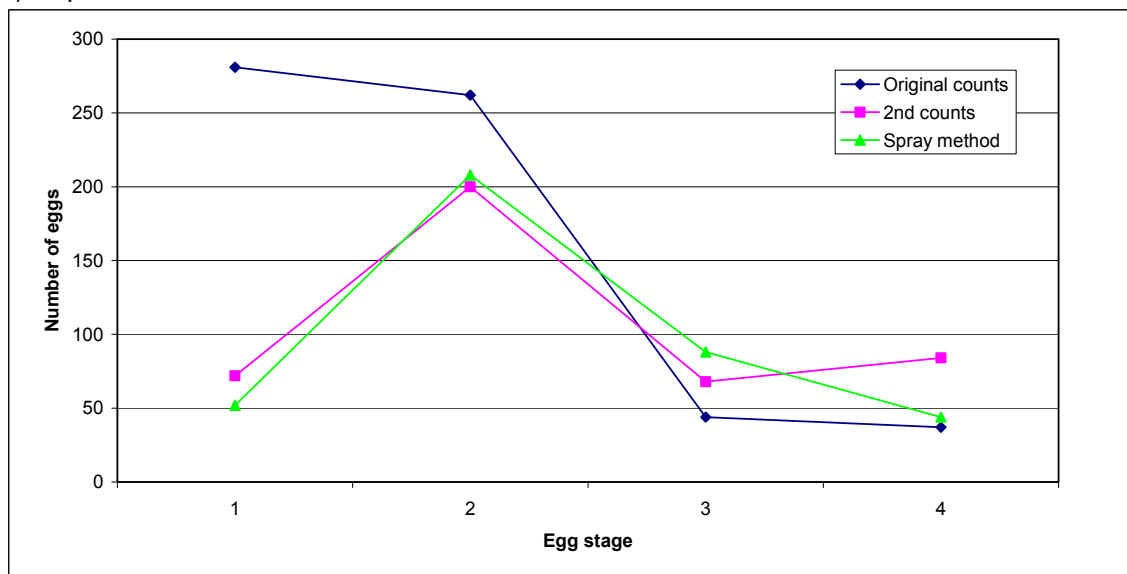
a) Sample 1.



b) Sample 2.



c) Sample 3.



Section 7.1 ANNEX A

The procedures employed by WGMEGS participants for the sorting and staging of mackerel and horse mackerel eggs from plankton samples in 2001.

All countries preserve the plankton samples in buffered 4% formaldehyde at sea (except Portugal).

England

Eggs are only roughly sorted from the samples at sea when approaching the western end of a transect. This is in order to determine the edge of the distribution before progressing to the next east/west transect. The eggs are left in the formaldehyde solution for at least 12 hours before sorting commences to allow the eggs to become opaque and easier to see. All samples are returned to the laboratory where the eggs are fully sorted, identified and staged.

If a sample contains many hundreds of eggs, (at least 400 eggs of either mackerel or horse mackerel) the sample is sub-sampled using a Folsom splitter. Small aliquots of plankton are placed under a microscope and all species of fish eggs are removed from the sample (or sub-sample). Once all the eggs have been removed from the sample (or sub-sample) they are identified to species where possible. Where it is not possible to determine the species, the egg diameters (and oil globules) are measured. Mackerel and horse mackerel eggs are staged to six (or five) development stages, respectively. A maximum of one hundred eggs of each species are staged from each sample and the rest are apportioned between the stages.

Germany

The samples are left for at least 12 hrs before sorting begins, usually on board the research vessel. To aid sorting at sea, most of the preservative liquid is drained from the samples by means of a fine gauze sieve. When most of the fluid has been drained, aliquots of the moist plankton are put on a petri dish under a binocular microscope. Mackerel and horse mackerel eggs are picked out with soft forceps, counted and placed in separate jars. The sorting of the plankton sample is repeated until no more eggs are found.

Sub-samples of eggs are taken for staging and measuring (100-200 eggs per sample maximum). The numbers of eggs at each stage are then raised to the total numbers of each species.

Norway

The plankton samples are kept in 4% formalin for a minimum of two hours before sorting at sea commences. The samples are then rinsed with seawater and small amounts of plankton with lots of seawater are placed in a petri dish on a black background prior to sorting. The seawater is changed frequently and if the sample is large it will be sub-sampled. The samples are sorted using a magnifying glass. All fish eggs and larvae are removed from the plankton and kept in separated vials. The sample is checked by repeating the sorting over a white background. Once the sample (or sub-sample) is fully sorted the mackerel and horse mackerel eggs are identified. All other species of eggs will be counted but not identified. Between 100 and 200 mackerel eggs and the same number of horse mackerel eggs are selected at random and staged. The numbers of eggs at each stage are then raised to the total numbers of each species.

All the eggs and larvae of mackerel and horse mackerel, are kept in separate tubes. The larvae of other species are also kept in a separate tube, but not identified.

Scotland

1. Plankton samples remain in 4% buffered formaldehyde for a minimum of four hours before sorting begins at sea.
2. The samples are then washed, and diluted with seawater.
3. Aliquots of the sample are placed in a sorting tray with a black base.
4. The sample is sorted with the use of a magnifier and all fish eggs are removed, counted and placed in a vial with 4% formalin.
5. The plankton sample is condensed (using a 250µm sieve) and placed back in 4% formalin. It is left for at least 12 hours before re-sorting (using the procedures 2-4 described above).

6. Once the second sort has been completed, a sub-sample of 200-400 fish eggs are identified and staged under a microscope. The numbers of eggs at each stage are then raised to the total numbers of each species.

Ireland

1. Plankton samples remain in 4% buffered formaldehyde for a minimum of one hour before sorting begins at sea.
2. The samples are then washed, and diluted with seawater.
3. The samples are then sorted under a microscope, where the eggs are removed, identified and separated into mackerel, horse mackerel and other eggs.
4. All eggs are counted and mackerel and horse mackerel eggs are staged.
5. The remaining sample is filtered and placed back into the sample jar with 4% formaldehyde.
6. The same sample is resorted after 36 hours to ensure that all the eggs are removed.

Netherlands

Eggs are only roughly sorted from the samples at sea when approaching the western end of a transect. This is in order to determine the edge of the distribution before progressing on the next east/west transect. All samples are returned to the laboratory where the eggs are sorted, identified and staged.

If a sample contains many hundreds of eggs, the sample is sub-sampled using a Folsom splitter. The eggs are sorted from the rest of the plankton using a large magnifying glass. All mackerel and horse mackerel eggs are removed from the sample (or sub-sample). Mackerel and horse mackerel eggs are staged to 5 and 4 development stages, respectively. A maximum of one hundred eggs of each species are staged from each sample and the rest apportioned between the stages.

Portugal

All samples are returned to the laboratory where all the eggs and larvae are sorted under a microscope. All fish eggs are removed from the sample and are identified to species where possible. Where it is not possible to determine the species, the egg diameters (and oil globules) are measured. Mackerel, horse mackerel, sardine and anchovy eggs are staged.

Spain (IEO)

Eggs are initially sorted from the samples at sea to obtain some preliminary results of the most important commercial species. All the samples are returned to the laboratory where they are always fully sorted (sub samples are never taken). Eggs and larvae of species of commercial interest are separated and identified to species using a binocular microscope. Both eggs and larvae are counted and placed in 4% buffered formaldehyde in separate tubes. All the mackerel and horse mackerel eggs are staged (sub samples are never taken).

Spain (AZTI)

The plankton samples are returned to the laboratory where a full sort is always carried out for both fish eggs and larvae (no sub-sampling takes place). The full sample is analysed in small portions which are placed in a divided petri dish, on a black background and a magnifying glass is used to help see the eggs. The quality of sorting is checked on 10% of the samples.

The eggs and larvae are separated, identified to species, using a binocular microscope, counted and placed in 4% buffered formaldehyde in labelled separate tubes.

If hundreds of eggs are present, a random sub-sample is taken until a maximum of one hundred eggs of both mackerel and horse mackerel are staged. The remaining eggs are apportioned between the stages.

Recent amendment to AZTI's sorting procedure

A new method for separating pelagic fish eggs from plankton samples will be used by AZTI. This method was developed by RIVO-DLO, Netherlands (A. Eltink, personal communication), and has been satisfactorily checked by AZTI.

At sea, the plankton samples are placed onto a sieve and washed with sea water directly into a beaker. A spray pump is used to 3/4's fill the beaker with pressurised water gently directed on the beaker's wall to avoid plankton damages. The pressure and the spray cause aeration of the sample. The sample is left to stand for one minute during which air bubbles become trapped in the parts of the plankton that have projections (legs, antennas etc). The aerated plankton ascends and all smooth structures including the fish eggs sink to the bottom. The floating plankton and surface liquid are then siphoned from the top of the sample using a vacuum pump, leaving the fish eggs on the bottom of the beaker. This process, is repeated 3 times removing and counting the eggs each time.

At the laboratory the entire samples are resorted using the standard AZTI protocol (described above) to ensure that no eggs are left. Once all the eggs are removed from the sample, they are identified, counted and staged in exactly the same way as described in the standard AZTI protocol.

This method dramatically reduces the time required to sort a plankton sample with large numbers of eggs.

A summary of the various procedures employed by WGMEGS participants in 2001 for the analysis plankton samples is given in the text table below.

Participant	Time in formalin before sorting	Sorting of samples			Is plankton ever sub-sampled whilst sorting?	Are eggs ever sub- sampled staging?	Are eggs identified to species before being sub-sampled for staging?
		Where	How				
England and Wales (CEFAS)	Several days	In Lab	Microscope	Yes	Yes	Yes	
Germany (BFA)	>12 hours	At Sea	Microscope	No	Yes	Yes (according to protocol). No (according to exchange results).	
Norway (IMR)	>2 hours	At Sea	Magnifier	Yes	Yes	Yes	
Scotland (FRS)	>4 hours	At Sea	Magnifier	No	Yes	No	
Ireland (MI)	>1 hour	At Sea	Microscope	No	No	N/A	
Netherlands (RIVO)	Several days	In Lab	Magnifier	Yes	Yes	Yes	
Portugal (IPIMAR)	Several days	In Lab	Microscope	No	No	N/A	
Spain (IEO)	Several days	In Lab	Microscope	No	No	N/A	
Spain (AZTI)	Several days	In Lab	Magnifier	No	Yes	Yes	

7.2 Genetic identification of fish eggs by species specific DNA markers (Addressing TOR d)

MARINEGGS is an EU funded project with the main objective of improving estimates of spawning stock biomass (SSB) by plankton survey. The aim is to develop a polymerase chain reaction (PCR) based method for the accurate identification of the early stages of the eggs of 13 commercially important marine fish species: *Trachurus trachurus*, *T. mediterraneus*, *T. picturatus*, *Macrorhamphosus scolopax*, *Scomber scombrus*, *S. japonicus*, *Gadus morhua*, *Melanogrammus aeglefinus*, *Merlangius merlangus*, *Merluccius merluccius*, *Merluccius senegalensis*, *Lepidorhombus whiffiagonis* and *L. boscii*. These species have overlapping egg sizes, spawning areas and spawning periods. Their eggs can therefore easily be confused when collected in plankton samples.

The results of MARINEGGS indicate that eggs preserved in 4% formaldehyde are suitable for genetic analyses and a routine protocol for genetic identification of preserved fish eggs is being developed. A full explanation and analysis of results from the Marineggs project will be available in the final contract report, (Genetic identification of fish eggs by species-specific DNA markers for use in stock biomass assessments and detection of commercial fraud.(QLRT-PL1999-01157)) which is due for publication in the Spring of 2004.

7.3 An image processing method for the identification of mackerel and horse mackerel eggs

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This is a short description of a PC based image processing method presently being developed at the BFA-Fi to identify eggs from a mixed mackerel and horse mackerel egg sample. It is work in progress and this presentation is intended as a starting point for further discussion, to collect opinions on the work done so far and to assess options for further development of the software.

Material and methods

The image analysis is based on the ImageJ package, which has been developed by Wayne Rasband at the American National Institute of Health as public domain software which can be downloaded from the web. The program is widely used in medical sciences around the world. As a Java-program it is compatible with all platforms and operating systems. The program is free-ware and the source-code is available for free as well. An extensive documentation and a lot of plug-ins are available for download at <http://rsb.info.nih.gov/ij/>. At present the BFA-Fi's Institute for Sea Fisheries in Hamburg is developing a plug-in for the identification of mackerel and horse mackerel eggs which can be separated by the different egg and oil globule diameters. Our short term aim was to automatically detect and measure the egg and oil globule diameter to facilitate an identification of the species at a later stage.

Results and future prospects

The analysis program can only search for egg and oil globule outlines in a defined region. So the prerequisite for the location and correct measurement is the pre-sorting of the single eggs into a prepared dish with small cavities. So far the plug-in could only be tested on a mosaic composed of images with single eggs as a model of a pre-sorted dish (Fig.1). The reliability on the recognition of the egg and oil globule outlines is depending on the image quality. The hit rate for a correct egg outline is app. 90 percent, and 50 percent for getting the correct oil globule outline. Incorrectly recognised outlines can be adjusted manually. Automatic species identification in the resulting database is presently under development.

Procedure

1. Take a digital image of the sample of eggs in a standardised dish
2. Open image file in ImageJ(Fig 1)
3. Set scale for calibration
4. Start Hough Transform (Fig 2)
5. Check the outlines of eggs and oil globules (Fig 3)
6. Correct the outlines where necessary
7. Start measurements (Fig 4)

In future it will be possible to insert the egg stages into the result table and to export the whole table to Excel.

For the time being this is work in progress and any comments and helpful suggestions are highly welcome. After the final completion the program will be free for distribution to all workshop members.

E-mails to juergen.schlickeisen@ish.bfa-fisch.de
jens.ulleweit@ish.bfa-fisch.de

or

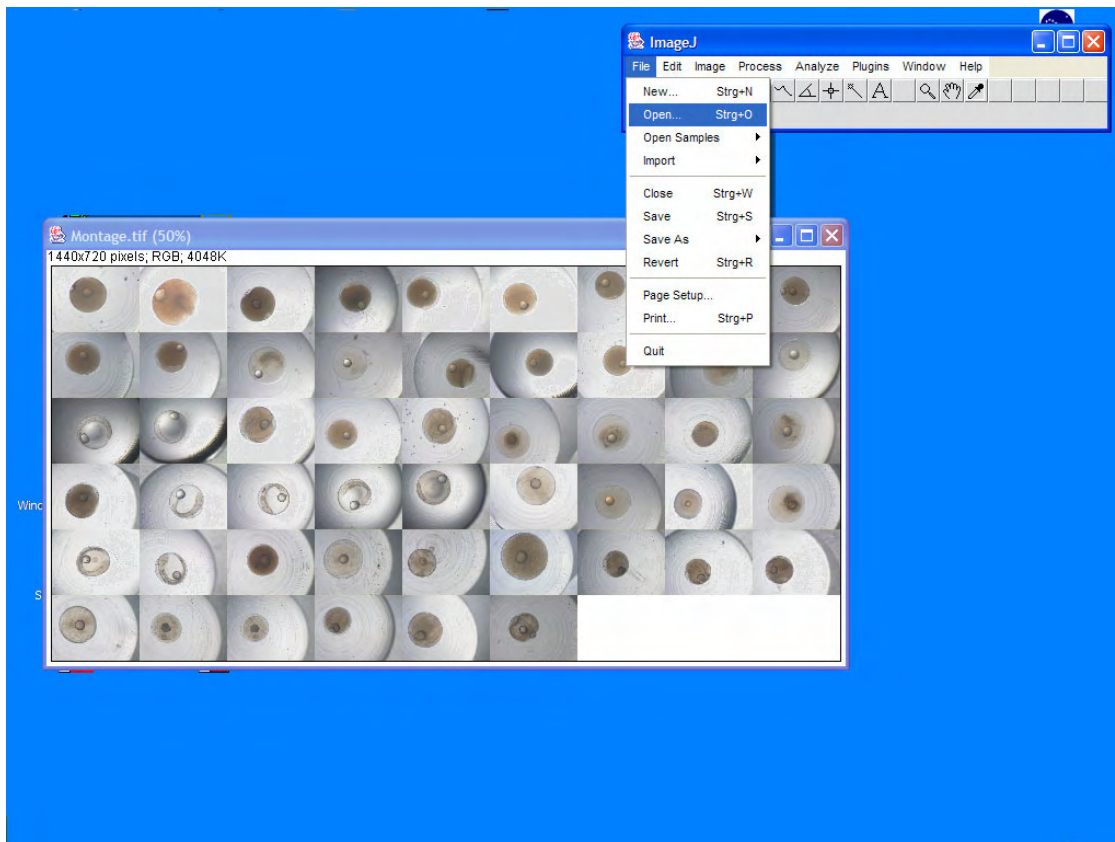


Figure1: Opening the image file

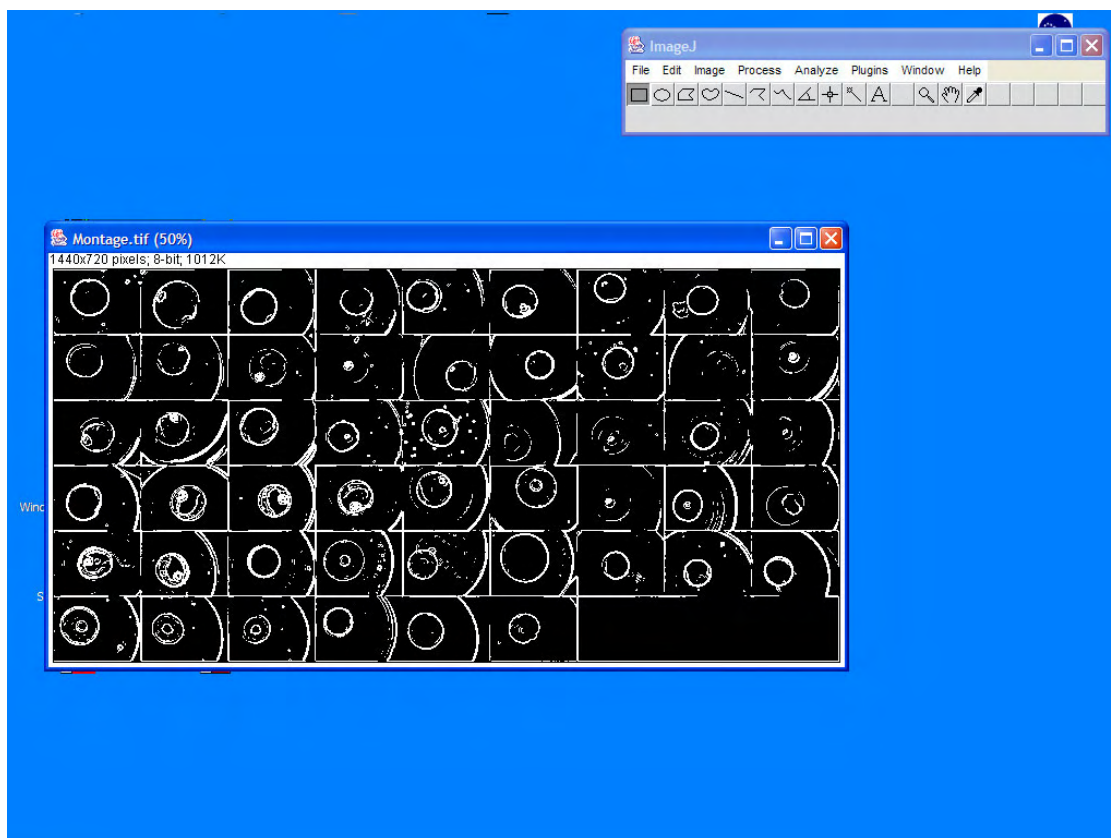


Figure 2: Processing of the image during Hough Transformation



Figure 3: Checking the egg and oil globulus outlines

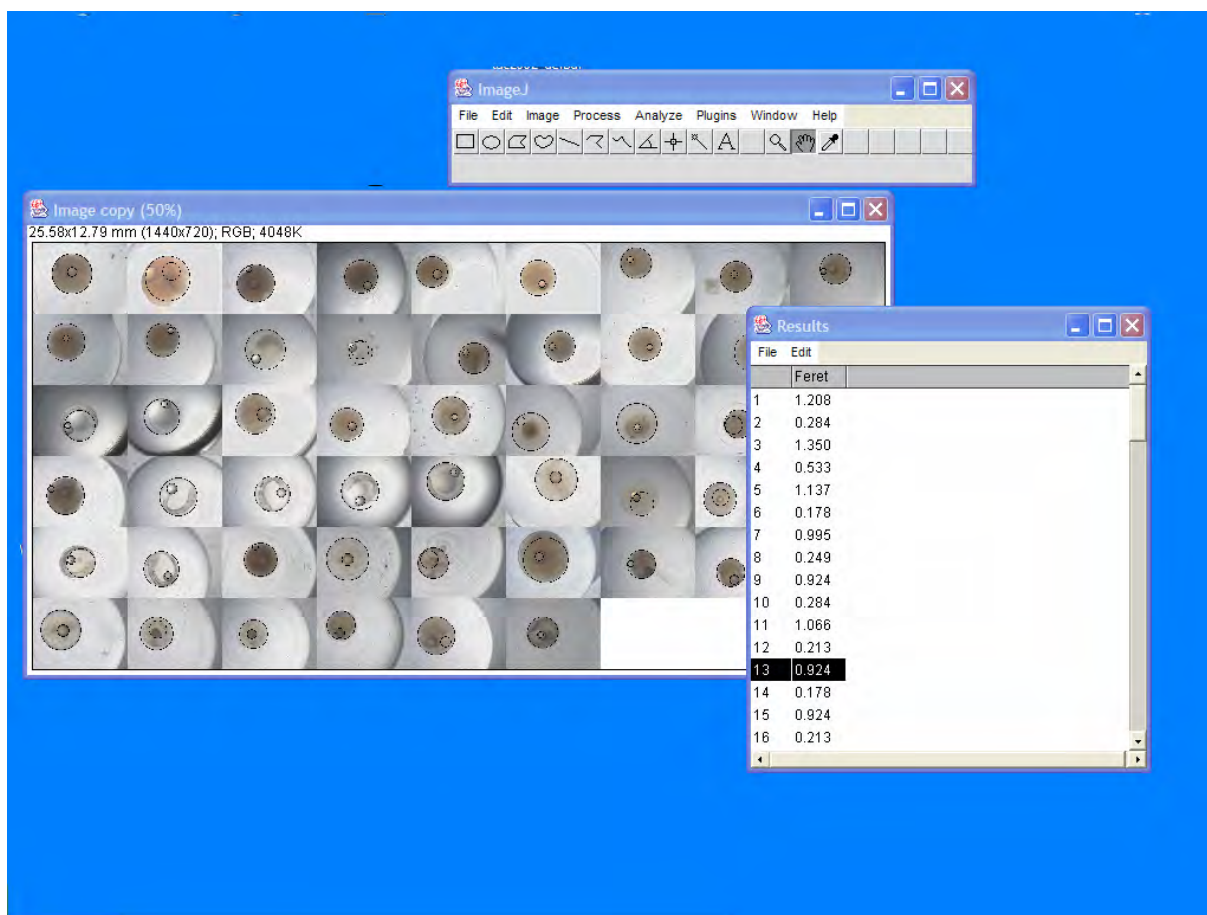


Figure 4: Recording of the results

8 APPENDICES

8.1 A plankton sampling manual for use during the ICES tri-ennial mackerel and horse mackerel surveys

S.P.Milligan

Sampling strategy, gear and procedures

The report of the mackerel, horse mackerel egg production workshop (WGMEGS, ICES, 1994, section 8) contains a manual for the conduct of egg surveys, targeted at the annual egg production method (AEPM). Those instructions are repeated in ICES 1997 (Sections 6.4.1 to 6.4.8) and incorporate changes, additions or clarifications, which are underlined.

This manual is designed to enhance those described above by providing a protocol on the collection and handling of plankton samples at sea and the processing of samples in the laboratory. In addition, the 1998 WGMEGS samples were used to fully describe the distribution and abundance of **all** species of fish eggs and larvae occurring throughout the sampling area at that time. The EU, in a project (97/017) designed to produce '*Ichthyoplankton-based indices of spring spawning commercial fish populations in Western European waters*' (INDICES), provided funding for this analysis. This manual encompasses the full analysis completed for the INDICES project.

Sampling areas and sampling effort

The spatial and temporal distribution of sampling is designed to ensure an adequate coverage of both mackerel (*Scomber scombrus* L.) and horse mackerel (*Trachurus trachurus* L.) spawning. Sampling effort is targeted at producing estimates of stage 1 egg production for both species.

The north-east Atlantic shelf area is sub-divided (by the WGMEGS) into 'western' and 'southern' areas for the purposes of estimating spawning stock biomass (SSB) of mackerel and horse mackerel. The 'southern' area is regarded as being from 36° N to 45° N. It includes southern Biscay, the Cantabrian Sea and from the Portuguese coast to 11° W. Sampling usually begins in January in this area and continues until June in the Cantabrian Sea.

The 'western' area is from 44° N to 58° N. It includes Biscay, the Celtic Sea and the shelf edge to the northwest of Scotland. Sampling is focussed along the shelf edge (200m contour) but also occurs from the French and Irish coasts out to 16° W. Sampling in this area usually begins in March and continues into early July.

Plankton samplers are deployed at the centre of half standard ICES rectangles which are 0.5° latitude by 0.5° longitude in the western area and to the west of Portugal. To the north of Spain (Cantabrian Sea) and to the south of Portugal the sampling rectangles are 0.25° latitude by 1° longitude because of the proximity of the shelf edge to the coast.

Sampling gear

The standard samplers acceptable for use on these surveys are national variants of the Gulf VII or Bongo high-speed plankton samplers (Nash *et al.*, 1998). These samplers generally incorporate conductivity, temperature, and depth probes (CTD's), and either contain mechanical or electronic Flowmeters to enable the volume of water filtered on each deployment to be calculated. These sensors either relay 'real time' environmental data back to a shipboard computer display or log the information ready for downloading once the station has been completed.

Although a mesh size of 500µm aperture is adequate for sampling mackerel and horse mackerel eggs, a nylon mesh with an aperture between 250µm and 280µm is recommended for these surveys. This allows the plankton samples to be more widely used for investigations on other species and taxa. If serious clogging occurs then a change to a 500µm aperture mesh can be made (this change has only rarely been made on any of the surveys).

The aperture on the Gulf VII type samplers should be 20cm diameter in order to ensure that an adequate volume of water is filtered. This ensures that the eggs of other species are quantitatively sampled, in particular the eggs of hake (*Merluccius merluccius* L.), which may be present at lower densities than those of mackerel or horse mackerel.

The aperture for the Bongo samplers should be either 40cm or 60cm diameter.

Sampler deployment

The Gulf VII sampler is deployed on a double oblique tow, at 4-5 knots, from the surface to within 3 metres of the bottom (or as near as bottom topography will allow) and return to the surface. The Bongo samplers are towed at 2-3 knots. Maximum sampling depth is restricted to 200m in deeper water off the shelf edge. In the presence of a thermocline greater than 2.5°C in 10m depth, sampling can be confined to a maximum depth of 20 m below the base of the thermocline.

The requirement is for an even, 'V' shaped dive profile, filtering the same volume of water per unit of depth. The aim is to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band.

At shallow stations, multiple double-oblique dives may be necessary to enable a sufficient volume of water to be filtered. A minimum sampler deployment time of 15 minutes is recommended.

Target Species

The WGMEGS tri-ennial sampling programme is targeted at mackerel and horse mackerel. An egg production estimate is calculated for both species in both the western and southern areas. In addition an egg production estimate for mackerel is calculated for the combined North East Atlantic area.

In 1998, an EU funded project (97-017) known as INDICES used the plankton samples collected to describe the distribution and abundance of all fish eggs and larvae. Seven commercially important target species (including mackerel and horse mackerel) were selected as suitable for further data analysis including geo-statistical techniques. These species (blue whiting, *Micromesistius poutassou* (Risso); hake, *Merluccius merluccius* (L.); megrim, *Lepidorhombus whiffiagonis* (Walbaum); sardine, *Sardina pilchardus* (Walbaum) and anchovy, *Engraulis encrasicolus* (L.) were all known to spawn in the same area and at the same time as mackerel and horse mackerel.

Plankton sample handling and processing

Sample fixation

It is recommended that the standard plankton samples collected for the SSB estimate will be analysed onshore and should be treated carefully when being fixed at sea. The procedure will be as follows:-

- a). Remove the end bag used on the station before washing down the net.
- b). Attach a clean end bag and **gently** wash down the net from both ends of the sampler, taking care to wash the lower surface of the net just in front of the end bucket.
- c). Always wash down from the nosecone end last.
- d). Make sure the net is clean, using more than one end bag if necessary.
- e). Make doubly sure that a clean end bag is left on the sampler ready for the next station.
- f). Wash the plankton from the end bags into a jar with the 4% formaldehyde solution in a wash bottle.
- g). Top up the jar with 4% formaldehyde, making sure that the volume of plankton does not exceed 50% of the volume of the jar.
- h). Any excess sample should be fixed separately in additional jars.
- i). Put labels containing station details in pencil into all jars.

The standard fixative for use on these surveys will be a 4% solution of buffered (pH 7 - 8) formaldehyde in either distilled or fresh water. (250g of sodium acetate trihydrate is dissolved in 10 litres of 30% formaldehyde to make a buffered stock solution. The stock solution is then diluted to 4% using distilled water). This solution is approximately iso-osmotic with seawater and will minimise damage and distortion of the eggs. The sample should be directly fixed

with the addition of the 4% formaldehyde solution and should not come into contact with formaldehyde strength in excess of 4%.

Sample sorting

Prior to sorting the plankton samples it is recommended that a small quantity of red, borax carmine, stain is added to the samples (Nichols, 1976). A few drops of this stain in each sample jar will stain most of the zooplankton (including fish larvae) a pink/red colour, but fish eggs will remain virtually unaffected. This technique makes sorting of the eggs much easier, and, done lightly, does not impair subsequent identification or staging. The samples should be stained for at least a day before being analysed.

Immediately before analysis it is recommended that the 4% formalin is drained from the sample and the sample washed gently with seawater. The sample can then be placed in a sorting/observation fluid (Steedman, 1976) which also acts as a preservative. The observation fluid stock solution is made with 50ml of propylene phenoxetol mixed with 450ml of propylene glycol (propane-1,2-diol). Before use, 5ml of the stock solution is diluted with 95ml of distilled water to produce a sorting fluid which is non-toxic and pleasant to use (odourless).

Whenever practicable the whole sample should be sorted in order to remove all the eggs of species, which may be present in low densities. All sorted eggs should be kept in tubes, in, 4% buffered formaldehyde, inside the sample container for future reference and use. Usually only the eggs of mackerel and horse mackerel need be identified to species and staged. However, in 1998, all fish eggs and larvae were identified to species where possible (for the INDICES project). The eggs of six of the seven target species were staged (Blue whiting eggs are not distinguishable from those of other species). The standard lengths of larvae of all the target species were measured.

Species identification

The eggs and larvae of most of the species found in the area are well described by Russell, 1976. This book is well known and used by all the participants of the ICES tri-ennial surveys. It is generally regarded as the definitive work on the subject in this area.

Some difficulties do occur, particularly with the identification of fish eggs, which do not show great differences in their morphological features. In some instances it is even difficult to recognise 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 (*Molva molva* L.) 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. However, some problems were experienced when using the SAT technique on fixed eggs during the INDICES project. The results were not always consistent and re-analysis of some samples became necessary.

Identification of fish larvae is not so much of a problem. However, some problems do occur where species are closely related e.g. Sprat (*Sprattus sprattus* L.), sardine, and anchovy, where further detailed analysis such as myotome counts may be required.

Positive identification is wholly reliant on the skills and experience of the analysts involved in processing the samples. Samples of mackerel and horse mackerel eggs are regularly passed between participants to help to standardise the identification criteria. In the INDICES project some samples were processed by at least two laboratories and in general a good level of agreement was reached.

Egg staging (ageing)

Within WGMEGS the eggs of mackerel are classified into one of five morphological stages (I, II, III, IV and V) (Lockwood *et al.*, 1981), following the development criteria described for plaice (Simpson, 1959). For horse mackerel the description of stages is the same with the exception of stage V which does not exist. Horse mackerel larvae hatch at the end of egg stage IV (Pipe and Walker, 1987). In the INDICES project, hake and megrim eggs were staged using the same criteria as that for mackerel. The eggs of pilchard and anchovy were both classified to eleven development stages using the descriptions provided by Ahlstrom (1943) and Moser and Ahlstrom (1985) respectively.

To help with consistency of staging, samples of mackerel and horse mackerel eggs are routinely passed between all participants of WGMEGS. Some discrepancies in the allocation of eggs to stages are always apparent and it is hoped that the situation can be improved by the setting up of regular ICES identification and staging workshops.

For the estimation of daily egg production for both mackerel and horse mackerel, only the counts of stage I eggs are used. This is recognised as a conservative estimate of the total eggs spawned because of mortality which occurs during development. However, until there is consistency in the identification of the other stages, between all countries, the other stages cannot be used for the estimation of mortality rates and backtracking to total eggs spawned.

Measurement of Larvae

Within INDICES the standard lengths of larvae of the seven target species were measured. The larvae were then assigned into one of three length classes according to the following morphological characteristics:

1. Length at hatching to yolk sac absorption.
2. Length at yolk sac absorption to post-larvae with recognisable features such as fins, characteristic pigmentation, spines etc.
3. Late post-larvae with additional physical characteristics such as fin rays, enhanced pigmentation, urostyle development etc.

This size categorisation was to overcome difficulties with different growth rates between species where the measurement of standard length might not reflect the overall pattern of larval growth.

Sub - sampling

Wherever practicable the whole plankton sample should be sorted. However, where large numbers of eggs and larvae occur in plankton samples it becomes impractical to sort the total sample. The recommended method for sub-sampling is by using a Folsom splitter. In this way, samples can be sub-divided repeatedly to achieve the optimum sampling level. It is recommended that 100 eggs of the target species (mackerel and horse mackerel) are present in the sub-sample. If more than 100 eggs of these species are sorted from the sample (or sub-sample) then only 100 need to be staged and the rest apportioned across the stages found in that particular sample. If 100 eggs of the target species are **NOT** found in 25% or less of the sample then the whole sample will have to be sorted.

In some samples the eggs of only one target species may be present in large numbers. These eggs can be sub-sampled using the procedure above, whilst the eggs of the other species should be sorted from at least 25% of the sample.

Where extremely large numbers of eggs of other (non-target) species occur or where the total plankton volume is large, it may be impractical and time consuming to sort through the whole sample for a few eggs of the target species. In these cases it may be practical to only sort 25% of the sample.

Fish larvae can be sub-sampled completely independently of the eggs if required. It is recommended that a minimum of 100 fish larvae of one species in a sub-sample would be required to provide a length/frequency distribution. All fish larvae sorted from a sub-sample should be identified.

Estimation of daily egg production

To convert abundance of eggs into daily egg production, data on the rate of development is required. For mackerel the relationship between egg development rate and temperature was described by Lockwood *et al.*, (1977, 1981a). This has been used as the basis for calculating daily production of stage I eggs on all the surveys from 1977. For horse mackerel similar egg development data are given by Pipe and Walker (1987) and have also been used for the calculation of stage I egg production since 1977.

The formula for calculating the age of **stage I mackerel eggs** from the sea temperature (T°C) is:

$$\text{Log}_e \text{ time (hours)} = -1.61 \log_e (T^\circ\text{C}) + 7.76$$

For calculating the age of **stage I horse mackerel eggs** the formula is:

$$\text{Log}_e \text{ time (hours)} = -1.608 \log_e (T^\circ\text{C}) + 7.713.$$

Temperature / depth profiles are obtained at each sampled position from the CTD's mounted on the plankton sampler bodies. When available the temperature at 20m depth should be used for the calculation of egg stage duration. If that is not available then the sub-surface temperature (ca. 3 m) should be used.

To estimate the daily egg production of the target species in the INDICES project, the rate of egg development with regard to temperature would need to be determined experimentally. This data is available for the development of hake eggs (Coombs and Mitchell, 1982), where the age of **stage I hake eggs** can be derived from the formula:

$$\text{Log}_e \text{ time (hours)} = \text{Log}_e 1263.9 - 1.411 \log_e (T^\circ\text{C})$$

The development rate of Bay of Biscay anchovy eggs with relation to temperature and egg stage is given by Motos (1994). The model is given below:

$$y_{i,t} = 15.45 e^{(0.115 T - 0.147i)} i^{2.071}$$

where **y** is the age in hours, **T** is the incubation temperature and **i** is the development stage.

The same data is available for eggs of the Iberian sardine population (Miranda et al., 1992):

$$y_{i,t} = 17.52 e^{(-0.136 T - 0.173i)} i^{2.222}$$

- Ahlstrom, E.H. 1943.** "Studies on the Pacific pilchard or sardine. IV Influence of temperature on the rate of development of pilchard eggs in nature". Spec. scient. Rep. U.S. FishWildl. Serv., (23): 26pp.
- Armstrong, M.J., Connolly, P., Nash, R.D.M., Pawson, M., Alesworth, E., Coulahan, P.J., Dickey-Collas, Milligan, S.P., O'Neill, M., Witthames, P.R. and Woolner, L. 2001.** An application of the annual egg production method to estimate the spawning biomass of cod (*Gadus morhua* L.), plaice (*Pleuronectes platessa* L.) and sole (*Solea solea* L.) in the Irish Sea. ICES Journal of Marine Science, 58: 183 - 203.
- Cameron, P.; Berg, J. 1992.** Morphological and chromosomal aberrations during embryonic development in dab *Limanda limanda*. Mar. ecol. prog. ser., vol. 91, no. 1-3, 163-169.
- Cameron, P; Berg, J; Dethlefsen, V; Westernhagen, H. v. 1992.** Developmental defects in pelagic embryos of several flatfish species in the southern North Sea, *Neth. J. Sea Res.*, vol. 29, no. 1-3, 239-256.
- Cameron, P; Berg, J; Westernhagen, H. v. 1996.** Biological effects monitoring of the North Sea employing fish embryological data. *Environ. Monit. Assess.*, vol. 40, no. 2, pp. 107-124.
- Cameron, P; Westernhagen, H. v. 1997.** Malformation rates in embryos of North Sea fishes in 1991 and 1992. *Mar. Pollut. Bull.*, vol. 34, no. 2, 129-134.
- Coombs, S.H. 1994.** Identification of eggs of hake, *Merluccius merluccius*. *J. Mar. biol. Ass.U.K.* (1994), 74, 449-450.
- Coombs, S.H. and Mitchell, C.E. 1982.** The development rate of eggs and larvae of the hake, *Merluccius merluccius* (L.) and their distribution to the west of the British Isles. *J. Cons. Int. Explor. Mer*, 40: 119-126.
- D'Ancona et al. 1956.** Fauna e Flora del Golfo di Napoli, Monographia 38: Uova, larve e stadi giovanili di Teleosti, Pubblicata dalla Stazione Zoologica di Napoli, 4 vols.
- Dethlefsen, V; Westernhagen, H. v.; Cameron, P. 1996.** Malformations in North Sea pelagic fish embryos during the period 1984-1995. In: Daan, N. ; Richardson, K.; Colijn, F.; Hislop, J. R. G.; Pedersen, B.; Pope, J. G.; Serchuk, F. M. (eds): *Changes in the North Sea Ecosystem and Their Causes: Aarhus 1975 Revisited. Proceedings of an ICES International Symposium held in Aarhus, Denmark.. ICES J. Mar. Sci.* Vol. 53, no. 6, 1024-1035.
- Ehrenbaum, E. 1905-1909.** Eier und Larven von Fischen. *Nordisches Plankton*, 1, 413pp. Lipsius & Tischer, Leipzig.
- Fahay, M. P. 1983.** Guide to the early stages of marine fishes occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf, *Journal of Northwest Atlantic Fisheries Science*, 4, 423pp.
- Froese, R. and Pauly, D. (Eds.) 2003.** FishBase, WWW Pulication, www.fishbase.org , version 12 October 2003
- Holt, E. W. L. 1893.** Survey of fishing grounds, west coast of Ireland, 1890-91: on the eggs and larval and post-larval stages of teleosts, *Scientific Transactions of the Royal Dublin Society*, Ser. 2, 5, 121pp.
- Holt, E. W. L. 1898.** Notes on the reproduction of teleostean fishes in the south-western district, *J.M.B.A.*, 5:107-155.
- ICES, 1994.** Report of the Mackerel / Horse Mackerel Egg production Workshop. Vigo, Spain 31 January–4 February 1994. 58 pp. ICES CM 1994/H4.
- ICES, 1997.** Report of the Working group on Mackerel and Horse Mackerel Egg Surveys. Lisbon, Portugal 3–7 February 1997. 48 pp. ICES CM 1997/H4.
- ICES, 2001.** Mackerel and Horse Mackerel Egg Staging and Histology Workshop. ICES CM 2001/G:01.
- ICES, 2002.** Report of the Working group on Mackerel and Horse Mackerel Egg Surveys. ICES CM 2002/G:06.

- Johnstone, J., Scott, A. and Chadwick, H. C. 1934.** The Marine Plankton, Hodder and Stoughton Limited, London, 194pp.
- Lockwood, S.J., Nichols, J.H. and Coombs, S.H. 1977.** The development rates of mackerel (*Scomber scombrus* L.) eggs over a range of temperature. ICES CM 1977/J:13, 8pp.
- Lockwood, S.J., Nichols, J.H. and Dawson, W.A. 1981.** The estimation of a Mackerel (*Scomber scombrus* L.) spawning stock size by plankton survey. J. Plankton Research, No 3 (2): 217-233p.
- Marrale, D., Alvarez, P., and Motos, L. 1996.** Development and identification of European hake, *Merluccius merluccius* L., embryonic and yolk-sac larval stages. Ozeanografika, 1: 5-26.
- Meneses, I., Vendrell, C. and Stratoudakis, Y. 2003.** Mackerel (*Scomber scombrus*) eggs parasitized by *Ichthyodinium chabelardi* in the north-east Atlantic: an overlooked source of mortality. J. Plankton Research, Vol 25 (9): 1177-1181p.
- Milligan, S., Warnes, S. and Nichols, J. (In prep.).** The identification and development rates of common megrim (*Lepidorhombus whiffiagonis*) and four-spot megrim (*L. boscii*) eggs over a range of temperatures.
- Miranda, A., Cal, R.M. and Iglesias, J. 1992.** Effect of temperature on the development of eggs and larvae of sardine, *Sardina pilchardus* W., in captivity. BOL.-INST.-ESP.-OCEANOGR. 1992 vol.8, no. 1, p.233.
- Moser, H.G., and Ahlstrom, E.H. 1985.** Staging anchovy eggs. In R.Lasker (ed.), An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*), p37-41. U.S. Dep.Commer., NOAA Tech.Rep.NMFS 36.
- Motos, L. 1994.** Estimación de le biomasa desovante de la población de anchoa del golfo de Vizcaya, *Engraulis encrasicolus* L., a partir de su producción de huevos. Bases metodológicas y aplicación. PhD dissertation, Universidad del País Vasco 1994.
- Nash, R.D.M., Dicky-Collas, M. and Milligan, S.P. 1998.** Descriptions of the Gulf VII / PRO-NET and MAFF / Guildline unencased high-speed plankton samplers. J.Plankton Res. Vol.20 no.10 pp 1915-1926, 1998.
- Nichols, J.H. 1976.** Bulk staining of small marine zooplankton using borax carmine. In Zooplankton fixation and preservation, The UNESCO Press pp287.
- Pipe, R.K. and Walker, P. 1987.** The effect of temperature on the development and hatching of scad (*Trachurus trachurus*, L.) eggs. J. Fish Biol., 31: 675-682p.
- Porebski, J. 1975.** Application of the surface adhesion test to identify the eggs of the hake *Merluccius* spp. Colln. Scient Papers: Int Commission for SthEast Atlantic Fisheries 1975. Vol 2 102-106p.
- Russell, F.S. 1976.** The eggs and planktonic stages of British marine fishes. Academic Press Inc. (London) Ltd., 524 p.
- Shaw, M. D. 2003.** *Personal communication.*
- Simpson, A.C. 1959.** The spawning of plaice (*Pleuronectes platessa*) in the North Sea. Fish. Invest., Lond., (Ser.II), 22, No 7, 111 pp.
- Steedman, H.F. 1976.** Miscellaneous preservation techniques. In Zooplankton fixation and preservation, The UNESCO Press pp175-183.
- US Fish and Wildlife Service, 1978.** Development of Fishes of the Mid-Atlantic Bight, Fish and Wildlife Service, U.S. Department of the Interior, FWS/OBS-78/12, 6 vols.
- Westernhagen, H. v. 1988.** Sublethal effects of pollutants on fish eggs and larvae. In: Hoar, WS; Randall, DJ (eds): Fish Physiology. Volume 11. The Physiology of Developing Fish. Part A: Eggs and Larvae., pp. 253-346.

- Westernhagen, H. v.; Dethlefsen, V.; Cameron, P.; Berg, J.; Fuerstenberg, G. 1988.** Developmental defects in pelagic fish embryos from the western Baltic. *Helgol Meeresunters.*, vol. 42, no. 1, 13-36.
- Westernhagen, H. v.; Cameron, P.; Dethlefsen, V.; Janssen, D. 1989.** Chlorinated hydrocarbons in North Sea whiting (*Merlangius merlangus* L.), and effects on reproduction. 1. Tissue burden and hatching success. *Helgol. Meeresunters.*, vol. 43, no. 1, 45-60.