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Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS)

20–24 April 2009

Hamburg, Germany

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CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS), chaired by Jens Ulleweit (vTI-SF, Germany), met in Hamburg on April 20-24, 2009, to plan the Mackerel and Horse Mackerel Egg Survey in 2010. For the first time the Faroe islands will contribute to the triennial survey with their own vessel in addition to the participation of Portugal, Spain, Scotland, Ireland, The Netherlands, Norway and Germany. The main aim of the survey is to relate the number of freshly spawned eggs found in the water to the number of females having produced these eggs. Knowing the fecundity of the females and sex ratio provides an estimate for the spawning-stock biomass.

As in 2007, the 2010 survey has been based on six sampling periods, again commencing with the Portuguese DEPM survey for horse mackerel (Section 2 of the report). As in 2007, there continues to be no participation from CEFAS at a time when the temporal and spatial widening of mackerel and horse mackerel distribution during spawning has led to an expansion of the potential survey area. This, combined with other ship-time restrictions, has already created difficulties with the 2007 survey with respect to defining egg distribution boundaries for both species. The working group anticipates that these problems might become more prominent in future. In this context, the working group welcomes the participation of the Faroese Islands in next year's survey. Therefore, a good spatial coverage over most survey periods could be achieved just for 2010. However, the group stresses, that in future triennial surveys only the participation of more states exploiting mackerel and horse mackerel will allow sufficient coverage of the spawning area over the complete reproductive periods of both species.

Furthermore, NEAFC and the Coastal states declared in late 2008 that there is a requirement for coordinated research and surveys on the seasonal distribution and migration of the mackerel stock in the Northeast Atlantic (Section 1.3). The scientific meeting dealing with this request emphasized the importance of the triennial mackerel egg survey for mapping the distribution of adults during the spawning period.

Sampling protocols for mackerel and horse mackerel fecundity analysis were established during the meeting (Section 3), either by adoption of the sampling schemes used during the 2007 MEGS or by modification of those where necessary. It is planned that during the 2010 survey 900 female mackerel and 495 horse mackerel samples will be taken and analysed for fecundity. Fecundity analysis will be conducted by Norway (IMR), the Netherlands (IMARES), Scotland (MSML), Ireland (MI) and Spain (IEO and AZTI). Sampling procedures will be finalized during the fecundity workshop in San Sebastian 1–4 December, 2009.

Following a recommendation of WGWIDE, the group established a standard procedure in case all the samples could not be processed in time (Section 4). In order to produce reliable preliminary egg production estimates, any participant unable to process samples to the required deadline will have to achieve an agreement about the selection of egg sample subsets in liaison with the survey data coordinator, WGMEGS Chair and an independent referee.

In preparation of the 2010 survey a workshop dealing with egg identification and staging will be held in IJmuiden 5–9 October, 2009. Procedures for fecundity and atresia estimation will be standardized and training conducted on the fecundity workshop in San Sebastian in December (Section 5). Once again the working group

points out that these workshops are essential to quality assurance of the mackerel and horse mackerel egg surveys.

The survey manual (Annex 3) was updated where necessary. It will be further improved during the workshops in autumn. Standardization of survey gears has achieved a high level (Section 7).

The manual on fecundity and atresia estimation was also updated (Annex 4). It is planned to be further improved during the workshop in San Sebastian.

Between 2 June and 5 July 2008 the Netherlands and Norway conducted the Mackerel Egg Survey in the North Sea (Section 6). The main spawning area was detected in the Southwest of the surveyed area but egg production was also more abundant further north and east than in 2002 and 2005. Only 14 mackerel ovaries could be collected for potential fecundity estimation because the survey only took place during peak spawning and prespawning females were rare. The estimated potential fecundity was low, and atresia was high suggesting that realized fecundity was less than 50% of the normal. The working group was concerned about the low fecundity but regrets that only few fecundity data could be collected. WGMEGS recommends that mackerel fecundity should be monitored annually in the North Sea and evaluated as a possible indicator for ecosystem health. The North Sea mackerel SSB was calculated as 154,000 tons.

The use of the WGMEGS survey to provide an abundance index for horse mackerel was evaluated (Section 8). The sampling design of the MEGS is unsuitable for the standard Daily Egg Production Method (DEPM) that estimates fecundity from the composition of the various oocyte types. Determination of batch fecundity at earlier developmental stages, when larger vitellogenic oocytes start separating from the standing oocyte stock seems to provide an alternative method. A procedure for the collection of ovary samples during the MEGS for an alternative DEPM estimate for horse mackerel was presented. For the southern stock only, IPIMAR (Portugal) will conduct in 2010 a DEPM survey directed at horse-mackerel (the first DEPM occurred in 2007).

1 Introduction

1.1 Terms of Reference

At the ICES Annual Science Conference in Halifax, Canada, September 2008 it was decided that the Working Group on Mackerel and Horse Mackerel Egg Surveys [WGMEGS] (Chair: J. Ulleweit, Germany) will meet in Hamburg, Germany, 20–24 April 2009 to:

a) Coordinate the timing and planning of the 2010 Mackerel/Horse Mackerel Egg Survey in the ICES Subareas VI to IX;
b) Coordinate the planning of the sampling programme for mackerel/horse mackerel fecundity and atresia;
c) Review and report on procedures for egg sample sorting, species identification and staging;
d) Review and report on procedures for fecundity and atresia estimation.
e) Analyse and evaluate the results of the 2008 mackerel egg survey in the North Sea.
f) Update the survey manual and make recommendations for the standardization of all sampling tools, survey gears and procedures.
g) Evaluate and report on the use of the Triennial egg survey to provide an abundance index for horse mackerel, and make recommendations for the 2010 survey for data collection and analysis.

WGMEGS will report by 1 June 2009 for the attention of SCICOM and TGISUR.

1.2 Participants

A list of participants is given in Annex I of this report.

1.3 The Bergen meeting on mackerel distribution and migration

Scientists from Denmark, Faroe Islands, Ireland, the Netherlands, Norway, Russia, Spain, Sweden and UK (Scotland, England and Wales) met in Bergen, 31.03–02.04.2009, to deal with the following requests from NEAFC and the Coastal states:

NEAFC:
In order to provide a comprehensive overview of seasonal distribution and migration of the mackerel stock in the Northeast Atlantic, coordinated research and surveys are needed. In order to investigate the feasibility for this, the Contracting Parties have agreed that a coordinated scientific survey programme should be organized.

Coastal states:
Mapping and describing the seasonal distribution and migration of the stock. The Delegations agreed to encourage their respective authorities to both advance activities through coordination of relevant research and to make financial means available from relevant resources.

A drafted version of the report from this meeting was available for presentation at WGMEGS. Annexed to this report is a lot of valuable information from surveys/investigations related to mackerel biology, ecology, abundance and distribution as well as identification of mackerel by multifrequency acoustics using the Large Scale Survey System (LSSS).

Data collated from egg surveys in the western and the southern areas during the period 1992–2007 demonstrate that the largest average concentration of eggs in this

time-series (7200 eggs/m^2/day) were in the region 49^{o}N and 11^{o}W. The average numbers of eggs per ICES statistical rectangle for this time-series were presented (Figure 1.3.1). This shows the shift in the egg distribution and abundance through an average spawning year.

The spawning area in the North Sea was investigated for the first time in 1968, with a single survey, with the first multiple surveys being conducted in 1980. There has been a significant shift in the main spawning area from the central North Sea in 1980 and 1983, to the western North Sea in 2005 and 2008 (Figure 1.3.2).

Main conclusions from the Bergen meeting:

The group could neither propose a new survey, nor a survey protocol, which would cover the entire distribution of mackerel in the Northeast Atlantic. Significant resources are already deployed towards the triennial mackerel egg surveys, which maps the distribution of adults in the spawning period. Beyond the spawning period, mackerel behave in a variety of ways. For example, in midsummer, they either: school close to the surface in the Norwegian Sea; or occur as dispersed individuals throughout the water column in the North Sea; or they may be close to the seabed (e.g. along the western continental shelf). There is currently no single method that will universally cover the whole distribution of mackerel at any time other than the spawning period. It is also currently impossible to combine the different methods which are tailored specifically to any one of the different behaviours.

The group recognized that there is scope to coordinate and standardize existing surveys and methods to provide new and valuable information on the distribution and migration of mackerel. A number of surveys were examined and listed in the report as be capable of providing information on the ecology, distribution and abundance of mackerel at various stages of their life cycle.

The group made some recommendations pertinent to these surveys, which would allow for data on mackerel to be more comparable.

The group also recommended that tagging studies and stock identification methods should be investigated.

Discussion on the merits of future extensive mackerel surveys could be dealt with at ICES WGWIDE.

It is recognized that planning, conduct and assessing the result of proposed future surveys would require technical expertise of various subgroups, along the lines of operation of WGMEGS for the egg surveys.

The spatial and temporal distribution of the stock has changed historically and this continues to be a dynamic feature of the stock. This property has to be taken into account when planning the present egg, and proposed future surveys, meaning that more ship time has to be allocated to continually cover areas beyond those currently defined. This is a problem which WGMEGS finds increasingly difficult to address with existing resource allocations.

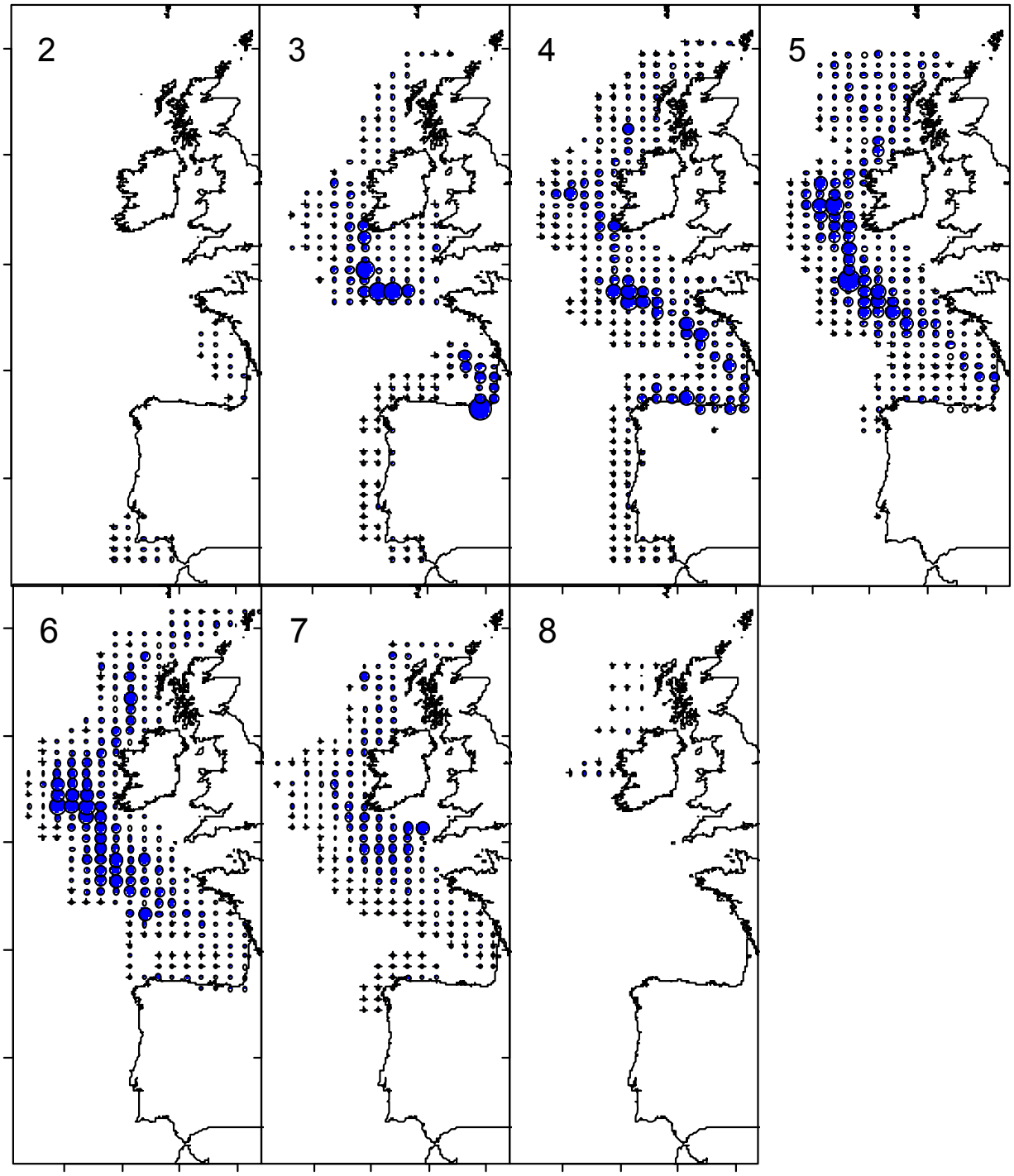

Figure 1.3.1. Average distribution of mackerel eggs by ICES statistical rectangle for each period 2–5 (some years 6–8) in the time-series 1992–2007.

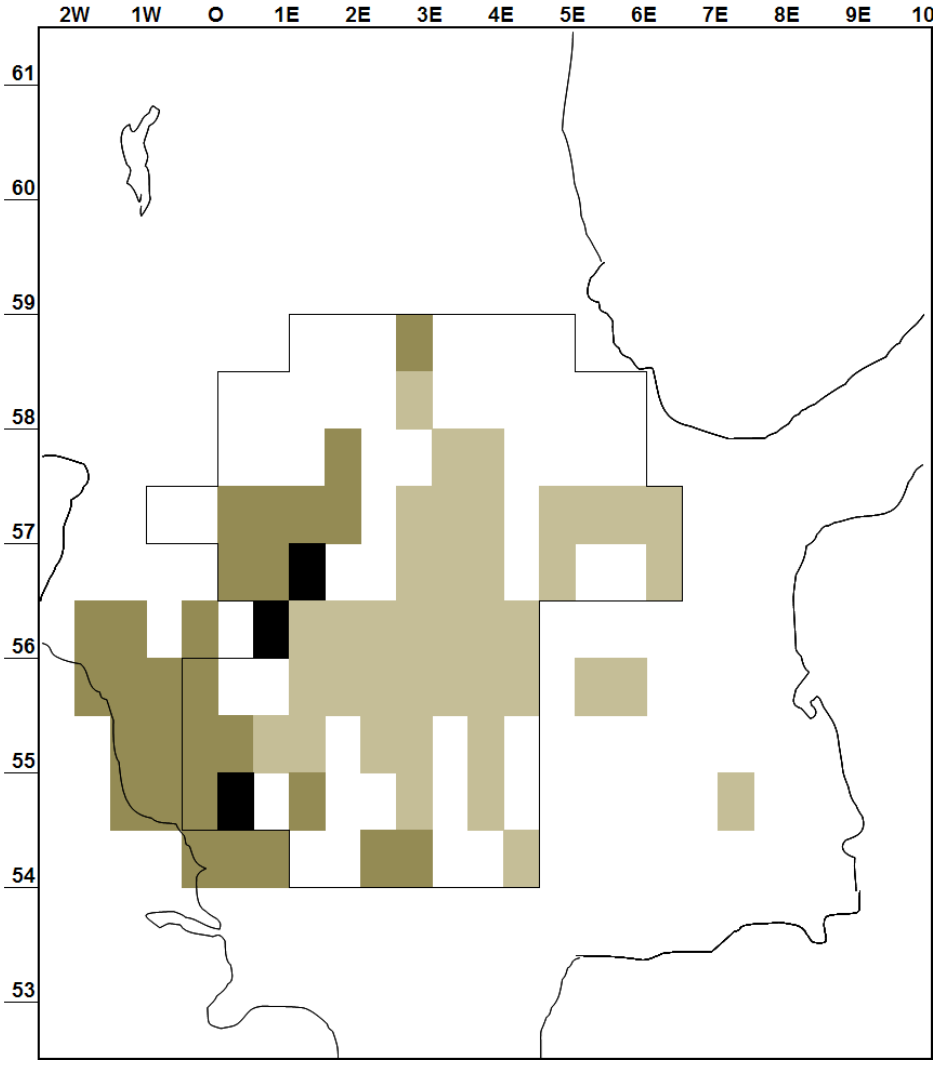

Figure 1.3.2. Main spawning area in 1980, 1983, 2005 and 2008 defined as rectangles with at least 50 mackerel eggs/m²/day. Light grey: 1980 and 1983, dark grey: 2005 and 2008. Black: overlapping area. The common sampling area for all four years is delineated.

2 Planning of the 2010 Mackerel and Horse Mackerel Egg Survey in the Western and Southern Areas (referring to ToR a)

2.1 Countries and Ships Participating

Germany, Ireland, Netherlands, Scotland, Portugal, Spain, Spain/Basque Country, Norway and also the Faroe Islands will participate in the mackerel/horse mackerel egg surveys in the western and southern area in 2010. Provisional dates (where possible) as well as vessel details for the forthcoming surveys can be found below in Table 2.1.1. As in 2007, it is disappointing to note that there continues to be no participation from CEFAS (UK, England and Wales). The reduction in survey effort is to some degree offset by the timely inclusion of the Faroese into the group and a commitment to devote 2 weeks of ship time to the 2010 survey in the western area. Although this additional survey is extremely welcome the 2007 results highlighted several challenges, in particular an expansion of the western mackerel and horse mackerel spawning area during the period of peak spawning. The result was an inability to fully survey the whole area for all periods at the minimum required level of one station per sampling rectangle and several boundaries remained unsecured. With no net increase in survey days available for 2010 this situation is set to continue. These challenges as well as recommendations are more fully described in Sections 9 and 10. Survey coverage of the western and southern area is given by area and period in Table 2.1.2. Detailed maps of survey coverage by period are given in Figures 2.1.1 – 2.1.5. Both vessel availability and area assignments are provisional and will be finalized by the survey coordinator at the appropriate times.

The survey coordinator for the 2010 survey will be Finlay Burns, Marine Scotland - Marine Laboratory, Aberdeen.

2.2 Survey Design

In keeping with 2007, the survey will be split into six sampling periods. The survey design and survey deployment plan for 2010 is almost identical with that used in 2007. The only significant change being the inclusion of the Faroese survey in May to replace the additional survey undertaken by Scotland in 2007. As already mentioned there is almost no change in the number of survey days available for 2010 compared with 2007. The first period (approximately January and February) will include a survey in ICES area IXa only, with fuller coverage starting in period 2 (March). In 2010 the survey effort in area IXa will again be targeted on a single extended DEPM survey (see Section 2.4). The timing and design of the survey in Period 1 is almost identical with that completed in 2007 and no sampling will take place in area IXa thereafter. Sampling of the western area will commence in period 2. During period 2 the survey will cover the full western area plus the Cantabrian Sea and Galicia. Sampling off Galicia will cease after period 3 and from period 5 onwards coverage will only be of the western area north of the Cantabrian Sea. Some spawning is expected in the Cantabrian Sea during this period, and it has been surveyed later in previous years, but as in 2007 no vessels are available. In periods 5 and 6 the surveys are designed to identify a southern boundary of spawning and to survey all areas north of this boundary. The deployment of vessels to areas and periods is summarized in Table 2.1.1.

Table 2.1.1. Countries, vessels, areas assigned, dates and sampling periods for the 2010 surveys.

Country	Vessel	Areas	Dates	Period
Portugal	Noruega	Cadiz, Portugal & Galicia	January - February (35 Days)	1
Spain (IEO)	Cornide de Saavedra	Cantabrian Sea & Biscay	14 Mar – 05 Apr	2
		Biscay & Cantabrian Sea	15 Apr – 12 May	3
Germany	W. Herwig III	West Ireland & W Scotland Celtic Sea & Biscay	24 Mar – 12 Apr	2
			13 – 30 Apr	3
Netherlands	Tridens	Celtic Sea & Biscay	3 – 20 May	4
		Celtic Sea & Biscay	1 – 19 June	5
Spain (AZTI)	Investigador	Biscay	24 March - 13April	2
		Biscay & Cantabrian Sea	May (20 Days)	4
Norway	Johan Hjort	West Ireland & West of Scotland	May/June (21 days)	4
Ireland	Celtic Explorer / Charter	Celtic Sea	March (20 Days)	2
		Celtic Sea, West Ireland & West of Scotland	July (20 Days)	6
Scotland	Scotia/Charter	West Ireland & West of Scotland	April (22 Days)	3
		West Ireland & West of Scotland	June (22 Days)	5
Faroe Islands	Charter	West of Scotland	May (14 Days)	4

In the western area maximum deployment of effort is during the second, third and fourth sampling periods. These periods coincide with the expected peak spawning of both mackerel and horse mackerel in the area. As a result of the expansion of the spawning area that was observed in 2007 the emphasis will be even more focused on area coverage. Cruise leaders have been asked to cover their entire assigned area using alternate transects (see Annex 2) then use any remaining time to fill in the missed transects. If time is short this should be concentrated in those areas identified as having high egg abundance on the first sweep of the survey. Particular points to note are:

Period 1

The southern area will only be surveyed in period 1.This is to accommodate the changes that were made to the Portuguese survey which was condensed from 3 surveys into a single extended (horse mackerel DEPM based – see Section 2.4.) survey.

Period 2

Period 2 marks the commencement of the western area surveys. For reasons which relate to the control of the period 3 survey it would be preferable for the German vessel to start and finish surveying at the southern boundary (51°30N) of her designated survey area (Figure 2.1.2).

Period 3

There are 3 vessels available for period 3. The German vessel will commence sampling in the Celtic Sea along the Northern boundary of the designated survey area (50°30N). It will then continue south into Biscay until the southern boundary is reached at 47°N. The Spanish vessel will complete the survey coverage in Biscay to the south of that covered by the German survey. In the area between 46°30N — 47°N,

6°– 10°W the west – east direction of the shelf break at these latitudes requires careful sampling to avoid having large samples at the edge of the survey area. It is therefore imperative that during these two surveys this area receives comprehensive coverage in order to define the edge of the spawning distribution. It should also be noted that the Spanish vessel will probably not have to survey in the area 45°N - 46°N, 5°- 10°W. This area is over deep water and very few eggs are normally found here. Given that the Spanish vessel will start its survey in Vigo, it is recommended that the survey be carried out as follows (Figure 2.1.3):

Survey to the east through the Cantabrian Sea, occupying alternate north/south transects

Move to 46° 45′ N and complete that transect then survey to the south, occupying all east/west transects

Survey to the west through the Cantabrian Sea, occupying the remaining north/south transects

Period 4

There are 4 vessels available during this period to survey the western area. AZTI will be carrying out a targeted DEPM survey for anchovy in Biscay and although it provides mackerel and horse mackerel egg samples as well, the design of this survey is constrained in that purpose. In 2007, there was virtually no sampling in the Cantabrian Sea during this period and significant horse mackerel spawning activity was almost certainly missed in this region as a result. AZTI will endeavour to expand their survey west into the Cantabrian Sea in order to try to secure a southern boundary for horse mackerel during this period although the success of this objective is by no means assured. The IMARES vessel north of this will commence its survey at 49°45N. The North and Western boundary was similarly not well defined during the 2007 surveys for this period. In an effort to address this issue WGMEGS has asked the Faroese vessel to survey the area from 56°45N to 60°45N. This expands the area coverage in the north. In 2007 this survey encountered a pulse of mackerel spawning activity which continued out past 13°W. The main priority of this survey is therefore to secure the western boundary during this period (Figure 2.1.4).

Period 5

In period 5, two vessels have to cover the entire area of spawning from northern Biscay to the West of Scotland. Alternate transects are recommended. The IMARES vessel covering the Biscay area will commence the survey along the southern boundary of the designated area although its exact latitude will depend on the results from period 4. The survey coordinator will advise the IMARES cruise leader prior to the survey. (Figure 2.1.5)

Period 6

In period 6, only one vessel will be available, and will have to cover the entire spawning area. This assignment will once again be undertaken by Ireland. As with period 5 the southern starting location will be dictated by the results of the previous period. Irrespective of this an alternate transect design will be necessary. (Figure 2.1.6).

Table 2.1.2. Periods and area assignments for vessels by week for the 2010 survey. Area assignments and dates are provisional.

		Area						
week	Starts	Portugal, Cadiz & Galicia	Cantabrian Sea	Biscay	Celtic Sea	North west Ireland	West of Scotland	Period
1	25-Jan-10	PO1(DEPM)						1
2	1-Feb-10	PO1(DEPM)						1
3	8-Feb-10	PO1(DEPM)						1
4	15-Feb-10	PO1(DEPM)						1
5	22-Feb-10	PO1(DEPM)						1
6	1-Mar-10	PO1(DEPM)						1
7	8-Mar-10				IRL1			2
8	15-Mar-10		IEO1		IRL1			2
9	22-Mar-10		IEO1	AZTI-1	IRL1	GER	GER	2
10	29-Mar-10		IEO1	AZTI-1		GER	GER	2
11	5-Apr-10			AZTI -1		GER	GER	2
12	12-Apr-10		IEO2		GER	SCO1	SCO1	3
13	19-Apr-10		IEO2	IEO2	GER	SCO1	SCO1	3
14	26-Apr-10		IEO2	IEO2	GER	SCO1	SCO1	3
15	3-May-10		IEO2	IMARES 1	IMARES 1			3
16	10-May-10		AZTI-2(DEPM)	AZTI-2(DEPM)	IMARES 1	IMR	IMR	4
17	17-May-10			AZTI-2(DEPM)	IMARES 1	IMR	FAR	4
18	24-May-10		AZTI-2(DEPM)			IMR	FAR	4
19	31-May-10			IMARES2	IMARES 2	SC02	SC02	5
20	7-Jun-10				IMARES 2	SC02	SC02	5
21	14-Jun-10			IMARES2	IMARES 2	SC02	SC02	5
22	21-Jun-10							5
23	28-Jun-10							6
24	5-Jul-10				IRL2	IRL2	IRL2	6
25	12-Jul-10				IRL2	IRL2	IRL2	6
26	19-Jul-10				IRL2	IRL2	IRL2	6
27	26-Jul-10							6

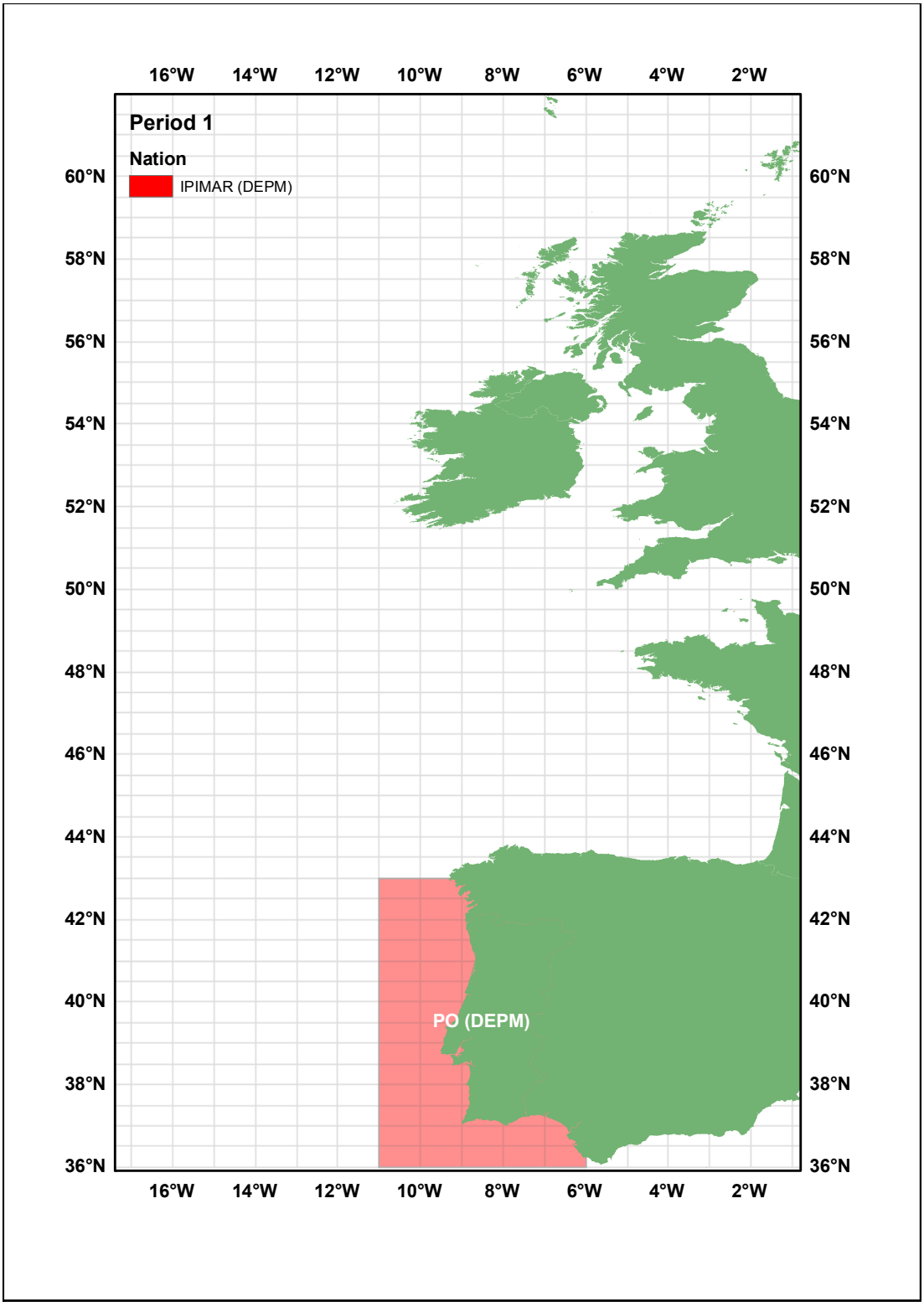

Figure 2.1.1. Survey plan for Period 1.

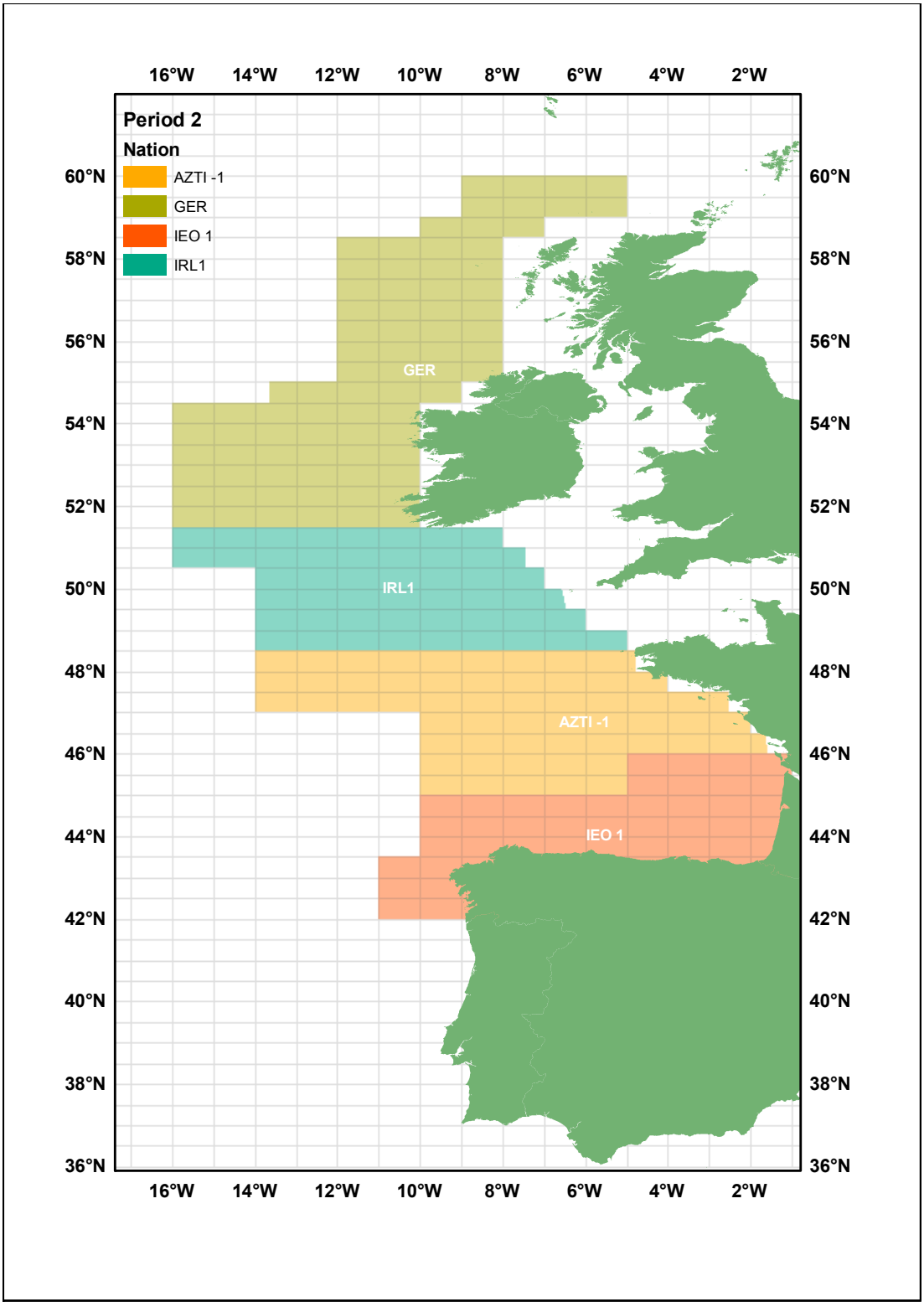

Figure 2.1.2. Survey plan for Period 2.

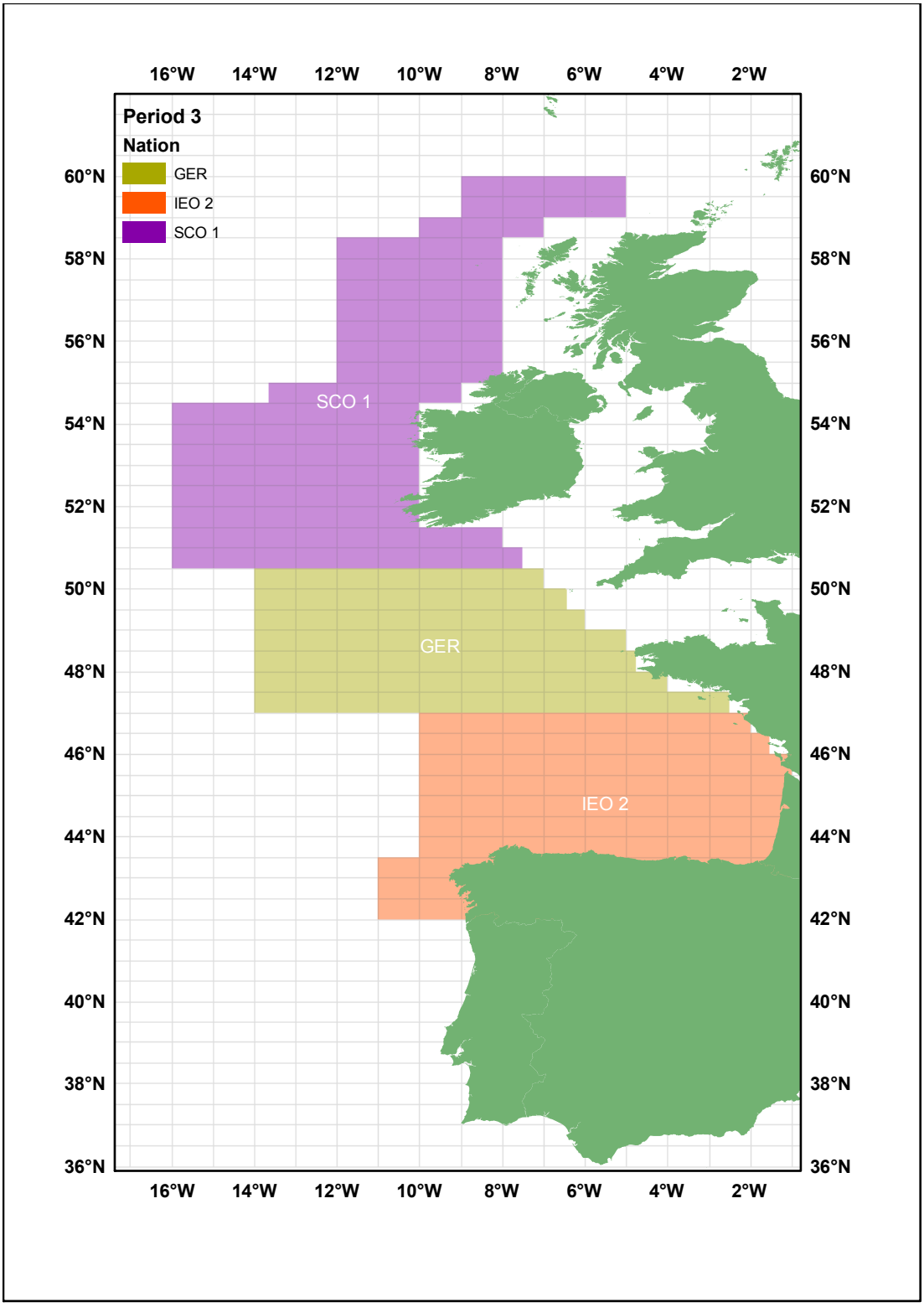

Figure 2.1.3. Survey plan for Period 3.

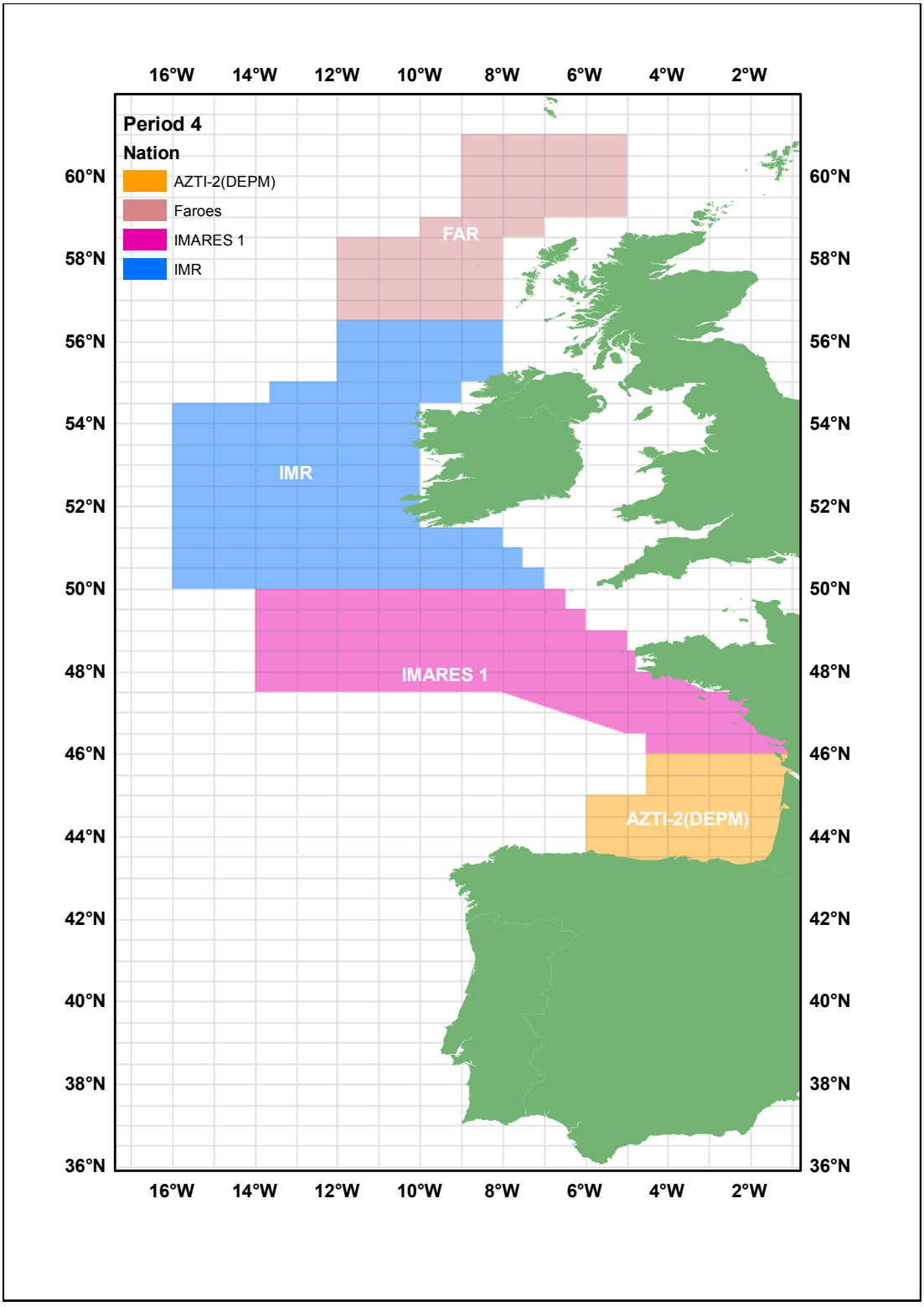

Figure 2.1.4. Survey plan for Period 4.

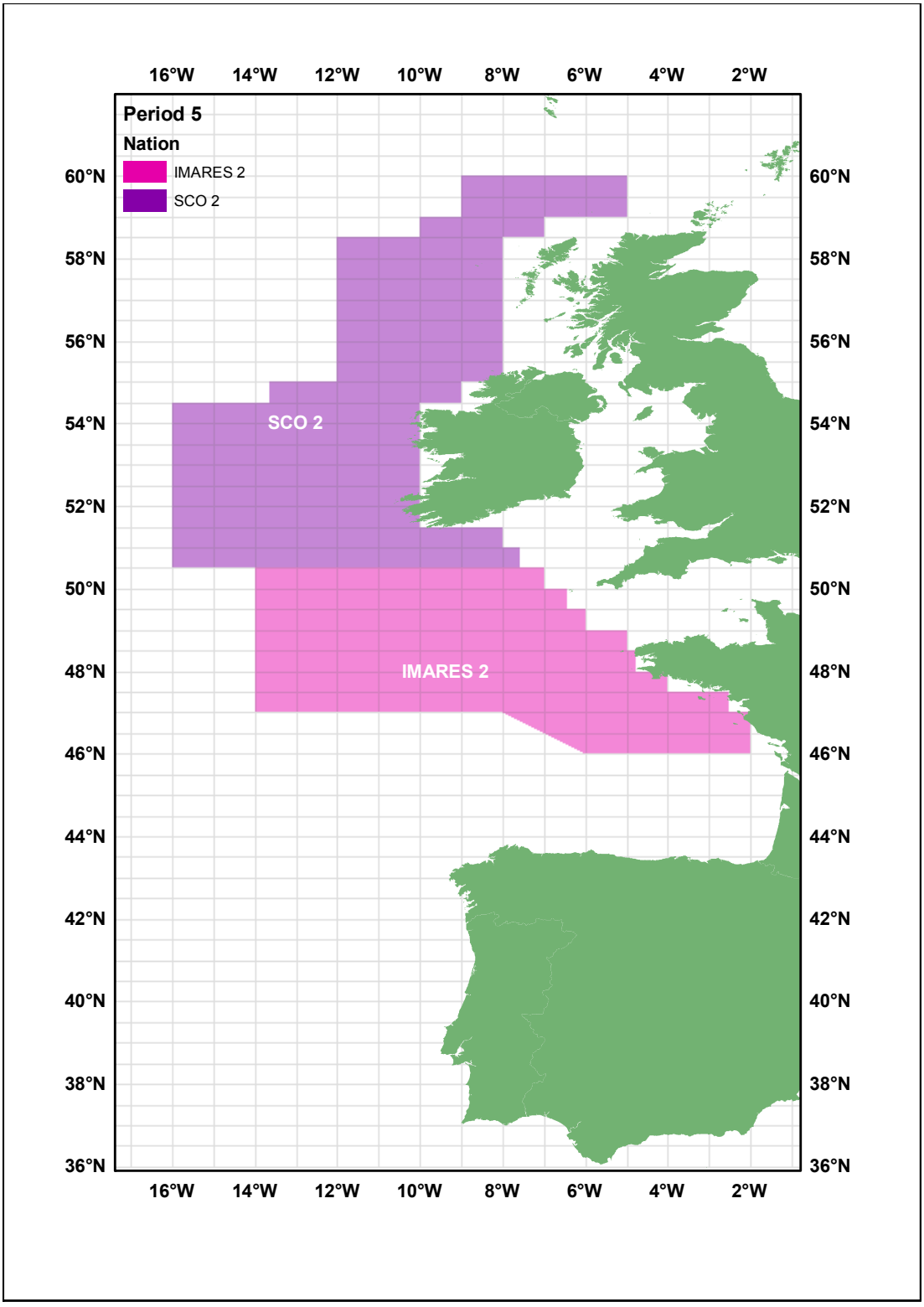

Figure 2.1.5. Survey plan for Period 5.

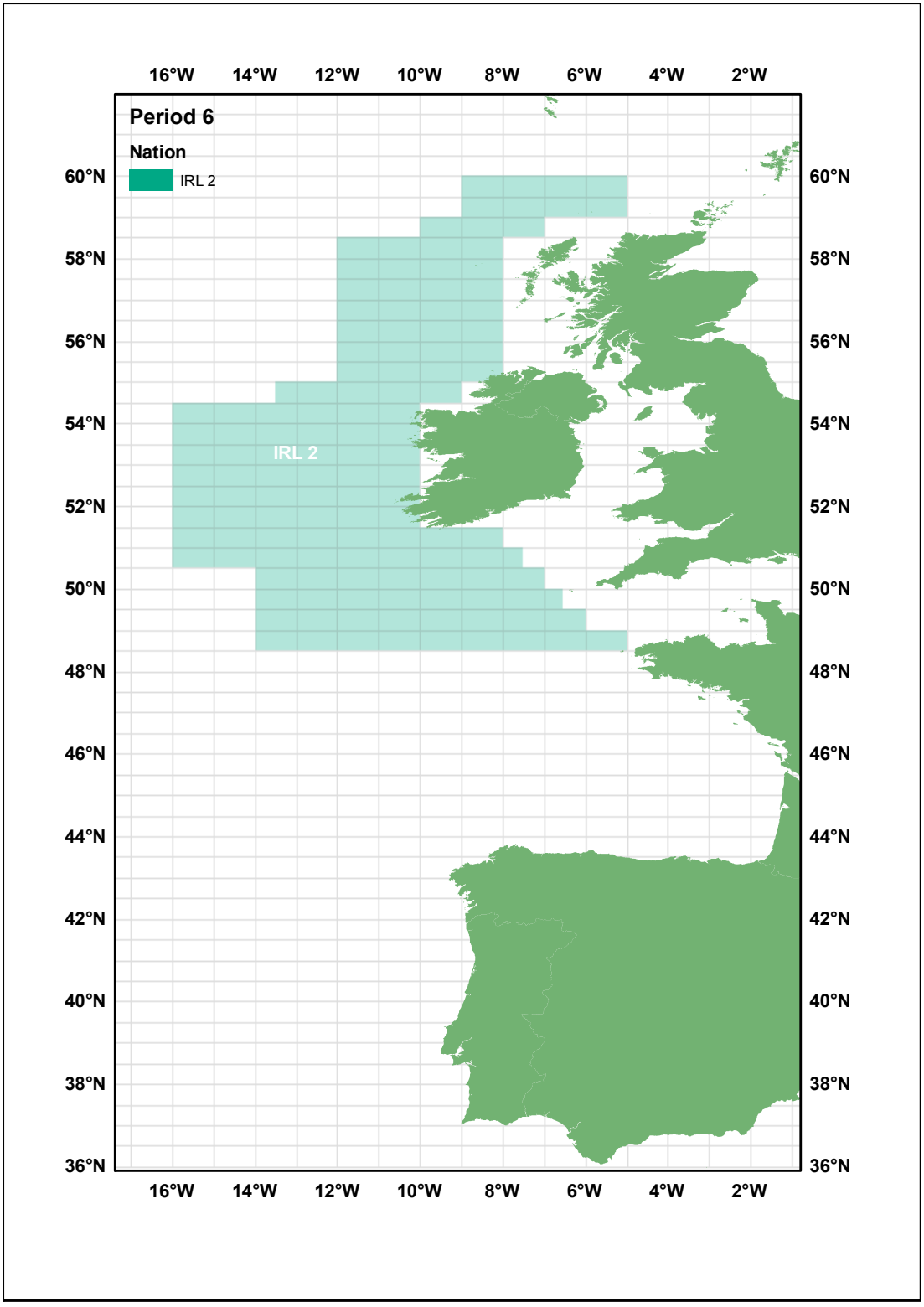

Figure 2.1.6. Survey plan for Period 6.

2.3 Sampling Areas and Sampling Effort

As in previous years it was decided that the spatial and temporal distribution of sampling would be designed to try to ensure adequate coverage of both mackerel and horse mackerel spawning and that estimates of stage 1 egg production would be made for both species.

Since the surveys began in 1977 considerable changes have been made to the standard sampling area and these have been described in Section 8.4 (ICES, 1994). In 1995 changes were made to the western boundaries of the western area because of the unusual westerly distribution of mackerel eggs which occurred in period 3, 1992. Examination of the 1995 egg distributions prior to the 1998 survey resulted in the addition of further rectangles to the standard sampling area. A total of eight rectangles were added at the northern edge and twenty five on the western edge between latitude 45°30'N and 51°N (ICES, 1997). Examination of the 1998 survey data showed that the distribution of mackerel and horse mackerel spawning in both the western and southern areas was adequately covered with the exception of mackerel spawning from mid May to July at the northern edge of the western standard area. As a result some additional rectangles were added to the standard area north of latitude 58°30'N.

Based on this steady growth of the "standard area" every survey, the Working Group agreed at the Dublin meeting (2002) to reconsider its use. It was agreed that the existing "standard area" should be retained **only as a guide** to the core survey area for cruise leaders, and that the extent of coverage should be decided based on finding the edges of the egg distribution only i.e. boundaries should be set based on the adaptive sampling guidelines (Annex 3). The core areas for the western and southern surveys for both species are presented in Figure 1 of Annex 3. A more detailed survey map of the Iberian areas as surveyed by IEO and IPIMAR can be found in Figures 2 in Annex 3 and 2.4.1. Section 2.4 also provides a description of the Portuguese DEPM survey.

The sampling area in the south has been modified from the design used in 2001 and previously. The stations have been placed closer together in the onshore/offshore direction and further apart in the alongshore direction. As stated above the limits of the survey in both areas should be established on the basis of two consecutive zero samples, and not by the boundaries on this map.

2.4 Horse mackerel DEPM survey in ICES Division IXa

During January-February 2010 IPIMAR (*RV* "Noruega") will conduct the horse mackerel DEPM survey for the area of its southern stock, Gibraltar to Finisterre.

The main icthyoplankton sampler will be the adapted CalVET system (2 nets, Ø 25 cm, mesh 150 μm + CTDF + flowmeters). Bongo and CUFES samples will be taken for complementary sampling. Plankton surveying will be undertaken following a predefined grid of sampling stations along-transects perpendicular to the coast and separated by 12 nmiles (Figure 2.3.1). About 560 CalVET stations (vertical hauls), 3 nmiles apart, are planned along the 48 transects. The grid of stations is designed in an attempt to cover the whole potential spawning area with good spatial resolution within the surveying time available. Some adaptive decisions on stations spacing may be taken during the course of the campaign if needed. The oblique tows using Bongo net (standard gear for the AEPM) will be carried out for selectivity comparisons between Bongo and CalVET hauls (~ 2 per transect). In the laboratory, all horse mackerel eggs will be counted and staged according to the 11 stage key of developmental stages (Cunha *et al.*, 2008). *Scomber* spp. eggs will be sorted and counted. Temperature data from the CTD casts will be used for the egg ageing procedure.

Sampling for adult horse mackerel will take place during the same cruise. On average two bottom-trawl hauls will be conducted per day, covering the whole survey area. From each trawl, a sample of 100 fish will be randomly selected and sampled biologically on-board. The biological data will be used to estimate the mature fraction of the population and to estimate the sex ratio and female mean weight for each haul. Sex, maturity stage, lipid content and stomach fullness will be recorded. For the first 30 females encountered the gonads will be immediately collected and preserved in formaldehyde solution. In addition, extra effort will be dedicated to obtain spawning females for batch fecundity estimation. In order to complement sampling, fish from commercial vessels will be obtained at 2 or 3 ports along the coast during the period of the campaign. The procedure for samples from commercial vessels will be adjusted according to the fishing operation (gear and time before reaching the port), facilities on-board and at the ports. At the laboratory the gonads preserved will be weighed, a tissue sample taken from one of the lobes, then dehydrated with alcohol and embedded in paraffin. The resulting blocks will be sectioned (3–5 μm thick), mounted on slides and stained according to Harris' Hematoxylin and Eosin procedure. The analysis of the slides will produce information on (1) microscopic maturity stage, (2) presence of hydrated oocytes, (3) stage of oocytes migratory nucleus development (4) presence and staging of post-ovulatory follicles (POFs) and (5) incidence of atresia. Spawning fraction will be estimated based on the presence/absence of oocytes at the migratory nucleus stage, hydrated oocytes and PFOs. Staging/ageing of POFs will be accomplished using morphological and biometrical criteria. Hydrated ovaries containing new post-ovulatory follicles will not be used to estimate batch fecundity. Batch fecundity will be estimated for each fish using the gravimetric method, three pieces of 0.10 g each will be cut from one lobe of the ovary, weighed, and the hydrated oocytes counted (Hunter *et al.*, 1985).

Data analyses for daily, egg production and fecundity and SSB estimation will be undertaken using adapted versions of the R packages available at ichthyoanalysis (http://sourceforge.net/projects/ichthyoanalysis) and routines developed at IPIMAR (ICES, 2008; Murta and Vendrell, 2009).

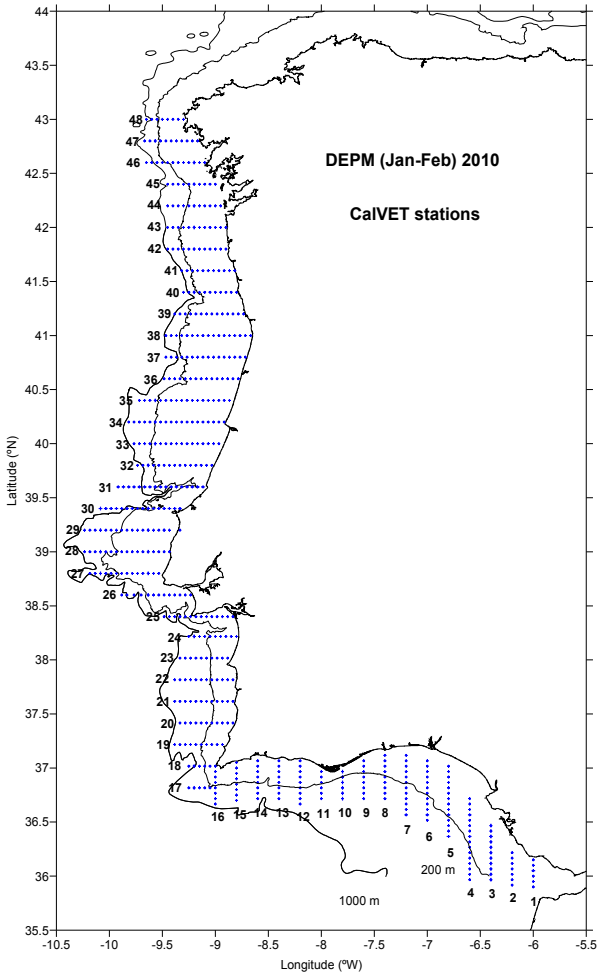

Figure 2.4.1. Sampling grid for CalVET stations.

3 Planning and sampling programme for mackerel and horse mackerel fecundity and mackerel atresia. (Referring to ToR b)

3.1 Sampling for mackerel potential fecundity and atresia in the Western and Southern areas

Following the WGMEGS decision to use only formaldehyde fixative (ICES 2003) it will be possible to provide a unified sampling scheme for fecundity and atresia for use in the 2010 survey. In consequence of the 2007 survey experience the following changes have been recommended for the 2010 survey (Table 3.1.1). The fecundity and atresia sampling manual for mackerel and horse mackerel keeps record of all the changes in earlier surveys (Annex 4).

Table 3.1.1. Major changes for 2010 compared with 2007. A complete list of changes is given in the fecundity manual.

2007	2010
Stereometric method	IMR will try to develop a new profile method. At the workshop it will be decided which method will be used. If profile counting is chosen then only a small part of the ovary needs to be brought back to the lab for atresia analysis.
Fecundity method	Of 10 mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.

Ovaries should be weighed and subsamples taken by pipette before fixing in 3.6% buffered formaldehyde solution on board. Participants are encouraged to attend the egg and/or fecundity workshop to learn the correct use of the pipettes. Participants should check the pipettes and plungers to see if they are working correctly prior to the survey. Ovary subsamples should be stored in formaldehyde in Nunc tubes. Care should be taken that oocyte samples are completely covered by formaldehyde. Participants should regularly check for evaporation from the samples and top up formaldehyde if necessary.

Samples for estimation of mackerel potential fecundity and atresia will be mostly taken on vessels participating in the egg survey or from commercial fishing vessels by observers. Recognizing the constraints of the egg survey cruise leaders should try to distribute trawl stations across the survey area aiming to complete a wide spread sampling regime for adults demonstrated in Tables 3.1.2 a-b. The purpose of this table is not to exactly specify the time and location of trawl hauls but to give an impression of how trawl hauls should be dispersed in time and space and the numbers of mackerel required for the estimation of realized fecundity and atresia.

If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes (see fecundity manual). In order not to concentrate the sampling on spawning fish it is desirable that trawling should avoid the 200 metre depth contour. Instead it should be adapted to fit in conveniently with the egg survey along the transects on the continental shelf. Details of preparation for fecundity sampling at sea are demonstrated in the fecundity manual.

Prior to cruise departure **Norway (Merete Fonn)** will coordinate the analysis of mackerel fecundity samples and assign tube reference numbers to cruise leaders for labelling the Nunc tubes used on their cruises.

Procedures to follow at sea to collect samples and for sample analysis in the laboratory are demonstrated in Tables 3.1.3. Provisional reporting of estimates for potential fecundity and atresia are required for the 2010 WGWIDE group in September. The final results will be presented to WGMEGS in spring 2011. If participants or coordinator are unsure of the data quality they should pass on their concerns to the survey coordinator (Finlay Burns, MSML).

Tab. 3.1.2a: Desired temporal and spatial distribution of the mackerel fecundity sampling in the Southern Area

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

MACKEREL

Week	Date	Period*	Lon ° 11W	10	9	8	7	6	5	4	3	2	1	Lat ° 36N	37	38	39	40	41	42	Total
4	25.01.2010	**1**	60 (prespawning (purseine/trawl)											10							10
5	01.02.2010	**1**														10					10
6	08.02.2010	**1**																			60
7	15.02.2010	**1**														10					10
8	22.02.2010	**1**																	10		10
9	01.03.2010	**1**																			0
10	08.03.2010	**2**																			0
11	15.03.2010	**2**	20																		20
12	22.03.2010	**2**	20						10												30
13	29.03.2010	**2**	20						10												20
14	05.04.2010	**2**							10												10
15	12.04.2010	**3**	10																		10
16	19.04.2010	**3**	10																		10
17	26.04.2010	**3**	10																		10
18	03.05.2010	**3**	20																		20
19	10.05.2010	**4**																			0
20	17.05.2010	**4**																			0
21	24.05.2010	**4**																			0
22	31.05.2010	**5**																			0
23	07.06.2010	**5**																			0
24	14.06.2010	**5**																			0
25	21.06.2010	**5**																			0
26	28.06.2010	**6**																			0
27	05.07.2010	**6**																			0
28	12.07.2010	**6**																			0
29	19.07.2010	**6**																			0
																					230

* Note that period **1**/**2** is dominated by prespawning fish; in periods **3** to **5** = atresia sampling

		per period			
		1	2	3	4
10	IEO	60	60	50	0
10	AZTI		30		0
60	IPIMAR	40			
10	Total:	100	90	50	0

Tab. 3.1.2b: Desired temporal and spatial distribution of the mackerel fecundity sampling in the Western Area

Fecundity sampling **Western Area**

MACKEREL Lat °

Week	Date	Period*	44N	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	Total
4	25.01.2010	**1**																			0
5	01.02.2010	**1**																			0
6	08.02.2010	**1**																			0
7	15.02.2010	**1**																			0
8	22.02.2010	**1**																			0
9	01.03.2010	**1**																			0
10	08.03.2010	**2**					20		20												40
11	15.03.2010	**2**						20		10											30
12	22.03.2010	**2**		25			10				20		20			10					85
13	29.03.2010	**2**	10		25												10				45
14	05.04.2010	**2**		20	20						20			20							80
15	12.04.2010	**3**					20														20
16	19.04.2010	**3**				20											20				40
17	26.04.2010	**3**		20				20			10		10		20						80
18	03.05.2010	**3**		20			20		20		10		10								80
19	10.05.2010	**4**				20						10		10				10			50
20	17.05.2010	**4**	20		20		20			10		10				10					90
21	24.05.2010	**4**									10		10					10			30
22	31.05.2010	**5**				20								10			10				40
23	07.06.2010	**5**					20		10		10		10								50
24	14.06.2010	**5**				20							10			10					40
25	21.06.2010	**5**																			0
26	28.06.2010	**6**																			0
27	05.07.2010	**6**											20			10					30
28	12.07.2010	**6**						10				20			10			10			50
29	19.07.2010	**6**					10			10											20
																					900

* Note that period **1/2** is dominated by prespawning fish; in periods **3** to **5** = atresia sampling

	per period					
	1	2	3	4	5	6
AZTI		90	0	40		
vTI		100	60			
MI		80				100
FRS			100		70	
IMARES			20	40	60	
IMR				60		
IEO		10	40			
FAR				30		
	0	280	220	170	130	100

Table 3.1.3. Adult mackerel sampling program – Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2010

MACKEREL SAMPLING

Estimation of potential fecundity in pre-spawning fish and the estimation of atresia for realised fecundity

Sampling at Sea (for details on cruises see Table 2.2)

Area	Sampling by	Period/samples 1	2	3	4	5	6	total no. of samples
Southern	POR/IPIMAR	40						40
	ESP/IEO	60*	60	50				170
	ESP/AZTI		30					30 **240**
Western	ESP/AZTI		90		40			130
	GER/vTI SF		100	60				160
	IRL/MI		80				100	180
	ESP/IEO		10	40				50
	FAR/FFS				30			30
	SCO/MSML			100		70		170
	NED/IMARES			20	40	60		120
	NOR/IMR				60			60 **900**

* Additional samples of 60 mackerel obtained by market and/or onboard sampling

a) obtain length, weight and maturity distribution (***carefully!***) of larger sample (>100 fish) → length, sex, maturity, weight distribution

b) select mature, late pre-spawning, spawning or spent (stages 3-6) females (20 per station in 4 weight classes [<250/-400/-550/>550 g]), measure, weigh (total), gut, take otoliths (***carefully!***) → lenght, weight (total), sex, maturity, age

c) remove ovaries ***undamaged!***, weigh ovaries
take 3 (6)* parallel pipette samples of one ovary **(25 µl)** in pre-filled individually coded Nunc tubes and → ovary, liver, guts, carcass weight
put the other ovaryin formalin jars**, weigh liver, guts (including contents)
guts (including contents)and carcass

d) send each one of the parallel samples according to the instructions given before the 2010 survey to either Vigo, Aberdeen, Bergen, Galway, San Sebastian, IJmuiden

histological analysis at IEO, IMR, AZTI, IMARES → potential fecundity, atresia, realised fecundity

***From two fish on each single survey every institute should take 6 parallel pipette samples to provide material for a histological ring test between IEO, MSML, IMR, MI, IMARES and AZTI!**

**** There might be changes regarding taking the ovaries due to changes in the astresia estimation. Please refer to the survey instructions which wil be send to all particpants before the 2010 survey!**

For the desired temporal and spatial distribution of the fecundity samples please refer to Table x.x.x; for further instructions please refer to paragraphs x.x.

Collection of samples for genetic population analysis (recommended)

a) Collect a larger sample of mackerel

b) Cut a tissue sample (about 10*5*5mm) of the muscle, put each tissue sample in a 1.5ml tube in absolute alcohole

c) Store the tubes until further notice

Sample analysis targets for Ireland, Norway, Scotland, Spain (IEO and AZTI) and Netherlands participating in estimation of mackerel fecundity and atresia are shown in Table 3.1.4. Each country carrying out the various cruises listed in Table 3.1.2.a-b is responsible for distributing the samples collected to the countries carrying out the fecundity analysis. Norway will coordinate mackerel fecundity and atresia sample analysis.

Table 3.1.4. Overview on the laboratory analysis for mackerel.

PROTOCOL FOR LABORATORY ANALYSIS OF MACKEREL FECUNDITY SAMPLES		
Tasks	Countries	Timing for work completion
Training	Ireland, Norway, Scotland, Netherlands and Spain (IEO and AZTI)	December 2009 Workshop
The fecundity manual will be revised during the 2009 Workshop based on procedures developed during the 2007 survey and ongoing research.	Ireland, Norway, Scotland, Netherlands and Spain (IEO and AZTI)	December 2009
Fecundity analysis	Ireland, Norway, Scotland, Netherlands and Spain (IEO and AZTI)	Completed by end of august 2010
Atresia analysis	Norway, Netherlands and Spain (IEO and AZTI)	Provisional results completed for 2010 Assessment Working in September Completed results for WGMEGS 2011

3.2 Western Horse mackerel fecundity

During the 2010 survey horse mackerel will be collected from trawl hauls on the Southern and Western spawning components selecting fish of maturity stages 3–5 (Walsh scale) as shown in Table 3.2.1. As with mackerel, the tables are only indicative of the range in temporal and spatial coverage to guide cruise leaders and are not in any way to be taken as a strict rule about the timing in relation to spatial coverage of the plankton sampling grid. Details of the horse mackerel sampling over the spawning season giving the best latitudinal coverage of fish and fish processing are shown in the flow chart below (Table 3.2.1). If one of the hauls fails to catch fish the number of fish taken can be increased in the next trawl haul.

Tab. 3.2.1: Desired temporal and spatial distribution of the horse mackerel fecundity sampling in the Western Area

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

HORSE MACKEREL Lat ° Lon °

Week	Date	Period	44N	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	11W	10	9	8	7	6	5	4	3	2	1	Total
4	25.01.2010	1																														0
5	01.02.2010	1																														0
6	08.02.2010	1																														0
7	15.02.2010	1																														0
8	22.02.2010	1																														0
9	01.03.2010	1																														0
10	08.03.2010	2					5		5																							10
11	15.03.2010	2						5		5																10						20
12	22.03.2010	2		10			10				10		10			5								5			5					55
13	29.03.2010	2	5		10													5						5			5					30
14	05.04.2010	2		10	10						10			10																		40
15	12.04.2010	3					10																									10
16	19.04.2010	3				10												10								10						30
17	26.04.2010	3		10				10			5		5			5										10						45
18	03.05.2010	3		10			10		5		5		5													10						45
19	10.05.2010	4				10						5		5					5								10					35
20	17.05.2010	4	10		10		10			5		5				5																45
21	24.05.2010	4									5		5						5								10					25
22	31.05.2010	5				10								5				5														20
23	07.06.2010	5					10		5		5		5																			25
24	14.06.2010	5					10						5			5																20
25	21.06.2010	5																														0
26	28.06.2010	6																														0
27	05.07.2010	6											5			5																10
28	12.07.2010	6						5				5			5			5														20
29	19.07.2010	6					5			5																						10
																																495

* Refer to Tab.3.1.2a for the area Cadiz to Galicia

	per period					
	1	2	3	4	5	6
AZTI		50		40		
vTI		50	30			
MI		30				40
FRS			40		35	
IMARES				30	30	
IMR				30		
IEO		25	50			
FAR				15		
total:	0	155	120	115	65	40

Protocols for horse mackerel sampling preparations, sampling at sea and analysis in the laboratory are shown in Tables 3.2.2 and 3.2.3 and Annex 4 respectively. 10 samples will be analysed by all countries for quality assurance but at least 2 subsamples should be analysed for all the remaining fish.

Ovaries should be weighed and subsamples taken by pipette before fixing in 3.6% buffered formaldehyde solution on board. Participants are encouraged to attend the egg and/or fecundity workshop to learn the correct use of the pipettes. Participants should check the pipettes and plungers to see if they are working correctly prior to the survey. Ovary subsamples should be stored in formaldehyde in Nunc tubes. Care should be taken that oocyte samples are completely covered by formaldehyde. Participants should regularly check that the samples are in sufficient amount of formaldehyde.

Prior to cruise departure **Cindy Van Damme (Netherlands)** will coordinate the analysis of horse mackerel fecundity samples and assign tube reference numbers to cruise leaders for labelling the Nunc tubes used on their cruises.

Table 3.2.2. Overview on the laboratory analysis for horse mackerel.

PROTOCOL FOR LABORATORY ANALYSIS OF HORSE MACKEREL		
Tasks	Countries	Timing for work completion
Training	Ireland, Netherlands Norway and Spain (IEO and AZTI)	December 2009 Workshop
Fecundity analysis	Ireland, Netherlands Norway and Spain (IEO and AZTI)	December 2010

3.3 Collection of samples for genetic population analysis

IMR will apply for a national project to investigate the genetic structure of the different NEA mackerel spawning components. The egg survey in 2010 will be a useful opportunity to obtain samples for this project for the southern and western spawning components. All the samples should be stored at the respective institutes until funding is found for further analysis.

Table 3.2.3. Flow chart for selecting and processing horse mackerel samples.

Mackerel and Horse Mackerel Egg Survey 2010

HORSE MACKEREL SAMPLING

Estimation of standing stock fecundity and lipid content in relation to spawning status

Sampling at Sea (for details on cruises see Table 2.2)

Stock Comp.	Sampling by	Period/samples 1	2	3	4	5	6	total no. of samples
Western	ESP/AZTI		50		40			90
	GER/vTI SF		50	30				80
	IRL/MI		30				40	70
	ESP/IEO		25	50				80
	SCO/MSML			40		35		80
	NED/IMARES				30	30		60
	FAR/FFS				15			
	NOR/IMR				30			30 **495**

(a) obtain length, weight and maturity distribution (***carefully!***) of larger sample (>100 fish) ----------► length, sex, maturity, weight distribution

(b) select females in maturity stages 3-5 (Walsh scale) >= 25cm, Be sure to divide the females equally into the 4 weight categories < 150g, 151-250g, 251-350g and >350g. Measure, weigh (total), gut, weigh (gonad, carcass), take otoliths (***carefully!***) ----------► lenght, weight (total, gonad, carcass, [gut]), sex, maturity (1-6, Walsh scale), age

(c) take 3 (6)* parallel pipette samples of one ovary **(25 µl)** in pre-filled individually coded Nunc tubes and make sure that all the ovary samples are covered with formaldehyde

(d) send each one of the parallel samples according to the instructions given before the 2010 survey to either Vigo, Aberdeen, Bergen, Galway, Umuiden, San Sebastian

histological analysis at AZTI, IEO, IMARES, IMR, MI, MSML ----------► vitellogenic oocyte frequency presence of POFs/atretic oocytes

***From two fish on each single survey every institute should take 6 parallel pipette samples to provide material for a histological ring test between IEO, MSML, IMR, MI, IMARES and AZTI!**

For the desired temporal and spatial distribution of the fecundity samples please refer to Table 3.2.2; for further instructions please refer to paragraph3.2.

4 Review procedures for egg sample sorting, species ID, staging, data submission and subsampling (referring to ToR c)

4.1 Planning for egg sample sorting, species identification and staging workshop

It is recommended that each institute participating in the 2010 mackerel and horse mackerel egg survey has at least one scientist/technician at the egg staging workshop (WKMHMES) to be held at IMARES, IJmuiden between 5 and 9 October, 2009. It is essential that this representative is the same person who will analyse the majority of their institute's plankton samples from the 2010 egg survey.

In particular the workshop will:

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

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

c) update a set of standard pictures and descriptions for species identification and egg staging; because the southern congeners of *S. scombrus* (southern *S. colias*) and *T. trachurus* (southern *T. mediterraneus* and *T. picturatus*) are becoming increasingly important in the southern area, particularly pictures and specimens of eggs of those species will be examined;

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

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

f) make each participant aware of the need for compliance with the standard operational procedures described in the WKMHMES and WGMEGS survey manuals;

g) carry out pipette sampling exercises for fecundity analysis in particular for those participants that do not take part in the fecundity workshop held in San Sebastian.

The workshop will attempt to standardize analytical procedures as far as possible. To help with this, the workshop will address each step of the plankton analysis, separately.

4.2 Sorting, identification and staging of eggs, submission of survey results

Sorting, identification and staging of eggs should be done strictly following the WGMEGS survey manual. Analytical results should be submitted to the survey coordinator, using the standard excel spreadsheets (Figure 4.1), no later than one month before the WGWIDE meeting.

Recent information from AZTI (see Section 3 and ICES 2008c) has highlighted the overlap in the temporal and spatial distribution of mackerel species *Scomber scombrus* and *Scomber colias* within ICES areas VIIIb and VIIIc. The northern limits of the distribution of both species appear to have been shifting gradually to the north and it is possible that the extent of spatial overlapping of these species has increased.

In NE Atlantic waters the relative abundances of the three co-occurring horse mackerel species (*T. trachurus*, *T. picturatus* and *T. mediterraneus*) appear to show some degree of seasonal and interannual variability (see Section 3).

For correct application of egg production methods it is essential that the early life stages of species can be distinguished accurately and no misidentification is carried out. However, within the genera of *Scomber* and *Trachurus*, the eggs of the different species are difficult to separate. As a result it is very likely that potential problems could arise during the identification process.

Marine Eggs project (QLK5-CT, 1999–01157) partially focused on solving these egg identification problems using genetic analysis, but was not able to obtain satisfactory results. The use of formaldehyde as an egg preservative appeared to be a problem in the application of this genetic method for species identification. However, more advanced genetic techniques are now available. These newer methods are more robust and will hopefully provide consistent results in the identification of *Scomber* and *Trachurus* eggs to species level.

WGMEGS acknowledges that there is a need for development of comparative egg descriptions from fertilization experiments of these various species. In addition it is possible that genetic analyses of egg samples from surveys would help to assess the degree of potential misidentification and also species overlap.

WGMEGS therefore recommends that the following work is conducted during the next survey in 2010:

i) Participants should attempt to compile information on spawning periods for all the target species, including all species of mackerel and horse mackerel

ii) Participants should attempt to collect information during the 2010 surveys on the macroscopic reproductive stages for all target species.

iii) Participants should attempt to compile photos and obtain egg samples from fertilization experiments and produce comparative egg descriptions for presentation at the next WKMHMES in 2009.

iv) Participants should look into the feasibility of conducting genetic analyses on egg samples from the whole survey area to assess degree of misidentification between species with very similar eggs.

4.3 Processing of subsets of samples

Selection and processing of an unrepresentative subset of plankton samples led to a serious underestimation in the preliminary calculation of SSB for the southern component of mackerel in 2007. In order to avoid major revisions of such preliminary estimates in future, WGMEGS recommends that processing of subsets of samples should only be done in liaison with the working group. All participants should attempt to meet the deadline of survey result submission (see Section 4.2 above) and processing of subsets of samples should be avoided, in order to provide a reliable estimate of the SSB index. Should it prove impossible for a participating institute to provide their survey results in time, the survey coordinator and the WGMEGS Chair should be notified as soon as possible. The survey data coordinator, WGMEGS Chair and Steve Milligan (CEFAS) as an independent referee will then liaise with the participant about selection of a representative subset of samples that can be processed as a priority.

Figure 4.1. Example of the Excel spreadsheet to be used for data submission to the survey coordinator upper panel: columns A to X, lower panel: columns Y to BA.

							Date			Time Deployed (GMT)		Position (not - decimal)					Flowmeters(FlowmeterB columns used only for twin bongo users where both nets are analyzed and therefore values are needed from both flowmeters. Otherwise leave blank!)				Depth		
Period	Country code	Vessel code	Gear code	Haul number	Aperture (m^2)	Efficiency factor	Day (dd)	Month (mm	Year (yyyy)	Hour (hh)	Minutes (m	Latitude (d	Latitude (m	Longitude	Longitude (r	East/West	Flowmeter (rev)	Flowmeter calibration (rev/m)	FlowmeterB (rev)	FlowmeterB calibration (rev/m)	Sampler depth (m)	Bottom depth (m)	Volume filtered (m^3)
Period	**Country**	**Vessel**	**Gear**	**Haul**	**Ap**	**Effic**	**Day**	**Month**	**Year**	**Hour**	**Minutes**	**LatDeg**	**LatMins**	**LongDeg**	**LongMins**	**E/W**	**FlowRevs**	**FlowCal**	**FlowRevsB**	**FlowCalB**	**Sdepth**	**Bdepth**	**VolFilt**
1	PO	CAPR	B	1	0,125663706	1	15	1	2004			36	5	5	55	W	2028	3,363503304			78	80	75,7680231
3	SP	COSA	B	8	0,125663706	1	20	3	2004	11	45	42	44,78	9	39,91	W	45743	36,68452381			225	1271	156,6937311
3	SP	INVE	B	66	0,125663706	1	7	4	2004			43	35	4	45	W	4329	3,855050116	4523	3,47705146	220	1000	304,5783377
5	NE	TRID	G7	598	0,031415927	1	26	5	2004			43	53	7	27	W	217219	37,2			144	152	128,0519922

Temperature(ºC)						Non - Raised Mackerel Egg Numbers/Stage						Raised Numbers Mackerel Eggs/Stage					Non - Raised Horse Mackerel Egg Numbers/Stage					Raised Numbers Horse Mackerel Eggs/Stage				Other Eggs		
at 5 m	at 20 m	at 50 m	at 100 m	at bottom	Salinity 20m	Mac Stage 1	Mac Stage 2	Mac Stage 3	Mac Stage 4	Mac Stage 5	ing Factor (if ap	Mac Stage 1	Mac Stage 2	Mac Stage 3	Mac Stage 4	Mac Stage 5	Hom Stage 1	Hom Stage 2	Hom Stage 3	Hom Stage 4	sing Factor (if ap	Hom Stage 1	Hom Stage 2	Hom Stage 3	Hom Stage 4	on-raised total othe	Factor others (if appr	Raised total others
Temp5m	**Temp20m**	**Temp50m**	**Temp100m**	**TempB**	**Sal20m**	**Mac1**	**Mac2**	**Mac3**	**Mac4**	**Mac5**	**MacFactor**	**MacStage1**	**MacStage2**	**MacStage3**	**MacStage4**	**MacStage5**	**Hom1**	**Hom2**	**Hom3**	**Hom4**	**HomFactor**	**HomStage1**	**HomStage2**	**HomStage3**	**HomStage4**	**Others**	**OthFactor**	**RaisedOthers**
15,66	15,66					2	4	0	0	0	1	2	4	0	0	0	1	1	0	0	1	1	1	0	0	4	1	4
13,7979	13,6463					2	8	0	0	0	1	2	8	0	0	0	51	3	16	0	1	51	3	16	0	3	1	3
	12,4					1	3	3	4	6	1	1	3	3	4	6	0	0	0	0	1	0	0	0	0	3	1	3
14,21	14,04					14	10	10	7	3	1	14	10	10	7	3	31	13	16	12	1	31	13	16	12	5	1	5

5 Review of procedures for fecundity and atresia estimation (referring to ToR d)

5.1 Planning for fecundity workshop

It is recommended that each institute participating in the 2010 mackerel and horse mackerel egg survey has at least one scientist/technician at the fecundity workshop (WKMHMES) to be held at AZTI, San Sebastian from 1 to 4 December 2009 (4 days inclusive). It is essential that this representative is the same person who will analyse the majority of their institute's fecundity and atresia samples from the 2010 egg survey. The workshop will attempt to standardize analytical procedures as far as possible. To help with this, the workshop will focus on each step of the fecundity analysis listed under the bullet points below. Participants should bring a laptop because this will be used for scoring images prepared from horse mackerel and mackerel whole mounts and slides from mackerel. All participating institutes will bring pictures of the fecundity samples from the 2007 surveys and slides stained with Toluidine blue, Haematosin and Eosin and PAS Mallory respectively.

Objectives for the fecundity and atresia workshop:

- Fecundity sampling using the Wiretrol pipette. AZTI will provide fresh or frozen ovary samples for demonstration and testing.
- Data collection and data format.
- Standardisation of whole mount and slide staining protocols to estimate fecundity and atresia respectively.
- Use of image analysis hardware and software to achieve reproducible data. IMR will prepare the necessary ImageJ (open source image analysis software) macros and procedures which will be distributed at the workshop.
- Standardisation of whole mount interpretation to identify spawning markers and follicle measurement
- Standardisation of slide interpretation to estimate 3 classes of early alpha atresia (Yolk vesical, Yolk vesical /Yolk Granule and Yolk granule).
- Update and improve the fecundity manual. Discuss improvements to the manual: Is diameter measurements of early maturing oocytes necessary? Can we use profile counting for estimation of artesian? Optimal image resolution. Optimal sample density in sample tray when working up fecundity samples

5.2 Issues relating to Atresia and spawning duration and it's persistence

Methods of data analysis to discount the production of atretic follicles defined in ICES 1996 rely critically on the duration of spawning (D) and the early alpha atretic atresia stage Ad referred to in the equation 3 below.

1 $$SSB = \frac{E}{Fr}$$

Where E = Population annual egg production
Fr = Realised fecundity (eggs / g female)

2 $$Fr = Fp - Ap$$

Where Fp = Potential fecundity (vitellogenic follicles / g measured just prior to spawning)
Ap = Atretic follicles (per g female produced over the spawning cycle of the average female

3 $$Ap = Ai \,.\, P \,.\, D \,.\, Ad$$

Where Ai = intensity of atresia (standing stock of atretic follicles per g female)
P = prevalence of atresia (proportion of spawning females containing atretic follicles)
D = Spawning duration of the average female
Ad = duration of the atretic follicle takes to regress

At present WGMEGS uses values of 7.5 and 60 days for early alpha atresia and spawning duration, respectively (ICES 1996), but these values are not supported by strong experimental evidence and there is no variance term to be included in the calculation of the overall SSB variance. It is therefore recommended to address these issues in future.

5.3 Experimental study of growth and reproduction in Atlantic horse mackerel

600 Horse mackerel were caught in September 2005 in the Matre fjord, Norway, and brought into two land-based tanks for experiments on oocyte development, growth and egg production. The fish survived and matured in captivity, but final maturation and spawning did not happen. Analysis of the oocyte samples showed that horse mackerel is a typical asynchronous spawner. The experiment also indicated that temperature is an important trigger for initiation of spawning. The results of the experiment were published in the ICES journal (Ndjaula *et al.*, 2009).

5.4 Fecundity database

For the 2010 survey each institute will send their fecundity and atresia data in Excel format to the coordinating institutes; IMR for Mackerel and IMARES for Horse mackerel. Excel templates will be provided from the respective coordinating institutes. It is important to stick to the templates strictly! Because the fecundity and atresia data are a part of a time-series it must be emphasized that the data formats stays as similar as possible over time. The coordinating institutes will be responsible for storing and maintaining the time-series. It is important that also the manuals for each survey are stored.

6 Analysis and evaluation of the results of the 2008 mackerel egg survey in the North Sea (referring to ToR e)

6.1 Spatial and temporal coverage

During the period 2 June-5 July 2008 the Netherlands, "Tridens", and Norway, "Håkon Mosby", carried out mackerel egg surveys in the North Sea to estimate egg production and spawning-stock biomass (SSB). The spawning area in the North Sea has been surveyed most years since 1968. Since 1980 the spawning area has been surveyed several times during the spawning season. Since 1996 the North Sea mackerel egg surveys have been carried out on a triennial basis.

The original plan was to survey the spawning area four times in 2008. The first and last periods were to be carried out by one vessel and periods 2 and 3 by the two vessels in cooperation. Unfortunately, because of bad weather and technical problems Norway was not able to participate during the second period.

6.2 Sampling and data analysis

The data collecting and the handling of the samples were carried out according to ICES (1997/H:4). RV "Håkon Mosby" carried out the survey with a Gulf VII deployed on double oblique hauls from the surface to 70 m (more than 20 m below the thermocline) or 5 m above the bottom. RV "Tridens" also used a Gulf VII in double oblique hauls from surface to 5 m above the bottom or 20m below the thermocline. The timing and the results of the surveys are given in Table 6.2.1. During the third period "Håkon Mosby" worked the area north of 56° 30'N and "Tridens" worked the area south of this latitude.

The eggs were sorted from each of the sampled stations using the spraying method (Eltink, 2007) and their ages were estimated according to development stage and to the observed temperature at 5 m. Only eggs in development stages 1A and 1B were used in the egg production calculations. The staging of the eggs and calculation of their respective ages were carried out according to Lockwood *et.al.* (1981). The number of eggs produced per m^2 per day was calculated for each statistical rectangle of 0.5 ° latitude * 0.5° longitude (Figure 6.3.1). The samples were collected from the middle of each of these rectangles. The egg production was calculated for the total investigated area for each of the four survey periods (Table 6.2.1).

6.3 Mackerel egg distribution

The distribution of daily egg production per m^2 surface is shown for each of the periods in Figure 6.3.1. The standard interpolation rules were applied and interpolated rectangles are shadowed in the figure. The interpolated values accounted for 23%, 16% and 20% of the daily egg production respectively for periods one, three and four. The results show that the main spawning still takes place in the southwest of the area but the production was more abundant further north and east than in 2002 and 2005. Based on the three successful survey periods an egg production curve was drawn (Figure 6.3.1).

6.4 Potential fecundity and atresia of North Sea mackerel

As a result of the relative low egg production in the North Sea the fecundity has only been investigated twice, 1982 and 2005 (Iversen and Adoff, 1983, Krüger-Johnsen, 2006). The fecundity was observed to be rather similar 1401 and 1359 egg/g/female

respectively in 1982 and 2005. The fecundity obtained in 1982 has been applied as a standard one to convert the egg production to SSB for all the years.

During the survey female mackerel were collected for fecundity purposes both by "Tridens" and "Håkon Mosby". "Håkon Mosby" had problems catching mackerel. Mainly small fish were caught and they may therefore not reflect the actual length distribution in the SSB. Because ovary collection took place during the peak of the spawning season it was only possible to collect 14 prespawning ovaries that could be used for potential fecundity estimation. In addition 84 spawning ovaries were collected and used for atresia estimation. The potential fecundity was low (684 eggs/g/female, range 279 - 1319) and the atresia was high, giving a realized fecundity that was less than 50% of the standard one.

The WG was rather concerned about the low fecundity observed and regrets that too few data were available. The fecundity might be an indicator reflecting the state of the ecosystem. The WG recommends that the mackerel fecundity in the North Sea should be monitored on a yearly basis to study the dynamics of this parameter and evaluate it as an ecosystem indicator. Ovary samples have to be obtained in May-June. At this time there is no directed fishery for mackerel in the North Sea. Samples have therefore to be obtained from bycatches in other fisheries and from research vessels.

6.5 Mackerel egg production and spawning stock estimate

By integrating the egg production curve in Figure 6.5.1 and applying the parameters in Table 6.5.1 the total egg production was estimated at $108*10^{12}$ eggs, which was 32% less than in 2005 (Table 6.5.2). This is 19% higher than the provisional estimate given by WG-WIDE (ICES, 2008b). The reason for this is that the provisional estimate was based on the assumption that all the Dutch data represented full samples. However, several plankton samples were sub sampled and therefore the number of eggs in the subsamples had to be raised accordingly.

The egg production is still underestimated because the sampling was never carried out until zero values were obtained in any period. Particularly the unsampled areas outside the southwestern part of the survey area might be an important part of the spawning area. Also, the Skagerrak was not part of the investigated area and earlier surveys have indicated that the egg production in the Skagerrak accounts for about 5% of the total production.

Due to limited data and the small size of sampled mackerel in 2008 there is concern that the ovary samples collected might not reflect the average spawning fish in the North Sea. WGMEGS therefore agreed with WGWIDE (ICES, 2008) to apply the standard fecundity of 1401 eggs/g/female for estimating the SSB giving a SSB of 154,000 tons.

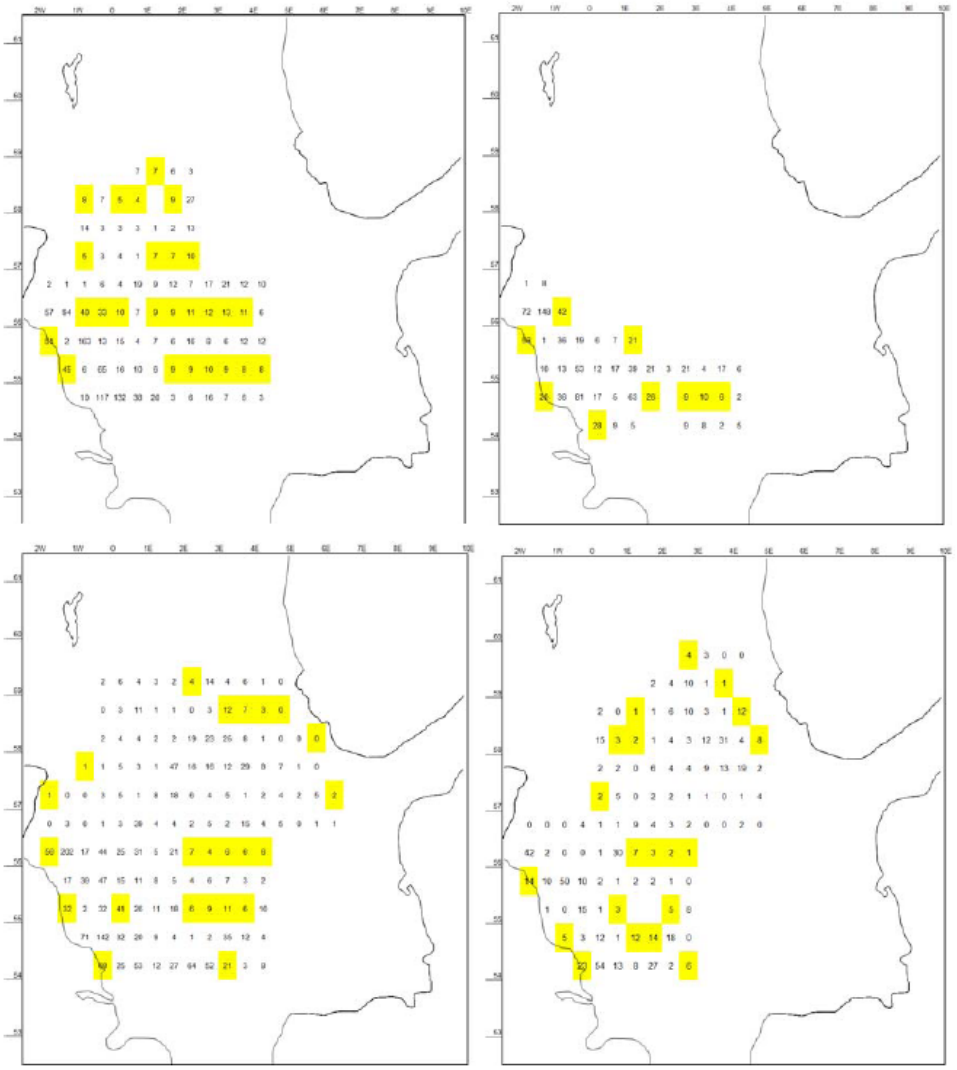

Figure 6.3.1. Daily mackerel egg production/m² during the four survey periods in 2008 (shadowed rectangles with interpolated values).

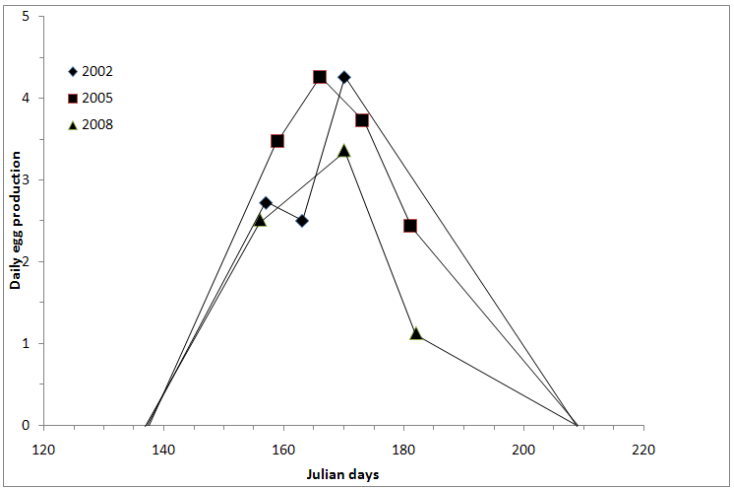

Figure 6.5.1. Daily egg production (eggs * 10–12) of North Sea mackerel during the 2002, 2005 and 2008 surveys.

Table 6.2.1. The 2008 mackerel egg survey in the North Sea.

Coverage	1	2	3	4
"Tridens"	2-6 June	9-11 June	14-19 June	-
"Håkon Mosby"	-	NA	14-23 June	26 June-3 July
Midpoint of survey	4 June	10 June	18 June	30 June
Julian day	156	162	170	182
Total daily egg production x 10^{-12}	2.53	(1.17)[1]	3.37	1.13
Interpolated daily egg production x 10^{-12}	0.57	(0.23)	0.54	0.23

[1]Represents only the southern part of the spawning area

Table 6.5.1. Parameters and formulas used in the egg production and SSB estimates.

Parameter	Value/formula	Reference
Age of stage 1A+1B eggs	Age = $Temp^{-1.61} * e^{7.76}$	Lockwood et.al. 1981
Fecundity North Sea	1401 eggs/g female	Iversen and Adoff 1983
Sex ratio	1 : 1	as in previous years
Spawning period (Julian days)	17 May - 27 July (137-208)	as in previous years, excl.1990[1]
Number of spawning days	72	as in previous years, excl.1990[1]

[1]A limited production of $0.02*10^{12}$ eggs was observed in the south eastern part of the North Sea during 23.04-3.05.1990

Table 6.5.2. Egg production estimates from egg surveys in the North Sea and corresponding SSB based on a standard fecundity of 1401 eggs/g/female.

Year	Egg prod $*10^{-12}$	SSB$*10^{-3}$ tons
1980	60	86
1981	40	57
1982	126	180
1983	160	228
1984	78	111
1986	30	43
1988	25	36
1990	53	76
1996	77	110
1999	48	68
2002	147 (118)	210 (168)
2005	155	223
2008	108	154

7 Updates on the survey manual and standardization of sampling tools and survey gears (referring to ToR f)

7.1 General overview

An update on the survey manual and standardization of sampling tools and survey gears is included in this report as Annex 2. Annex 2 also presents recommendations and highlights recent changes to the previous version of the manual which was presented in the ICES 2008 report. The survey manual with be further discussed and where necessary updated during the egg staging and fecundity workshops (WKMHMES) to be held in autumn 2009. The survey manual will also be prepared as a stand-alone document to be available at the workshop and for the 2010 survey.

7.2 Standardization of survey gears and sampling methods

Since the 2004 surveys a high level of standardization of sampling equipment has been achieved for the mackerel and horse mackerel egg surveys. According to the Table 7.2.1 all Gulf VII type samplers used by the respective participants are more or less comparable with respect to their dimensions and therefore also their sampling performance. Provided that calibration of flowmeters is carried out carefully and the sampling manual is strictly followed it can be assumed that there is no sampler related bias.

Table 7.2.1. Gulf type "high-speed" plankton sampler designs as used by WGMEGS survey participants.

Institute	IMARES	IMARES	vTI	MI	CEFAS	MSML	MSML	IMR
Country	Netherlands	Netherlands	Germany	Ireland	England	Scotland	Scotland	Norway
Torpedo type	Gulf III	Gulf VII	Nackthai*	Gulf VII	Gulf VII	Gulf III	Gulf VII	Gulf VII
Years	Before 2004	After 2004	2004, 2007**	Pre 2004	Since 1995	before 2007	2007	2007
Frame	Encased	Open	Open	Open	Open	Encased	Open	Open
Total length (cm)	224	275	275	272	278	230	273	273
Length frame (without nosecone) (cm)	199	215	221	214	215	199	213	213
Length nosecone (cm)	35	60	54	59	63	31	60	60
Length of streched planktonnet (cm)	165	180	173	177	193	177	177	180
Diameter frame (cm)	50	50	43	53	53	50	53	50
Diameter planktonnet (cm)	41	40	38	50	45	46	46	38
Diameter codend (mm)	80	70	92	95	80	75	75	80
Diameter nosecone (cm)	19	20	20	20	20	19	20	20
Flowmeter position	internal	internal and external	internal and external	internal and external	internal and external	internal and external	internal and external	internal
Flowmeter brand/type		Valeport	Hydro-Bios	Valeport	Valeport	In-house design	Valeport-replica	Valeport
Flowmeter blade diameter (cm)			7.5		12.5			5
Mechanical/electronic	Mechanical	Electronic	Electronic	Electronic	Electronic	Mechanical	Electronic	Electronic

*** Modified Gulf VII; ** A similar type but shorter was used the years before.**

Portugal (IPIMAR) used a CalVET-net in the 2007 surveys and will continue to do so for the 2010 survey. Spain (AZTI and IEO) use 40 cm Bongo nets. All specifications are listed in Table 7.2.2. As with the Gulf VII samplers it can be assumed that no

sampler related bias is present provided that the WGMEGS manual is strictly followed.

Table 7.2.2. Plankton sampler designs as used by WGMEGS survey participants in the southern area.

Country	Net	Diameter (cm)	Shape	Mesh size (µm)	Total length (cm)
Spain (IEO)	Bongo	40	Cylinder-cone	250	248
Spain (AZTI)	Bongo	40	Cylinder-cone	250	284.3
Portugal (IPIMAR)	CalVET	25	Cylinder-cone	150	150

7.3 Alternatives to the Pronet system and future developments of Gulf VII plankton samplers

There has been a concerted effort by WGMEGS in the last five years to standardize the plankton sampling equipment used by the various participants in the triennial egg surveys. One of the systems chosen (Pronet, by Spartel, UK) and used by both Ireland and the Netherlands is now becoming increasingly difficult to maintain and the software has become outdated. A replacement system is therefore required and Kees Bakker (IMARES) gave a presentation of a new monitoring system currently being developed by IMARES, the Netherlands.

This new system utilizes an electrically cored towing cable which provides power to a Seabird CTD, flowmeters and altimeter, as well as allowing real time data transfer to a PC display and data storage. The graphical display is provided by LABVIEW software which provides both the operator and winch-man with a real time display of the depth of the sampler, height from the seabed, shape of the dive profile and various environmental parameters.

The development of such an updated system is very welcome and all participants are encouraged to consider using such a system should their current sampling systems become unsustainable. The LABVIEW application can be provided free by IMARES to all WGMEGS participants once a LABVIEW licence has been obtained.

7.4 Current status of spray method

A 'spray technique' (Eltink, 2007), for the separation of fish eggs from plankton samples, was first trialled by some participants during the 2004 triennial survey. The technique was then enhanced and tested during the egg-staging workshop in 2006 (ICES, 2006). Following these successful trials WGMEGS recommended the use of the 'spray technique' for the removal of fish eggs from plankton samples by all participants of the 2007 surveys. This remains a recommendation for the 2010 surveys.

The 'spray technique' will be further evaluated at the next WKMHMES, workshop to be held at IMARES, IJmuiden, Netherlands in October 2009.

8 Development of a relative index of horse mackerel abundance in the western area (referring to ToR g)

Horse mackerel is believed to be an indeterminate spawner and therefore, since 2007, IPIMAR has adopted the DEP methodology (DEPM=Daily Egg Production Method) for the species in the area of the southern stock (ICES IXa). The IPIMAR survey in period 1 is directed at horse mackerel DEP although the mackerel eggs collected during that survey will be used for the AEP method in the western and southern area.

In the western area the egg survey is directed at the AEP method which produces an index of mackerel SSB whereas for horse mackerel the egg data are collected to provide a relative indicator of its spawning-stock biomass. The procedures used (and resources available) for the AEP method do not allow for a DEP estimation of horse mackerel in the western area. It is necessary to have a good spatial coverage of both egg and adult sampling in a short period for the DEP method to be effective. In the western area, the requirement is to cover as much of the egg production as possible, both temporally and spatially, and resources are not available for intensive adult sampling.

The data from the samples collected in the western area might be used for a modified DEPM for horse mackerel. The 'traditional' DEPM estimates batch fecundity from the composition of migratory nucleus oocytes or hydrated oocytes and post-ovulatory follicles (POF's) for the estimation of the spawning fraction. The duration of these stages is short in horse mackerel and with the current limited sampling effort in the western area it is not possible to use these stages for determining batch fecundity. The modified method will determine the batch fecundity at an earlier stage, where the batches of larger vitellogenic oocytes start to separate from the standing oocyte stock (Figure 8.1). Using this modified method on the 2007 survey results gave a batch fecundity estimate of 132 oocytes per gram female. This is similar to the batch fecundity found in 2007 in the southern area (WGMEGS, 2008a) and similar to a study on horse mackerel in Greek waters (Karlou-Riga and Economidis, 1997). Estimating spawning fraction, assuming the females are spawning when the oocytes in the batches are bigger than 250 μm, gives a spawning fraction of 25%. This is also similar to the results obtained from the southern area and from Greek waters.

For the 2010 survey WGMEGS will continue to develop this modified DEPM approach and try to use this modified DEP method for estimating horse mackerel spawning stock size.

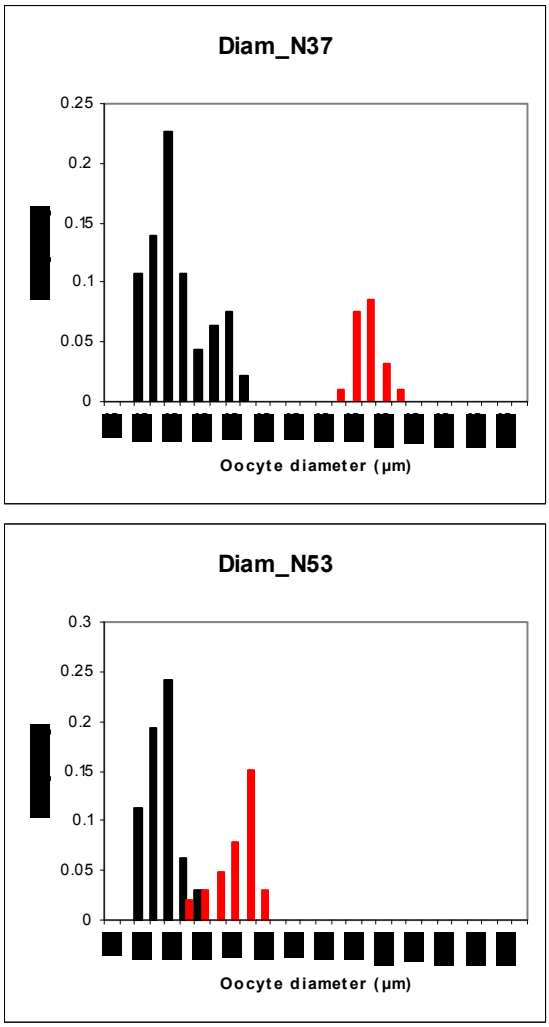

Figure 8.1. Oocyte frequency distribution. In sample N_37 the maturing batch is clearly separated from the standing stock of vitellogenic oocytes. In sample N_53 the batch is starting to form.

9 Deficiencies

Due to selection and processing of an inappropriate subset of samples, significant discrepancies were encountered between the preliminary egg production figures provided in September 2007 and the final results as presented to the group in April 2008. This initially led to a serious underestimation of the mackerel SSB in 2007. The rules for processing adequate subset of samples have now been established and are included in the survey manual (Annex 3) for future surveys.

Spatial changes in the spawning behaviour of mackerel and horse mackerel have resulted in an expansion of the perceived survey area. This, combined with the current constraints on shiptime, has previously created difficulties during the surveys where spawning boundaries for both species were not always secured satisfactorily, especially during the periods of peak spawning. The working group anticipates these problems becoming more prominent especially as sea temperatures continue to increase. The group wishes to emphasize that this deficiency will only be addressed by broadening participation in the triennial survey to include **all** of the states currently exploiting mackerel and horse mackerel.

10 Recommendations

RECOMMENDATION	FOR FOLLOW UP BY:
1. The group is extremely concerned about the limited resources that are available to complete the 2010 egg survey. The spawning area of horse mackerel and particularly mackerel is extending into new areas and WGMEGS is finding it increasingly difficult to survey all spawning areas satisfactorily given the allocated ship time. WGMEGS therefore encourages the coastal states to discuss whether fishing rights might be coupled with an obligation to participate in the triennial egg surveys and in the work analysing egg and fecundity data after the surveys.	All Atlantic mackerel and horse mackerel fishing countries
2. The Working Group is concerned with the low fecundities estimated during the 2008 North Sea mackerel egg survey. Fecundity may be an indicator of the state of the ecosystem. WGMEGS recommends that mackerel fecundity in the North Sea should be monitored on an annual basis to study its dynamics, and evaluated as an ecosystem indicator. Ovary samples should be collected from May to June. At present there is no directed fishery for mackerel in the North Sea, therefore samples will have to be collected from by-catch in other fisheries, and from research surveys.	National laboratories
3. The group reiterates the need to continue with the egg identification/staging and fecundity workshops prior to the egg surveys. WKMHMES are crucial refreshers for scientists and technicians who participate in the triennial egg surveys. Therefore, WGMEGS recommends that all survey participants are also participating in the workshops. The group recommends investigating the possibility of securing DCR funding to assist with the cost of these workshops.	National laboratories, PGCCDBS
4. The group notes the mixing of mackerel (*S. scombrus* with *S. colias*) and horse mackerel (*T. trachurus*, with *T. mediterraneus* and *T. picturatus*) species in the Southern area. This may also become an issue further north in future. The Group recommends that WGWIDE report commercial catches at species level to allow a realistic apportioning of the stock.	WGWIDE
5. The group recommends collecting samples of eggs, in the southern area, for genetic analysis. This will help determine the percentages of the southern mackerel and horse mackerel species in that area.	National laboratories

6. The group recommends the collection of genetic samples from the adult fish to determine the magnitude of different spawning components of mackerel.	National laboratories
7. The working group supports and recommends the PGEGGS initiative to establish a central expert group for ichthyoplankton surveys at ICES (rationales in Annex 5).	ICES
8. The working group recommends the proposed ICES Theme session on Ichthyoplankton surveys at the annual science conference in 2010. (rationales in Annex 6).	ICES
9. WGMEGS recommends that the following work is conducted during the next survey in 2010: i. Participants should attempt to compile information on spawning periods for all the target species, including all species of mackerel and horse mackerel ii. Participants should attempt to collect information during the 2010 surveys on the macroscopic reproductive stages for all target species. iii. Participants should attempt to compile photos and obtain egg samples from fertilization experiments and produce comparative egg descriptions for presentation at the next WKMHMES in 2009. iv. Participants should look into the feasibility of conducting genetic analyses on egg samples from the whole survey area to assess degree of misidentification between species with very similar eggs.	National laboratories

11 Working documents presented to the Working Group

1) Abundance of mackerel and horse-mackerel eggs South of 47°N in summer

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Abstract

A total of 127 plankton stations were analysed during summer of 2008 to identify mackerel and horse mackerel eggs in the area of the Bay of Biscay. Eggs were staged and daily production was estimated using the abundance of stage I. Maps of spatial distribution showed a marked presence of eggs, for both species, in the area of the Cantabrian Sea and very scarce abundance in French waters. The mean egg production for mackerel was $2.61*10^{11}$ and for horse mackerel was $1.07*10^{12}$. Compared to Southern mackerel mean daily stage I egg production in 2007 this value is similar to that obtained for period 3 (ICES, 2008a). For horse mackerel this values was also low, although higher than for mackerel. As a result of the similarity of eggs and larvae of both species of mackerel and horse-mackerel which inhabit the southern part of the Bay of Biscay, a global analysis on the affect of Spanish mackerel (*S. colias*) and Mediterranean horse mackerel (*T. mediterraneus*) was carried out. Data on reproductive cycle and seasonal landings of Spanish mackerel and Mediterranean horse-mackerel imply that a high percentage of eggs identified as mackerel and horse-mackerel in summer in the Bay of Biscay could likely be Spanish mackerel and Mediterranean horse-mackerel. In any case, the egg production estimated for the period (June-July) was low, as it should correspond to a small population of these species inhabiting these waters.

2) Seasonal occurrence of mackerel and horse mackerel eggs in the Cantabrian Sea (ICES Division VIIIc) in 2007

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Abstract

This work presents the results of a monthly ichthyoplankton sampling along 2007, at three locations off the North Iberian Peninsula. These transects are located in front of La Coruña, Gijon and Santander ports in the N Spanish coast (ICES Division VIIIc). The aim of this paper is the study of the yearly seasonal cycle of spawning of mackerel and horse mackerel eggs present in the plankton in order to know of the end-point of mackerel and horse mackerel egg production south of 47ºN during 2007.

Mackerel eggs appeared from February to July and the peak of spawning occurred in March. Horse mackerel spawning was more extended and took place from January to October and the peak of spawning was from April to June.

3) Planning the 2010 Portuguese DEPM survey for horse mackerel

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Abstract

The Annual Egg Production Method (AEPM) has been applied triennially since 1995 to the western and southern stocks of horse mackerel *Trachurus trachurus* in the ICES area (Abaunza *et al.,* 2003), assuming that fecundity for the species was determinate. Recent work suggested that horse mackerel has indeterminate fecundity, i.e. potential annual fecundity is not fixed prior to the onset of spawning and unyolked oocytes continue to mature during the spawning season (Gordo *et al.,* 2008). Following these developments the Daily Egg Production Method (DEPM), more appropriate to species with indeterminate fecundity, was adopted for the southern stock (ICES IXa - Gilbraltar-Finisterre, ICES, 2005) by IPIMAR (Portugal) the Institute responsible for surveying in this area (ICES, 2008a). DEPM application requires the estimation of daily fecundity (batch fecundity and spawning fraction) and daily egg production estimates (Stratoudakis *et al.,* 2006). The first survey directed at the DEPM was undertaken in 2007 involving several changes on the methodology used prior to that. Modifications introduced included a new plankton sampling gear and design, and laboratorial and data analyses developments for egg and adult samples (ICES, 2008a). In 2010 IPIMAR will conduct the second horse mackerel DEPM survey for the southern stock. This document summarizes the planning for plankton and adult surveying.

4) Measuring and staging eggs using a new ImageJ application

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Abstract

A new application for ImageJ-ObjectJ for measuring and staging fish eggs has been developed for the 2008 North Sea mackerel egg survey. The application developed for fresh eggs at IMR was taken and modified for formaldehyde fixed eggs. Unfortunately the image analysis program cannot distinguish between the egg scale and the yolk-sac boundary in formaldehyde fixed eggs therefore the new application is semi-automatic.

On board the research vessel pictures are taken with a SLR camera mounted on a dissecting microscope light source. (See the table below for the specifications of the SLR camera and the computer screen used.) This picture is imported into the ObjectJ folder in ImageJ. Using ObjectJ allows for all the measurement to be stored in a different layer and not in the original picture. With the mouse a circle is drawn around the circumference of the egg and the egg diameter is measured. The same in done for the oil globule. Then egg species and development stage are entered using numbers 1 to 12 for 12 different species and 1 to 6 for the development stages 1A, 1B, 2, 3, 4 and 5. The data can be exported from ImageJ per sample to for example an excel file.

ImageJ and ObjectJ are free available software and this fish egg staging and measuring application is free to use for an egg survey participants.

During the 2008 egg survey comparisons were carried out between the analysis using a dissecting microscope and the analysis in ImageJ. 26 samples were analysed in both manners and produced very similar results.

Specification of the SLR camera:
Camera: Canon 40D
Lens: Canon EF-s 60mm, maximum magnification 1:1

Settings of the camera:
ISO: 200
Aperture: f/6.3
Shutter speed: 1/640s
White Balance: manual 3400K

Specifications of the computer screen:
HP LP3065 30" Widescreen TFT Monitor speed 6ms 3–3–3
HP LP3065 – flat screen - TFT - 30"
Size: 69.2 cm x 24 cm x 49 cm
Maximum resolution: 2560 x 1600 / 60 Hz
Colour: 24-bit
Height-width 16:10
Screen clarity: 300 cod/m2

Interface: DVI

5) An updated system for the real time monitoring of Gulf VII plankton sampler deployments

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Abstract

A new plankton sampler monitoring system was needed to replace an outdated PRONET system. This new system utilizes an electrically cored towing cable which provides power to a Seabird CTD, flowmeters and altimeter, as well as allowing real time data transfer to a PC display and data storage. The graphical display is provided by LABVIEW software, which provides both the operator and winch-man with a real time displays of the depth of the sampler, height from the seabed, shape of the dive profile and various environmental parameters.

This application has been developed by IMARES to run within the LABVIEW software package. This application is simple to extend or modify. The current development interfaces environmental data from a Seabird CTD, flowmeter data (allowing calculation of volume of water filtered) and an altimeter (providing height off the seabed). These data are then displayed graphically on a PC monitor together with GPS position and echosounder information from the ship. The display shows the shape of the dive profile in real time and provides the winch operator with a useful guide for the control of the sampler in the water column particularly when approaching the seabed. It is hoped that future developments will include an application capable of allowing safe, automatic winch control.

6) Use of historical survey data to estimate the stock abundance of mackerel in the North Sea

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Abstract

Although mackerel is a species of high commercial interest, up to now the only available data on the stock abundance in the North Sea comes from the triennial egg survey. An annual abundance index for mackerel is a high priority of international fisheries management. Historical acoustic surveys might be used to estimate the stock abundance of North Sea mackerel and to find out more about their seasonal distribution and migration in the North Sea and the Northeast Atlantic.

In this study the data from the annual North Sea acoustic herring survey was used to identify mackerel resulting in a relative biomass index. School detection was based on an algorithm developed by the Fisheries Research Institute in Scotland (FRS). An algorithm for single target detection was considered but proved impossible to be used for a mackerel biomass estimate.

The relative biomass estimate was then compared to the estimate from the triennial egg survey to show the usefulness of the acoustic survey estimate, in an attempt to provide more information on North Sea mackerel and to get more value from existing acoustic surveys.

7) Egg production and spawning stock size of mackerel in the North Sea in 2008

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Abstract

During the period 2 June-5 July 2008 the Netherlands and Norway carried out egg surveys in the North Sea to estimate the spawning-stock biomass (SSB) of mackerel. The plan was to cover the spawning area four times. Due to bad weather and technical problems Norway was not able to participate in period 2. The data collecting and the handling of the samples were carried out according to the standard procedures. The total egg production was estimated at 108*1012 eggs. This is 32% less than in 2005. The egg production was underestimated because the sampling in none of the four coverages was carried out until zero values were obtained in all directions. During the survey only 14 ovaries of the trawled mackerel were in the right prespawning maturity stage and 84 ovaries in the spawning stage. These ovaries have been analysed respectively for potential fecundity and the atresia. The potential fecundity was low and the atresia was high resulting in a realized fecundity less than 50% of the standard one. Due to few data and that the caught mackerel might not reflect the average spawning fish in the North Sea the standard fecundity of 1401 eggs/g/female was applied to estimate the SSB at 154,000 tons.

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Annex 1: List of participants

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Annex 2: Proposed Terms of Reference for 2010

The **Working Group on Mackerel and Horse Mackerel Egg Surveys** [WGMEGS] (Chair: J. Ulleweit, Germany) will work by correspondence in 2009–2010 to:

a) examine the results of the IJmuiden and San Sebastian workshops (October and December 2009) on mackerel and horse mackerel egg staging and identification and histology, and incorporate these into the Survey Manual for the 2010 survey;

b) fine-tune survey execution in 2010.

WGMEGS will report by 1 June 2010 for the attention of the SCICOM and TGISUR.

Supporting Information:

Priority:	Essential. Terms of Reference are set up to provide ACFM with the information required for responding to requests for advice/information from NEAFC and EC DGXIV.
Scientific justification and relation to action plan:	Action Plan No: 1. The egg survey provides the only fishery-independent stock estimate for North-east Atlantic mackerel and for both the western and the southern horse mackerel stocks. The surveys provide the most essential indices for the tuning of the VPAs. The survey is based on a time-series since 1977. The ToRs for this year are largely routine ones, as the group does not meet in the year of a survey. **Term of Reference a)** WGMEGS has previously sponsored pre-survey Workshops in 2000, 2003 and 2006. These are essential to standardize many aspects of the survey protocol, but particularly egg sample collection, sorting, species ID and staging. In 2003 the workshop was expanded to provide the same standardization for the histological work required for the survey estimates; fecundity and atresia. As the surveys are held only once every 3 years it is vital to have all participants working in concert. The workshop will make recommendations for survey procedures and analysis, and these will be assimilated into the survey manual and used for the 2010 survey **Term of Reference b)** The 2009 report of WGMEGS outlined the provisional plan for the 2010 surveys. The group will maintain a watching brief on how this transpires in practice. The main actions are to ensure that the best coverage is obtained for the survey in the six survey periods. Problems with weather, vessels etc must be taken account of. The group will also maintain oversight of the adult sampling aspects of the work, to ensure the best temporal and spatial coverage of these samples.
Resource requirements:	None. The surveys are all part of the national programmes. The surveys and associated meetings are also partially funded under the EU data directive
Participants:	Far, N, NL, P, ESP, UK (E), UK (Scot), D, IRL. Usually 25 – 30 participants
Secretariat facilities:	None.
Financial:	No financial implications.
Linkages to advisory committees:	ACOM.
Linkages to other committees or groups:	Reports to the Living Resources and the Resource Management Committees, as well as WGMHSA. Other less formal links with SGRESP, WKSAD, and WGACEGG
Linkages to other organizations:	There are or have been a number of associated EU funded projects which make reports to the Group

Secretariat marginal cost share:	ICES: 100%.

Annex 3: Survey Manual

[All changes to the last version of the survey manual (ICES, 2008a) and recommendations are highlighted in bold text].

A manual for the conduct of egg surveys, targeted at the AEPM, is given in Section 8 of the Report of the Mackerel/Horse Mackerel Egg Production Workshop (ICES, 1994). Those instructions are repeated in ICES 1997 (Sections 6.4.1 to 6.4.8) and incorporate changes, additions or clarifications, which are underlined. Additional changes and recommendations for further standardization between participants are given in Section 3.3 of ICES, 2003.

This annex incorporates the current protocols (together with recent changes) for the collection and analysis of adult fish parameters required for the AEPM method. It is recommended that this annex is updated on a regular basis and is distributed for use by all participants on the **2010** and future triennial surveys. **It should also be made available to participants of WKMHMES and the associated fecundity workshop, which will both be held in autumn of 2009.**

1. 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 Northeast Atlantic shelf area is subdivided (by 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 36º N to 45º N. It includes southern Biscay, the Cantabrian Sea and the Portuguese coast and Gulf of Cadiz. Sampling usually begins in January in this area and continues until June in the Cantabrian Sea.

The 'western' area is from 44º N to 60º N. It includes Biscay, the Celtic Sea and the shelf edge to the northwest of Scotland. Sampling is focused along the shelf edge (200m isobath) but also occurs from the French and Irish coasts out to 16º W. Sampling in this area usually begins in March and continues into early July.

In the western area plankton samplers are deployed at the centre of half standard ICES rectangles, which are 0.5º latitude, by 0.5º longitude. To the north of Spain (Cantabrian Sea) and Galicia (North of 42°N) the sampling positions are separated by 10' latitude and 20' longitude because of the proximity of the shelf edge to the coast. The Portuguese survey in ICES IXa will be undertaken following a predefined grid of sampling stations along-transects perpendicular to the coast and separated by 12 nmiles

Since the surveys began in 1977 considerable changes have been made to the 'standard' sampling area and some of these were described in Section 8.4 (ICES, 1994). Based on the expansion of the "standard area" since 1977, it was agreed (ICES, 2002a) to reconsider its use. It was agreed that the existing "standard area" **(described above)** should be retained only as a guide to the core survey area for cruise leaders, and that the extent of coverage should be decided based on finding the edges of the egg distribution only. i.e. boundaries should be set based on the adaptive sampling guidelines given below (Section 2.). The core areas for the western and southern sur-

veys together, are presented in Figure 1. The sampling area in the south has been modified from the design used in 2001 and previously (Figure 2). Figures 1 and 2 are provided as a planning guide only. The limits of the survey in both areas should be established on the basis of two consecutive zero samples, and not by the boundaries on these maps.

2. Sampling strategy

The sampling strategy in the western and southern areas will be targeted at the AEPM only. However, Portugal will collect both plankton and adult fish samples to produce a DEPM estimate for horse mackerel in the area ICES IXa (Portugal, Gulf of Cadiz and W Galicia), in **2010. The DEPM methodology is not described in this manual.**

Two important factors needed to be considered when planning the survey strategy. First, a set of rules must be established in order to decide when to stop sampling along a given transect, in order to ensure that the whole area of egg distribution is sampled with no effort wasted outside the spawning area. Second, some guide-lines need to be provided to cruise leaders on the number and spacing of transects which may be omitted in order to best match available effort to the size of the area to be surveyed. As a first guide to planning the distribution of sampling effort, historical egg distributions should be reviewed with particular reference to the latest WGMEGS reports. The main areas of egg abundance, identified for each of the different sampling periods, should always be sampled to the north/south and east/west limits although individual transects may be omitted. When sampling along-transects, shipboard enumeration of results should be undertaken several rectangles before the limit of the core area is reached. The introduction of the 'Spray technique' (**Eltink, 2007**) should allow a rapid assessment of the numbers of eggs present in each station. Sampling will be completed along a transect when two consecutive stations contain no mackerel or horse mackerel eggs. In some cases it may be necessary to sample beyond the core area limits (Figure 1).

The amount of ship time available and the size of the area to be covered will determine the spacing and omission of sampling transects. During periods when several ships are available it should be possible to sample all transects, whereas at other times it may be necessary to omit several, at least during the first pass over the designated sampling area. No more than one consecutive transect should ever be omitted. Given that the area to be covered is more or less known, as is ship time, cruise leaders should be able to estimate fairly accurately the number of the full transects they will be able to make. It is strongly recommended that, where practical, and even where total coverage is expected, a first pass over the area be made on alternate transects. The intervening transect should be sampled on the return leg. If time is limited on the return leg, sampling should be concentrated in areas where high egg densities were observed in the first pass. The cruise leader should be aware of edge definition problems where the contours run east-west. In this way, weather problems, equipment failure and vessel breakdown need not seriously prejudice results. Such a strategy, furthermore, allows better evaluation of distributional change with time, which is likely to be important in modelling the results. An example of an appropriate sampling strategy where one in two transects is fully sampled is given in Figure 6.16 in ICES (1994).

Where possible, additional sampling should be carried out in areas where high densities of either mackerel or horse mackerel eggs are encountered. This will allow an estimate of sampling error to be calculated.

3. Standardisation of survey gears

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

It would be preferable to use a standard survey sampler for the triennial surveys. **A review of the design of sampling equipment (including flowmeters) used by each participating nation was last conducted and presented at WGMEGS in 2008 (Section 4.3.2, ICES, 2008a).** Nash *et al.,* 1998, provides a comprehensive description for a Gulf type sampler, which they call a Gulf VII. A useful review of Bongo designs and a suggested standard is given by Coombs *et al*. (1996) in an annex to the final report of EU AIR project AIR3 CT94 1911.

The estimation of volume of water filtered by each sampler is critical in the calculation of egg abundance. Again, the suggestions provided by Nash *et al*. (1998), and Coombs *et al.* (1996) provide an acceptable standard. It is recommended that participants follow these standards as closely as possible**.** It is also critical that participants understand the importance of calibrating flowmeters, and changes in flowmeter performance, when they are mounted in the apertures of plankton samplers (EU AIR3 CT94 1911). It is recommended that all participants review the performance of their flowmeters and regularly their check their calibration in-situ (i.e. within the sampling device). The current flowmeters used in the survey are largely considered as state-of-the-art; however, new developments are being made in non-intrusive flowmeters. It is recommended that participants investigate the utility and cost–benefits of these and report back to WGMEGS as appropriate.

Although a mesh size of 500 micron aperture is adequate for sampling mackerel and horse mackerel eggs, a nylon mesh with an aperture between 250 and 280 microns is the recommended size for these surveys. This allows the plankton samples to be more widely used for investigations on other species and taxa. In the North Sea surveys, where clogging is a problem, a 500 micron aperture mesh is used by both the Netherlands and Norway. Norway is the only participant to use 500 micron aperture mesh in the western (or southern) area.

The aperture on the Gulf type sampler should be 20 cm in diameter in order to ensure that an adequate volume of water is filtered. The aperture of the Bongo samplers should be either 40 cm or 60 cm diameter. It is recommended that no ad hoc changes take place.

Different mouth openings for Bongos do not seem to make a difference in sampling efficiency or performance, although 60 cm nets (vs. 40 cm) are apparently more prone to clogging **if the filtering area of the net isn't adjusted adequately**. Portugal used a 60 cm Bongo until the 2004 survey, but in 2007 they used a 40 cm diameter Bongo, similar to that used by AZTI and IEO, Spain for all their triennial surveys.

4. Plankton sampler deployment

It is recommended that the Gulf type samplers are deployed on a double oblique tow, at 4 knots, from the surface to maximum sampling depth (see below) and return. The Bongo samplers are deployed at 2–3 knots on similar, double oblique tows. The aim is

for an even (not stepped) 'V' shaped dive profile, filtering the same volume of water from each depth band. The aim is to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band (ICES, 2001). At shallow stations, multiple double-oblique dives may be necessary to allow a sufficient volume of water to be filtered. A minimum sampler deployment time of 15 minutes is recommended.

Norway uses the Gulf type samplers in the western area but deployed a Bongo in the North Sea until the 2005 survey when a Gulf VII sampler was used. Both Norway and the Netherlands now use Gulf VII samplers on the North Sea surveys and this is now the recommended sampling device for this survey. Norway has also changed from a stepped tow profile (used with the Bongo) to the recommended double oblique tow used by all other nations.

Recommended maximum sampling depth is to 200m, or to within 5m of the bottom where the bottom is less than 200m. In the presence of a thermocline greater than 2.5°C **across a 10m depth interval**, sampling can be confined to a maximum depth of 20m below the base of the thermocline.

Vessels can only achieve the high frequency of samples taken at exactly the recommended maximum depth if they have automatic devices controlling the sampling depth, or by samplers fitted with real time pressure sensors. As a result, and because depth is an important parameter when calculating egg densities, the working group recommends that depth measurements are recorded carefully, with the use of real time depth, flowmeter and temperature monitoring systems.

5. Plankton sample collection and fixation

It is recommended that the standard plankton samples collected for the SSB estimates will be handled carefully and preserved as soon as practicable. The recommended 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 freshwater. (420g of sodium acetate trihydrate is dissolved in 10 litres of 4% formaldehyde, ICES, 2001). **This solution is only slightly hyper-osmotic to seawater but much less than formaldehyde-seawater solutions and will, therefore,** minimize 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%.

The volume of plankton in the sample jar must never exceed 50% of the volume of the jar. Excess sample should be fixed separately in additional jars. Details of an alternative fixative, giving better definition of egg development stage, for a more precise estimate of elapsed time because spawning, were given in ICES (1988). That fixative is 9.5 parts ethanol (95%); 1 part formalin (10%); 0.5 part glacial acetic acid.

6. Plankton sample sorting

Following practical demonstrations and trials with a 'spray technique' for the removal of fish eggs from plankton samples at WKMHMES (ICES, 2004b), it was recommended that this technique was used on samples collected during the 2004 triennial survey. Since then, enhancements have been made to the equipment and methods (Eltink, 2007), **and the device** will again be evaluated at WKMHMES in **2009**. It is recommended, that where **possible,** the spray technique be used at sea to quickly remove the majority of fish eggs from plankton samples. This will allow a rapid decision to be made on whether to continue sampling along a transect or to move to the next transect line.

The eggs removed by the 'spray technique' can be stored in separate vials within the plankton sample jar. It is recommended that every sample is subjected to a manual sorting and removal of any remaining eggs, to ensure that all eggs are removed from each sample. The use of the spray technique will remove the need for any subsampling of the plankton samples collected.

Immediately before the manual sorting, 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 **once the eggs are sufficiently fixed with formaldehyde**. 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 non target species such as hake, megrim, maurolicus and sardine, which may be present in lower concentrations than the target species. 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**. Where large numbers of eggs have been removed from a plankton sample, a minimum 100 eggs of each of the target species must be identified and staged from the sorted sample. The rest of the eggs must then be apportioned across the appropriate species and stages. If 100 eggs of one of the target species are NOT found in 25% of the sample, then the whole sample will have to be sorted. For a more detailed description of plankton sorting and identification, in particular for work in the laboratory, the plankton sampling manual (ICES 2004, Appendix 1) should be consulted.**

The results of the egg analysis should be submitted to the survey data coordinator, using the standard excel spreadsheets, within a month of the end of each cruise.

All participants should attempt to meet the deadline for the submission of survey results (see Section 4.2). The processing of subsets of samples should be avoided in order to provide a reliable preliminary estimate of the SSB index. If, it becomes obvious that a participating institute will fail to provide their survey results on time, then the survey coordinator and the WGMEGS Chair should be notified as

soon as possible. The survey coordinator, WGMEGS Chair and Steve Milligan (CEFAS), as an independent referee, will then liaise with the participant about selection of a representative subset of samples that can be processed as a priority.

7. Egg identification and staging

This is a key area for standardization and has been the subject of considerable attention by the working group. Egg staging was the subject of a detailed workshop held at Cefas, Lowestoft in 2000 (WKMHMES, ICES, 2001). This workshop produced a detailed manual on plankton sample handling and analysis, which was used by all survey participants during the 2001 surveys. A subsequent exchange programme on plankton sorting, species identification and staging revealed some deficiencies, mainly in the species identification (**ICES, 2001**, Section 9.3). Based on these findings further WKMHMES (ICES, 2004b and **ICES, 2006**) workshops were held, which included, sample sorting, species identification and egg staging. The results of these workshops were very re-assuring and a further WKMHMES is planned for **2009**, to train and evaluate the performance of the plankton analysts involved with the **2010** survey. The results of this workshop will be presented to ICES by the end of **2010**.

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 triennial surveys. It is generally regarded as the definitive work on the subject in this area**. Descriptions of the eggs of mackerel, horse mackerel and species with similar eggs can also be found in Munk and Nielsen (2005).**

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 recognize differences between mackerel and horse mackerel eggs when the segmentation of the yolk is not distinct in the latter.

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

Within WGMEGS the eggs of mackerel are classified into one of five morphological stages (I, II, III, IV and V; Lockwood *et al.*, 1981; Figure 3), 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).

For the estimation of daily egg production for both mackerel and horse mackerel, only the counts of stage I eggs are used. This is recognized 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.

8. Calculation 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, 1981). This has been used as the basis for calculating daily egg 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 duration of stage I mackerel eggs from the sea temperature (T°C) is:

Log_e time (hours) = -1.61 $\log_e$ (T°C) + 7.76

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

Log_e time (hours) = -1.608 $\log_e$ (T°C) + 7.713

The temperature at 20 m depth (5m for the North Sea) should be used for the calculation of egg stage duration. If that is not available then the subsurface temperature (ca. 3m) should be used.

9. Standardisation of plankton data analysis

Detailed procedures for the post analysis of egg abundance data to produce daily and, finally, annual egg production estimates are given below. **A designated data co-ordinator**, F. Burns, **MS**, Aberdeen will **collate** and manage the results for the entire **2010** survey. This analysis is subject to examination and approval by the full working group and ensures a standard approach and methodology. It is recommended that participants supply their plankton data in a standard MS Excel spreadsheet, to be distributed by the data co-ordinator.

To convert the number of eggs in each sample (or subsample) to the number of eggs per m^2, the following calculations are made. First the volume of seawater filtered by the sampler during the haul is calculated.

$$\textbf{Volume filtered (m3)} = \frac{\textbf{Flowmeter-revs x Aperture}}{\text{Flowmeter calibration}} \times \textbf{Efficiency Factor}$$

The number of egg m^{-2} is calculated from the formula:

$$\text{Eggs/m}^2 = \frac{\text{Eggs counted x Factor}}{\text{Volume Filtered (m}^3\text{)}} \times \text{Depth Sampled}$$

Where:

Flowmeter-revs.	=	Number of revolutions of the flowmeter during tow
Aperture	=	The area of the mouth opening of the sampler in m^2
Flowmeter calibration	=	The number of flowmeter revolutions per metre towed, obtained from the flume or sea calibration in free flow.
Eggs counted	=	Number of eggs in subsample
Factor	=	Raising factor from the subsample to the whole sample
Depth Sampled	=	The maximum depth of the sampler during the tow in metres

Efficiency Factor = The sampler efficiency from flume or towing tank calibration

Numbers of eggs per m^2 are raised to number per m^2 per day using development equation for both species in the following way:

For stage I **mackerel** eggs:

$$Eggs/m^2/day = 24 \times Eggs/m^2 / \exp [-1.61 \log_e (T°C) + 7.76]$$

For stage I **horse mackerel** eggs:

$$Eggs/m^2/day = 24 \times Eggs/m^2 / \exp [-1.608 \log_e (T°C) + 7.713]$$

Eggs/m^2/day are then raised to the area of the rectangle they represent. The rectangle values are summed to give numbers of stage 1 eggs per day over the survey area for each sampling period. Rectangle areas are calculated by each ½° row of latitude using the formula:

$$Area (m^2) = (\cos(latitude) \times 30 \times 1853.2) \times (30 \times 1853.2)$$

The next stages in the estimation of annual egg production are:

- Estimating the daily egg production for each survey period in turn
- Integrating the daily egg production histogram, to give annual egg production
- Calculating the variance of the estimate of annual egg production

The method was modified for use in the analysis of the 1995 survey data. It is fully described in Section 5.3.3 of the report of those surveys (ICES, 1996b). The same methods will be used for the analysis of the **2010** survey data. It is recommended that the flowmeters and sampling devices **are calibrated prior to the survey**, in terms of the volume of water filtered. There are two aspects to calibration. The first requirement is to know and understand the relationship between flowmeter revolutions and distance travelled through the water. The second is to relate flowmeter revolutions, (while mounted *in-situ* in the aperture of a plankton sampler), to volume filtered by the sampler. The only way in which the second aspect can be accurately determined is to calibrate the flowmeter and sampler under controlled conditions in a circulating water channel or in a large towing tank. These facilities provide independent measures of water or towing speed and also allow water velocity to be measured extremely accurately at numerous positions across the sampler aperture (EU AIR CT94 1911). Such facilities are extremely expensive and alternative methods to calibrate flowmeters in-situ have been employed by various participants. This usually involves calibration at sea using a reference flowmeter mounted outside of the sampler and two tows in opposite directions to overcome the effects of tides or currents on ship

and sampler speed through the water. Such calibrations will provide a crude estimate of volume filtered (under non-clogged net conditions) but it must be remembered that there are differences in water velocity across the aperture of any sampler and that this water velocity profile may change as clogging of the net progresses. However, it is recommended that participants conduct calibrations of their flowmeters *in-situ* over a range of towing speeds at least at the beginning and end of each survey.

There is also a well defined protocol to interpolate egg densities for some unsampled rectangles which fulfil the following criteria. In order to qualify for an interpolated value an unsampled rectangle must have a minimum of two sampled rectangles immediately adjacent to it. Once qualified, the sample values of all surrounding rectangles, both immediately adjacent and diagonally adjacent are used to calculate the interpolated value. The interpolated value is the arithmetic mean of all those surrounding rectangles. Once calculated, interpolated values are not used in order to calculate values for other unsampled rectangles, or to qualify those rectangles for interpolation. No values are to be extrapolated outside the sampled area. As a general recommendation, the cruise leader should try to avoid situations where interpolation is going to be problematic.

On some occasions and in particular where multiple observations are made within a rectangle sampling positions may fall on a dividing line between rectangles. When this occurs the sample is allocated to the rectangle to the north of the line of latitude and to the west of the line of longitude. However, it must be remembered that sampling should be attempted at the centre of the designated rectangles wherever possible.

10. Standardization of adult sampling – data collection and analysis

The working group prepared an updated protocol for the collection and analysis of adult parameters; fecundity, atresia, and parameters for condition and feeding for horse mackerel. These are detailed in Sections 3.4 to 3.6 (ICES, 2003). The analysis of these samples, particularly with reference to fecundity estimation, the use of the auto-diametric approach and oocyte diameter determination, were standardized at WKMHMES (ICES, 2004). This fecundity and atresia manual will again be updated at the next meeting of WKMHMES to be held at **AZTI, San Sebastian, Spain in December 2009.**

In 2004 and 2007, some participants experienced problems with formaldehyde evaporating from the recommended Eppendorf tubes. WGMEGS now recommends that all participants use Nunc tubes with screw on caps to prevent evaporation of the liquid from the ovary samples collected.

10.1. Sampling for mackerel potential fecundity and atresia in the Western and Southern areas

Following WGMEGS decision to use only formaldehyde fixative (ICES 2003) it **has been** possible to provide a unified sampling scheme for fecundity and atresia for use since the 2007 survey. **An auto-diametric method was used for an assessment of fecundity during the 2004 survey,** and although useful where the fecundity subsample weight is not known, this method does produce more variable fecundity data compared with the Gravimetric method (Hunter *et al.,* 1989). The Working Group therefore recommended that the **Gravimetric method should be used during the 2007 and subsequent surveys**. All changes in the sampling protocol and methods between the 2007 and 2010 surveys are given in Table 10.1.1.

Table 10.1.1. Changes for 2010 compared with 2007.

2007	2010
Stereometric method	**IMR will try to develop a new profile method. At the workshop it will be decided which method will be used. If profile counting is chosen then only a small part of the ovary needs to be brought back to the lab for atresia analysis.**
Fecundity method	**Of 10 mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.**

Ovaries should be weighed and subsamples taken by pipette before fixing in 3.6% buffered formaldehyde solution on board. Participants are encouraged to attend the egg and/or fecundity workshop to learn the correct use of the pipettes. Participants should check the pipettes and plungers to see if they are working correctly prior to the survey. Ovary subsamples should be stored in formaldehyde in Nunc tubes. Care should be taken that oocyte samples are completely covered by formaldehyde. Participants should regularly check that the samples are in sufficient amount of formaldehyde.

Samples for estimation of mackerel potential fecundity and atresia will be mostly taken on vessels participating in the egg survey or from commercial fishing vessels by observers. Recognizing the constraints of the egg survey cruise leaders should try to distribute trawl stations across the survey area aiming to complete a wide spread sampling regime for adults shown in Tables 3.1.2 a-b of this report. The purpose of this table is not to exactly specify the time and location of trawl hauls but to give an impression of how trawl hauls should be dispersed in time and space and the numbers of required for the estimation of realized fecundity.

If a limited size range of fish is caught, the remaining sample quota should be taken from the more abundant classes to fill the weight classes (see fecundity manual). In order not to concentrate the sampling on spawning fish it is desirable that trawling should avoid the 200 metre depth contour. Instead it should be adapted to fit in conveniently with the egg survey along the transects on the continental shelf. Details of sampling fish for fecundity at sea are described in the fecundity manual.

Prior to cruise departure Norway (Merete Fonn) will coordinate the analysis of mackerel fecundity samples and provide cruise leaders with tube reference numbers for labelling the Nunc tubes used on their cruises.

Table 10.1.2 shows the procedures to follow for the collection of samples at sea, and for sample analysis in the laboratory. Provisional estimates of potential fecundity and atresia are required for the 2010 WGWIDE group in September and final results are required for WGMEGS in spring of 2011. If the participants or coordinator are unsure of the data quality they should pass on their concerns to the Survey Coordinator (Finlay Burns MSML).

Table 10.1.2. Adult mackerel sampling program - Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2010

MACKEREL SAMPLING

Estimation of potential fecundity in pre-spawning fish and the estimation of atresia for realised fecundity

Sampling at Sea (for details on cruises see Table 2.2)

Area	Sampling by	Period/samples 1	2	3	4	5	6	total no. of samples
Southern	POR/IPIMAR	40						40
	ESP/IEO	60*	60	50				170
	ESP/AZTI		30					30 **240**
Western	ESP/AZTI		90		40			130
	GER/vTI SF		100	60				160
	IRL/MI		80				100	180
	ESP/IEO		10	40				50
	FAR/FFS				30			30
	SCO/MSML			100		70		170
	NED/IMARES			20	40	60		120
	NOR/IMR				60			60 **900**

* Additional samples of 60 mackerel obtained by market and/or onboard sampling

a) obtain length, weight and maturity distribution (***carefully!***) of larger sample (>100 fish) ⇢ length, sex, maturity, weight distribution

b) select mature, late pre-spawning, spawning or spent (stages 3-6) females (20 per station in 4 weight classes [<250/-400/-550/>550 g]), measure, weigh (total), gut, take otoliths (***carefully!***) ⇢ lenght, weight (total), sex, maturity, age

c) remove ovaries ***undamaged!***, weigh ovaries take 3 (6)* parallel pipette samples of one ovary **(25 µl)** in pre-filled individually coded Nunc tubes and put the other ovaryin formalin jars**, weigh liver, guts (including contents) guts (including contents)and carcass ⇢ ovary, liver, guts, carcass weight

d) send each one of the parallel samples according to the instructions given before the 2010 survey to either Vigo, Aberdeen, Bergen, Galway, San Sebastian, IJmuiden

histological analysis at IEO, IMR, AZTI, IMARES ⇢ potential fecundity, atresia, realised fecundity

***From two fish on each single survey every institute should take 6 parallel pipette samples to provide material for a histological ring test between IEO, MSML, IMR, MI, IMARES and AZTI!**

**** There might be changes regarding taking the ovaries due to changes in the astresia estimation. Please refer to the survey instructions which wil be send to all particpants before the 2010 survey!**

For the desired temporal and spatial distribution of the fecundity samples please refer to Table x.x.x; for further instructions please refer to paragraphs x.x.

Collection of samples for genetic population analysis (recommended)

a) Collect a larger sample of mackerel

b) Cut a tissue sample (about 10*5*5mm) of the muscle, put each tissue sample in a 1.5ml tube in absolute alcohole

c) Store the tubes until further notice

Each country carrying out the various cruises listed in Table 3.1.2.a-b is responsible for distributing the samples collected to the countries carrying out the fecundity analysis.

10.2 Sampling for horse mackerel fecundity in the Western area.

In the 2010 survey horse mackerel will be collected from the Southern and Western spawning components. Fish in maturity stages 3–5 (Walsh scale) will be selected and sampled on trawl hauls shown in Table 3.2.2a-b of this report. As with mackerel, the tables are only a guide to cruise leaders providing an indication of the range in temporal and spatial coverage and are not in any way to be taken as a constraint on the timing in relation to spatial coverage of the plankton sampling grid. Details of the horse mackerel sampling over the spawning season giving the best latitudinal coverage of fish and fish processing are shown in the flow chart below (Table 10.2.1). If one of the hauls fails to catch fish the number of fish taken can be increased in the next trawl haul.

Protocols for horse mackerel sampling both at sea and the analysis in the laboratory are shown in the fecundity and atresia manual. Ten samples will be analysed by all countries for quality assurance but at least 2 subsamples should be analysed for all the remaining fish. A procedure shown in Figure 10.2.1 should be used to minimize damage while separating the ovary from the fish.

Ovaries should be weighed and subsamples taken by pipette before fixing in 3.6% buffered formaldehyde solution on board. Participants are encouraged to attend the egg and/or fecundity workshop to learn the correct use of the pipettes. Participants should check the pipettes and plungers to see if they are working correctly prior to the survey. Ovary subsamples should be stored in formaldehyde in Nunc tubes. Care should be taken that oocyte samples are completely covered by formaldehyde. Participants should regularly check that the samples are in sufficient amount of formaldehyde.

Prior to cruise departure Cindy Van Damme (Netherlands) will coordinate the analysis of horse mackerel fecundity samples and provide cruise leaders with tube reference numbers for labelling the Nunc tubes used on their cruises.

Table 10.2.1. Flow chart for selecting and processing horse mackerel samples

Mackerel and Horse Mackerel Egg Survey 2010

HORSE MACKEREL SAMPLING

Estimation of standing stock fecundity and lipid content in relation to spawning status

Sampling at Sea (for details on cruises see Table 2.2)

Stock Comp.	Sampling by	Period/samples 1	2	3	4	5	6	total no. of samples
Western	ESP/AZTI		50		40			90
	GER/vTI SF		50	30				80
	IRL/MI		30				40	70
	ESP/IEO		25	50				80
	SCO/MSML			40		35		80
	NED/IMARES				30	30		60
	FAR/FFS				15			
	NOR/IMR				30			30 **495**

(a) obtain length, weight and maturity distribution (***carefully!***) of larger sample (>100 fish) ----------► length, sex, maturity, weight distribution

(b) select females in maturity stages 3-5 (Walsh scale) >= 25cm, Be sure to divide the females equally into the 4 weight categories < 150g, 151-250g, 251-350g and >350g. Measure, weigh (total), gut, weigh (gonad, carcass), take otoliths (***carefully!***) ----------► lenght, weight (total, gonad, carcass, [gut]), sex, maturity (1-6, Walsh scale), age

(c) take 3 (6)* parallel pipette samples of one ovary **(25 µl)** in pre-filled individually coded Nunc tubes and make sure that all the ovary samples are covered with formaldehyde

(d) send each one of the parallel samples according to the instructions given before the 2010 survey to either Vigo, Aberdeen, Bergen, Galway, Umuiden, San Sebastian

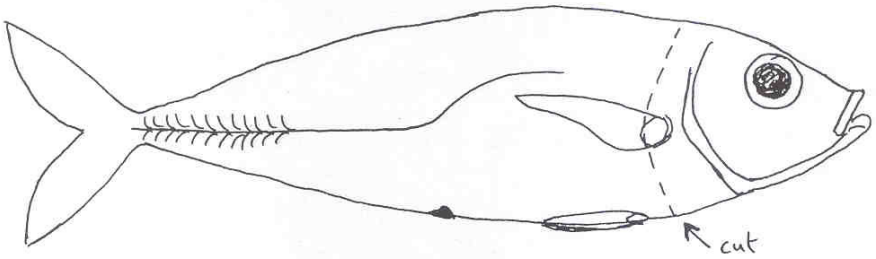
histological analysis at AZTI, IEO, IMARES, IMR, MI, MSML ----------► vitellogenic oocyte frequency presence of POFs/atretic oocytes

***From two fish on each single survey every institute should take 6 parallel pipette samples to provide material for a histological ring test between IEO, MSML, IMR, MI, IMARES and AZTI!**

For the desired temporal and spatial distribution of the fecundity samples please refer to Table 3.2.2; for further instructions please refer to paragraph3.2.

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

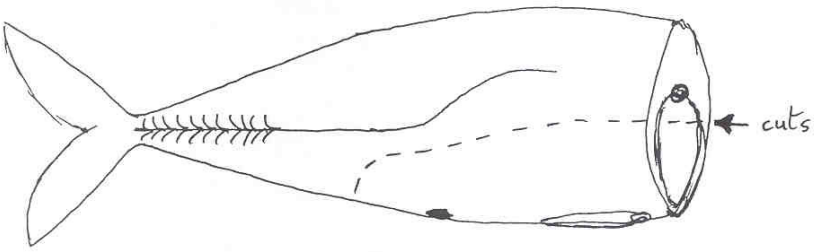
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral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



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

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

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



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

Figure 10.2.1. Procedure for collecting ovaries from horse mackerel.

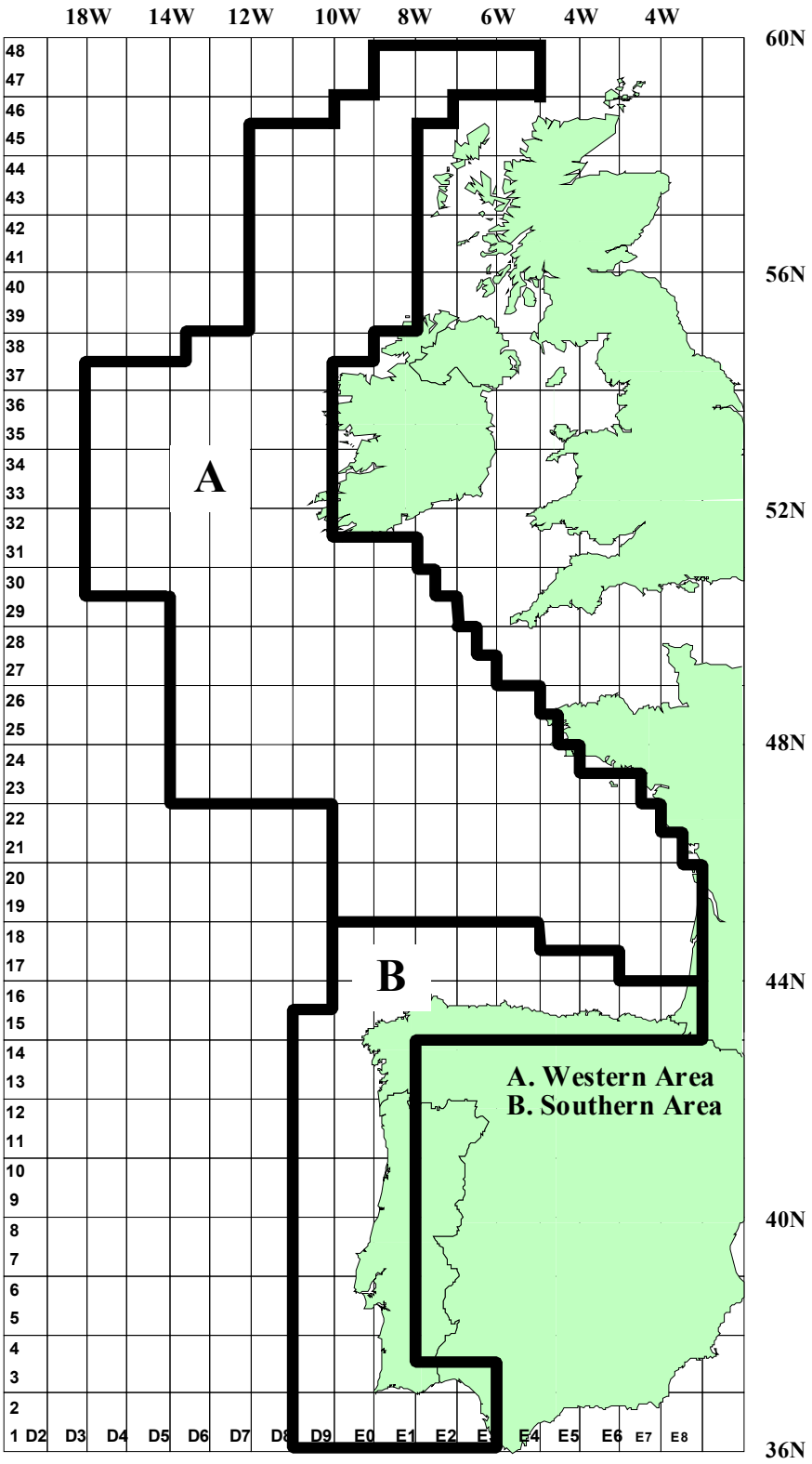


Figure.1. Core sampling areas for mackerel and horse mackerel eggs in the western and southern areas for 2004. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines.

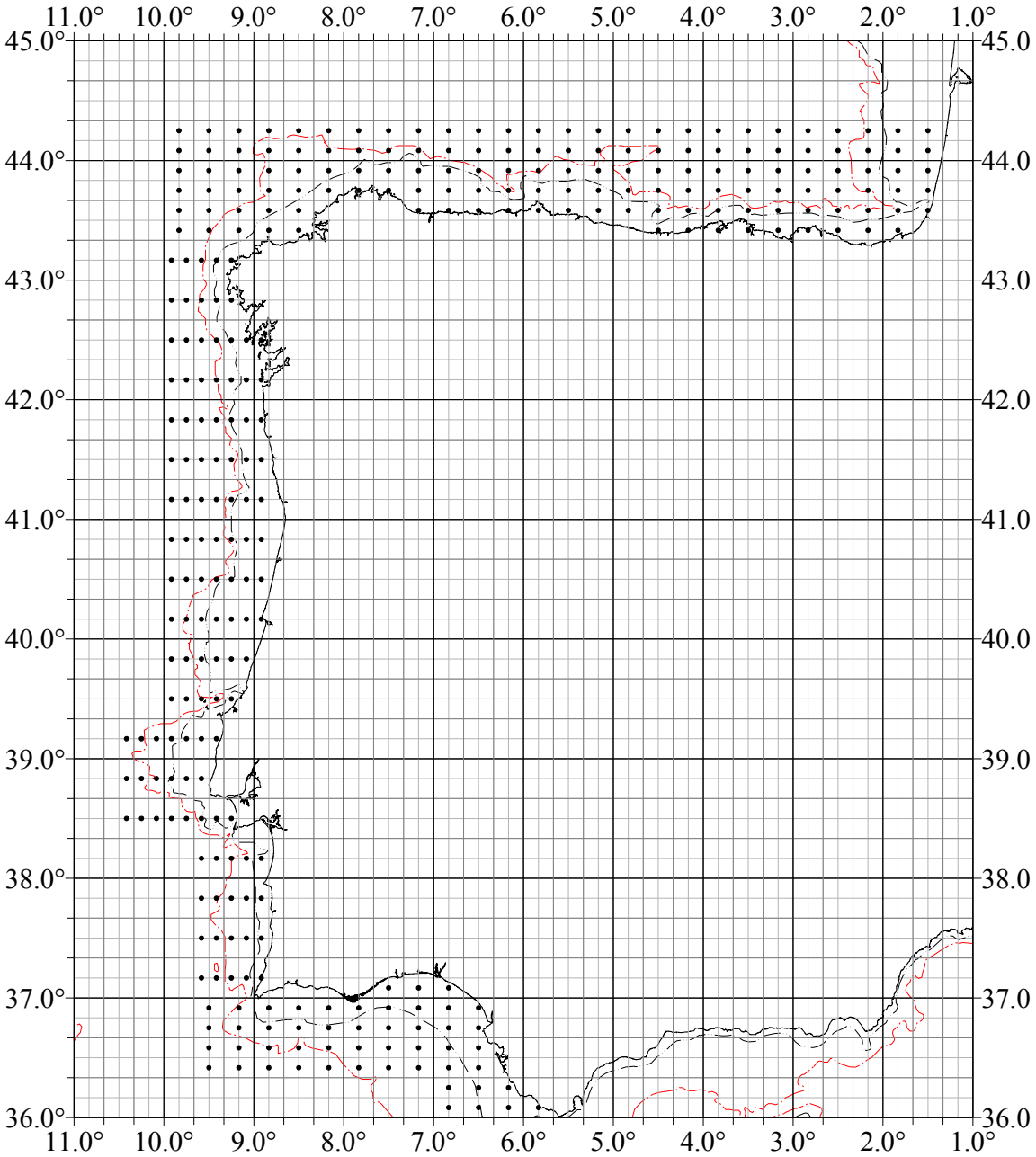


Figure 2. Provisional station location for mackerel and horse mackerel egg surveys in the southern area in 2004. Offshore boundaries will be based on two consecutive zero rectangles. Note that the sampling design for ICES area IXa has changed since the introduction of the Portuguese DEPM survey in 2007 (see Section 2.4 of the 2009 WGMEGS report).

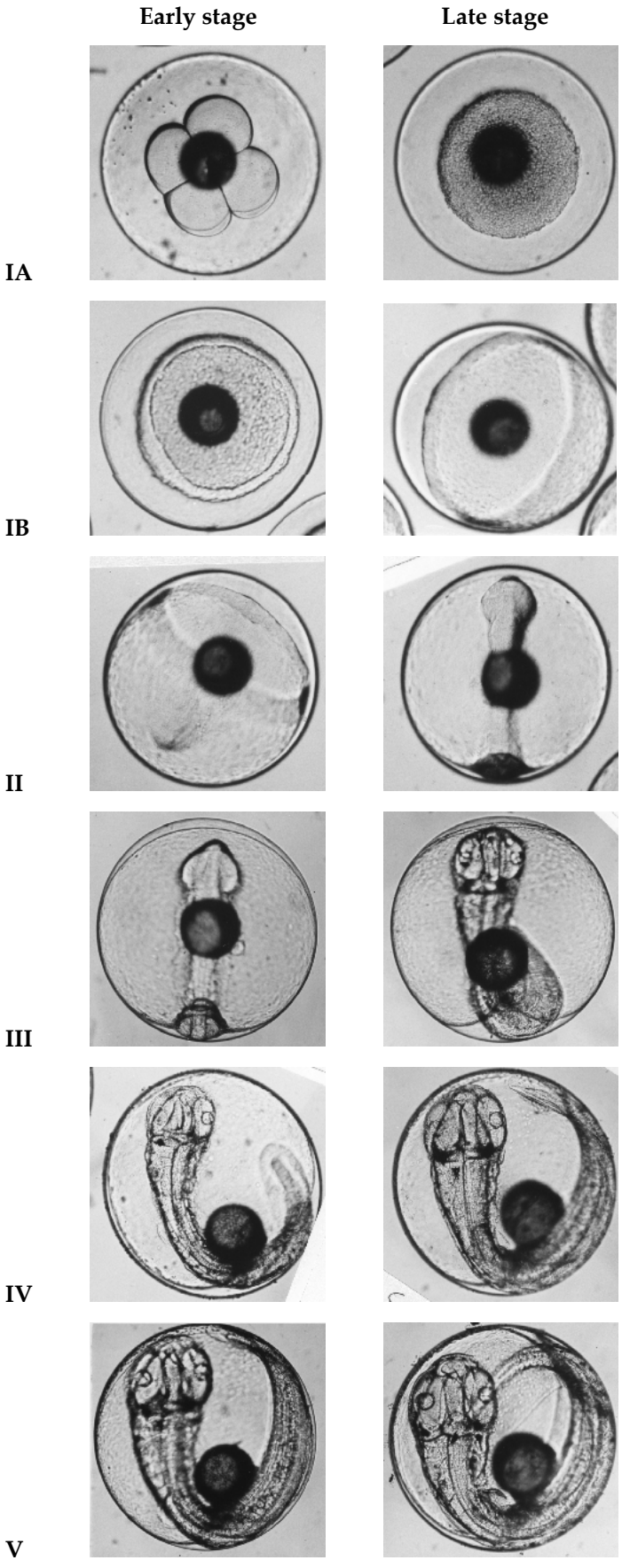
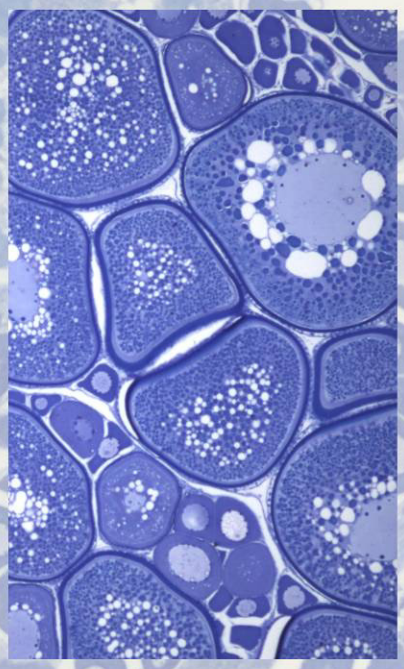


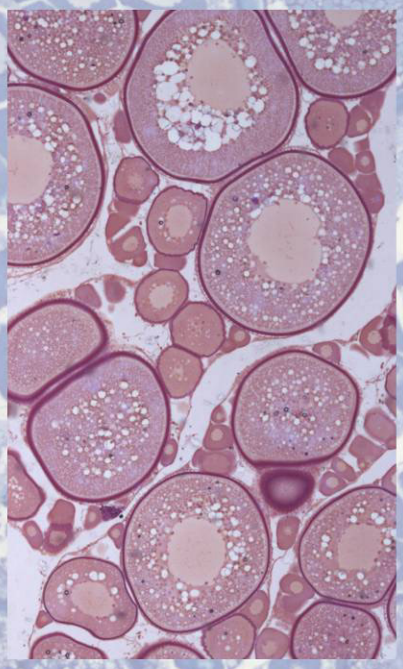
Figure 1. Mackerel eggs at the beginning and end of the six development stages.

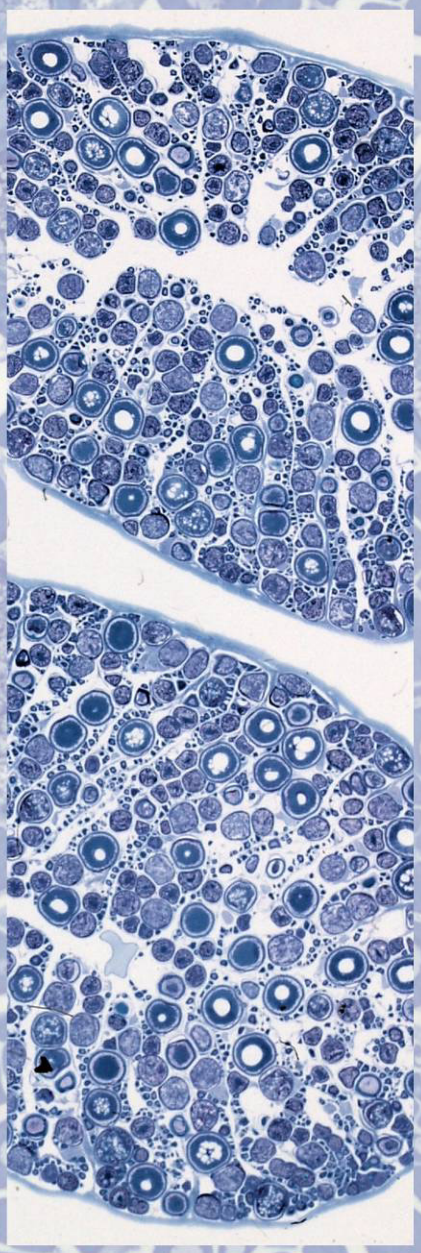
Annex 4: Fecundity Manual

A MANUAL FOR :

Sampling at sea, Mackerel and Horsemackerel Estimation of -fecundity and atresia in Mackerel -fecundity in Horsemackerel

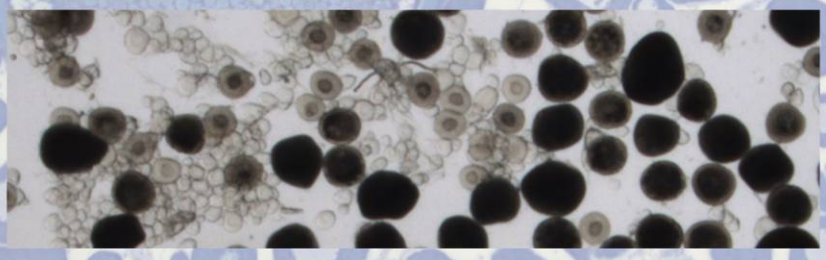






Editors M.Fonn, C.van Damme and A.Thorsen

April 2009
Version 8a



Version 7, April 2009

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

Contents Page

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

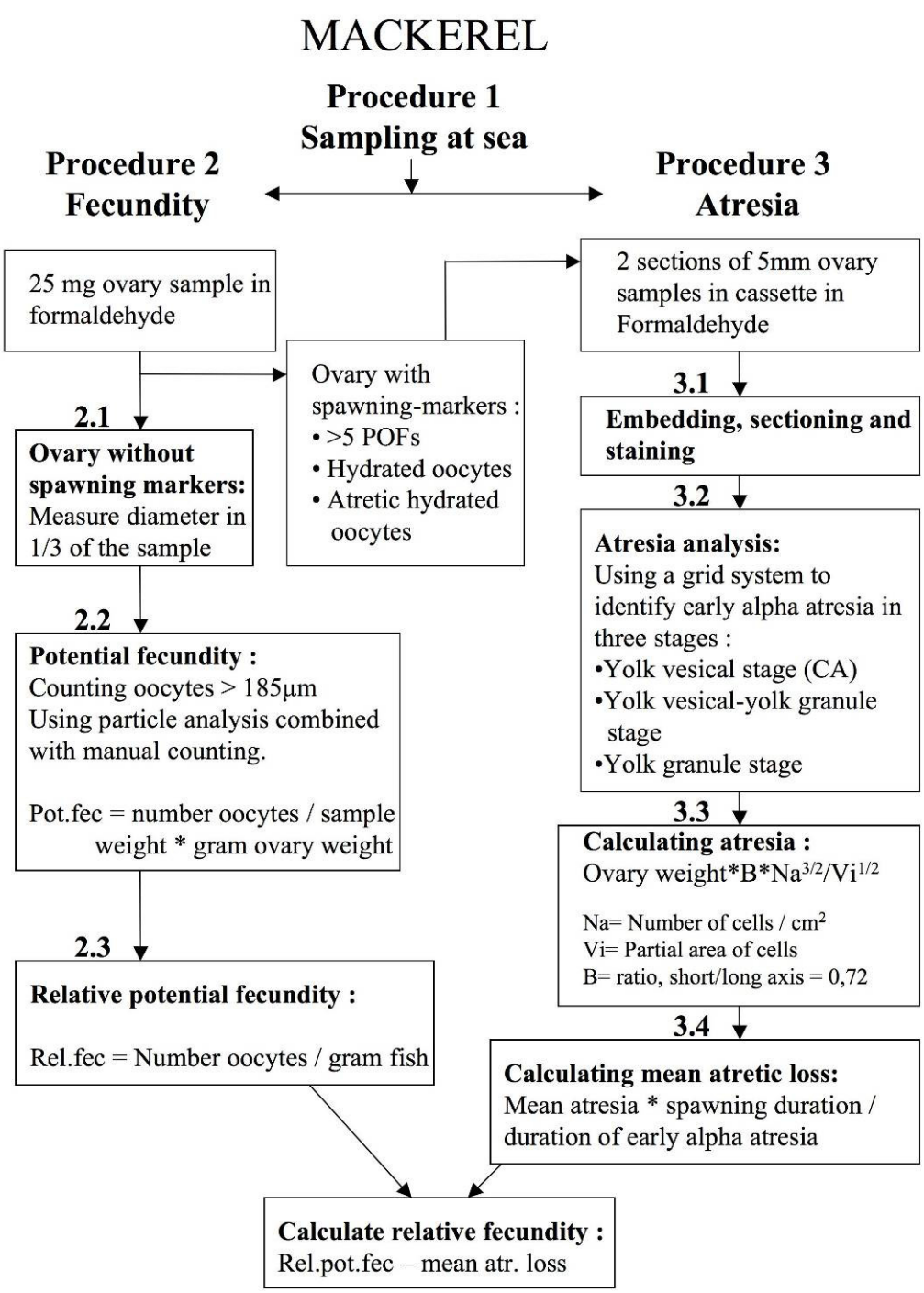
2001	2007	2010
Mackerel		
On board ovaries were collected whole and fixed in Gilson's fluid (for potential fecundity) and formaldehyde solution (for assessing spawning status and atresia)	On board ovaries are weighed and pipette subsamples of known volume and weight taken and fixed in formaldehyde solution	
Potential fecundity Count follicles > 130 µm after Gilson digestion	Gravimetric fecundity estimation Sub samples preserved in 3.6% buffered formaldehyde. F = O * C * S (F = fecundity, O = Ovary weight, C = count follicles > 185 µm in subsample, S = subsample weight; Hunter *et al.*, 1989)	
Atresia Stereometric method	Stereometric method	New method: profile? Discus at the wk
PAS stained sections	H&E -PAS – Toluidine blue	
		Fecundity samples: In 2007 count all oocytes >185 um and measure 1/3 of the oocytes. Suggestion: Count all oocytes >185 and use the automatic diameter measurements only for estimating leading cohort. Discus this at the fecundity wk
Mackerel and Horse mackerel		ImageJ and macros will be made available before the wk to all participants and use this for working up of the samples
		During the wk we need to discuss how many oocytes can be in the pictures for the image analysis. In 2007 it was shown that the pictures with the lower density showed higher fecundity estimates.
		At WK decide on the resolution of the pictures used for image analysis.
		For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes
Horse mackerel		

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

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

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

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



UPDATE 12/2009: FIGURE NEEDS TO BE CHANGED IF METHOD CHANGES

Procedure 1

Mackerel sampling procedure at sea

Before the cruise:

Procure 25–50 µl capillary pipettes (Table 3.3.1) Test performance of the pipette by practise, taking 25 µl water samples and weighing the dispensed fluid before the survey.

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

During the cruise:

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

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

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

Measurements:

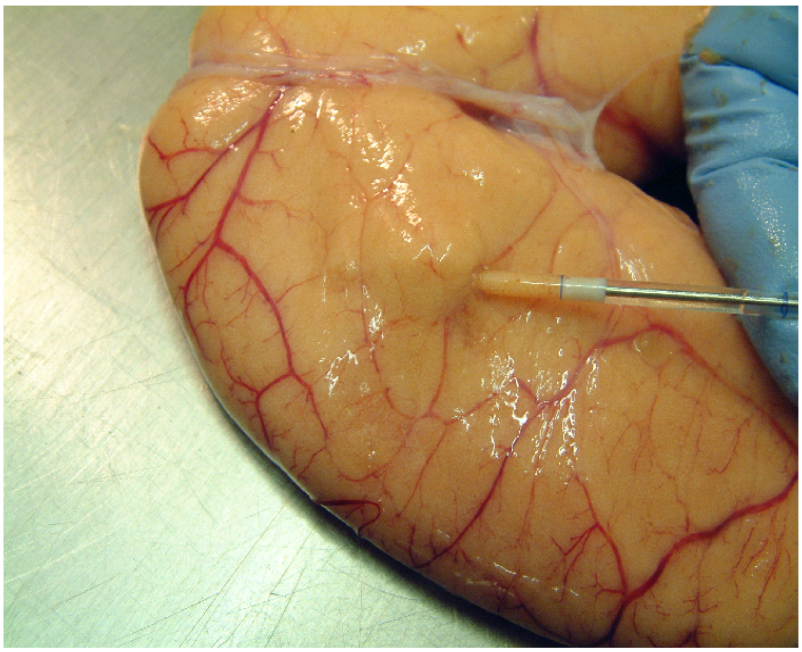
- Total length
- Total weight
- Maturity
- Otoliths
- Weight of gut, ovary and liver (If it is not possible to take these weights at sea, take the pipette and atresia samples and fix the remainder of the ovary and weigh the ovary in the lab. Gut and liver should be frozen and weighed in the lab. The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

DNA sampling:

- Cut a tissue sample (about 10*5*5 mm) of the muscle from the tick muscle behind the head, put each tissue sample in a 1.5 ml nunc tube in absolute 96% alcohol. All the samples should be stored at the respective institutes until funding is found for further analysis

Fecundity sampling:

- From one half of the ovary take 3 samples of each 25µl with a pipette and immediately put each sample in individual coded Nunc tubes. Make sure that all oocytes are covered in 3.6% buffered formaldehyde solution.



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

Atresia sampling:

- For artesian: Place the other half of the ovary in a labelled bottle (100–250 ml with wide opening) filled with 3.6% buffered (sodium phosphate) formaldehyde. If the profile method will be used it will only require to collect part of the ovary. This will be discussed at the fecundity workshop and an updated manual will be send around to all participants.
- Make sure that all the ovary samples are covered with formaldehyde
- Discuss at wk how to take the ovary weight if it is not possible to weigh them on board (cutting on board and take atresia samples later?? IMR will do some trials with the mackerel from the Matre experiment)
  ***UPDATE 12/2009:** Needs to be changed when decision is made about the method to use*

After the cruise:

From the fixed half ovary, cut two 5mm thick slices and put them in a labelled cassette. If the ovary is very big you may have to use 2 cassettes. Separate the cassettes into 4 colour coded leak proof bottles filled with 70% ethanol. Pack the consignments for each country with a maximum volume of 1000 ml solution in each package. On the outer cover of the package indicate the volume of fixative and that it is within the limits for unclassified transport. Send the cassettes and nunc samples for analysis to the different institutes referring to Table 2. IMR will be bringing a sample of the cassettes to the wk.

Table 2.

COLOUR CODE ORDER AND CHECK THE LABELS	COUNTRY	INSTITUTE AND ADDRESS	RESPONSIBLE PERSON
Blue	Norway	IMR, Nordnesgaten 50,PB 1870, 5817 Bergen-Nordnes, Norway	Merete Fonn
Red	Ireland	MI, Rinville, Oranmore, Co. Galway, Ireland	Brendan O'Hea
Yellow	Scotland	FRS, Marine Laboratory, Victoria Road, Torry, Aberdeen, AB9 8DB, Scotland	Finlay Burns
White- Even numbers	Spain	IEO, Apartado 1552, Cabo Estay, Canido, 36280-VIGO (Pontevedra), Spain	Jose Ramon Perez
White- Un-even numbers	Spain	AZTI, Foundation Herrera Kaia, Portu-alde z/ g20110 Pasaia, Basque Country, Spain	Paula Alvarez
	Netherlands	IMARES, Haringkade 1, 1976 cp Ymuiden, Netherlands	Cindy van Damme

Procedure 2

Fecundity whole mount analysis procedure for mackerel

2.1 Spawning markers and atretic oocytes

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

Under the microscope check for spawning markers, if there are hydrated oocytes or ≥ 5 POFs in the whole sample, it should not be analysed for fecundity. For mackerel these sample should be analysed for atresia.

Show at the WK how to transfer the oocytes from the tube to the dish

2.2 Potential fecundity

Distribute the sample randomly in the tray.

Measure the oocyte diameters automatically.

Count all the oocytes >185µm in the sample.

Potential fecundity:

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

2.3 Relative potential fecundity

Relative potential fecundity:

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

Procedure 3

Atresia analysis for mackerel

UPDATE 12/2009: If method is changed this needs to be changed

3.1 Embedding, sectioning and staining

Preparing resin blocks

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

STEP	INFILTRATION SOLUTION	DURATION	PROCESS TEMPERATURE
1	90% ethanol	2 hours	Room temperature
2	Pour out the liquid and add fresh 90% ethanol	1 hour	Room temperature
3	90% ethanol + Technovit 7100 (1:1 ratio) prepared by diluting Technovit 7100 (from used in steps 4).	2 hours or overnight	Store cool (+5°C) after the orbital shaker
4	Replace the liquid with Technovit 7100 (from step 5).	2–3 days	Store cool (+5°C) after the orbital shaker
5	Replace the liquid with freshly prepared Technovit 7100.	1 day	Store cool (+5°C) after the orbital shaker
6	Transfer the sections from the cassettes to the moulds. Store tissue with catalysed resin in moulds in the freezer.	2–3 hours or overnight	-6°C
7	Polymerise by adding Technovit 7100: hardener (15:1) in the freezer.	2 hours	-6°C
8	Leave overnight	overnight	Room temperature
9	Block up using Technovit 3040.	15 minutes	Room temperature

Store the blocks in a box containing 70% glycerol.

Disposal of waste resin (in the fume cupboard)

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

Sectioning the blocks

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

Staining the sections

Recipe 2% Toluidine blue

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

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

3.2 Atresia analysis

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

For mackerel we score only the early alpha atretic stage.

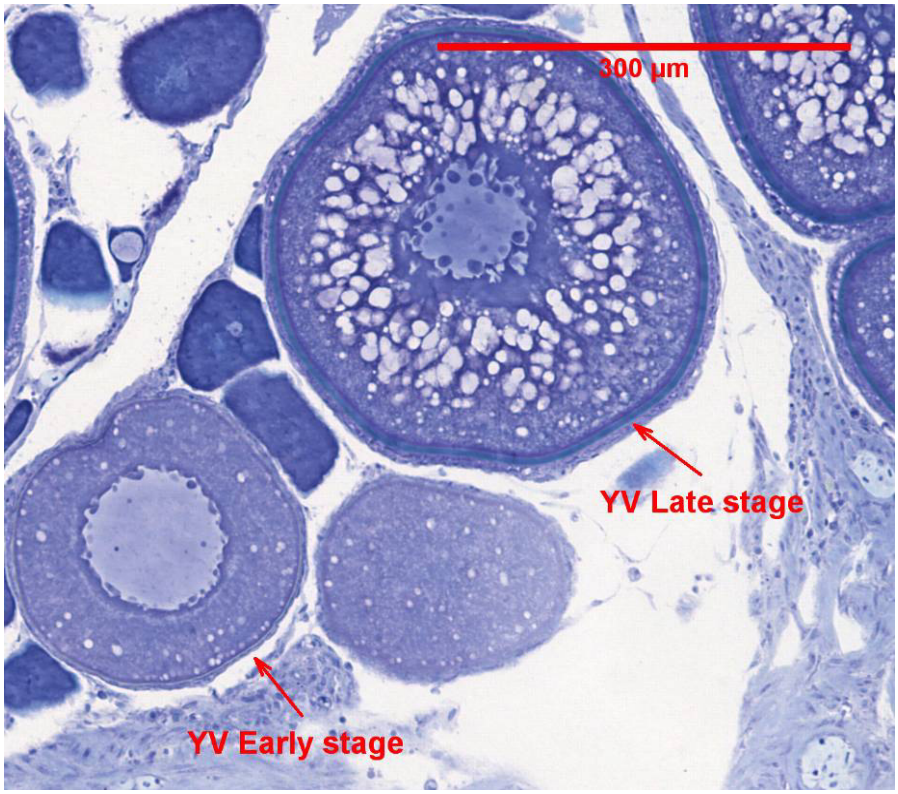
The oocytes are divided into 3 different stages:

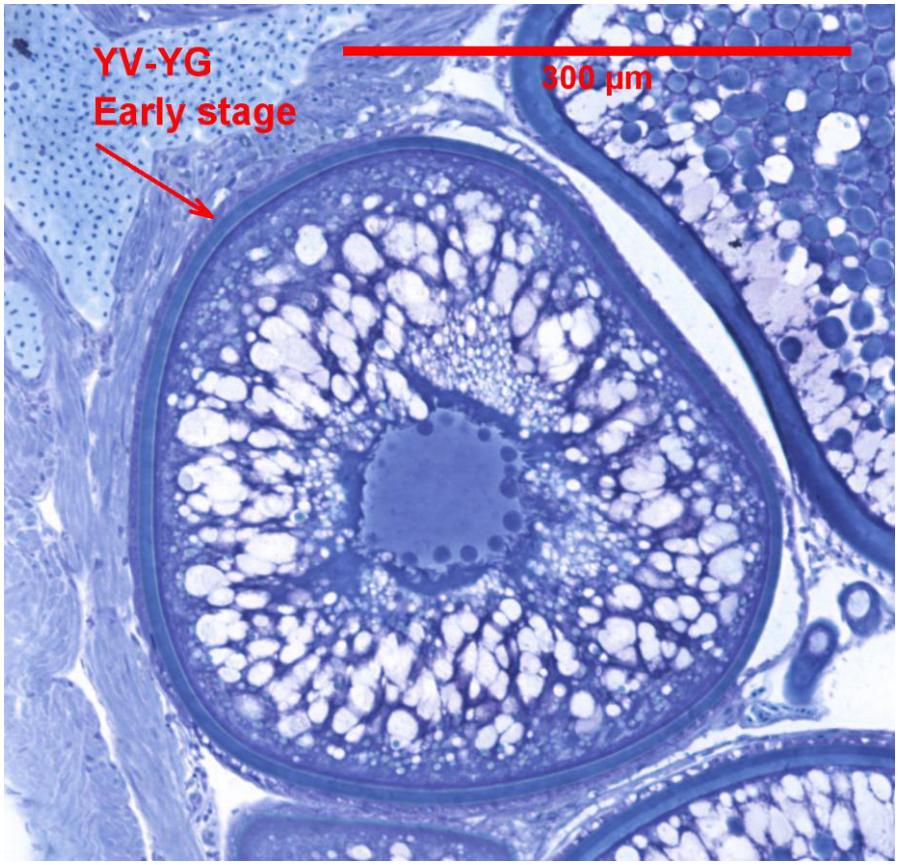
YV (yolk vesical stage): arises from the smallest vitellogenic oocytes making up the potential fecundity ranging in size from 175 (appearance of corticale alveolie) to 325μm when a complete ring of vacuoles extends throughout the oocyte cytoplasm.

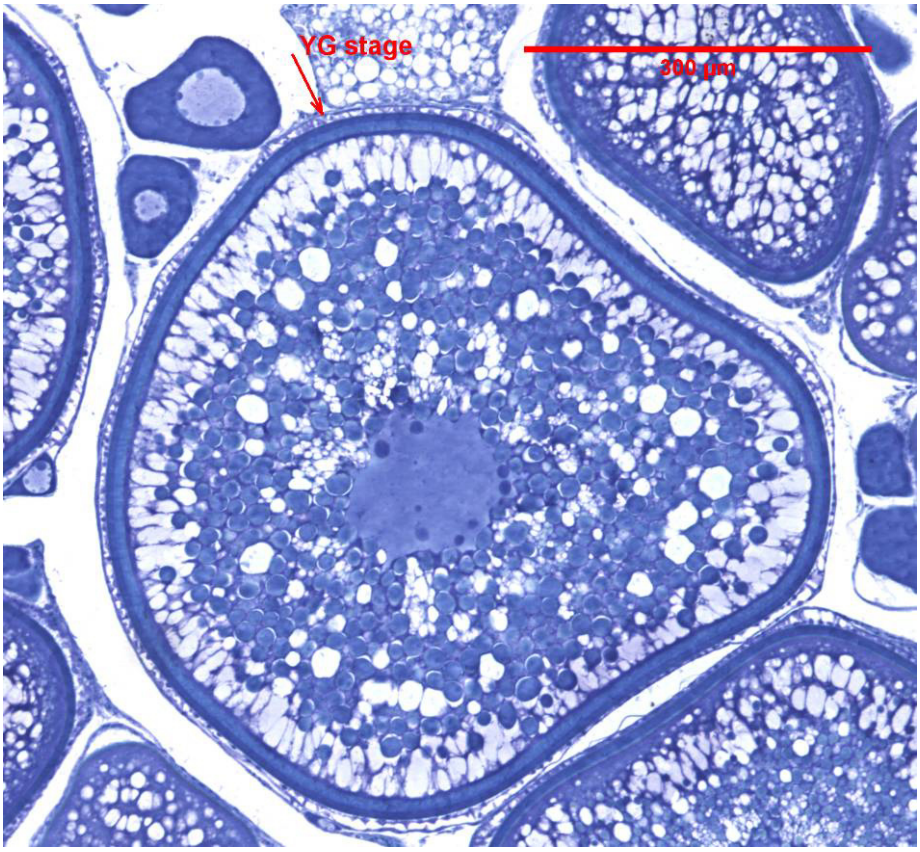
YV-YG (yolk vesical to yolk granule stage): the oocytes range in size from 325 to 525μm and contain yolk granules that slowly enlarge and start to fill the cytoplasm.

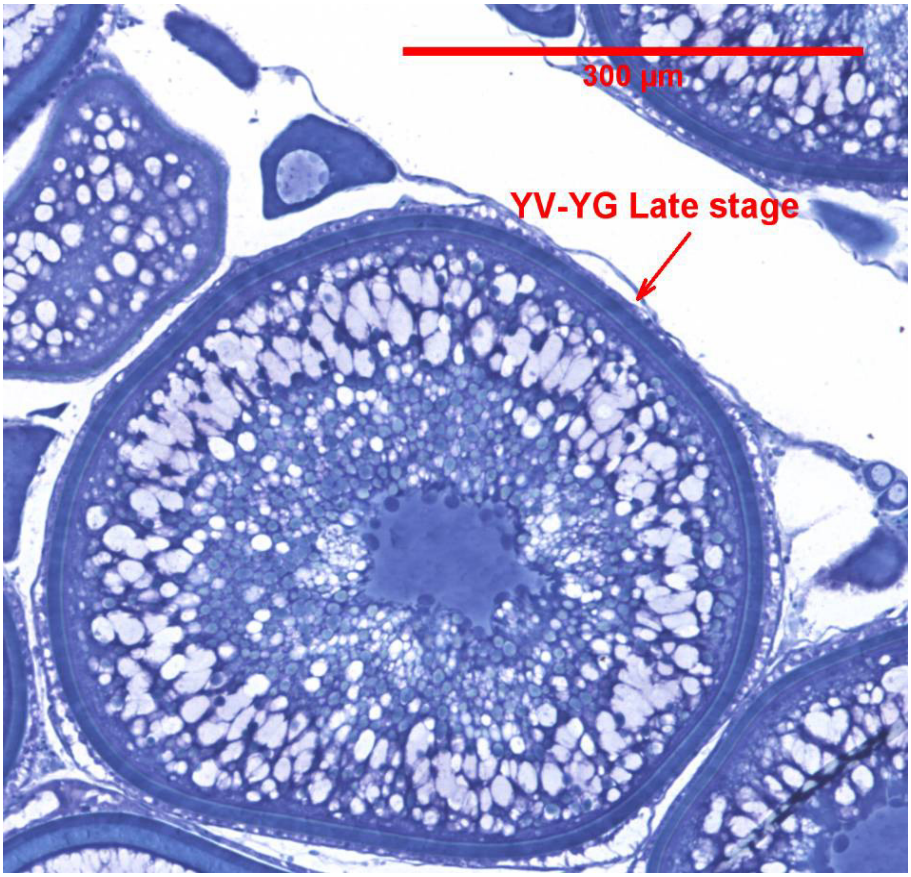
YG (yolk granules): yolk granules occur throughout the full depth of the cytoplasm. This stage also includes the largest oocytes making up the potential fecundity up to oil droplet formation and the migratory nucleus stage.

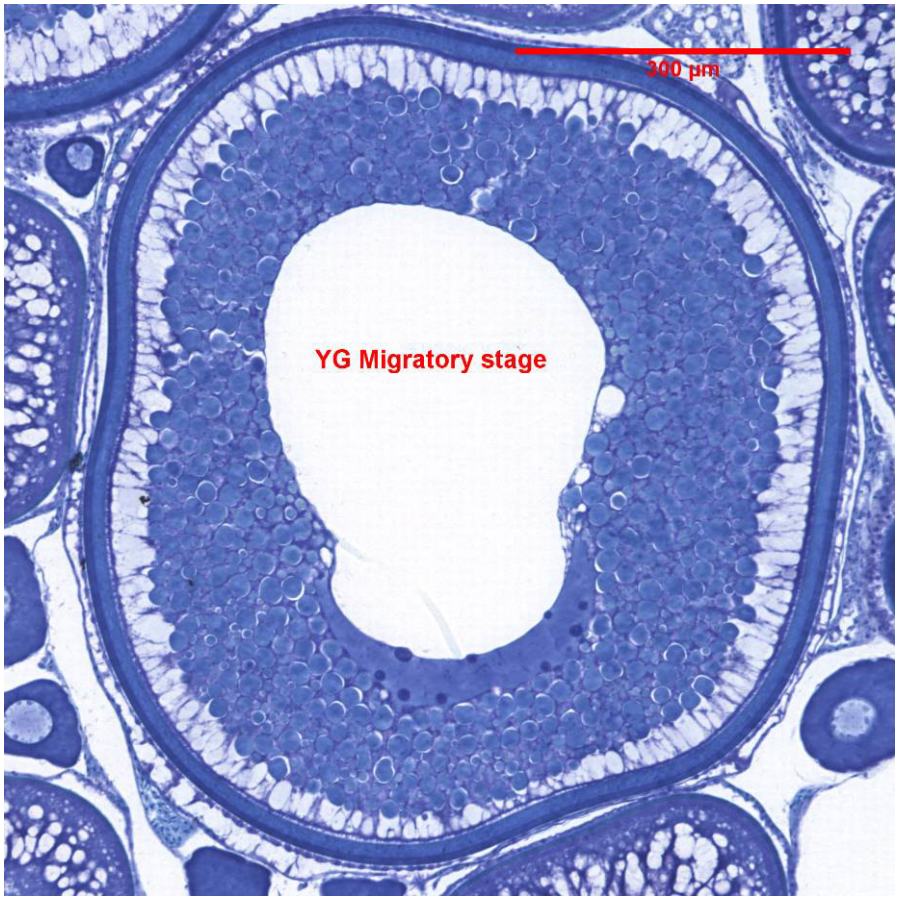
Pictures of the 3 different stages in normal oocytes stained with toluidine blue.

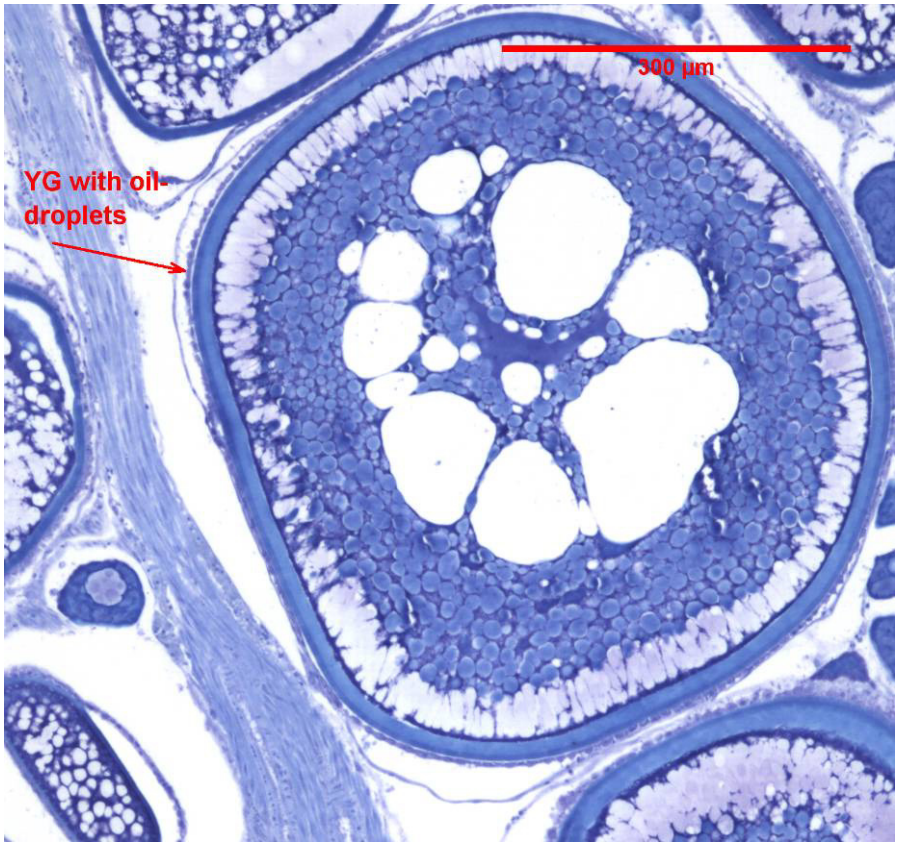




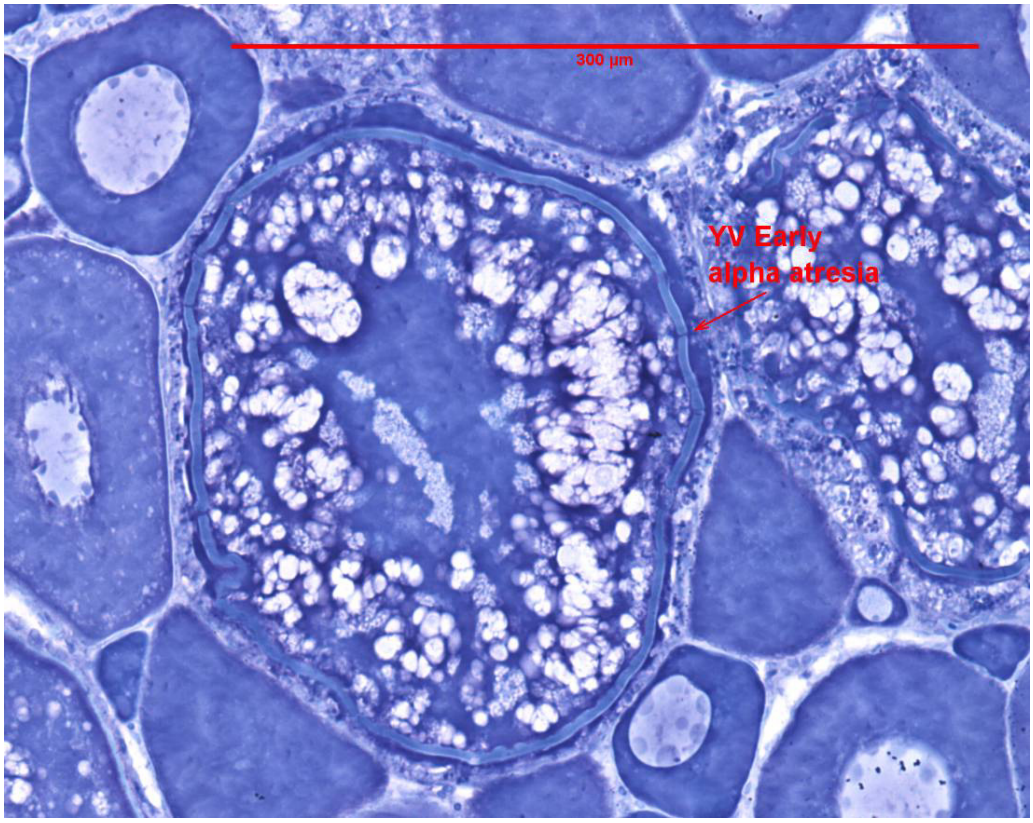

YG stage
300 µm

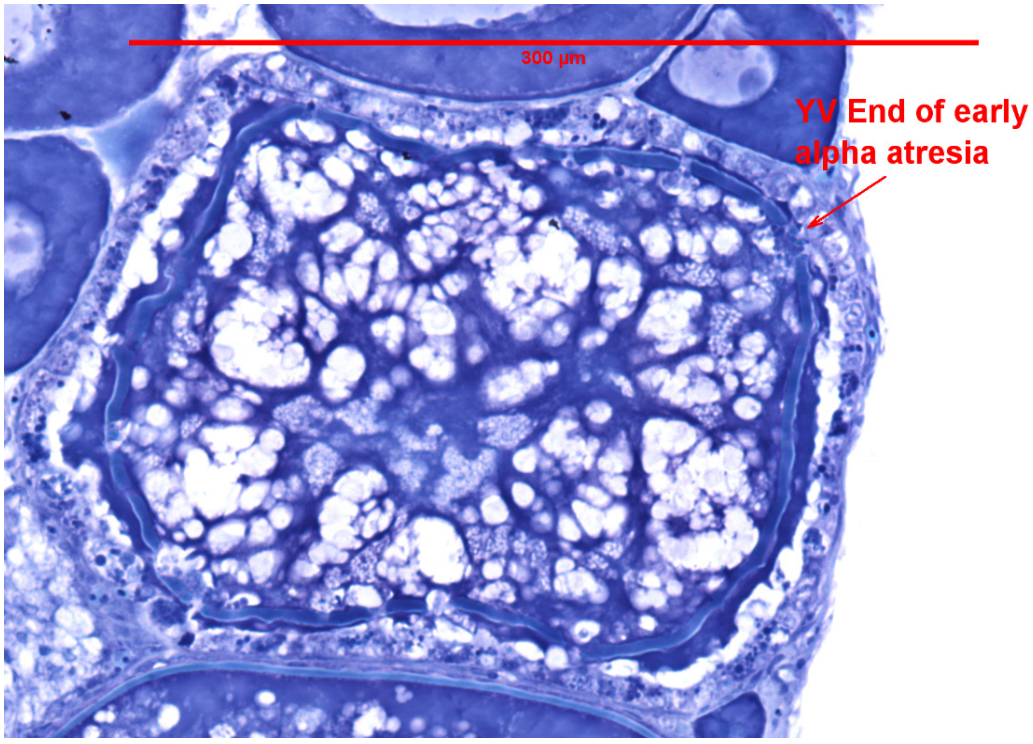

300 µm
YV-YG Late stage

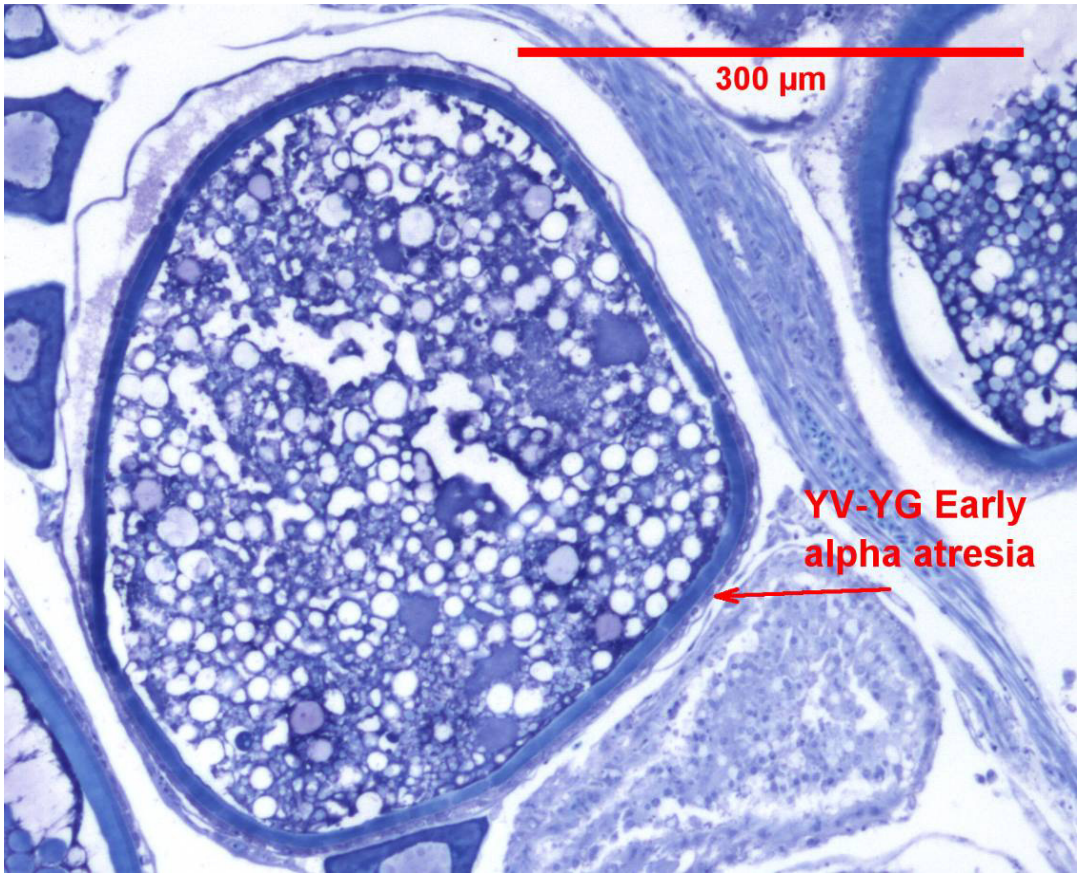

300 µm
YG Migratory stage


300 µm
YG with oil-droplets

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

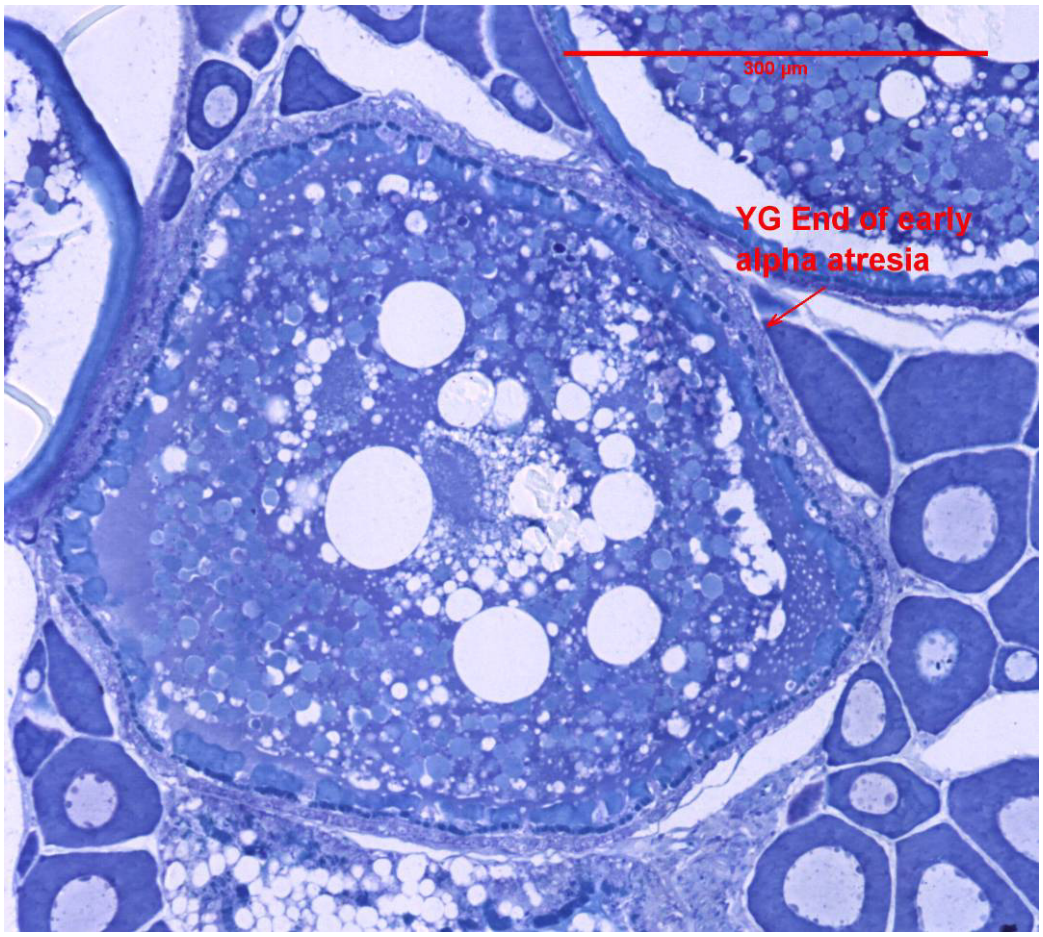





300 µm
YV-YG Early
alpha atresia


300 µm
YV-YG End of
early alpha atresia


300 µm
YG Early
alpha atresia


300 µm
YG End of early
alpha atresia

Measurement of VI:

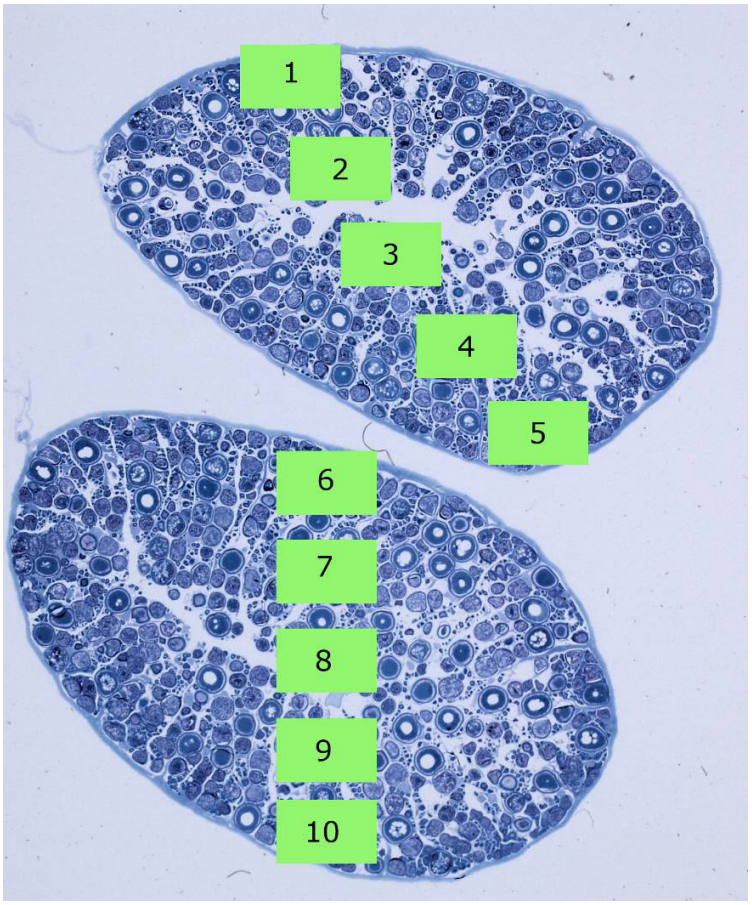
Vi = partial area of vitellogenic oocytes in the histological section.

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

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

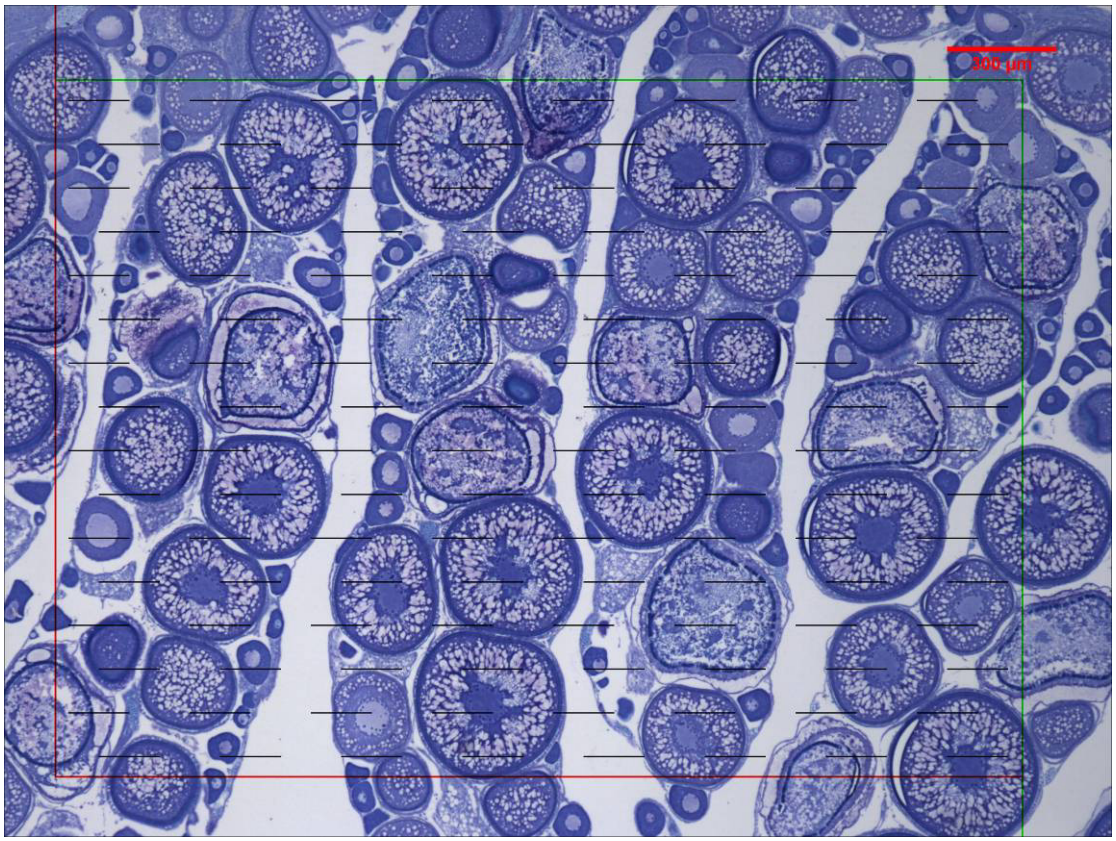
Ovary weight (g)	**Approximate area to be analysed**	**Number of fields to be analysed if the area is 0,05 cm²**
2–9	0,3 cm²	6
10–19	0,4 cm²	8
20–29	0,6 cm²	12
>30	0,7 cm²	14

The outer grids should include area occupied by the ovary tunica and points lying outside the ovary should be discounted (negative grid).



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

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



Count the point that hit early alpha atretic oocyte in each of the three stages: YV, YV-YG, YG.

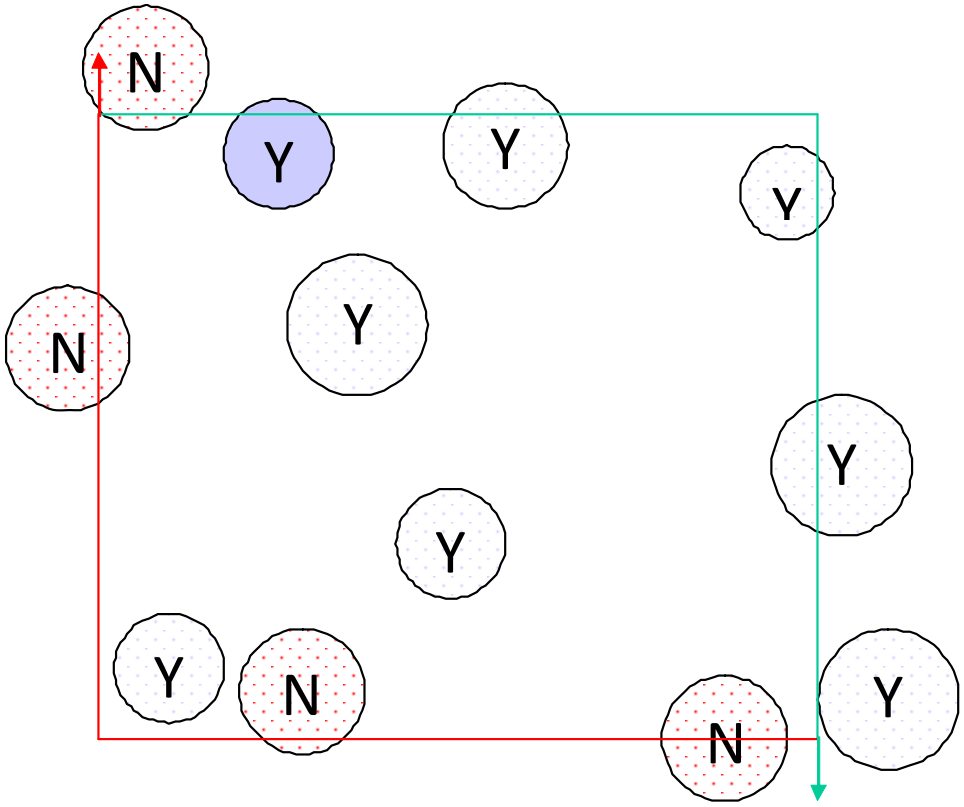
Calculate Vi for each stage using the following equation:

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

Measurement of Na:

Na = number of vitellogenic oocyte transactions per unit area.

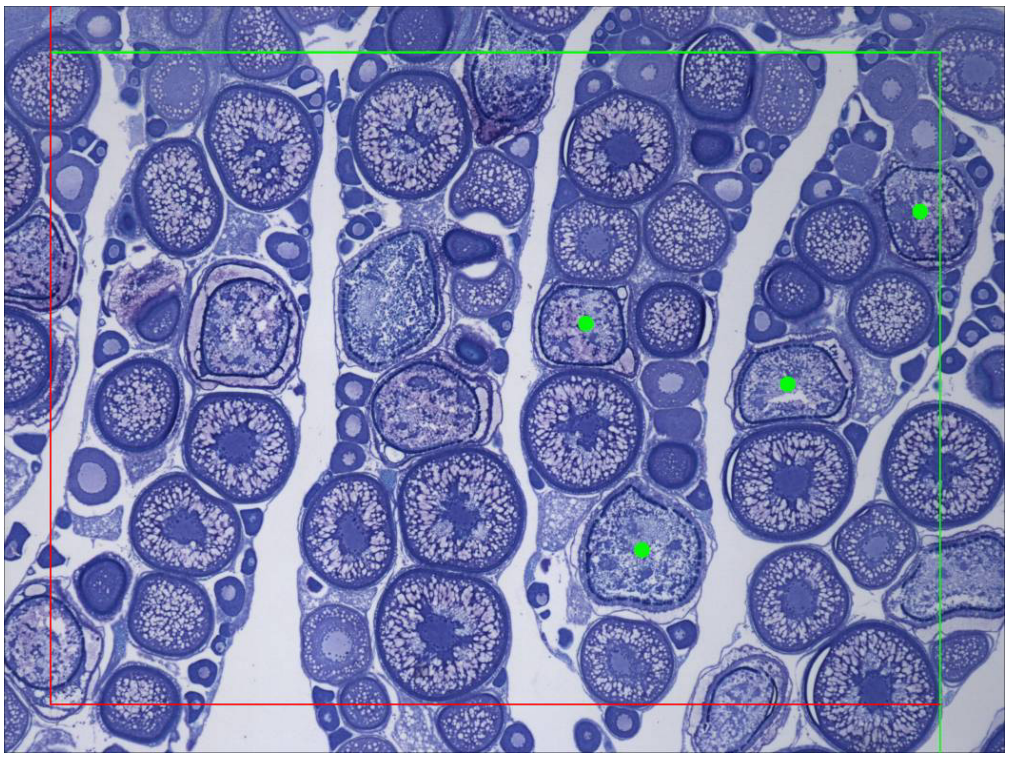
A frame is superimposed over the section and the number of early alpha atretic cells in each class of oocyte counted using the rules shown in the illustration below. Oocytes touching the forbidden line (red) or extended red line will not be counted (N). Oocytes inside the frame or touching only the green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



Calculate Na for each stage using the following equation:

Na = Number of profiles / field area

In the example below 4 early alpha atresia cells in the stage (YV-YG) are counted. The area inside the frame is 0.053 cm^2, Na for YV-YG will be 4 / 0.053 = 75.5 profiles / cm^2.



3.3 Calculation of atresia

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

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

Ov = ovary weight in gramme

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

K = 1 (constant value for atretic oocytes)

Calculate relative atresia:

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

Summerize F_{atr} for the 3 stages

Calculate the mean atresia from all the fish examined.

3.4 Calculation of mean atretic loss

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

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

Spawning duration = 60 days

Duration of early alpha atresia = 7.5 days

Procedure 4

Horse mackerel sampling procedure at sea

Before the cruise:

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

During the cruise:

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

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

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

Measure or take:

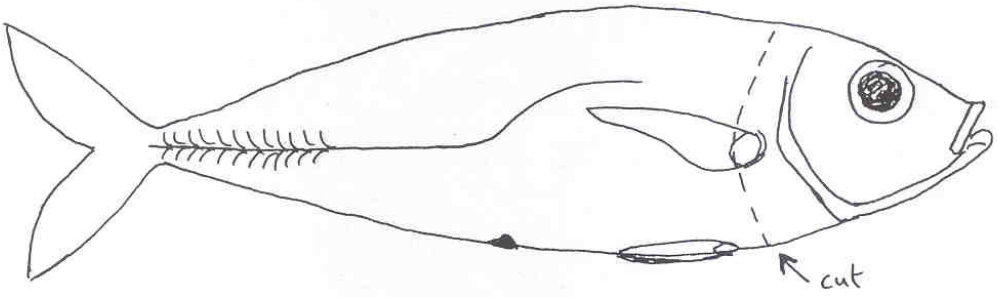
- Total length
- Total weight
- Maturity
- Otoliths for age reading
- Weight of gut, ovary and liver (If it is not possible to take these weights at sea, take the pipette and atresia samples and fix the remainder of the ovary and weigh the ovary in the lab. Gut and liver should be frozen and weighed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

Ovary sampling:

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

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

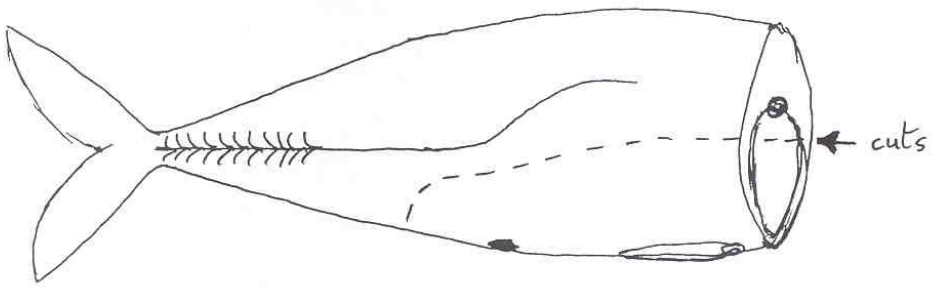
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral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



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

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

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



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

Figure 3.2.1. Method to remove undamaged ovaries from horse mackerel.

After the cruise:

Send the nunc samples for analysis to the different institutes referring to Table 1.

Ipimar to send the manual of their DEPM method.

UPDATE 12/2009 with comment from Maria: Method summarized in Section 2.4. Manual doesn´t apply for the IPIMAR´s DEPM

Colour code ***WK: Sort out the coding***	**Country**	**Institute and address**	**Responsible person**
Blue	Norway	IMR, Nordnesgaten 50,PB 1870, 5817 Bergen-Nordnes, Norway	Merete Fonn
Pink	Ireland	MI, Rinville, Oranmore, Co. Galway, Ireland	Brendan O'Hea
Green	Netherlands	IMARES, Haringkade 1, 1976 cp IJmuiden, Netherlands	Cindy van Damme
White	Spain	IEO, Cabo Estay-Canido, 36280 - VIGO (Pontevedra) Spain	Jose Ramon Perez
	Spain	AZTI, Foundation Herrera Kaia, Portualde z/ g20110 Pasaia, Basque Country, Spain	Paula Alvarez

Procedure 5

Fecundity whole mount analysis procedure for Horse mackerel

5.1 Spawning markers

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

Under the microscope check for spawning markers, if there are hydrated oocytes or ≥ 5 POFs, the sample should not be analysed for fecundity.

5.2 Potential fecundity

Distribute the sample randomly in the tray and automatically measure the oocyte diameters for the whole sample.

Count all the oocytes >185µm in the sample.

Potential fecundity:

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

5.3 Relative potential fecundity

Relative potential fecundity:

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

Annex 5: Expert ichthyoplankton group

During the December 2008 meeting of the ICES PGEGGS group it was proposed to constitute an expert group on ichthyoplankton surveys. This proposal has been discussed during the Science Committee meeting in January. The Committee agreed such a group was needed within ICES and PGEGGS was asked to draft a proposal together with other ichthyoplankton experts groups to put forward during the ICES Annual Science Conference in September 2009. It was also suggested to write a proposal for a theme session on added values of ichthyoplankton surveys for the 2010 ICES ASC.

Below is the text from the proposal as it went into the PGEGGS meeting report.

There are a number of ichthyoplankton surveys currently being conducted in the ICES area, primarily for providing information that can be used in stock assessments (see Table 5.1). The surveys are targeted at a number of species such as plaice, cod, herring, anchovy, sardine, mackerel and horse mackerel. The surveys are targeted at sampling either eggs or larvae and use a variety of types of sampling equipment with a range of sampling protocols. In addition the surveys are either national programmes or consist of a variety of levels of international cooperation.

The surveys have a variety of goals, and with them come a variety of levels of complexity in the sampling programmes. There are a number of herring larvae surveys where the abundance of larvae is used as an index of the Spawning Stock Biomass (SSB) i.e. North Sea Autumn Spawning, Irish Sea Autumn Spawning and Norwegian Spring-spawning herring. In one instance (Rügen herring in IIIa) the production of young herring larvae is used as an index of herring recruitment. In this case assumptions need to be made on larvae growth and mortality rates.

The abundance of later larvae is often used as an index of recruitment such as for North Sea Autumn Spawned herring and Irish Sea gadoids. In all of these cases the sampling equipment has been standardized to Methot Isaac Kidd (MIK) trawls.

There are also a number of egg surveys that are used to provide indices of SSB. The surveys currently undertaken involve anchovy, sardine, mackerel, horse mackerel, cod and plaice in areas such as the western margin of the northeastern Atlantic, the Bay of Biscay to off Cadiz, North and Irish Seas. In these surveys the egg abundances are used to estimate egg productions and through estimates of fecundity back to the SSB. These techniques involve a greater level of data as they require not only pelagic egg data but also information on the reproductive potential for individuals in the stock. These analyses often come under the heading of Annual Egg Production (AEP) or Daily Egg Production (DEP) Methods. As with larvae production methodology these techniques also require information or assumptions on egg development rates and egg mortalities.

Table 5.1. Summary of current ichthyoplankton surveys undertaken in the ICES area, primarily for use in stock assessments. Note this list is not exhaustive and does not include surveys being undertaken over limited time periods (years) solely for process studies.

Survey	Quarter	Area	Target species	Years/ frequency	Types of equipment	Purpose	Notes
Plaice and cod eggs (PGEGGS)	1	North Sea	Cod, Plaice	2004, 2009	Gulf, Bongo	Egg distributions (spawning locations), plaice EP	International
Demersal egg surveys	1	Irish Sea	Plaice and Cod	1995, 2000,2006, 2008,2010	Gulf	Eggs to EP to SSB	International, now England and Northern Ireland
International Herring Larvae Survey (PGIPS)	4 and 1	North Sea	Herring	1973– / Annual	Gulf	Larvae as SSB index	International
Northern Irish	4	Irish Sea	Herring	1993– / Annual	Gulf	Larvae as SSB index	Only Northern Ireland
Mackerel Egg (WGMEGS)	2	North Sea (North/ Central)	Mackerel	Every 3 years	Gulf	Eggs to EP to SSB	International
Rügen Herring	1	IIIa, Baltic	Herring	1977 / Annual	Bongo	Larvae to recruitment	Only German
MIK	1	North Sea	Herring	1976-/ Annual	MIK	Recruitment index	International
MIK	2	Irish Sea	Gadoids	1993– / Annual	MIK	Recruitment Index	Only Northern Ireland
North Sea CUFES	1	Southern North Sea/ English Channel	Eggs	2006– / Annual	CUFES, Vertical WP2	?	Only France, done in conjunction with IBTS.
Baltic eggs and larvae		Baltic (Bornholm Basin)	Cod eggs and larvae	Annual	?	EP?	Only Denmark, Germany?
Malformed eggs	1	South-eastern North Sea	Plaice eggs	Annual since 1980s	?		Only Germany
Mackerel and Horse mackerel egg surveys	1–2–3	North-east Atlantic	Mackerel and Horse mackerel eggs	1977– / Every three years	CalVET Bongo 40 Gulf VII	Eggs to EP to SSB	International
Anchovy and Sardine acoustic survey (WGACEGG)	2	Biscay to the Gulf of Cadiz	Anchovy and Sardine eggs and larvae	Annual since 1995	CUFES	Acoustic Biomass	International
Sardine DEPM egg survey (WGACEGG)	1–2	Biscay to the Gulf of Cadiz	Sardine eggs	Every 3 years	Pairovet	Eggs to EP to SSB	International

Survey	Quarter	Area	Target species	Years/ frequency	Types of equipment	Purpose	Notes
Anchovy DEPM egg survey (WGACEGG)	2	Biscay	Anchovy eggs	Annual	Pairovet	Eggs to EP to SSB	Only Spain (AZTI)
Anchovy DEPM egg survey (WGACEGG)	3	Gulf of Cadiz	Anchovy eggs	Every 3 years	Pairovet	Eggs to EP to SSB	Only Spain (IEO)
NVG Sild surveys	1 or 2	Norwegian coastal zone	Norwegian Spring Spawned herring	Annual since 1982	Gulf and Vertical hauls	Larvae	Only Norway
Herring larvae	2	Stettin lagoon and Vistula lagoon	Herring larvae			Larvae	Poland
Russian surveys?		Barents Sea					

There is a need for the various groups working on ichthyoplankton surveys to communicate on a number of topics. These include discussions on developments and problems in sampling, sampling equipment, protocols and data archiving and formats. There is also a need to keep informed on new or novel techniques for e.g. species identification using e.g. genetic probes or automated procedures. A number of these surveys are being undertaken at a regional level and as such there is also a need to bring people together so that surveys are not undertaken in isolation. Experience gained in one area can be transferred to others and there can be some semblance of standardization across all similar surveys.

We recommend that a central group for ichthyoplankton surveys is constituted which may not necessarily take the form of a Working Group. A suggested name for this group would be 'Standards in Ichthyoplankton Surveys (SIPS). The group will be required to provide coordination that ensures that every three years the group (encompassing all regional and species based ichthyoplankton survey planning, working and study groups) either meets or a special session is requested for the ASC. The final product for this group will be the cross fertilization of ideas and standards for ichthyoplankton surveys in the ICES area.

Annex 6: Theme session on ichthyoplankton surveys at ASC 2010

Theme Session Ichthyoplankton Surveys – value added beyond assessment

Conveners: Cindy van Damme, Matthias Kloppmann, Steve Milligan

Within ICES coordinated work a number of ichthyoplankton surveys have been carried out for many years. Many of them already constitute a long time-series that would allow for data analysis beyond estimates of annual indices of recruitment or annual egg production. Some of those surveys also may have undergone changes in methodology or have adopted new techniques. This session invites contributions that analysed ichthyoplankton survey data with respect to changes in distribution, size and stage composition of species in relation to the changing physical and biological environment. Descriptions and analysis of change of methodology and the adoption of new techniques, and how they affected the survey results are also expected. Because most of those surveys are carried out under the supervision of different ICES Working Groups it is expected that this theme session will also promote positive influences between different ichthyoplankton groups.

Contributions addressing the following topics are encouraged:

- The effect of changes in methodology on survey results
- Adoption of new techniques and their benefits for improvement survey results
- Changes in abundance and distribution patterns in relation to changing ecosystem
- Changes in size and/or stage composition
- Species composition, parasitisation of eggs and other issues of interest.