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

By Correspondence

ICES International Council for the Exploration of the Sea

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Executive summary

The Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS) is primarily responsible for the planning and data analysis of the ICES Triennial mackerel and horse mackerel egg surveys. In 2010 as the year of the actual survey the WG carries out its activities by correspondence. The outcomes for 2010 are as follows:

The results of the two workshops on i) mackerel and horse mackerel egg staging and identification (5–9 October in IJmuiden) and ii) on fecundity and atresia estimation (1–4 December in San Sebastian) were considered and incorporated into the 2010 survey. The recommendations and the actions taken on these are detailed in the present report. In general all the recommendations were accepted and will be employed on the surveys in 2010.

The survey execution in 2010 was fine-tuned. Although the broad planning of the 2010 surveys was carried out at the 2009 planning WG, the detailed conduct required coordination within the survey year. Most importantly this involved ensuring that the coverage, in time and space, was as complete as possible with the vessel resources available.

Furthermore, with Iceland one more participant could be incorporated in the survey activities. In 2010 Portugal, Spain, Ireland, UK/Scotland, Norway, the Netherlands, Germany, the Faroe Islands and Iceland are participating in the egg survey.

A general updated survey manual was compiled as a stand-alone document and can be found as an annex of the report.

The next meeting of WGMEGS will be held in San Sebastian AZTI (Spain – Basque Country), in April 2011.

1 Introduction

The Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS) is primarily responsible for the planning and data analysis of the ICES Triennial mackerel and horse mackerel egg surveys. The meetings are held in the years before and after the surveys themselves. As 2010 is an egg survey year, the WG carried out its activities by correspondence.

2 Terms of reference

At the ICES WGMEGS in Hamburg, Germany, in April 2009 it was decided that the Working Group on Mackerel and Horse Mackerel Egg Surveys [WGMEGS] (Chair: J. Ulleweit, Germany) will work by correspondence in 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 Science Committee and TGISUR.

3 Incorporation of WKMHMES recommendations

Two workshops for quality assurance of the mackerel and horse mackerel egg surveys were held in autumn 2009. The first workshop dealt with mackerel and horse mackerel egg staging and identification (5–9 October in IJmuiden), the second workshop with fecundity and atresia estimation (1–4 December in San Sebastian). Based on the outcome of these workshops a number of recommendations were made which are referring to the 2010 survey.

In the following the recommendations and consequent actions are described. The original recommendations are in italics and the response is in normal face.

ToR a) It is recommended that all participants carry out artificial fertilizations of any species, which have eggs similar to those of mackerel and horse mackerel. It would be useful if egg and oil globule diameters are measured and that photographs are taken of as many stages as possible. It would also be beneficial if the eggs were preserved at various stages of development and any morphological changes noted following fixation. These eggs should be made available for analysis during the next workshop (scheduled for 2012).

It is recognized that some species have similar structures and size ranges to the survey target species. All participants agreed that this is useful as the correct species identification of fish eggs in different development stages has a main impact on the quality of the survey.

ToR b) The Spray technique should be included as a method for sorting eggs from the rest of the plankton during the 2010 triennial surveys. Following the use of the 'Spray Technique' to remove the eggs, each sample should subsequently be resorted by hand to remove any remaining eggs.

All participants agreed that the 'spray technique' will be used for routinely removing fish eggs from plankton samples.

ToR c) All participants are reminded that the procedures described in the WGMEGS survey manual should be followed during the 2010 surveys. Particularly that 4% formaldehyde, buffered with sodium acetate tri-hydrate, is the standard survey fixative and that plankton samples should never come into contact with formaldehyde of a concentration greater than 4%. All participants are encouraged to check the pH of their fixative on a regular basis.

The use of correct buffered formaldehyde for the conservation of the plankton samples is fundamental to minimize damage and distortion of the eggs. All participants agreed to use the described chemicals.

ToR d) All participants should try to collect reference eggs from different species during the 2010 egg survey and keep them for the next workshop in 2012.

See ToR a).

ToR e) WGMEGS should consider whether stage 1A and 1B could be amalgamated into a single stage both for the survey samples and future workshops. These stages are combined for the TAEP estimate. Not all participants separate these two stages.

This recommendation will be forwarded to the 2011 WGMEGS meeting and included in the terms of reference for this meeting.

ToR f) All analysts who are engaged in the analysis of fecundity and atresia of mackerel and horse mackerel samples must complete the intercalibration exercise before starting the analysis of the 2010 Triennial survey samples.

The participants agreed that until the beginning of March an intercalibration exercise using real samples and images will be finished including both fecundity and atresia analysis.

ToR g) It is recommended that more data are collected for the comparison of the standard method and the alternative method for atresia estimation. All participants of the 2010 survey should collect an extra sample of the mackerels and send these to IMR.

A manual on the mackerel sampling procedure for fecundity samples including samples for the comparison was distributed in January 2010 by Merete Fonn from IMR. All participants agreed to collect the samples.

4 Fine-tuning of the 2010 Mackerel and Horse Mackerel Egg Survey in the Western and Southern Areas

4.1 Countries and ships participating

Germany, Ireland, Netherlands, Scotland, Portugal, Spain, Spain/Basque Country, Norway, Faroe Islands and also Iceland 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 4.1.1. Following on from 2007 there continues to be no participation from Cefas. The reduction in survey effort is to some degree offset once again by the inclusion of an additional third two week survey by Scotland in period 4 as well as commitment from the Faroe Islands and Iceland to each devote 2 weeks of ship time to the 2010 survey in the western area. While these additional surveys are 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 consequently several boundaries remained unsecured. These challenges as well as recommenda-

tions are more fully described in Section 10. Survey coverage of the western and southern area is given by area and period in Table 4.1.2. Detailed maps of survey coverage by period are given in Figures 4.1.1 – 4.1.5. Both vessel availability and area assignments are still adaptable and will be finalized by the survey coordinator during the survey based on preliminary results.

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

Table 4.1.1. 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	24 Mar – 12 Apr	2
		Celtic Sea & Biscay	13 – 30 Apr	3
Netherlands	Tridens	Celtic Sea & Biscay	3 – 20 May	4
		Celtic Sea & Biscay	1 – 19 June	5
Spain (AZTI)	Investigador	Biscay	23 Mar – 9 April	2
		Biscay & Cantabrian Sea	3 May – 26 May	4
Norway	Johan Hjort	West Ireland & West of Scotland	11 May – 5 June	4
Ireland	Celtic Explorer / Celtic Voyager	Celtic Sea	5 – 29 March	2
		Celtic Sea, West Ireland & West of Scotland	8 – 28 July	6
Scotland	Scotia/Charter	West Ireland & West of Scotland	20 April – 11 May (22 Days)	3
		NW Ireland & West of Scotland	19 May – 1 June	4
		West of Ireland & West of Scotland	14 June – 5 July	5
Faroe Islands	Magnus Heinason	Faroes & Shetland	19 May – 2 June	4
Iceland	Arni Fridriksson	Faroes & Shetland	9 – 22 June	5

4.2 Details of vessels participating

Annex 1 shows contact details for vessels taking part in the 2010 survey.

4.3 Survey design

In keeping with 2007, the survey will be split into six sampling periods. Regarding survey design and survey deployment the plan for 2010 is almost identical with that used in 2007. The only significant change being the inclusion of the Faroese and Icelandic survey in May and June which will expand the geographic range of the survey in the North during these periods. As in 2007 an additional third survey will be undertaken by Scotland during period 4. In terms of survey days this represents an overall increase for 2010 compared to 2007, however an expansion of the geographical survey area to the north during periods 4 and 5 mean that any net benefit to the survey in terms of increased survey effort is negligible. 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. Regarding period and design this is almost identical with that completed in 2007 and will again constitute survey period 1 and no sampling in area IXa will take place 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 in 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 at this time in previous years, but as in 2007 no vessels are available to survey it. 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 4.1.1.

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. Due to the expansion of the spawning area that was observed in 2007 the emphasis will be even more focused on area coverage and finding the edges of the egg distribution. 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 2009 WGMEGS report, 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 of her designated survey area (51°30N)(Figure 4.1.2).

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 this latitude requires careful sampling to avoid having large samples at the edge of the survey area. It is therefore **imperative** that between these two surveys that 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 4.1.3):

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. This was due to a significant pulse of mackerel spawning activity which continued west past 59°N 13°W. In an effort to address this issue, the Scottish survey will survey to the north of the Norwegian survey from 55°15N to 59°45N. In turn the Faroese vessel will then survey north of 59°45N. The result of this will be to significantly expand the range of the survey in the North and will attempt to secure this northern boundary should the spawning distribution found in 2010 mirror that of 2007 (Figure 4.1.4).

Period 5

In period 5, 2 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 Norwegian vessel – the period 4 survey overlaps into period 5 - will also be utilized during this period to survey part of what was originally the Scottish survey area North West of Scotland. Additional period 5 coverage for the Norwegian survey will commence on the 31 May at 58°45N and continue north on alternate transects for

the remainder of the survey. Any additional sampling in this area during period 5 will then be able to be completed by the Scottish vessel. The survey coordinator will advise nearer the time. This will allow the other vessels – notably the IMARES and Scottish vessels - to survey further south and permit the IMARES vessel to better secure a southern spawning boundary for period 5. In addition to these surveys Iceland will provide a 2 week survey in period 5 which will cover the area north of the area covered by Scotland and Norway at 60°45N. As in period 4 this will expand the survey range and attempt to secure a northern boundary within this period. See Figure 4.1.5 for survey areas, however these are provisional and definitive survey areas as well as starting positions will be provided by the survey coordinator and will largely be dependent on what is observed in period 4.

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 4.1.6)

Table 4.1.2. Periods and area assignments for vessels by week for the 2010 survey. Area assignments and dates are provisional.

week	Starts	Area							Period
		Portugal, Cadiz & Galicia	Cantabrian Sea	Biscay	Celtic Sea	North west Ireland	West of Scotland	Faroes & Shet	
4	25-Jan-10	PO1(DEPM)							1
5	1-Feb-10	PO1(DEPM)							1
6	8-Feb-10	PO1(DEPM)							1
7	15-Feb-10	PO1(DEPM)							1
8	22-Feb-10	PO1(DEPM)							1
9	1-Mar-10	PO1(DEPM)			IRL1				1
10	8-Mar-10				IRL1				2
11	15-Mar-10		IEO1		IRL1				2
12	22-Mar-10		IEO1	AZTI-1	IRL1	GER	GER		2
13	29-Mar-10		IEO1	AZTI-1		GER	GER		2
14	5-Apr-10			AZTI -1		GER	GER		2
15	12-Apr-10		IEO2		GER				3
16	19-Apr-10		IEO2	IEO2	GER	SCO1	SCO1		3
17	26-Apr-10		IEO2	IEO2	GER	SCO1	SCO1		3
18	3-May-10		IEO2\ AZTI-2(DEPM)	IMARES 1	IMARES 1	SCO1	SCO1		3
19	10-May-10		AZTI-2(DEPM)	AZTI-2(DEPM)	IMARES 1	IMR	IMR		4
20	17-May-10			AZTI-2(DEPM)	IMARES 1	IMR	SCO2	FAR	4
21	24-May-10		AZTI-2(DEPM)		IMR	IMR	SCO2	FAR	4
22	31-May-10			IMARES2	IMARES 2		IMR		5
23	7-Jun-10				IMARES 2			ICE	5
24	14-Jun-10			IMARES2	IMARES 2	SC03	SC03	ICE	5
25	21-Jun-10					SC03	SC03		5
26	28-Jun-10					SC03	SC03		5
27	5-Jul-10				IRL2	IRL2	IRL2		6
28	12-Jul-10				IRL2	IRL2	IRL2		6
29	19-Jul-10				IRL2	IRL2	IRL2		6
30	26-Jul-10								6

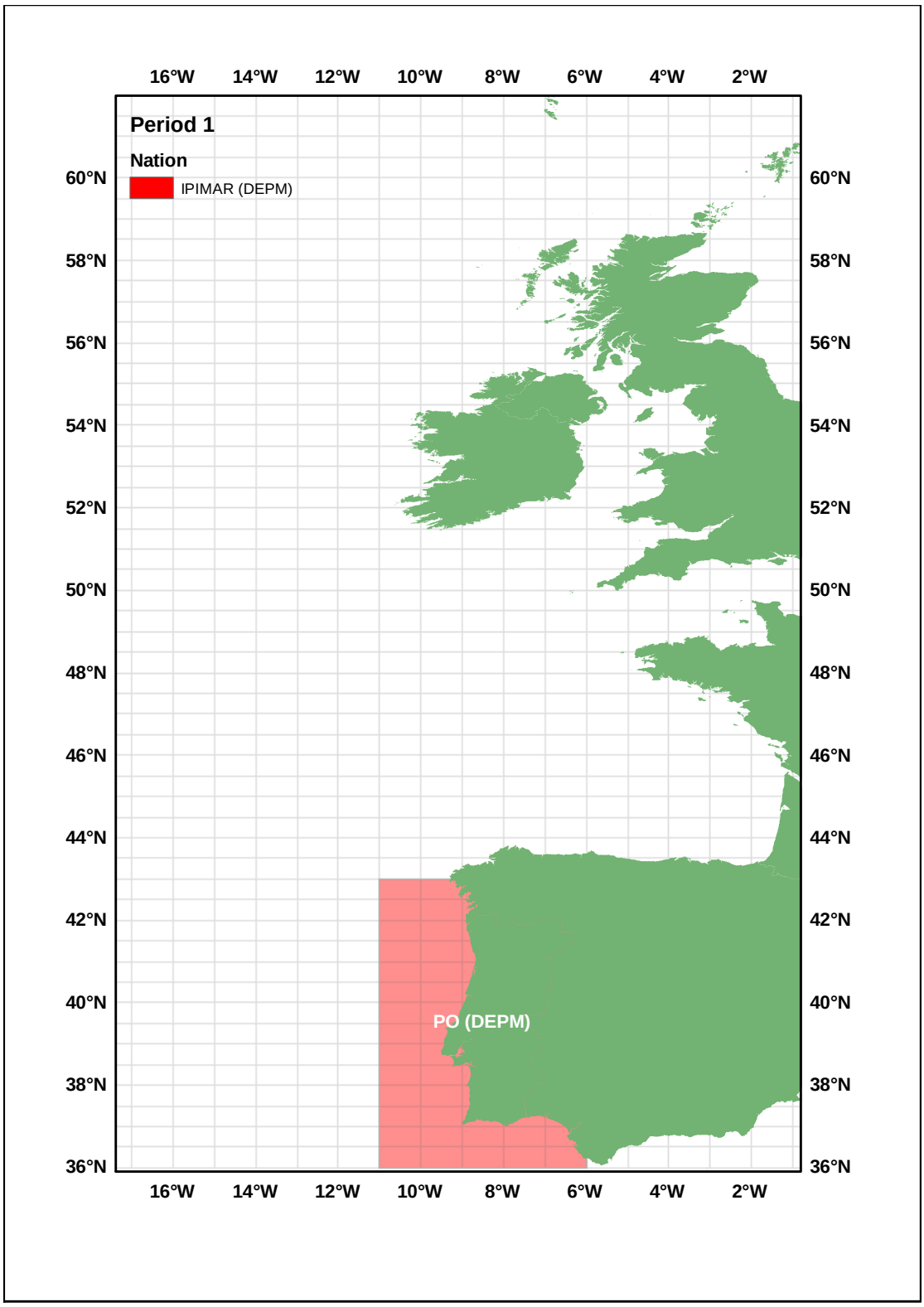

Figure 4.1.1. Survey plan for Period 1.

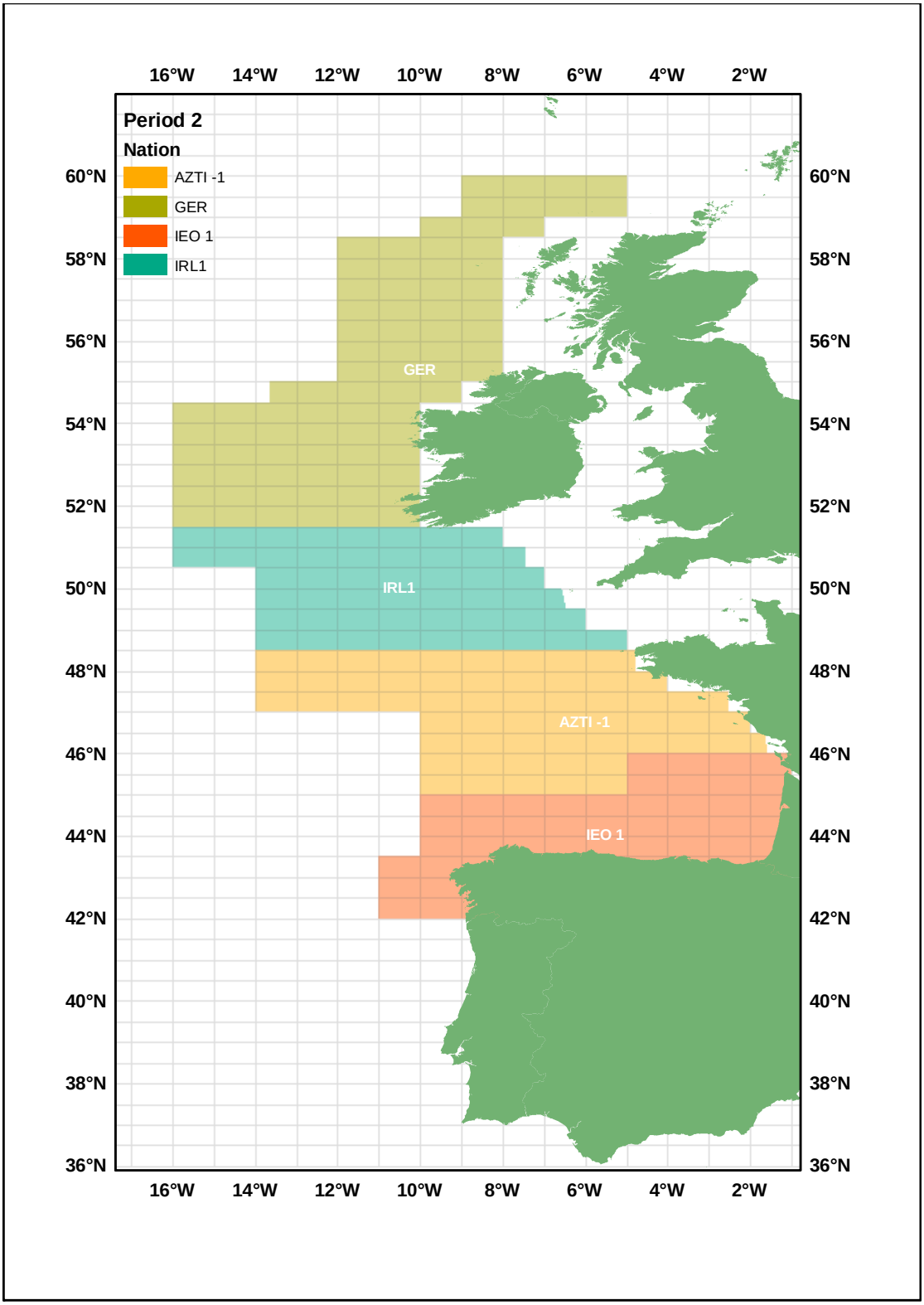

Figure 4.1.2. Survey plan for Period 2.

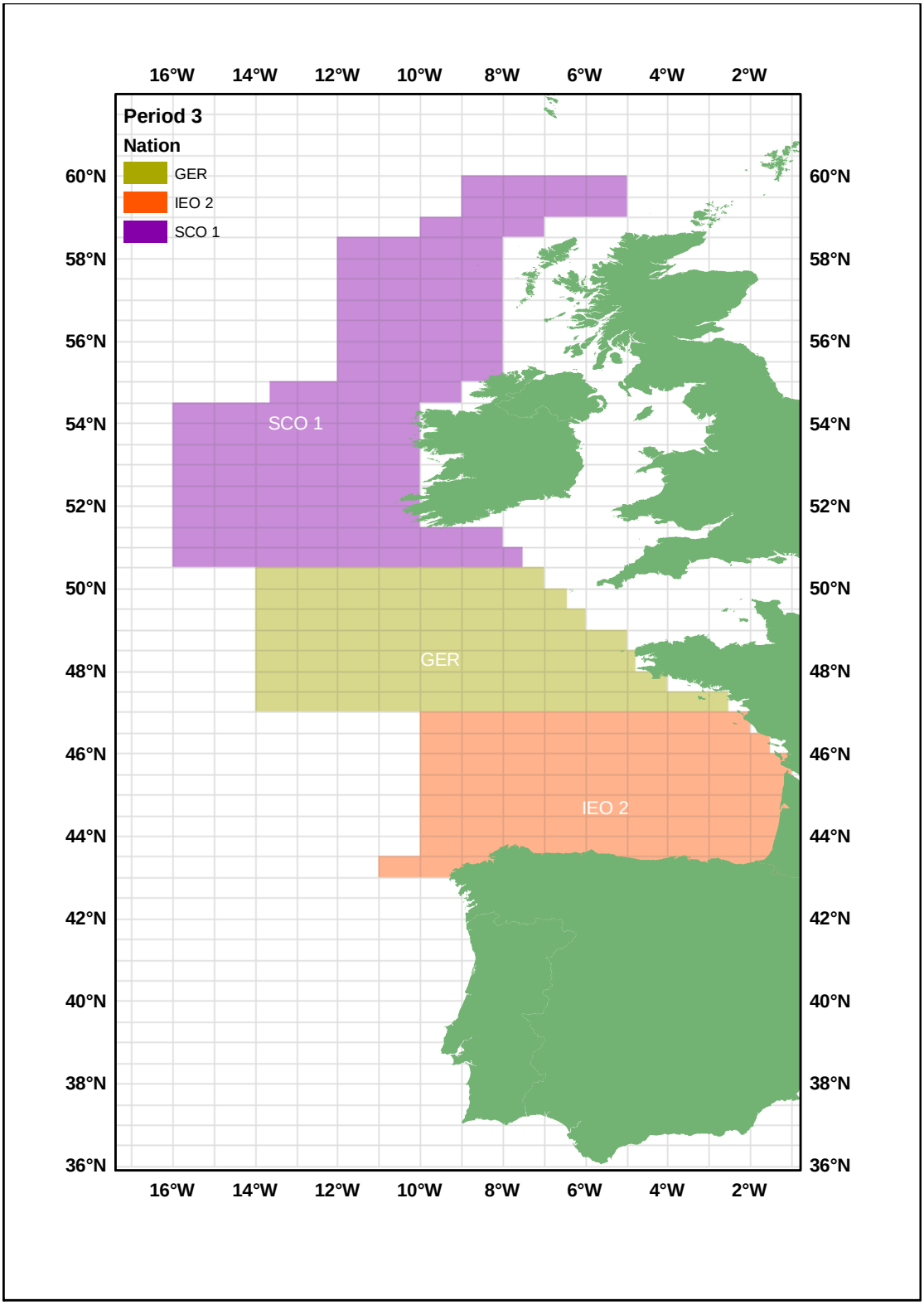

Figure 4.1.3. Survey plan for Period 3.

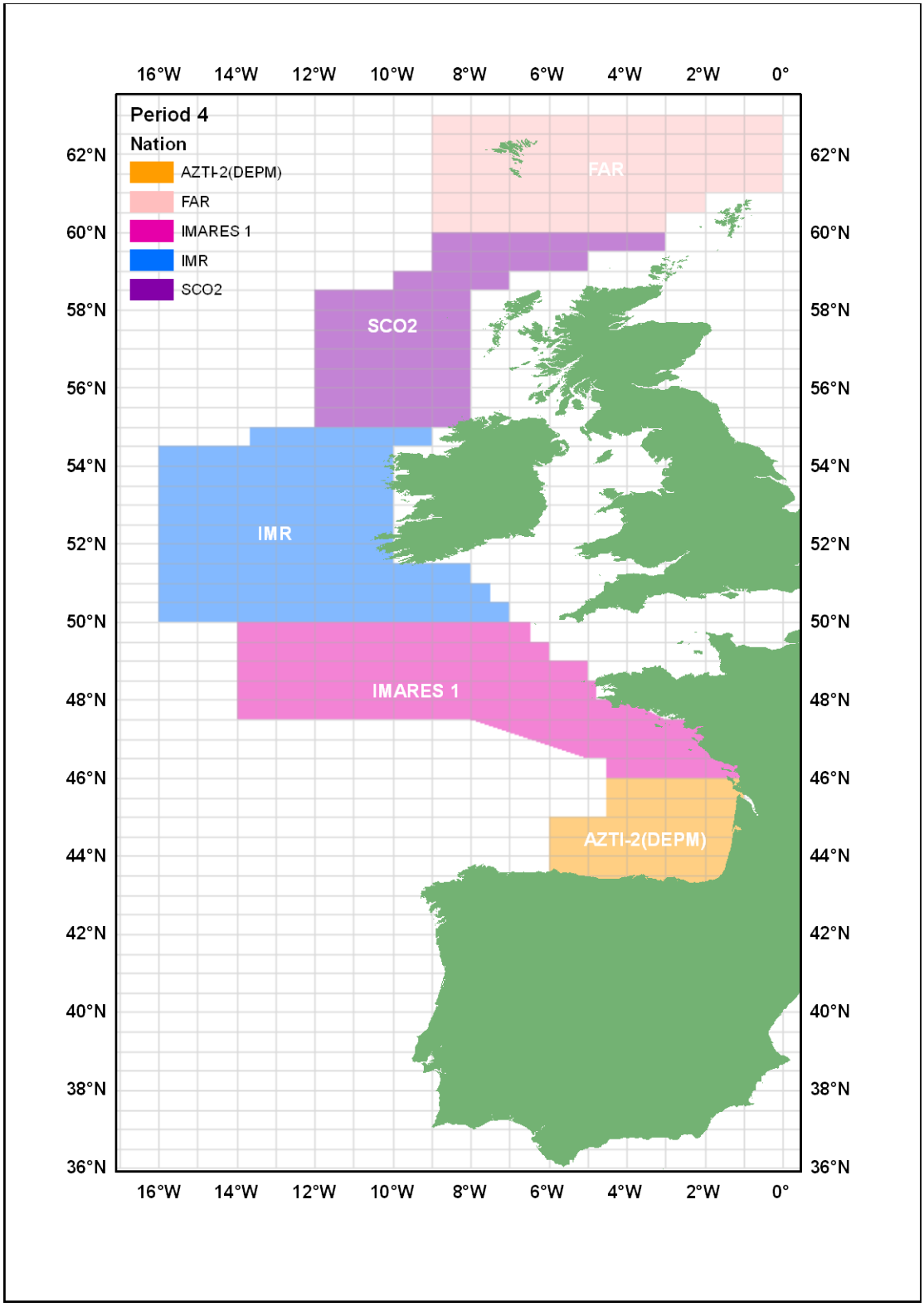

Figure 4.1.4. Survey plan for Period 4.

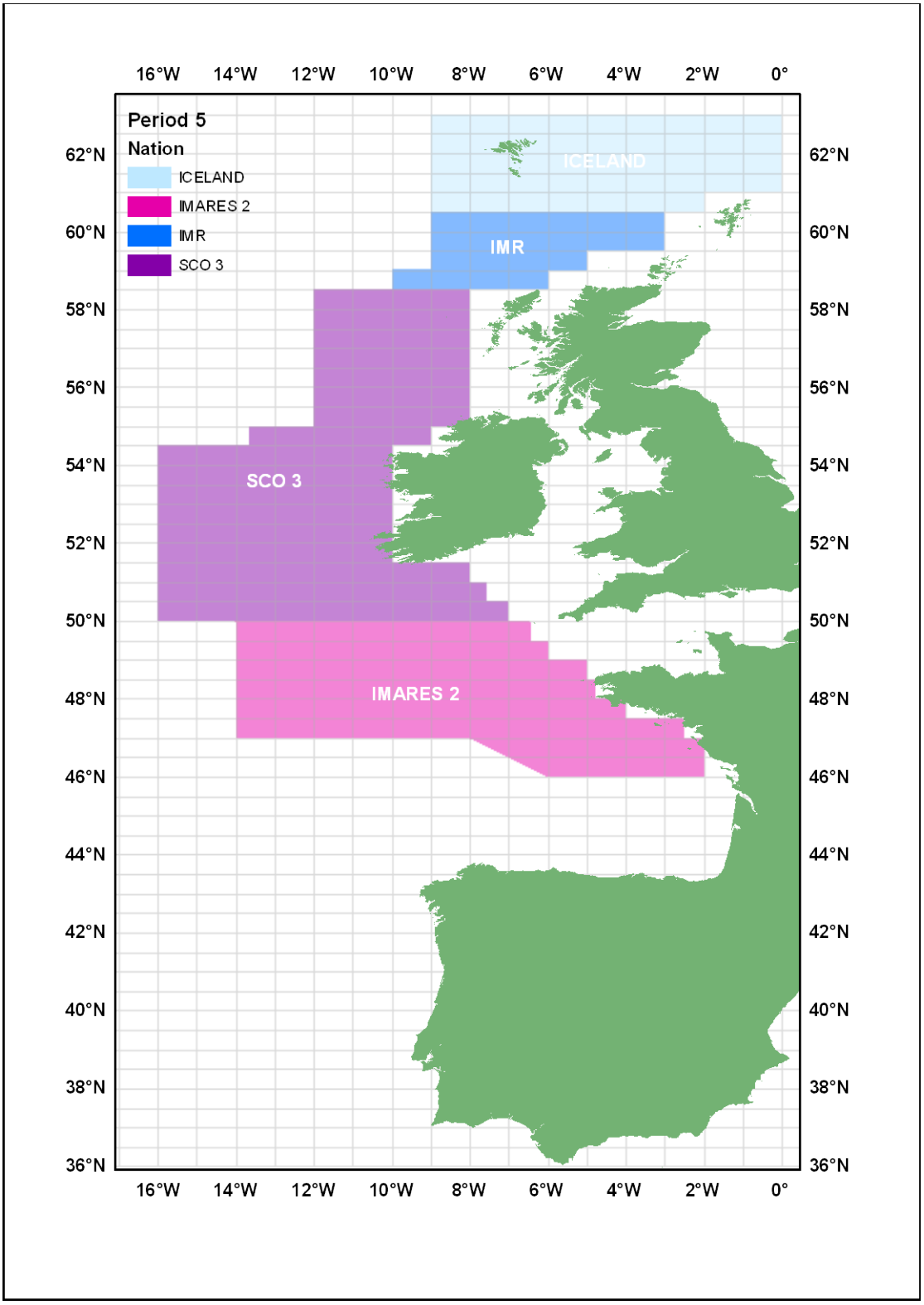

Figure 4.1.5. Survey plan for Period 5.

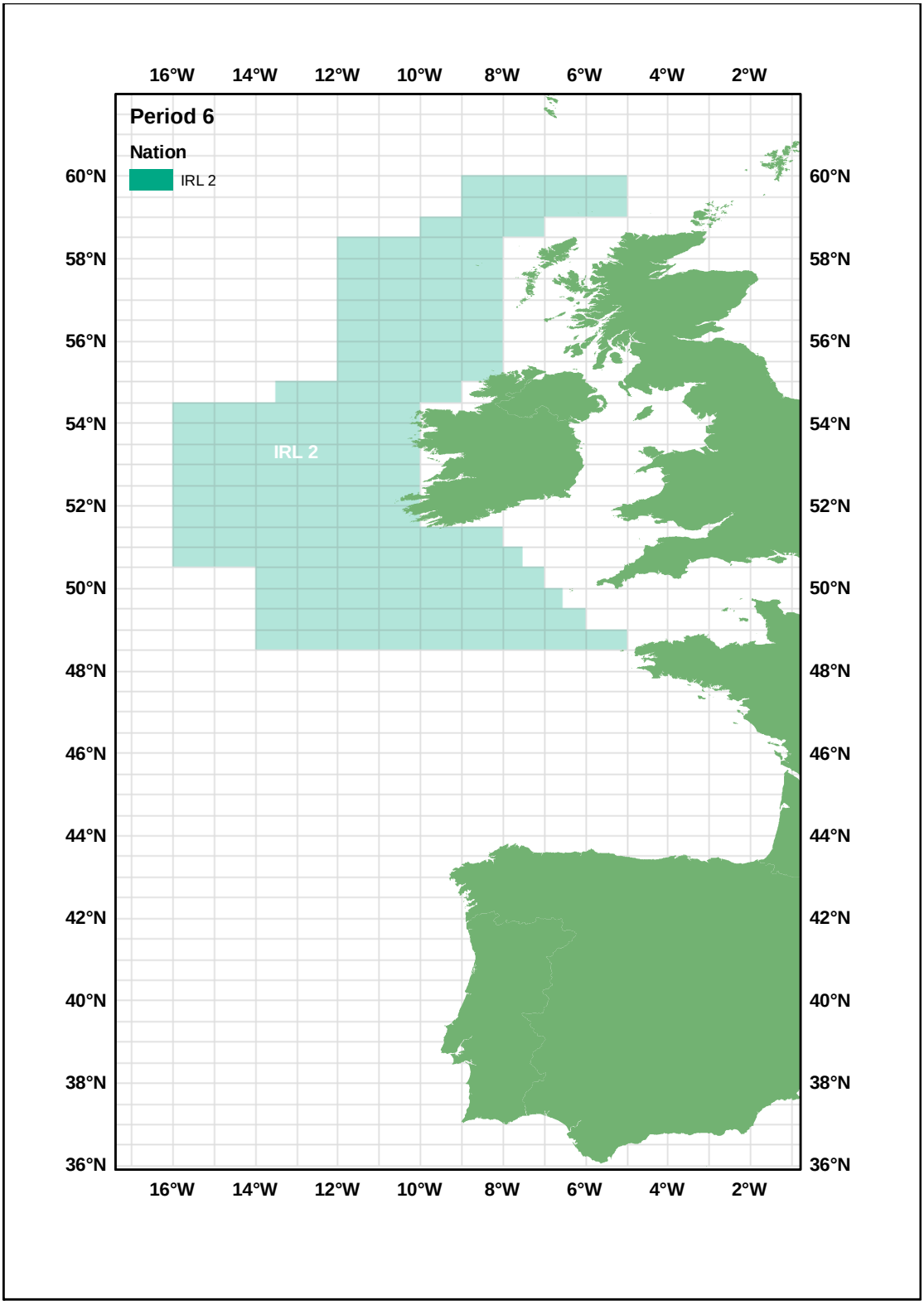

Figure 4.1.6. Survey plan for Period 6.

4.4 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 were started 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, 1997b). 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 2). The core areas for the western and southern surveys for both species are presented in Figures 4.4.1 and 4.4.2. A more detailed survey map of the Iberian areas as surveyed by IEO and IPIMAR can be found in Figure 4.4.3. Section 2.4 of the 2009 WGMEGS report 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.

4.5 Timing for work completion

WGMEGS was asked by WGWIDE to come up with a reliability estimate of the preliminary estimates of mackerel SSB submitted to WGWIDE in the year of the survey to avoid huge changes of SSB estimate in the year after the survey. During the 2010 WGMEGS worked by correspondence, it was decided by egg survey participants that an attempt would be made to analyse all plankton samples and a subsample of the mackerel fecundity and atresia samples in time for the WGWIDE 2010 meeting and that a preliminary estimate of spawning-stock biomass (SSB) for mackerel and a total annual egg production estimate (TAEP) for horse mackerel be available also in time for the WGWIDE 2010 meeting. WGMEGS will discuss the evaluation of the preliminary estimates at the next meeting in 2011.

In order to deliver a robust provisional biomass estimate for mackerel and an egg production index for horse mackerel to WGWIDE participants are asked to complete the analysis of the plankton and fecundity samples in time:

Plankton samples should be analysed, data checked, ready and submitted within 2 weeks of returning from the individual survey to the survey coordinator (Finlay Burns). If this is not possible the data of all participants with the exception of the Irish period 6 survey should submitted at the very latest by the end of July.

Fecundity samples should be sent out immediately after the individual surveys to the analysing institutes according to the sampling procedure sheets distributed by Cindy van Damme and Merete Fonn.

Preliminary estimates have to be supplied to WGWIDE by 23 August, with last revisions possible on 27 August. WGWIDE will then evaluate on their first meeting day (28 August) if they are using the preliminary data for the 2010 assessment or not.

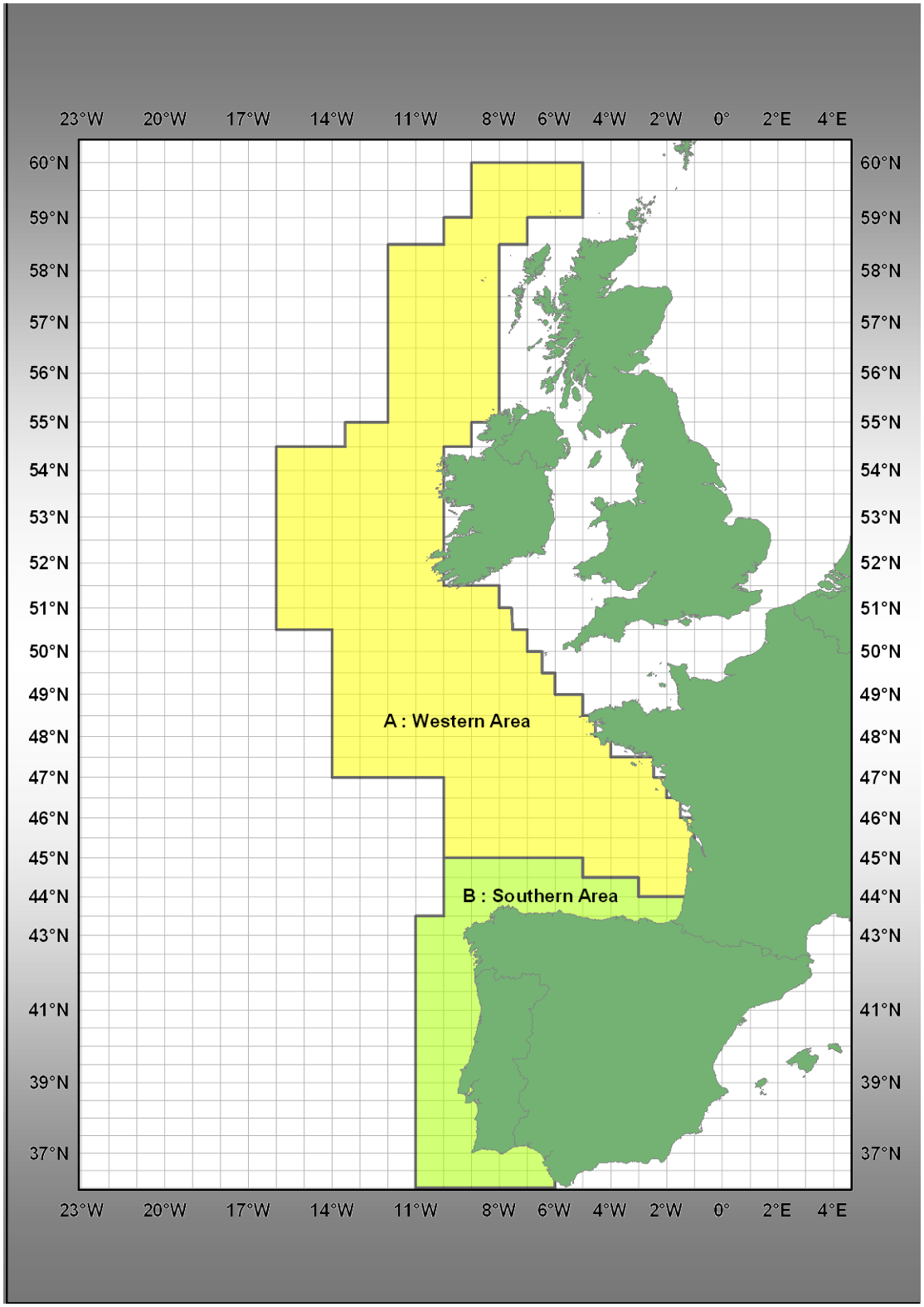

Figure 4.4.1. Core sampling areas for mackerel eggs in the western and southern areas for 2010. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines (Annex 2).

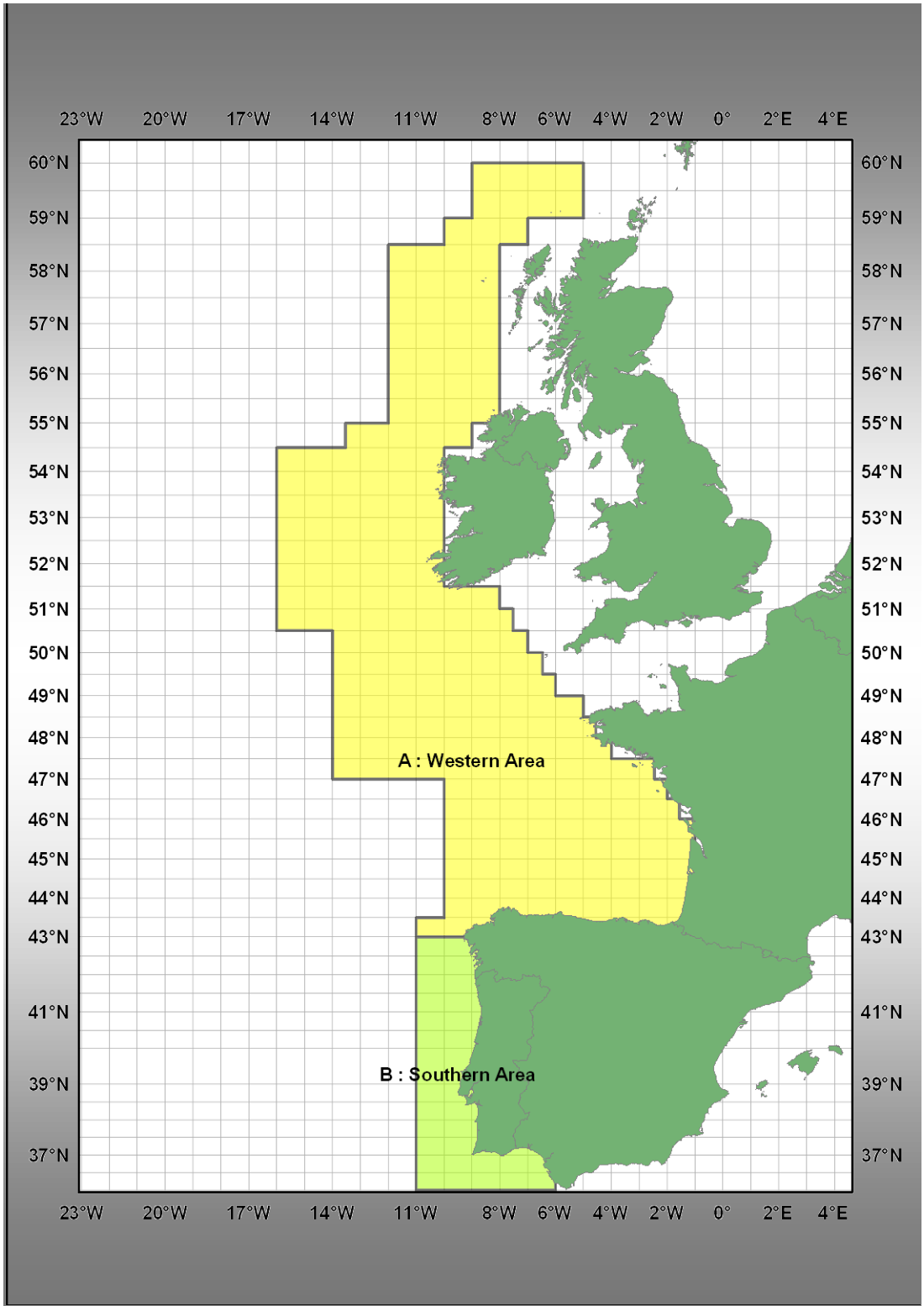

Figure 4.4.2. Core sampling areas for horse mackerel eggs in the western and southern areas for 2010 corresponding to the boundaries of the western and southern horse mackerel stocks. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines (Annex 2).

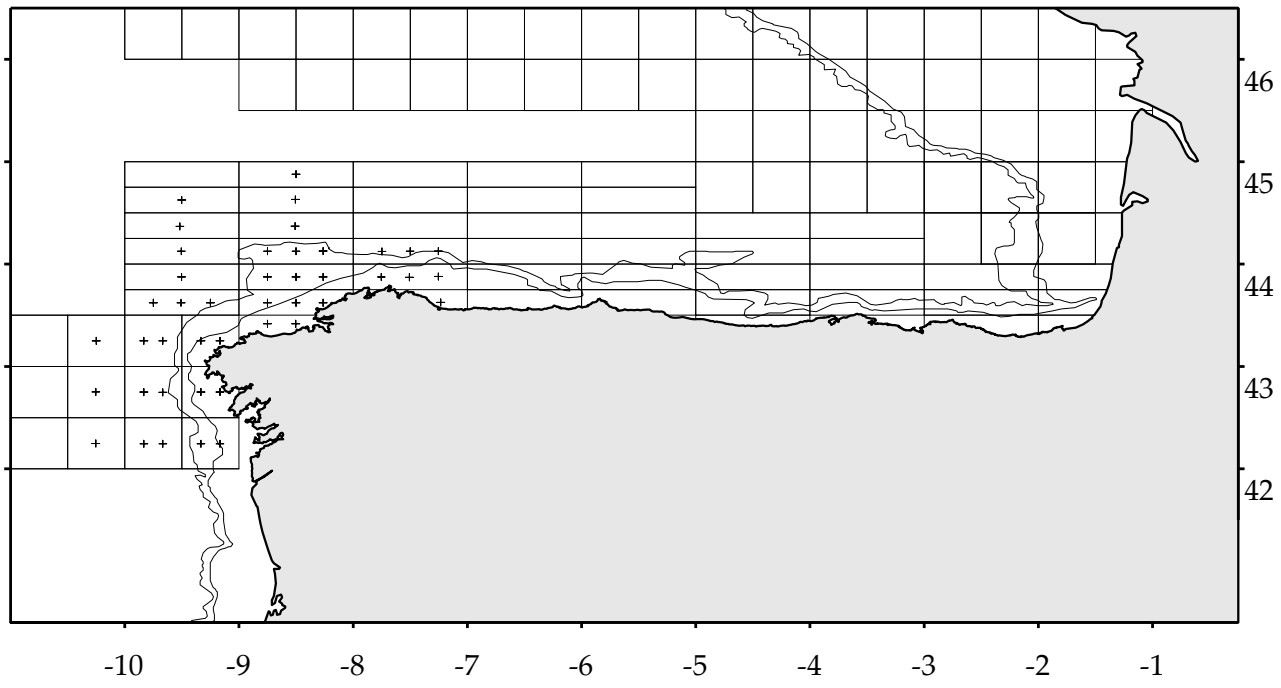

Figure 4.4.3. IEO sample locations for Galicia and the Cantabrian Sea.

5 Other changes and additions

5.1 Survey manual

A survey manual including the updated plankton and adult parameter sampling was compiled as stand-alone document and can be found as Annex 2 of this report.

5.2 Fecundity sampling

An excel template for the data entry of the mackerel and horse mackerel fecundity sampling parameters was distributed by Cindy van Damme (cindy.vandamme@wur.nl). All participants are asked to use the template to avoid time-consuming converting of different formats.

5.3 Collaboration with WGNAPES

The ICES Working Group on Northeast Atlantic Pelagic Ecosystem Surveys (WGNAPES) agreed that during the International ecosystem survey in the Nordic seas (IESN) bongo samples for mackerel eggs will be taken where spawning mackerel are found in order to support WGMEGS with information on mackerel spawning in the survey area. Contact person to WGMEGS will be Matthias Kloppmann (vTI-SF, Germany).

5.4 Stomach sampling programme

Following a recent hypotheses that adult prespawning, spawning, and post-spawning mackerel might feed on blue whiting eggs and larvae where the distribution areas are overlapping, a stomach sampling programme for adult mackerel was compiled. All survey participants are asked to take part in the sampling programme (Annex 4).

5.5 Possible collaboration with PHISHED

A potential collaboration between the project "Physics to Fishes at the Shelf Edge (PHISHED)" and the work of the International Mackerel and Horse Mackerel Egg Survey in 2013 was proposed. This collaboration would involve the participation of guest scientists on some vessels during the 2013 surveys in the Western areas. Contact person to WGMEGS will be Dave Reid (MI, Ireland).

Annex 1: Vessel details

Country	Vessel	Call sign	Cruise leader	sat tel number	mob number	fax sat	fax mob	email
Faroe Islands	Magnus Heinason	OW 2252	Høgni Debes	+871 623104120	+298 286092	+871 623104120		423104110@inmc.eik.com
SPAIN (AZTI)	INVESTIGADOR	EAJO	Paula Alvarez Maria Santos	00870762713140; 00871762712140; 00874762712140	639839401; 670716988			investigador.investigador@amosconnect.com
SPAIN	Cornide de Saavedra	EDSV	Ana Lago de Lanzós Concha Franco Gersom Costas	00871622476510 or 00871764356765	00 34 639677849 (Coastal only)	00871764356768	00 34 609 602 157	csaavedra@satellite-email.com cornide@vi.ieo.es
Ireland	Celtic Explorer	EIGB	Brendan O' Hea	00871 763066743	0035387 2044837	00871 763066741	0035387 6519288	celticexplorer@pomaritime.ie
Ireland	Celtic Voyager	EIQN	Brendan O' Hea	00871 761606474	0035387 9186786		0035387 2016046	celticvoyager@pomaritime.ie
Netherlands	Tridens	PBVO	Cindy van Damme and Kees Bakker	+31 207178825 or +31 207178826		+31 207178827		cindy.vandamme@wur.nl kees.bakker@wur.nl
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Annex 2: Survey manual

MANUAL FOR THE TRIENNIAL MACKEREL AND HORSE MACKEREL EGG SURVEYS

The Working Group on Mackerel and Horse Mackerel Egg Surveys

Introduction

The working group on mackerel and horse mackerel egg surveys coordinates the Mackerel and Horse Mackerel Egg Survey in the Northeast Atlantic and the Mackerel Egg Survey in the North Sea, both carried out triennially. Both surveys provide indices for the strength of the SSB of the both the western and North Sea stocks of Atlantic mackerel (*Scomber scombrus*) and a relative abundance index of horse mackerel (*Trachurus trachurus*) spawning stocks in the Northeast Atlantic. The survey for the western mackerel stock was initiated in 1977 by England (Lockwood *et al.*, 1981) joined only by France. Later the North Sea survey was added as well as the utilization of the Northeast Atlantic Survey for investigating the abundance of horse mackerel eggs. The survey was soon acknowledged for its usefulness in providing the only independent measure of SSB of western mackerel and more and more countries joined the survey. Consequently, and in order to achieve comparable data over the complete survey, regardless of participating nation, it became necessary to standardize methods applied during the survey.

A first manual for the conduct of egg surveys, targeted at the annual egg production method (AEPM), was presented in Section 8 of the Report of the Mackerel/Horse Mackerel Egg Production Workshop (ICES, 1994). Those instructions were repeated in ICES 1997 (Sections 6.4.1 to 6.4.8) and incorporate changes, additions or clarifications. Additional changes and recommendations for further standardization between participants were given in Section 3.3 of ICES (2003). At each working group meeting as well as during the workshops on egg staging and fecundity estimation, the manual is discussed and updated where necessary, and incorporated in the working group and workshop reports as an annex document. Other methods necessary for adequate storage and preservation of the samples, sorting, identification and staging of fish eggs are described in sections of the different workshops and working group meetings. In order to facilitate the ease of use of the survey manual and all other available descriptions of the standard operational procedures for the MEGS it was recommended on the 2009 WGMEGS meeting that all those descriptions necessary for a successful execution of the survey shall be combined in one stand alone document.

This manual 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 manual 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 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 areas. 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 an egg production index for horse mackerel.

Figure 1.1 shows the core sampling areas for mackerel eggs. The 'southern' area is regarded as being from 36º N to 45º N. It includes southern Biscay, the Cantabrian Sea and from the Portuguese coast to 11º W. 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.

The core sampling areas for horse mackerel eggs are slightly different from mackerel. The 'southern' area covers the area from 36° N to 43°N, the ``western' area is from 43° N to 60° N.

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. Usually, plankton samplers are deployed at the centre of half standard ICES rectangles, which are 0.5º latitude, by 0.5º longitude. However, to the north of Spain (Cantabrian Sea) and to the south of Portugal (south of 37ºN) the sampling positions are separated by 10' latitude and 20' longitude because of the proximity of the shelf edge to the coast. To the west of Portugal (from 37ºN to 43º 10'N) the station positions are separated by 20' latitude by 10' longitude to provide greater spatial resolution across the shelf break.

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, 2002) 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 surveys together, are presented in Figure 1.1. The sampling area in the south has been modified from the design used in 2001 and previously (Figure 1.2). Figures 1.1 and 1.2 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 their waters, 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 1.1).

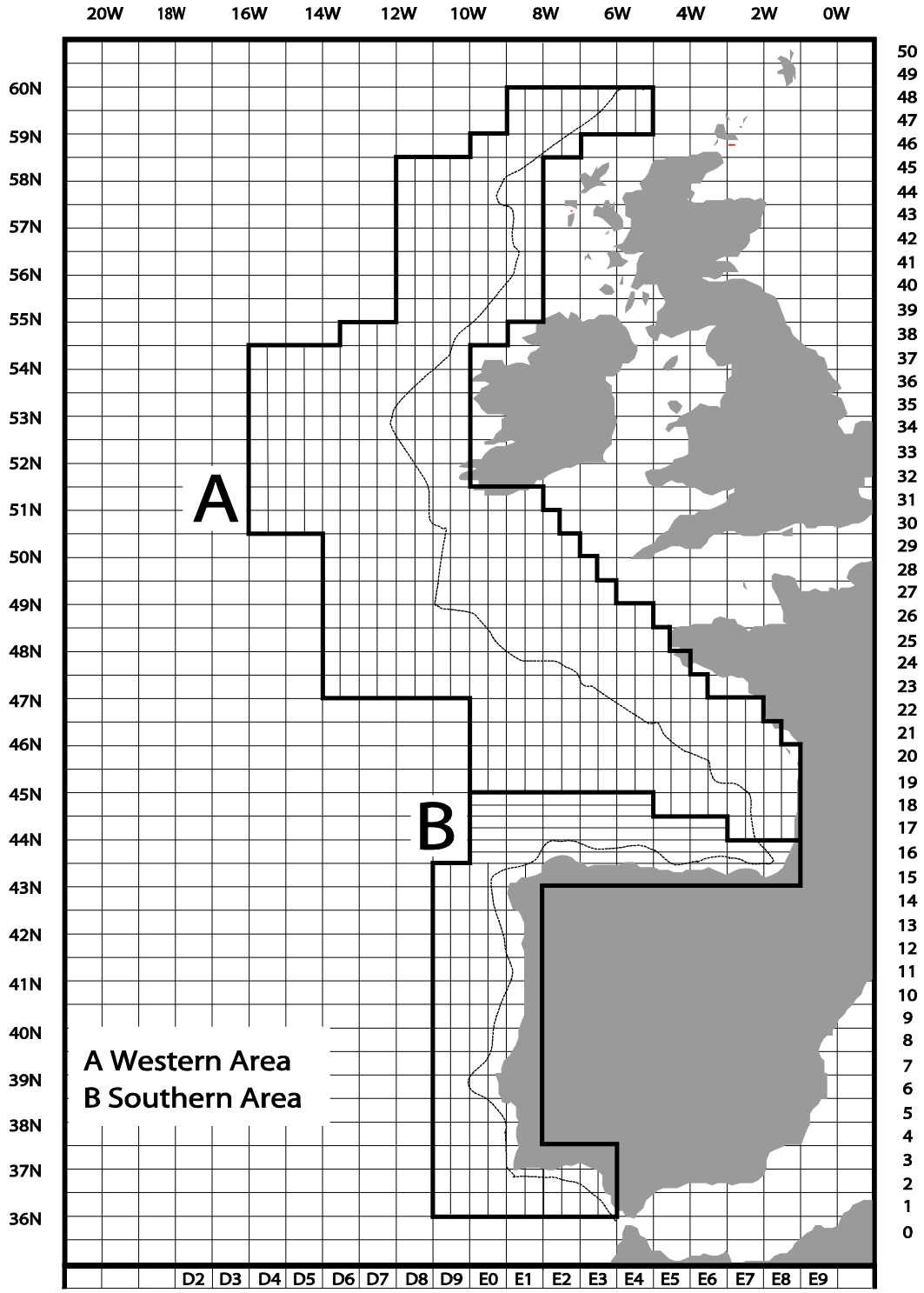


Figure 1.1. Core sampling areas for mackerel eggs in the western and southern areas for 2004. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines.

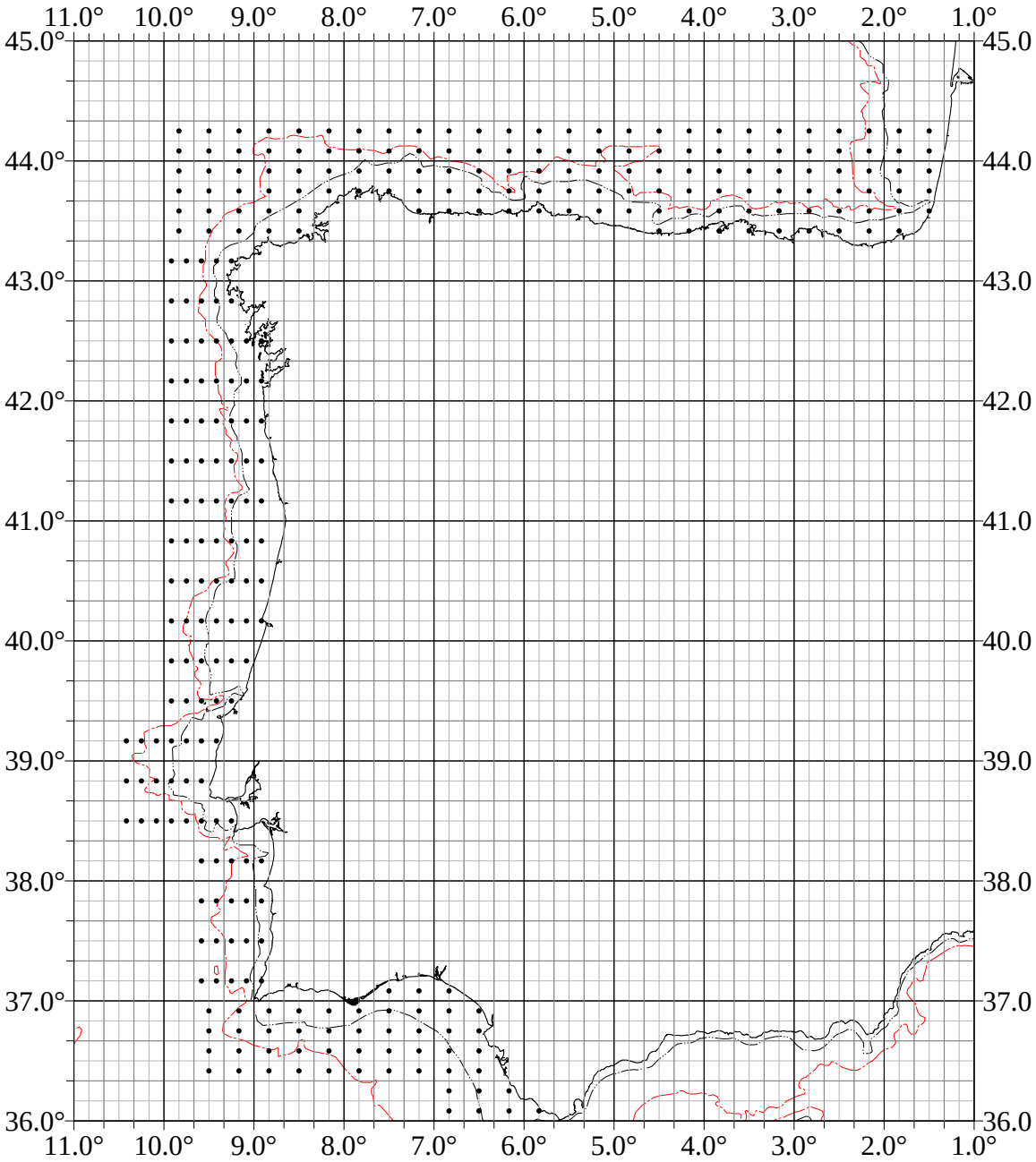


Figure 1.2. Provisional station location for mackerel and horse mackerel egg surveys in the southern area in 2004. Offshore boundaries will be based on two consecutive zero rectangles.

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, while 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, permits 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 permit 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 Bongo or 'Gulf type 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 permit 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, 2008).** Nash *et al*., 1998, provides a comprehensive description for a Gulf type sampler, which they call a Gulf VII. The Bongo net is sufficiently described in Smith and Richardson (1977) while 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 Smith and Richardson (1977) provide an acceptable standard. These standards should be followed as closely as possible**.** It is also critical that the importance of calibrating flowmeters, and changes in flowmeter performance, when they are mounted in the apertures of plankton samplers is understood (EU AIR3 CT94 1911). 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, (whilst 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 permit 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 on the outside 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.

It is recommended that all participants review the performance of their flowmeters and regularly 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 is 20 cm in diameter in order to ensure that an adequate volume of water is filtered. The aperture of the Bongo samplers is 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.

Since the 2004 surveys a high level of standardization of sampling equipment has been achieved for the mackerel and horse mackerel egg surveys (Table 3.1). According to the table presented below 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 3.1. 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 vertically deployed 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 (Table 3.2). All specifications are listed in the table below. 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 3.2. 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

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

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) Label jars with station details and put labels containing same 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 since 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, improvements 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. However, it is imperative 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 (normally after 48 hours in 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, pearlside (*Maurolicus muelleri*) 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.**

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, 2004 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 six morphological stages (Ia, Ib, 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).

7.1 Egg stage criteria

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

7.1.1 Stage Ia

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

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

7.1.2 Stage Ib

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

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

7.1.3 Stage II

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

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

7.1.4 Stage III

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

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

7.1.5 Stage IV

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

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

7.1.6 Stage V

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

Secondary characteristics: Pigmentation develops in the eye.

Hake and Horse mackerel never attain stage V.

NB

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

For the estimation of daily egg production for both mackerel and horse mackerel, only the counts of stage Ia and Ib 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.

7.2 Egg identification criteria

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

In addition to the published descriptions given in the text table (below), various other criteria are used by participants to help with egg identification based their own knowledge and experience. These criteria can be regarded as secondary characteristics and are described for each species below. Photographs of known mackerel eggs are shown in Figure 7.2-1 for comparison with horse mackerel eggs from the southern area shown in Figure 7.2-2.

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

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

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

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

7.2.3 Megrim *(Lepidorhombus whiffiagonis)*

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

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

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

7.2.5 Longspine snipefish (*Macrorhamphosus scolopax*)

- Egg spherical and transparent.

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

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

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

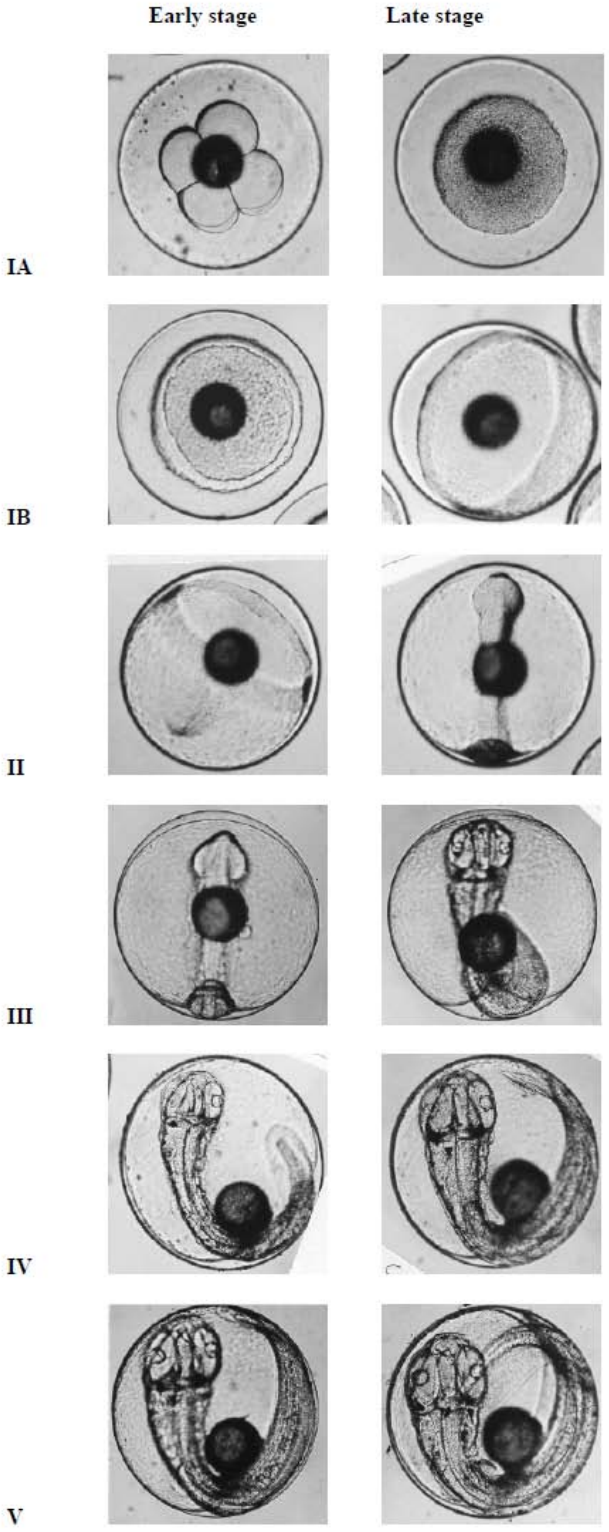


Figure 7.2.1. Mackerel eggs at the beginning and end of the six (IA, IB – V) development stages.

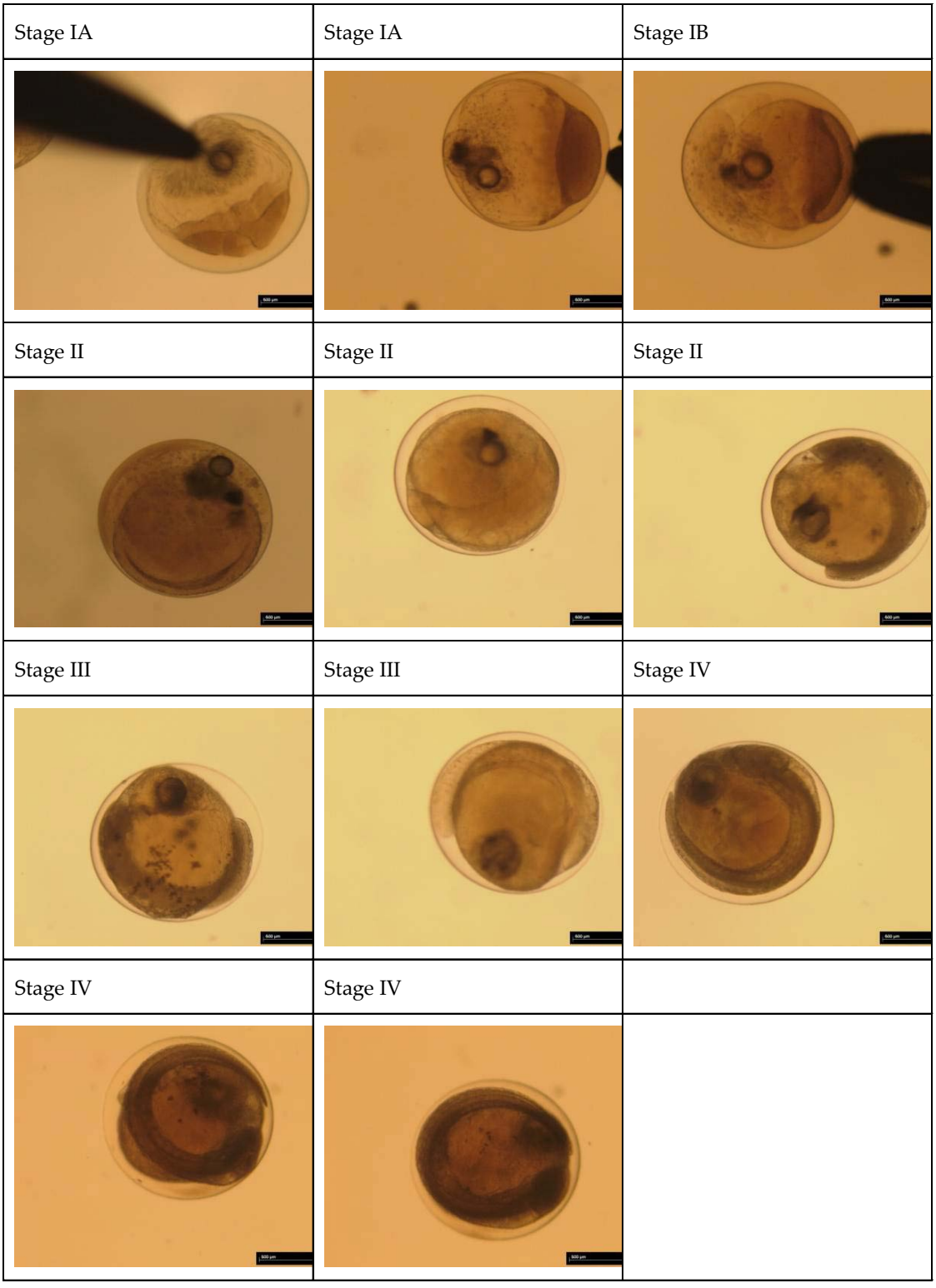


Figure 7.2.2. Horse mackerel eggs in each of the five development (IA, IB – IV) stages.

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.

$$V = \frac{r \cdot a}{cal} \cdot F ,$$

The egg abundance (in eggs m^{-2}) is calculated from the formula:

$$A_e = \frac{C_e \cdot S}{V} \cdot D$$

Where:

V	=	Volume filtered in m^3
r	=	Number of revolutions of the flowmeter during tow
a	=	Aperture: The area of the mouth opening of the sampler in m^2
cal	=	The number of flowmeter revolutions per metre towed, obtained from the flume or sea calibration in free flow.
A_e	=	Egg abundance in eggs m^{-2}
C_e	=	Number of eggs in subsample
S	=	Raising factor from the subsample to the whole sample
D	=	The maximum depth of the sampler during the tow in metres
F	=	The sampler efficiency from flume or towing tank calibration (ideally 1)

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

For stage I **mackerel** eggs:

$$EP = \frac{24 \cdot A_e}{e^{-1.61 \cdot \log(T)+7.76}}$$

For stage I **horse mackerel** eggs:

$$EP = \frac{24 \cdot A_e}{e^{-1.608 \cdot \log(T)+7.713}}$$

Where EP = egg production in eggs m^{-2} day^{-1} and T = temperature in °C at 20 m depth (5 m in the North Sea, and see above).

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:

$$A_R = \left(\cos(Lat) \cdot 30 \cdot 1853.2\right) \cdot \left(30 \cdot 1853.2\right)$$

where A_R = rectangle area in m^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.

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, cruise leaders 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

A detailed description of ship board methods for fecundity sampling is also given in the WGMES Fecundity Manual.

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 to 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 to 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.**

Ovaries of the maturity stages 3 – 6 (Walsh scale, Table 10.1.2) should be taken as laid out in Table 10.1.3. Ovaries should be weighed and subsamples taken by micropipette before fixing in 3.6% buffered formaldehyde solution on board. **The recipe for formaldehyde solution for both, mackerel and horse mackerel fecundity sampling is given in Section 10.3 below.** 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 tubes with sealed screw caps (such as Nunc tubes) in order to avoid evaporation of the fixative. Care should be taken that oocyte samples are completely covered by formaldehyde. Participants should regularly check that the samples are in a 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 10.1.2 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 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.

Tab. 10.1.2a: 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 Lon ° Lat °

Week	Date	Period*	11W	10	9	8	7	6	5	4	3	2	1	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																		20
13	29.03.2010	2	20																		20
14	05.04.2010	2																			0
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
																					210

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

	per period			
	1	2	3	4
IEO	60	60	50	0
IPIMAR	40			
Total:	100	60	50	0

210

Tab. 10.1.2b: 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	>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		35			10				20		20			10						95
13	29.03.2010	2	10		35												10					55
14	05.04.2010	2		20	30						20				20							90
15	12.04.2010	3					20															20
16	19.04.2010	3				20					10						10					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				5						85
21	24.05.2010	4									10		10			5				10		35
22	31.05.2010	5			20																	20
23	07.06.2010	5				20															5	20
24	14.06.2010	5				20			10		10		10								5	50
25	21.06.2010	5											10			10					5	20
26	28.06.2010	5							20													20
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
																						945

* 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		120	0	40			
vTI		100	60				
MI		80				100	
MSS			100	10	70		
IMARES			20	40	60		
IMR				60			
IEO		10	40				
FAR				20			
ICE					15		
	0	310	220	170	145	100	945

Table 10.1.2. Key for the determination of mackerel and horse mackerel maturity (Walsh Scale, Walsh *et al.*, 1990).

FEMALES	STAGE	MALES
Ovaries small, wine red and clear. Torpedo shaped. No sign of development.	**1** **Virgin**	Testes small, pale, flattened and translucent. No sign of development.
Ovaries occupying ¼ to ¾ body cavity. Opaque eggs visible, giving pale pink to yellowish colouration. Largest eggs without oil globule.	**2~** **Early ripening**	Testes occupying ¼ to ¾ body cavity, off-white, no milt running.
Ovaries occupying 3/5 to almost filling body cavity. Yellow to orange in colour. Largest eggs may have oil globule.	**3** **Late ripening/ partly spent (early)**	Testes occupying 3/5 to almost filling body cavity. Creamy white in colour.
Ovaries size variable from a full to ¼. Characterised by externally visible hyaline eggs, no matter how few or how early the stage of hydration. Ovaries with hyaline eggs only in the lumen are not included.	**4** **Ripe**	Testes filling body cavity. Milt freely running.
Ovaries occupying ¾ to <¼ of body cavity. Slacker than stage 3 and often blood shot.	**5** **Partly spent (late)**	Testes occupying ¾ to <¼ of body cavity, with free running milt and shrivelled at anal end.
Ovaries occupying ¼ or less of body cavity. Reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs.	**6** **Spent/Recovering spent**	Testes occupying ¼ or less of body cavity. Opaque with brownish tint and no trace of milt.

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.

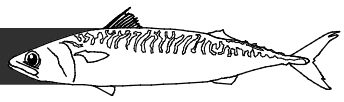
Table 10.1.3 shows the procedures to follow for the collection of samples at sea, and for sample analysis in the laboratory. Provisional estimates of potential fecundity and atresia are required for the 2010 WGWIDE group in September and final results are required for WGMEGS in spring 2011. If the participants or coordinator are unsure of the data quality they should pass on their concerns to the Survey Coordinator (Finlay Burns MSML).

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.

Table 10.1.3. 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 WGMEGS report)

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	**210**
Western	ESP/AZTI		120		40			160	
	GER/vTI SF		100	60				160	
	IRL/MI		80				100	180	
	ESP/IEO		10	40				50	
	FAR/FFS				20			20	
	SCO/MSS			100	10	70		180	
	NED/IMARES			20	40	60		120	
	NOR/IMR				60			60	
	ICE/HAFRO					15		15	**945**

* 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 parallel pipette samples of one ovary **(25 µl)** in pre-filled individually coded Nunc tubes and put the other ovary in formalin jars**, weigh liver, guts (including contents) ----------► ovary, liver, guts weight

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

histological analysis at IEO, IMR, AZT, IMA, MII, MSS ----------► potential fecundity, atresia, realised fecundity

**
Please refer to the sampling protocolls which were sent to all particpants before the 2010 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Table 10.1.2; for further instructions please refer to paragraphs 10.1.

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 10.2.1. 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 10.2.2). 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. 10 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 whilst separating the ovary from the fish.

Ovaries should be weighed and subsamples taken by pipette before fixing in 3.6% buffered formaldehyde solution in sealed vials (e.g. Nunc tubes) on board. The recipe for formaldehyde solution for both, mackerel and horse mackerel fecundity sampling is given in Section 10.3 below.

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

.

Tab. 10.2.2: 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

Week	Date	Period	Lat ° 44N	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	>61	Lon ° 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																						20
12	22.03.2010	2		10			10				10		10			5																55
13	29.03.2010	2	5		10													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										5															25
17	26.04.2010	3			10			10			5		5			5																45
18	03.05.2010	3			10			10		5		5		5				5														50
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																										10
23	07.06.2010	5					10																									10
24	14.06.2010	5				10			5		5		5			5					5											30
25	21.06.2010	5											5			5					5											10
26	28.06.2010	5												5			5															10
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
																																500

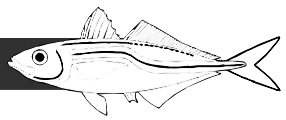
* Refer to Tab.3.1.2a for the area Cadiz to Galicia

	per period						
	1	2	3	4	5	6	
AZTI		50		40			
vTI		50	30				
MI		30				40	
MSS			40	5	40		
IMARES				30	30		
IMR				30			
IEO		25	50				
FAR				10			all periods
ICE					10		
total:	0	155	120	115	80	40	510

Table 10.2.2. Flow chart for selecting and processing horse mackerel samples.

Mackerel and Horse Mackerel Egg Survey 2010

HORSE MACKEREL SAMPLING



Estimation of standing stock fecundity in relation to spawning status

Sampling at Sea (for details on cruises seeWGMEGS report)

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				75
	SCO/MSS			40	5	40		85
	NED/IMARES				30	30		60
	FAR/FFS				10			10
	NOR/IMR				30			30
	ICE/HAFRO					10		10 **510**

(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), take otoliths (*carefully!*) ----------► lenght, weight (total, gonad, [gut]), sex, maturity (1-6, Walsh scale), age

(c) take 3 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, IJmuiden, Pasaia, Bergen, Galway

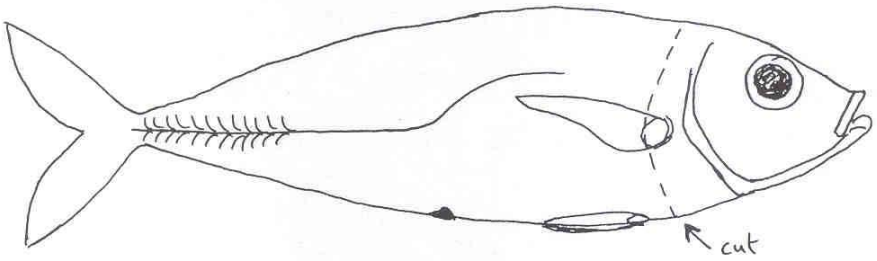
histological analysis at IEO, IMA, IMR, MII, AZT ► vitellogenic oocyte frequency presence of POFs/atretic oocytes

Please refer to the sampling protocolls which were sent to all particpants before the 2010 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Table 10.2; for further instructions please refer to paragraph 10.2.2.

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

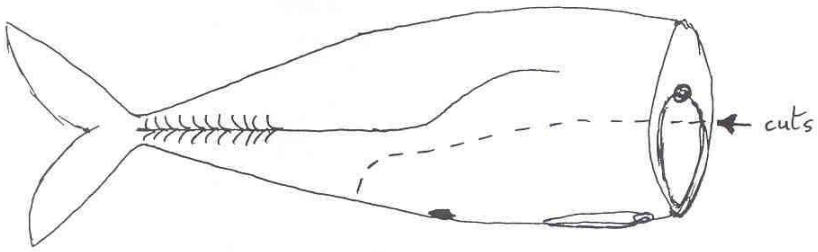
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral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

10.3 Formaldehyde solution for histological samples

All fecundity samples shall be fixed and preserved in a buffered formaldehyde solution suitable for later histological examination. Two types of phosphate buffers are utilized in order to obtain a stable pH. One agent is Sodium-Di-Hydrogene-Phosphate Hydrate (NaH_2PO_4-H_2O), the other is Di-Sodium-Hydrogene-Phosphate-Di-Hydrate (Na_2HPO_4-$2H_2O$). Two obtain 1L of fixative solution the following recipe as applied:

4.1 g NaH_2PO_4-H_2O, 8.2 g Na_2HPO_4-$2H_2O$ and 97 mL Formaldehyde 37% are filled up to 1L with distilled or de-ionised water and thoroughly mixed.

11. References

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

Coombs, S., Dunn, J.D. Eltink, A., Milligan, S. Nichols, J., and Schnack, D. 1996. .EU Concerted Action AIR3 CT94 1911. Co-ordination of the development of an improved method of measuring volume filtered by high-speed plankton samplers. Appendix 9.5 ICES Bongo Nets: Recommendations for design, construction and sampling protocol for ichthyoplankton surveys.

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

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

Eltink, A. 2007. The spray technique: a new method for an efficient separation of fish eggs from plankton. Journal of Plankton Res. Vol 29. No 10: 871–880.

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

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

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

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

Hunter, J. R., Macewicz, B. J., Kimbrell, C. A. 1989. Fecundity and other aspects of the reproduction of sablefish, Anoplopoma fimbria, in central California waters. *Rep.Ccofi.* **30,** 61–72.

ICES. 1988. Report of the mackerel egg and recruitment workshop. ICES CM 1988/H:3.

ICES. 1994. Report of the mackerel and horse mackerel egg production workshop. ICES CM 1994/H4.

ICES. 1996. Report of the working group on mackerel and horse mackerel egg surveys. ICES CM 1996/H:02.

ICES. 1997. Report of the working group on mackerel and horse mackerel egg surveys. ICES CM 1997/H:4.

ICES. 2001. Mackerel and horse mackerel egg staging and histology workshop. ICES CM 2001/G:01.

ICES. 2002. Report of the working group on mackerel and horse mackerel egg surveys. ICES CM 2002/G:06.

ICES. 2003. Report of the working group on mackerel and horse mackerel egg surveys. ICES CM 2003/G:07.

ICES. 2004. Workshop on mackerel and horse mackerel egg staging and identification. ICES CM 2004/G:13.

ICES. 2006. Workshop on mackerel and horse mackerel egg staging and identification. ICES CM 2006/LRC:17.

ICES. 2008. Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS), 7–11 April 2008, IJmuiden, Netherlands. ICES CM 2008/LRC:09 111pp.

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

Lockwood, S. J., Nichols, J. H., and Dawson, W. A. 1981. The estimation of a mackerel (Scomber scombrus L.) spawning stock size by plankton survey. J.Plank.Res., 3:217–233.

Munk, P., and J. G. Nielsen 2005: Eggs and larvae of North Sea fishes. Biofolia, Frederiksberg, DK, 215 pp.

Nash, R. D. M., Dicky-Collas, M., and Milligan, S. P. 1998. Descriptions of the Gulf VII / PRONET and MAFF / Guildline unencased high-speed plankton samplers. J.Plankton Res. Vol.20 no10: 1965–1926.

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

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

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

Simpson, A. C. 1959. The spawning of plaice (*Pleuronectes platessa*) in the North Sea. Fish Invest., Lond., (Ser II), 22, No 7, 111p.

Smith, P.E., Richardson, S. L. 1977. Standard Techniques for Pelagic Fish Egg and Larvae Surveys. FAO Fish. Tech. Pap. No. 175. FAO, Rome, 110 pp.

Steedman, H. F. 1976. Miscellaneous preservation techniques. *In* Zooplankton fixation and preservation, The UNESCO Press: 175–183.

Walsh, M., Hopkins, P., Witthames, P. R., Greer Walker, M., and Watson, J. 1990. Estimation of total potential fecundity and atresia in the western mackerel stock, 1989. CM 1990/H:31.

Annex 3: WGMEGS terms of reference for the next meeting

The **Working Group on Mackerel and Horse Mackerel Egg Survey (**WGMEGS) chaired by J. Ulleweit, Germany, will meet in San Sebastian, Spain, 11–15 April 2011 to:

a) analyse and evaluate the results of the 2010 mackerel and horse mackerel egg surveys of the western and southern areas;

i) calculate the total seasonal stage 1 egg production estimates for mackerel separately for the western and southern areas;

ii) calculate the total seasonal stage 1 egg production estimates for the western horse mackerel stock (AEPM) and for southern stock (DEPM);

iii) consider whether stage 1A and 1B could be amalgamated into a single stage both for the survey samples and future workshops.

iv) analyse and evaluate the results of the mackerel and horse mackerel fecundity and mackerel atresia sampling in the western and southern areas;

v) analyse and evaluate the results of the horse mackerel batch fecundity and spawning fraction in the southern stock;

vi) evaluate the results of studies on horse mackerel fecundity determination and proxies on the basis of data collected during the 2010 surveys and in other relevant work;

vii) provide estimates of the spawning-stock biomass of mackerel, using stage 1 egg production estimates and the estimates of fecundity and atresia, separately for the western and southern areas;

viii) provide estimates of the spawning-stock biomass of horse mackerel, using production estimates and the estimates of batch fecundity and spawning frequency for southern stock

ix) evaluate the quality and reliability of the 2010 survey in the light of the previous surveys and to evaluate the reliability of the preliminary estimates calculated in 2010 against the final estimates.

b) re-analyse the survey data under a survey design where the transects are spread out to allow covering a wider area but without increasing ship time. The analysis should aim to estimate the impact of such changes on bias and precision for both mackerel and horse mackerel estimates.

c) plan and coordinate the 2011 North Sea mackerel egg survey.

WGMEGS will report by 1 June 2011 for the attention of the SSGESST, WGISUR, ACOM and WGWIDE.

Supporting Information

Priority	Essential. The egg survey provides the only fishery-independent stock data used in the assessment for Northeast Atlantic mackerel and for both the western and the southern horse mackerel stocks. As part of the multiannual management plan the index for horse mackerel is directly used for the calculation of the TAC.

Scientific justification	The egg survey provides the only fishery-independent stock estimates for Northeast Atlantic mackerel and for both the western and the southern horse mackerel stocks. The survey is based on a time-series since 1977. Terms of Reference a): WGMEGS will finally analyse and evaluate the results of the 2010 egg survey and calculate the egg production indices, the fecundity estimates and biomass estimation for the Western and Southern stock components of mackerel and the Southern stock of horse mackerel used in the assessment. The final estimation is also essential to the documention of the reliabitliy of the preliminary estimates provided in the year of the survey. Term of Reference b): The temporal and spatial widening of mackerel and horse mackerel distribution during spawning might lead to an expansion of the potential survey area. Alternative survey designs and their impact on the results have to be investigated by WGMEGS. Term of Reference c): The North Sea mackerel egg survey is the only available information on the size of the North Sea component of the Northeast Atlantic mackerel stock.
Resource requirements	None. The surveys are all part of the national programmes. The surveys and associated meetings are also partially funded under the EU fisheries data directive.
Participants	ICE, 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	The survey data are prime inputs to the assessments which provide ACOM with information required for responding to requests for advice/information from NEAFC and EC DG MARE.
Linkages to other committees or groups	WKFATHOM, WGNAPES, SGSIPS, WKEPM.
Linkages to other organizations	There have been a number of associated EU funded projects and the cooperation with new projects is planned.

Annex 4: Mackerel stomach sampling protocol during the 2010 egg survey

Following recent hypotheses put forward by Faroes and Norway that adult prespawning, spawning, and post-spawning mackerel might feed on blue whiting eggs and larvae where the distribution areas overlap, a stomach sampling programme for adult mackerel has been initiated in 2010.

Below is presented a short sampling protocol. All survey participants are kindly asked to take part in the sampling programme during their routine sampling for fecundity analysis.

Before the cruise:

Obtain otolith envelopes and small plastic bags (7x10 cm zipper bags) for mackerel stomachs, and permanent marker to label the plastic bags.

During the cruise:

Numbers: 10 mackerel randomly from every trawl station or as part of the fecundity sampling.

Stomach sampling:

- Cut out the stomach as far forward towards the oesophagus as possible with scissors/knife, put stomach in plastic bag and zip. Label the plastic bag (or pre label the bags) with station and fish number. Individually freeze the stomachs as soon as possible (or place on ice during sampling).
- Collect all 10 stomachs and put them in a larger plastic bag labelled with trawl station, date and position, and freeze the sample.

Measurements: Total length (mm), total weight (g), sex, maturity stage, otoliths, stomach.

The otoliths should be put in labelled paper envelopes.

The preferred sampling method is to cut out the stomachs as part of the sampling as described above. However, if the work load is high on board or there is a shortage of manpower to sample fish individually, a sample of 10 whole mackerel to be frozen and properly labelled (station number, date and position) will do as an alternative.

After the cruise:

Shipping of samples: Frozen samples should be shipped to coordinator below.

Coordinator: Jan Arge Jacobsen, Faroe Marine Research Institute, Nóatún 1, FO-100 Torshavn, Faroe Islands. E-mail: janarge@hav.fo