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SISP 6 – MEGS V1.3

Manual for the mackerel and horse mackerel egg surveys (MEGS): sampling at sea

Version 1.3

The Working Group on Mackerel and Horse Mackerel Egg Surveys

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CIEM Conseil International pour l'Exploration de la Mer

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1 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 2013 and future triennial surveys.

2 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, except for the southern stock of horse mackerel where eleven egg stages are used to produce an estimate of numbers of eggs spawned.

The core areas for the western and southern surveys for both species are presented in Figures 2.1 and 2.2. A more detailed survey map of the Iberian areas as surveyed by IEO and IPIMAR can be found in Figure 2.3. The Northeast Atlantic shelf area is subdivided (by WGMEGS) into 'western' and 'southern' areas for the purposes of estimating spawning-stock biomass (SSB) of mackerel and horse mackerel. The 'southern' area for mackerel is regarded as being from 36° N to 44° N in the east and 45° N in the west (Figure 2.1). It extends from Cape Trafalgar in the Gulf of Cadiz, around the coast of Portugal to 11° W, the Cantabrian Sea and southern Biscay. Sampling usually begins in January in this area and continues until June in the Cantabrian Sea. The southern area for horse mackerel coincides with the limits of the southern 'stock', from the Gulf of Cadiz to Cape Finisterre at 43° N (Figure 2.2 with additional sampling detail provided in Figure 2.4).

The 'western' area for mackerel is from 44° N (45° N in the west) to 63° N (Figure 2.1). It includes Biscay, the Celtic Sea and the shelf edge to the northwest of Scotland. Sampling is focused along the shelf edge (200 m isobaths) but also occurs from the French and Irish coasts out to 16° W. Sampling in this area usually begins in March and continues into early July. The last survey in 2010 indicated that mackerel spawning continued into Faroese, Icelandic and international waters beyond 20° W and to 63° N. The western area for horse mackerel includes the Cantabrian Sea and is from 43° N to 63° N with same western boundary as for mackerel (Figure 2.2).

In most of the western area plankton samplers are deployed at the centre of half standard ICES rectangles, which are 0.5° latitude, by 0.5° longitude. To the north of Spain (Cantabrian Sea) three sampler deployments are undertaken (in an east-west direction) in each 0.25° latitude by 1.0° longitude rectangle because of the proximity of the shelf edge to the coast. For the limits of the southern horse mackerel stock the station distribution is along-transects 12 nml apart and the stations are occupied according to an adaptive strategy (depending on egg density) either every 3 nml or 6 nml (Figure 2.4).

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 "standard area" should no longer be used but that an adaptive sampling strategy be employed based on the distribution of eggs found in the previous survey. See Section 4 for the priority areas to be covered during the **2013** survey. The figures shown in this section are provided as a planning guide only but they do cover 90% of the mackerel egg production and 99% of horse mackerel egg production estimated from the 2010 survey. The limits of the survey in both areas should be established based on two consecutive zero samples if at all possible, and not by the boundaries on these maps.

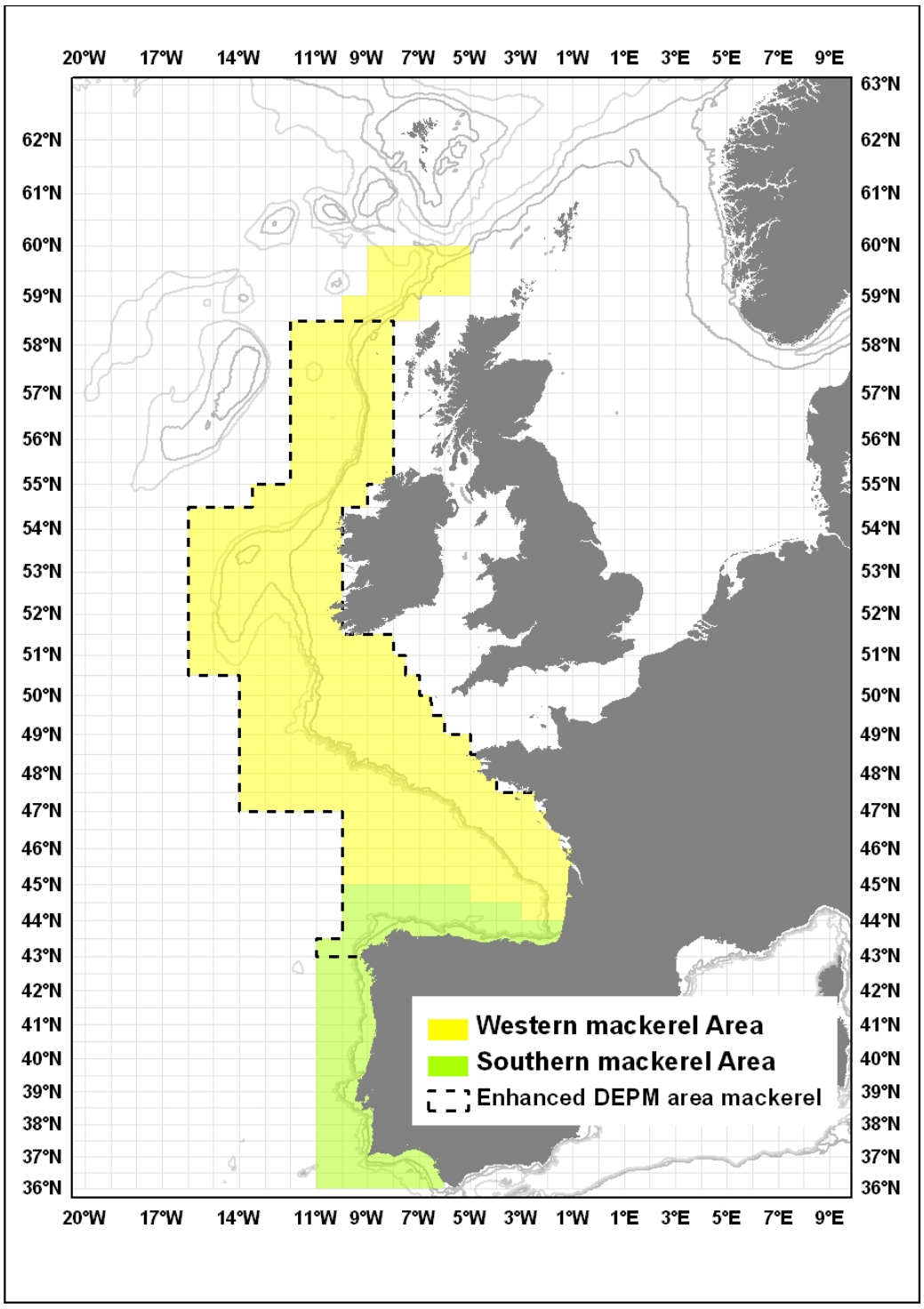


Figure 2.1. The priority areas for the sampling of mackerel eggs in both the western and southern areas. The dashed line delimits the 'enhanced' area for the sampling of adults for DEPM.

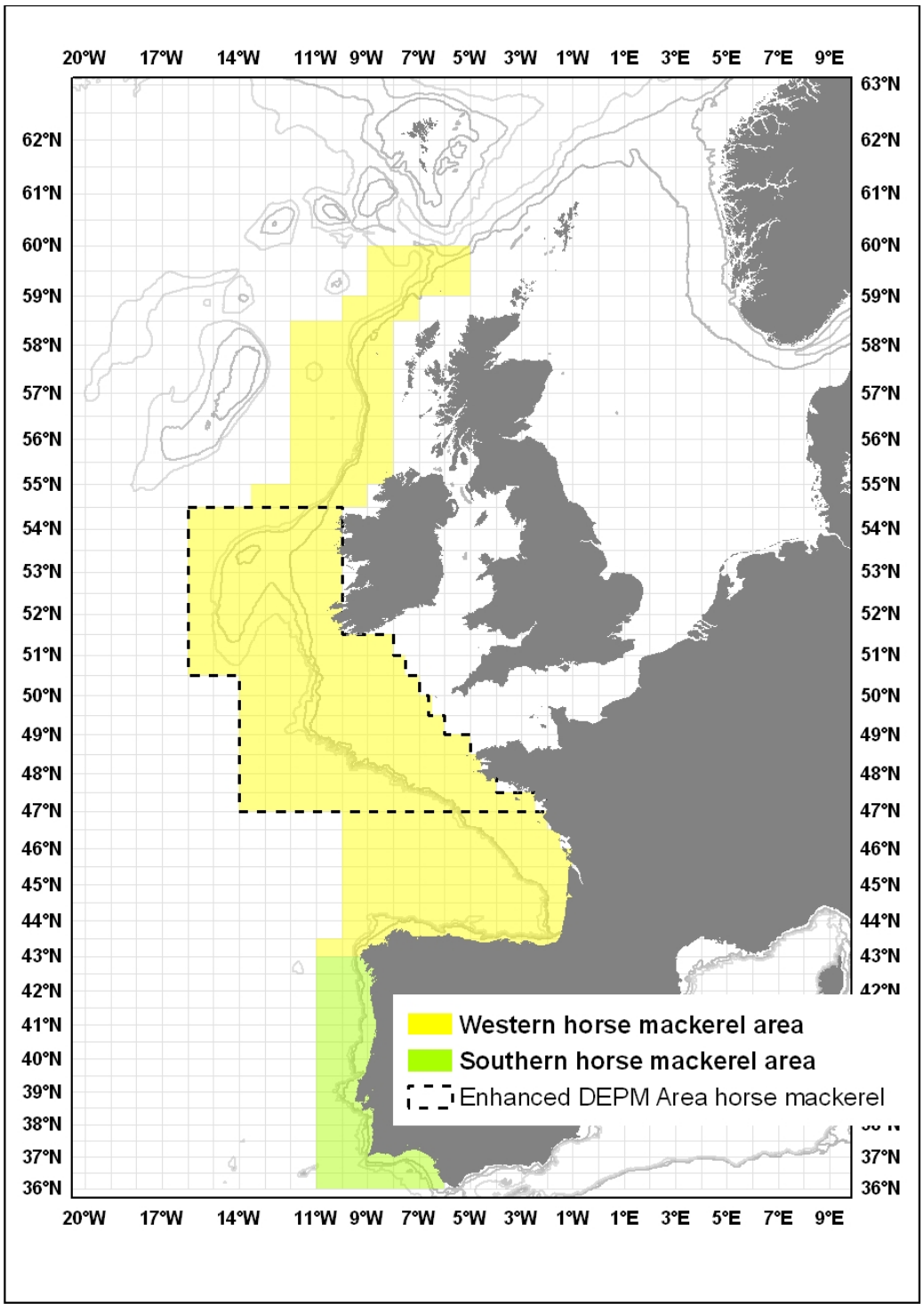


Figure 2.2. The priority areas for the sampling of horse mackerel eggs in both the western and southern areas. The dashed line delimits the 'enhanced' area for the sampling of adults for DEPM.

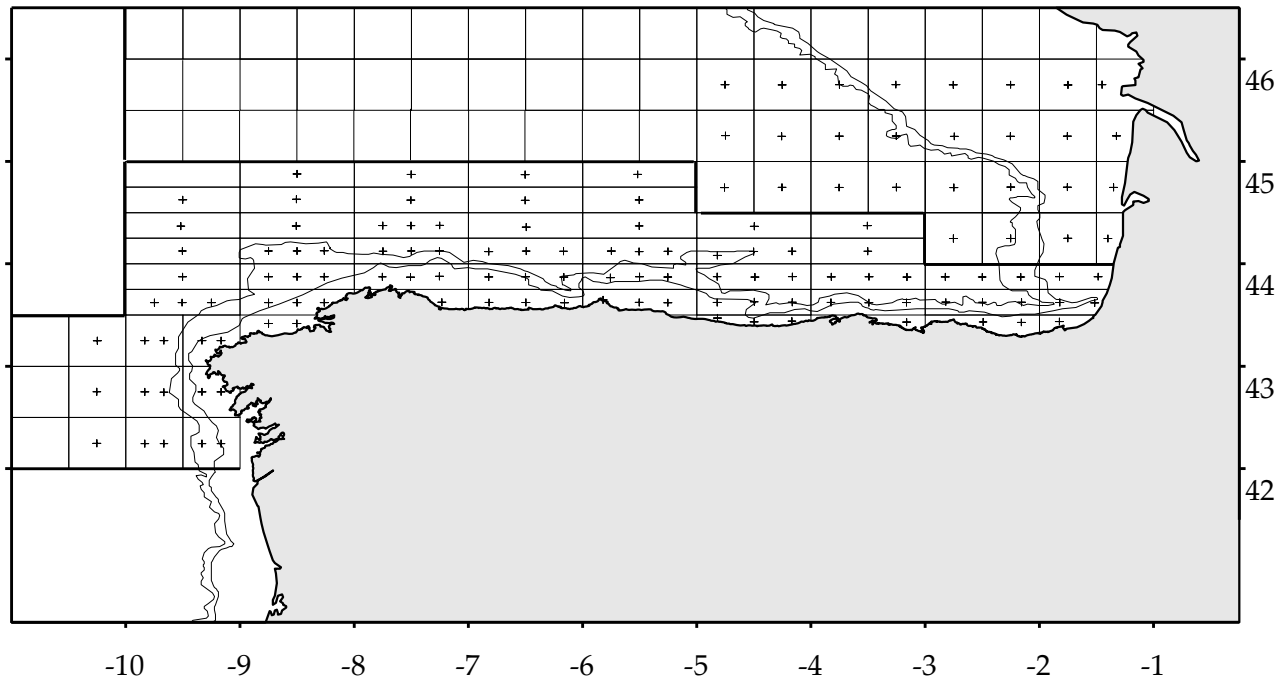


Figure 2.3. IEO sample locations for Galicia and the Cantabrian Sea.

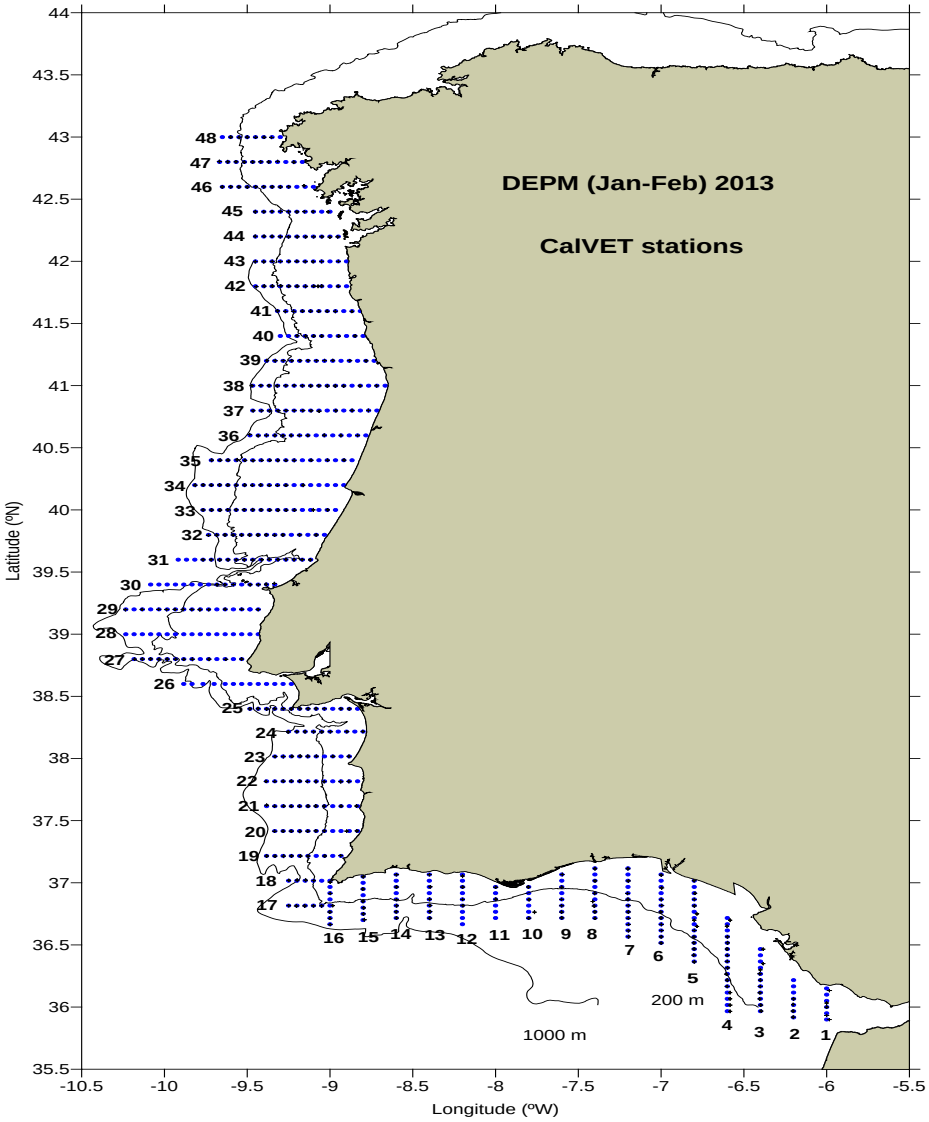


Figure 2.4. Distribution of planned plankton stations 2013, for the sampling of southern horse mackerel eggs.

3 Sampling strategy

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 guidelines 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, historic 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 stations 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 priority areas highlighted in Figures 2.1 and 2.2.

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. Furthermore, such a strategy enables better evaluation of distributional change with time, which is likely to be important in modelling the results. This procedure does not apply to the area surveyed only by Portugal.

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

4 Planning of the 2013 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, Iceland and the Faroe Islands will participate in the mackerel/horse mackerel egg surveys in the western and southern area in 2013. Provisional dates (where possible) as well as vessel details for the forthcoming surveys can be found below in Table 4.1.1. As in 2010, Iceland and the Faroe Islands have committed to devote 2 weeks of ship time to the 2013 survey in the Northern area, north of 60°N. While these additional surveys are extremely welcome, the 2010 results continued to highlight several challenges, in particular the significant expansion of the western mackerel spawning area from April to June. This resulted in an inability to fully survey the whole area for all periods even with the use of an alternate transect strategy several boundaries remained unsecured. With a net reduction in survey days available for 2013, this situation is set to continue. These challenges as well as recommendations are more fully described in deficiencies in Section 9. Survey coverage of the western and southern area is given by area and period in Table 4.2.1. Detailed maps of survey coverage by period are given in Figures 4.2.1–4.2.6. Both, vessel availability and area assignments are provisional and will be finalized by the survey coordinator at the appropriate times.

The survey coordinator for the 2013 survey will be Finlay Burns, Marine Scotland Science (MSS), Aberdeen.

Table 4.1.1 Countries, vessels, areas assigned, dates and sampling periods for the 2013 surveys.

Country	Vessel	Areas	Dates	Period
Portugal	Noruega	Cadiz, Portugal & Galicia	26 January – 2 March	1
Spain (IEO)	Angeles Alvarino	Cantabrian Sea & Biscay	1 – 24 March	2
		Biscay & Cantabrian Sea	1 – 24 April	3
Germany	W. Herwig III	West Ireland & Celtic Sea	20 March – 25 April	3
Netherlands	Tridens	Celtic Sea	6 – 23 May	4
		Celtic Sea & Biscay	3 – 21 June	5
Spain (AZTI)	Margalef	Biscay	25 March – 13 April	3
		Biscay & Cantabrian Sea	8 – 28 May	4
Norway	Johan Hjort	West Ireland & West of Scotland	15 May – 8 June	4
Ireland	Celtic Explorer	Celtic Sea & Biscay	18 February – 10 March	2
		Celtic Sea, West Ireland & West of Scotland	13 July – 2 August	6
Scotland	Scotia (IBTSQ1) Charter Scotia Charter	West of Scotland	19 February – 12 March	2
		NW Ireland & West of Scotland	14 – 27 March	2
		West of Ireland & West of Scotland	18 April – 8 May	3
		West of Ireland & West of Scotland	June (3 weeks)	5
Faroe Islands	Magnus Heinason	Faroes & Shetland	23 May – 2 June	4
Iceland	Bjarni Saemundsson	Faroes & Shetland	9– 23 June	5

4.2 Survey design in 2013

The AEPM survey design for mackerel and horse mackerel for 2013 will not change however, an attempt will be made to estimate DEPM (daily egg production method), adult parameters for both species and this will require additional sampling during the perceived peak spawning periods for both species as identified from the 2010 surveys during WKMSPA 2012. These were identified as period 3 for mackerel and period 5 for horse mackerel. As in 2010, the survey will be split into six sampling periods and the design and survey deployment plan for 2013 is very similar to that employed in 2010. Once again, the Faroe Islands and Iceland will participate in the survey during May and June respectively, which will expand the geographic range of the survey in the North during these periods. Period 1 (mid-January to mid-February) will include a survey in ICES area IXa only, with more extensive coverage starting in period 2. In 2013 the survey effort in area IXa will again be targeted on a **single** extended DEPM survey (see WGMEGS report 2012). No sampling in area IXa will take place after the end of period 1. In 2013 period 2 is being moved forward to commence in mid-February and period 3 is being extended to cover a six-week period. From period 4 onwards period timing and design is almost identical with that completed in 2010.

Sampling in the western area will commence in period 2. During period 2 the survey will concentrate on the Cantabrian Sea, Biscay and the Celtic Sea, West of Ireland and Scotland. Periods 3 and 4 will see sampling continue from the Cantabrian Sea north to the northwest of Scotland and Faroese waters towards the end of period 4. No sampling will take place in the Cantabrian Sea after period 4. In period 5 sampling will extend northwards from Biscay to Faroese waters with Iceland surveying the area north of 60°. 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 4.2.1.

In the western area, maximum deployment of effort will be during period 3. In 2010, the peak of mackerel spawning occurred in period 2. Moving the timing of period 2 forward and concentrating effort in period 3 is an attempt to ensure that this peak is adequately sampled at this time. Due to the expansion of the spawning area that took place in 2010 the emphasis in 2013 will once again be focused on maximizing area coverage. Cruise leaders will be asked to cover their **entire** assigned area using alternate transects and then use any remaining time to fill in the missed transects. If time is short, this should be concentrated in those areas identified from 2010 as having the highest densities of egg abundance. Particular points to note are:

Period 1

The southern area will only be surveyed in period 1. This will be the Portuguese DEPM survey, and it will extend into the early part of period 2 (Figure 4.2.1).

Period 2

Period 2 marks the commencement of the western area surveys. In 2010 this period commenced in early March. However, for 2013 this period has been brought forward to the middle of February. It is hoped this will help capture the start of mackerel spawning in the western area. The Irish survey will commence at the beginning of period 2. Limited opportunistic sampling will also be undertaken by the Scottish IBTS survey West of Scotland in period 2. The first dedicated Scottish survey will then commence later in period 2 and will complete the survey coverage in this area as well as to the west of Ireland. (Figure 4.2.2)

Period 3

With period 2 moving forward in time, period 3 has been extended to six weeks. During period 3, surveys will be carried out by Scotland, Spain (IEO), Spain (AZTI) and Germany. The second dedicated Scottish survey will – as in period 2 – survey the area west of Scotland as well as northwest Ireland. The two Spanish surveys will cover the Bay of Biscay and the Cantabrian Sea and Germany will cover the Celtic Sea and the west of Ireland.

Period 3 was identified as the peak of mackerel spawning in 2010 and the results of additional egg sampling undertaken by Scotland and Ireland in March 2012 provide strong evidence that this may be set to continue (WD to WGMEGS 2012). WGMEGS have undertaken to collect additional adult samples within the enhanced DEPM area as delineated in Figure 4.2.3 and instructions for collection of these additional samples can be found in the Section 11. It is also especially desirable that as far as is possible comprehensive survey coverage is achieved within this enhanced area and this should be the prime consideration when completing the second sweep of the survey area during this period.

Period 4

Period 4 occupies the same period as in 2010 and once again will be surveyed by four vessels. 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. The Netherlands will carry out sampling in the Celtic sea, and Norway will survey to the west of Ireland and west of Scotland. In turn, the Faroese vessel will then survey north of 59°45N (Figure 4.2.4).

Period 5

In period 5, two vessels have to cover the entire area of spawning from northern Biscay to the West of Scotland. 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. Scotland will survey to the west of Ireland and Scotland. The Norwegian vessel will undertake limited sampling in the early part of period 5 similar to that undertaken in period 5 in 2010 although this will be heavily dependent on the survey coverage achieved during period 4. In addition, Iceland will provide a 2-week survey, which will cover the area to the north. As in period 4 this will expand the survey range and attempt to secure a northern boundary within this period. See Figure 4.2.5 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 5 was identified as the peak of horse mackerel spawning in the western area in 2010 and as with the mackerel in period 3 WGMEGS have undertaken to collect additional adult samples within the enhanced DEPM area as delineated in Figure 4.2.5, information, and instructions pertaining to the collection of these samples can be found in Section 11. Providing adequate survey coverage during this period will be especially challenging given that there are only two vessels to cover the area from 47°N – 59°N, however as with period 3 every effort should be made to achieve as comprehensive coverage as is possible within this enhanced area.

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 4.2.6)

Table 4.2.1. Periods and area assignments for vessels by week for the 2013 survey. Dashed areas denote period and approximate area of enhanced DEPM sampling. Period 3 for mackerel and Period 5 for western horse mackerel. Area assignments and dates are provisional.

		Area							
week	Starts	Portugal, Cadiz & Galicia	Cantabrian Sea	Biscay	Celtic Sea	North west Ireland	West of Scotland	Northern Area	Period
1	21-Jan-13	PO1(DEPM)							1
2	28-Jan-13	PO1(DEPM)							1
3	4-Feb-13	PO1(DEPM)							1
4	11-Feb-13	PO1(DEPM)							1
5	18-Feb-13	PO1(DEPM)		IRL1	IRL1		SCO(IBTS)		2
6	25-Feb-13	PO1(DEPM)		IRL1	IRL1		SCO(IBTS)		2
7	3-Mar-13		IEO1	IRL1/ IEO1	IRL1		SCO(IBTS)		2
8	11-Mar-13		IEO1	IEO1		SCO1	SCO1		2
9	18-Mar-13		IEO1	IEO1		SCO1	SCO1		2
10	25-Mar-13			AZTI1	GER1	GER1			3
11	1-Apr-13		IEO2	AZTI1	GER1	GER1			3
12	8-Apr-13		IEO2	AZTI1	GER1				3
13	15-Apr-13		IEO2	IEO2	GER1	SCO2	SCO2		3
14	22-Ap-13		IEO2	IEO2	GER1	SCO2	SCO2		3
15	29-Apr-13					SCO2	SCO2		3
16	6-May-13		AZTI2(DEPM)	IEO2/AZTI2(DEPM)	NED1				4
17	13-May-13		AZTI2(DEPM)	AZTI2(DEPM)	NED1	NOR	NOR		4
18	20-May-13		AZTI2(DEPM)	AZTI2(DEPM)	NED1	NOR	NOR	FAR	4
19	27-May-13					NOR	NOR	FAR	4
20	3-Jun-13			NED2	NED2		NOR		5
21	10-Jun-13			NED2	NED2	SCO3	SCO3	ICE	5
22	17-Jun-13			NED2	NED2	SCO3	SCO3	ICE	5
23	24-Jun-13					SCO3	SCO3		5
24	1-Jul-13								6
25	8-Jul-13				IRL2	IRL2	IRL2		6
26	15-Jul-13				IRL2	IRL2	IRL2		6
27	22-Jul-13				IRL2	IRL2	IRL2		6
28	29-Jul-13								6

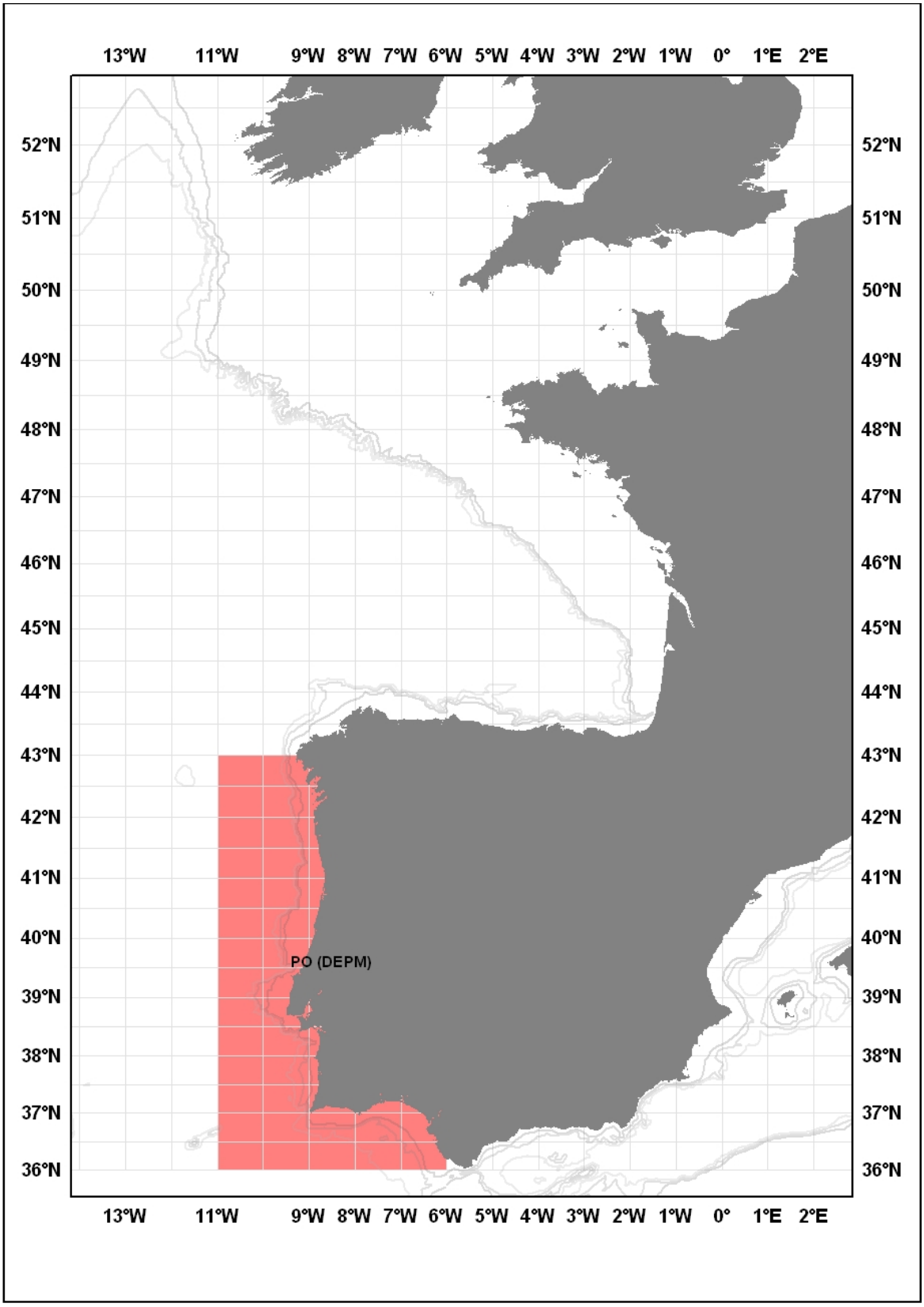


Figure 4.2.1. Survey plan for Period 1.

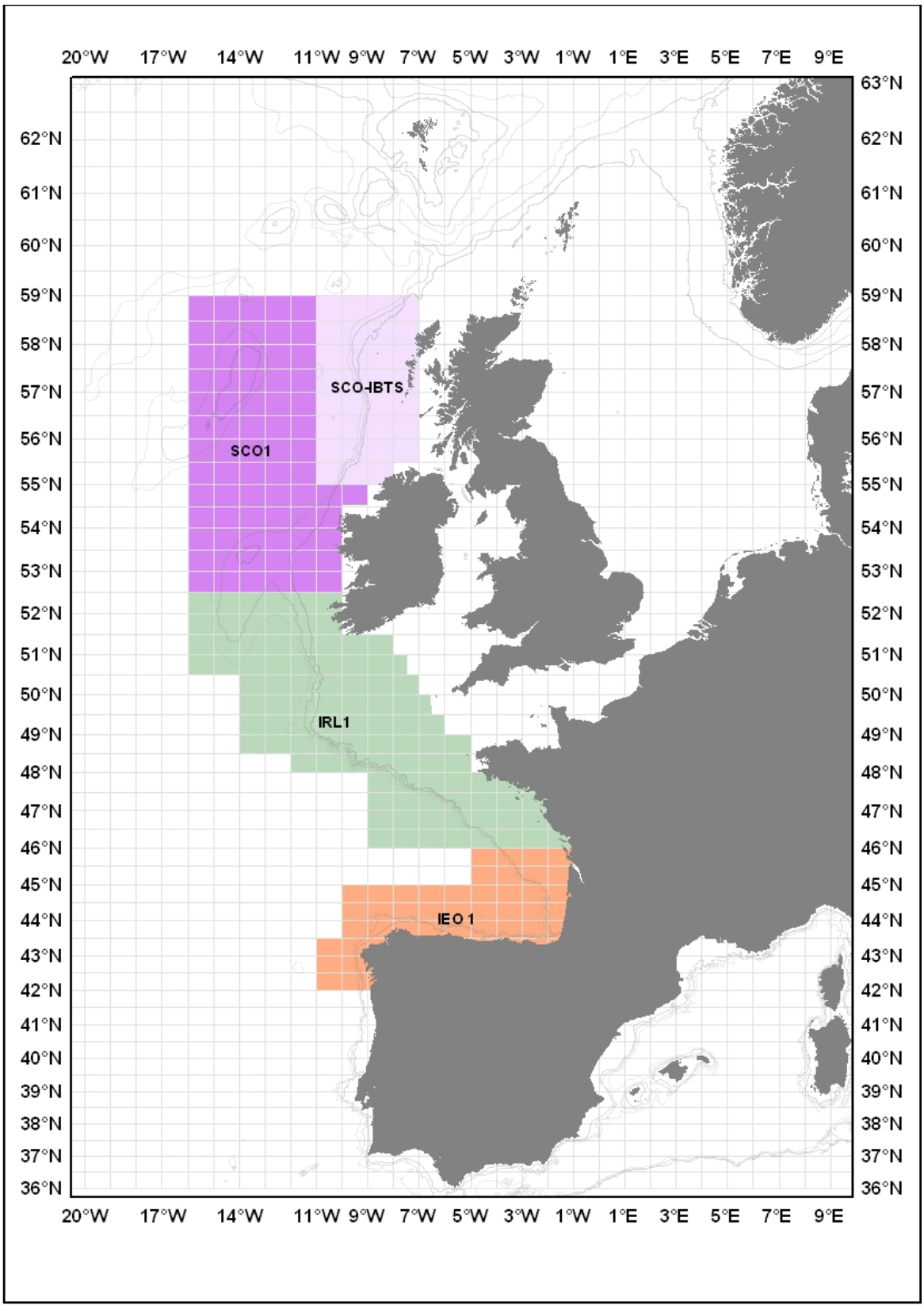


Figure 4.2.2. Survey plan for Period 2.

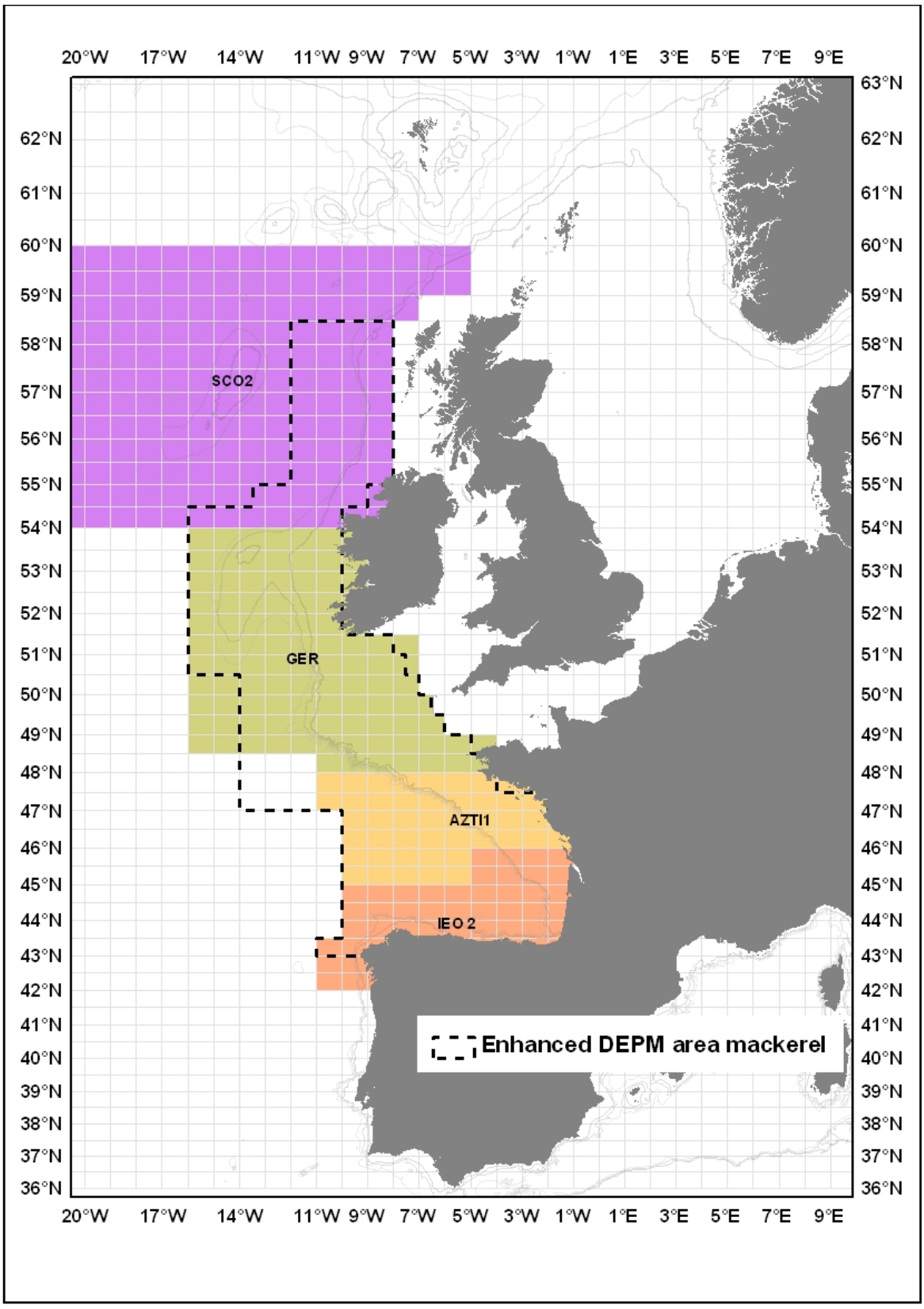


Figure 4.2.3. Survey plan for Period 3. Area within the dashed line represents the Enhanced DEPM sampling area for mackerel.

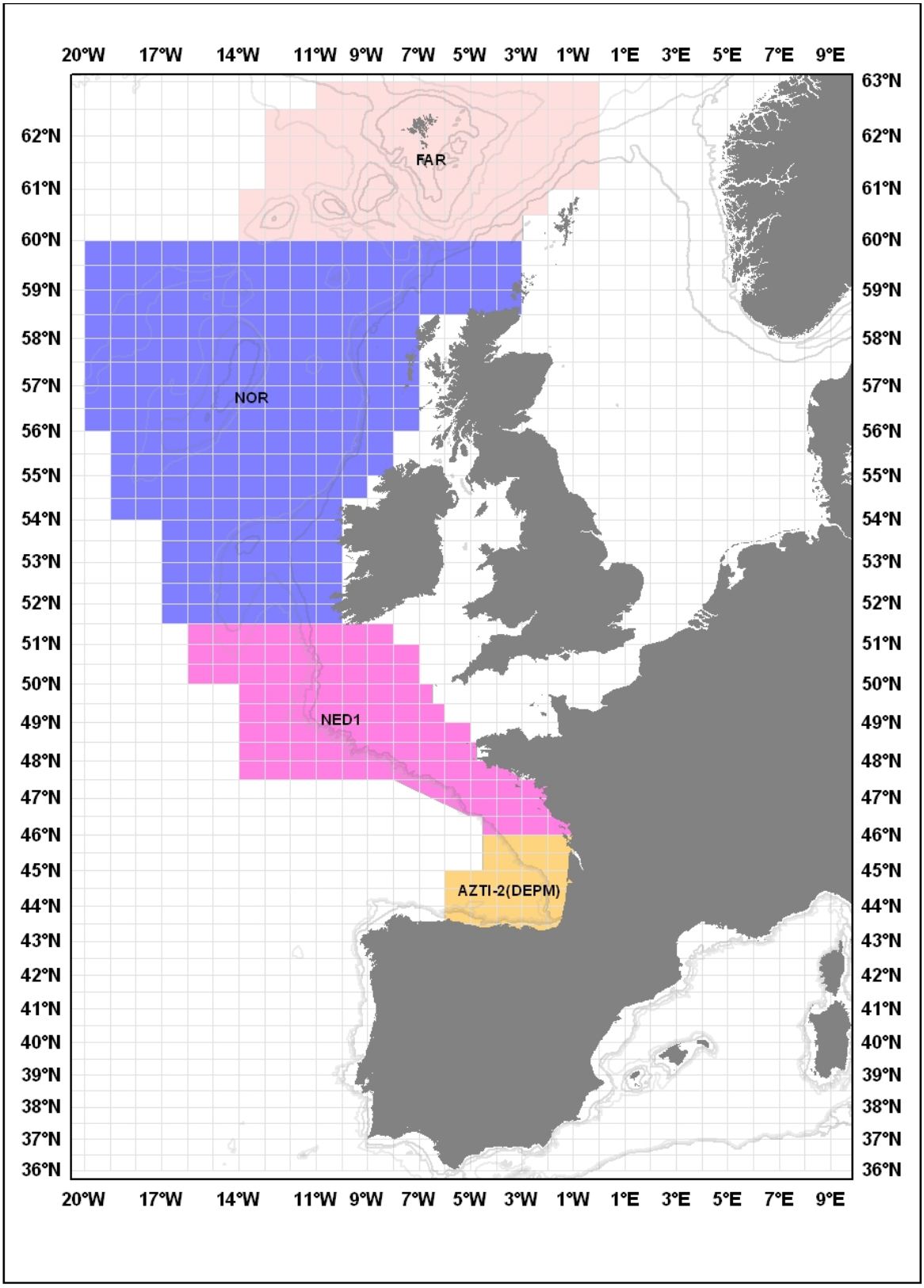


Figure 4.2.4. Survey plan for Period 4.

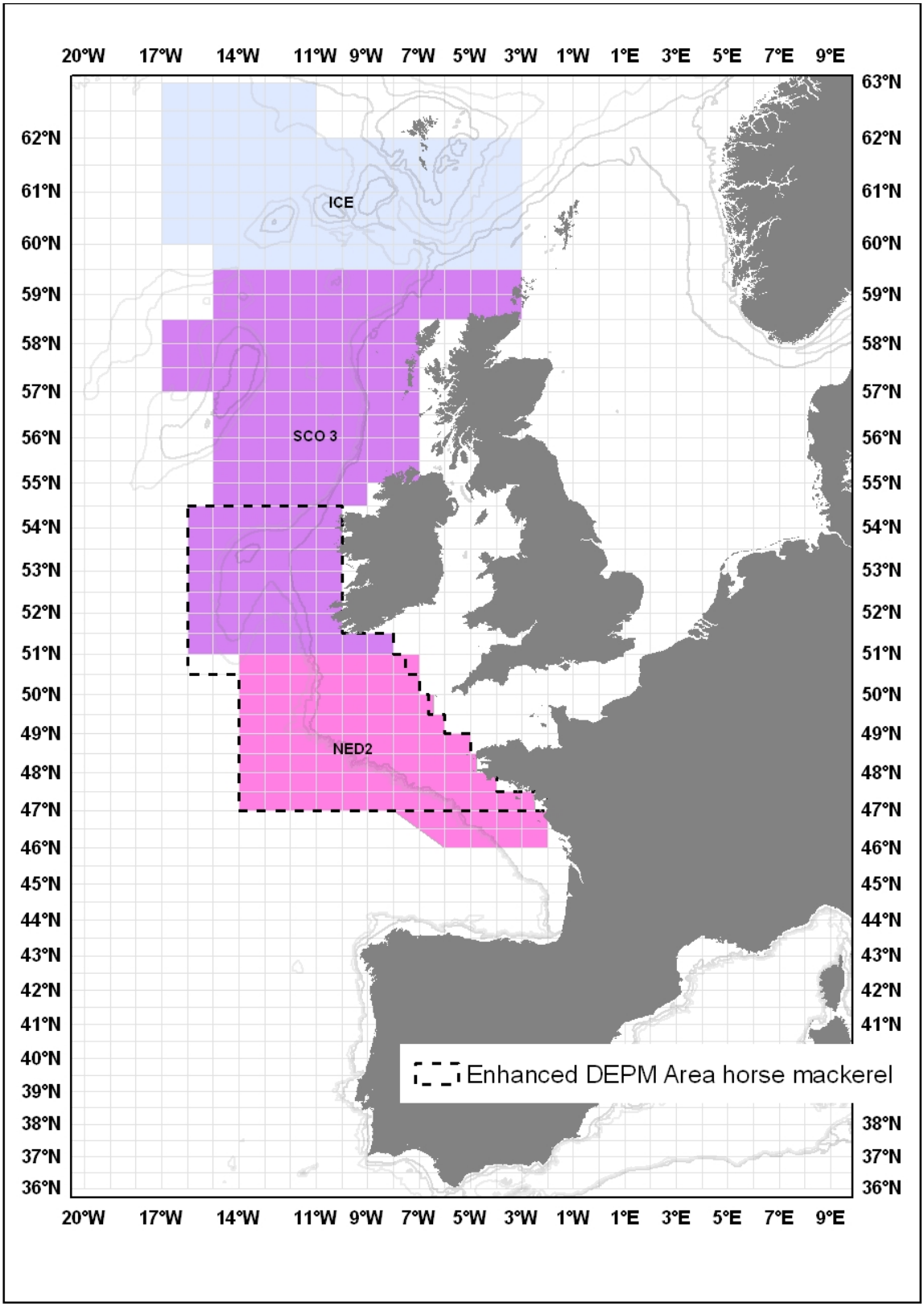


Figure 4.2.5. Survey plan for Period 5. Area within the dashed line represents the Enhanced DEPM sampling area for western horse mackerel.

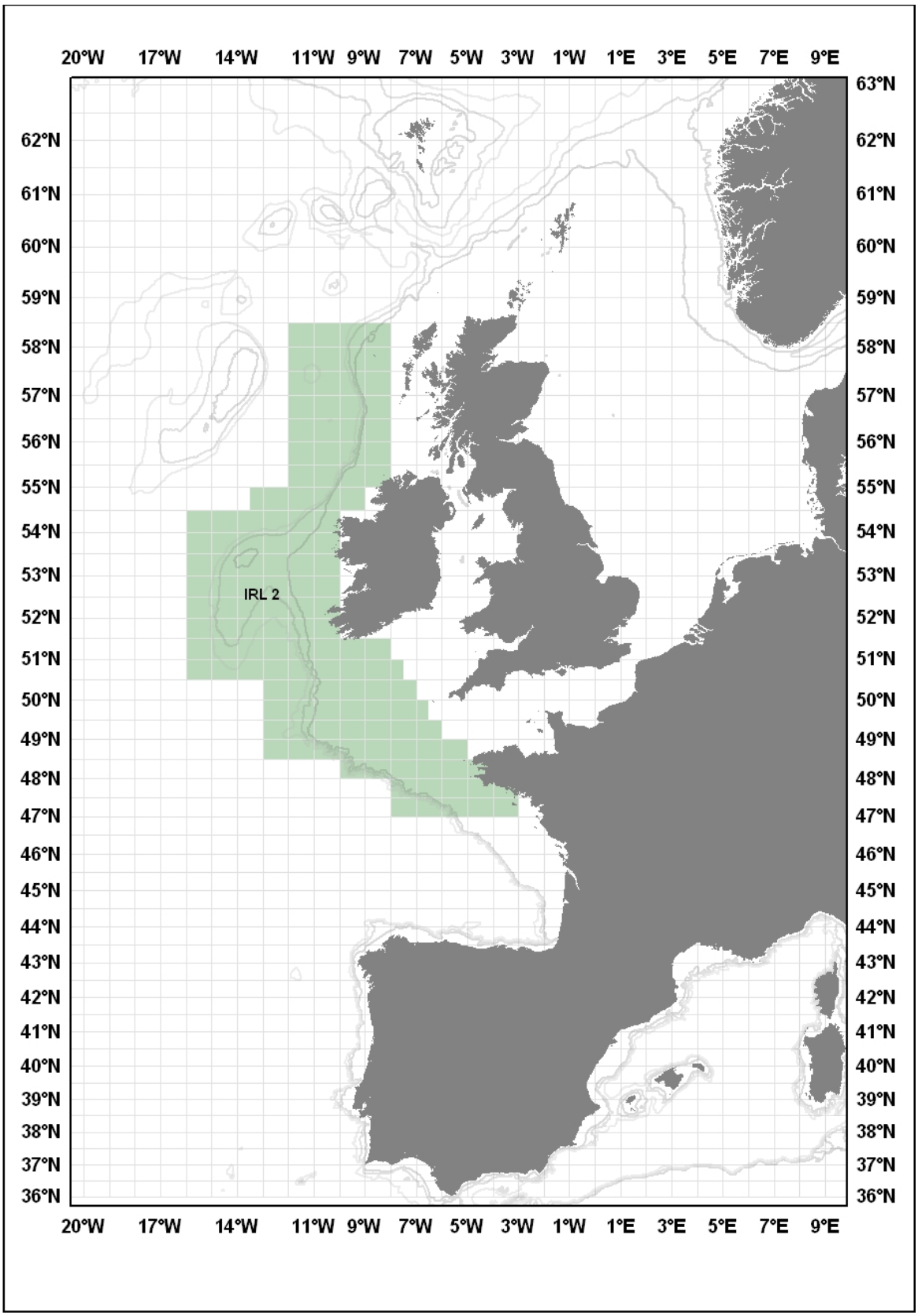


Figure 4.2.6. Survey plan for Period 6.

4.3 Data submission

Plankton samples should be analysed, data checked, 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 31 July.

An excel template for the data entry of the plankton data will be distributed by the survey coordinator prior to the survey (January 2013).

The screening (2–3 g) 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.

All participants are asked to use the templates to avoid time-consuming converting of different formats.

5 Standardization of survey gears

The standard plankton samplers for use on these surveys are mainly national variants of Bongo or 'Gulf type high-speed' samplers (Nash *et al*., 1998). Only Portugal (IPIMAR) continues to use a vertically deployed CalVET-net. All of these samplers generally have conductivity, temperature and depth probes (CTD's) attached to the frames and they are also fitted with either mechanical or electronic flowmeters to enable the volume of water filtered on each deployment to be calculated. These CTD 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 and Table 5.1 below).** 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, (although 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 sampler fitted with its flowmeter(s) 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 enable 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 of the sampler and two tows in opposite directions to overcome the effects of tides or currents on ship and sampler speed through the water. Such calibrations will provide a crude estimate of volume filtered (under non-clogged net conditions) but it must be remembered that there are differences in water velocity across the aperture of any sampler and that this water velocity profile may change as clogging of the net progresses. However, it is recommended that participants conduct calibrations of their flowmeters *in-situ* over a range of towing speeds at least at the beginning and end of each survey.

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 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. The CalVET-net deployed by Portugal currently has a double mouth aperture both of 25 cm. The mesh size of the CalVET-nets are 150 microns. 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 is not adjusted adequately**.

Since the 2004 surveys, a high level of standardization of sampling equipment has been achieved for the mackerel and horse mackerel egg surveys (Table 5.1). 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 5.1. 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

Portugal (IPIMAR) used a vertically deployed CalVET-net in the 2007 and 2010 surveys and will continue to do so for the 2013 survey. Spain (AZTI and IEO) use 40 cm Bongo

nets (Table 5.2). 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 5.2. 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

6 Plankton sampler deployment

It is recommended that the Gulf type samplers be deployed on a double oblique tow, at 4 knots through the water, from the surface to maximum sampling depth (see below) and return. The Bongo samplers are deployed at 2–3 knots through the water 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. If possible, the external flowmeter should be used to monitor the correct speed of the Gulf type sampler, approximately $2ms^{-1}$.

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 200 m, or to within 5 m of the bottom where the bottom is less than 200 m. 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.

The CalVET net is hauled vertically from the same maximum depths as described above.

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.

7 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.

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. (420 g 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.

8 Plankton sample sorting

Samples can be sorted for the first time after two hours of fixation in formaldehyde. However, complete fixation of the plankton sample will only occur after at least 12 hours in 4% formaldehyde. Therefore, a full check of the samples is required to be completed thereafter.

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 technique will again be evaluated at WKFATHOM prior of the survey. 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 50 ml of propylene phenoxetol mixed with 450 ml of propylene glycol (propane-1.2-diol). Before use, 5 ml of the stock solution is diluted with 95 ml of distilled water to produce a sorting fluid which is non-toxic and pleasant to use (odourless).

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. IPIMAR is currently sorting and identifying to stages all eggs of the target species collected by the CalVET nets.**

The results of the egg analysis should be submitted to the survey data coordinator, using the standard excel spread sheets, within a month of the end of each cruise, but at the latest by 31 July (3 weeks in advance of the WGWIDE meeting; see also Section 4.3).

All participants should attempt to meet the deadline for the submission of survey results. 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.

9 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; 2006; 2009) workshops were held, which included sample sorting, species identification and egg staging. The results of these workshops were very re-assuring and in future WKFATHOM (previously WKMHMES) will be held at the end of the year before the actual survey to provide quality assurance for the egg surveys. The results of these workshops will be presented to ICES.

The eggs and larvae of most of the species found in the area are well described by Russell, 1976. His book is well known and used by all the participants of the ICES triennial surveys. It is generally regarded as the definitive work on the subject in 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 9.1.1), 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 in this species (Figure 9.1.2). Horse mackerel larvae hatch at the end of egg stage IV (Pipe and Walker, 1987). For the southern horse mackerel, an eleven-stage scale adapted from Cunha *et al*. (2008) is in use.

9.1 Egg stage criteria

9.1.1 Egg staging criteria for the AEPM survey directed at mackerel and horse mackerel (Western stocks)

Because of discussions following the first round of egg staging the participants decided upon the following definitions of the developmental stages for mackerel, horse mackerel, hake and megrim. The primary characteristics are based on those presented in Lockwood *et al.* (1977) for mackerel (Figures 9.1.1 and 9.1.2), but now include some other (secondary) characteristics, which the participants thought were crucial in determining egg stage. Figures 9.1.3 and 9.1.4 shows the development stages for horse mackerel.

Stage IA

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

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

Stage IB

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

Stage II

Primary characteristics: From the first sign of the primitive streak, which begins as a cleft in the cell bundle, until closure of the blastopore. 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 cell bundle. Late in this stage, the head is still narrow and the eyes are not well formed.

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, and has lifted clear of the yolk sac.

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

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.

Stage V

Primary characteristics: Growth of the embryo until the tail is touching the nose or beyond.

Secondary characteristics: Pigmentation develops in the eye.

NB

The preservation of eggs can cause shrinkage and distortion of the embryo. Therefore, care should be taken when assessing the length of the embryo, as they do not always

remain around the full circumference of the yolk. They may also become distorted giving a false impression of development stage.

Horse mackerel and hake embryos hatch at the end of stage 4.

9.1.2 Egg staging for the Portuguese DEPM survey directed at horse-mackerel (southern stock)

Since 2007, the horse-mackerel southern stock, monitored by Portugal IPIMAR, has been surveyed according to a DEPM methodology. For the implementation of this method an egg development scale with 11 stages has been developed (Cunha *et al.*, 2008). A revised version of that classification is now in use (Figure 9.1.3):

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

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

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

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

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

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

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

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

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

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

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

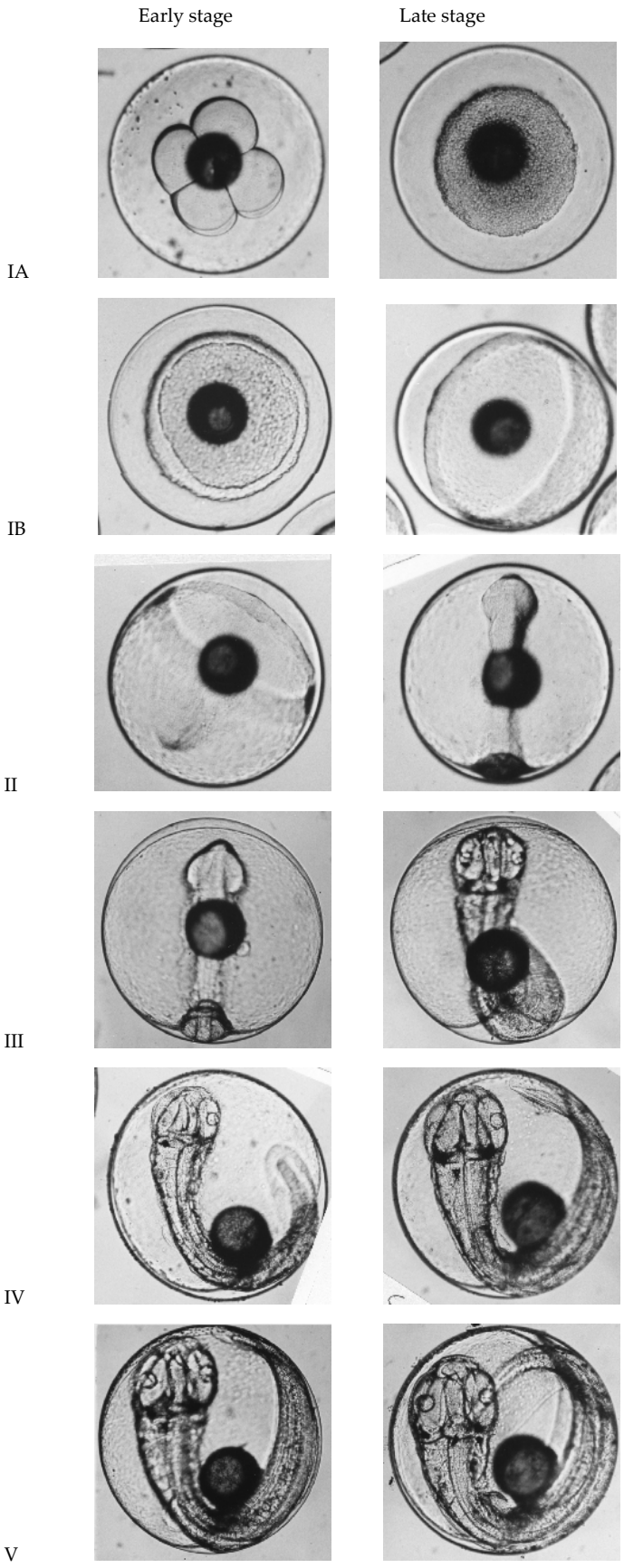
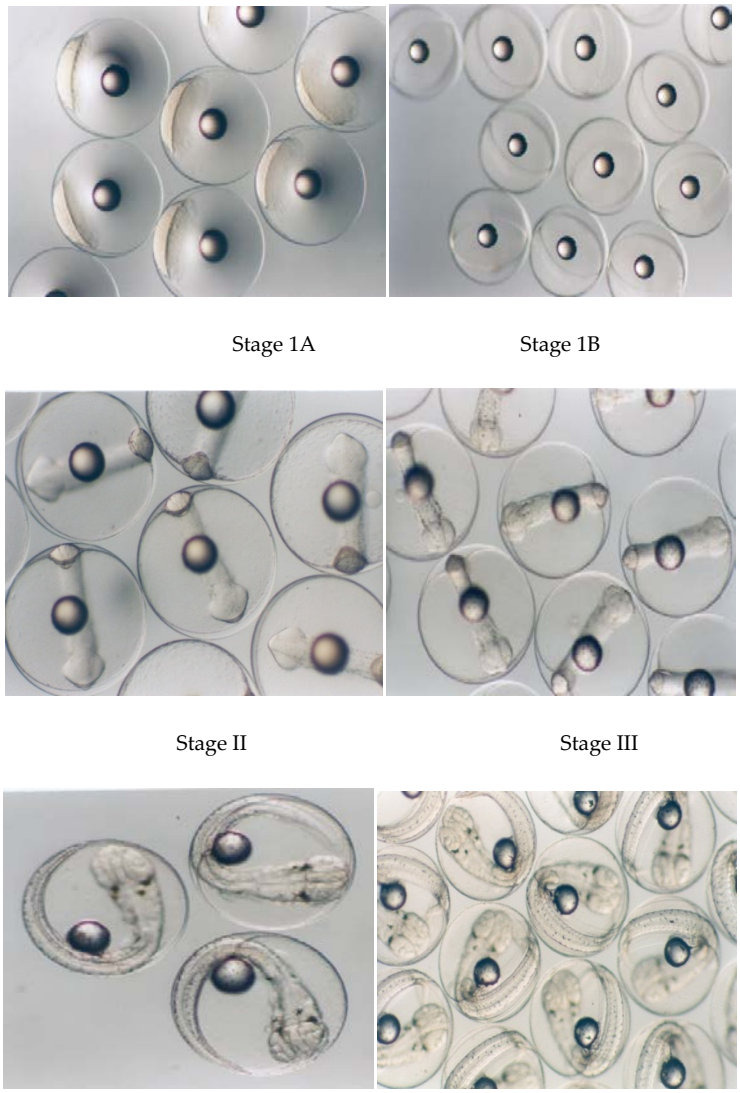


Figure 9.1.1. Mackerel eggs at the beginning and end of the six development stages.



Stage 1A Stage 1B

Stage II Stage III

Stage IV Stage V

Figure 9.1.2. Development stages of mackerel from fertilization experiments.

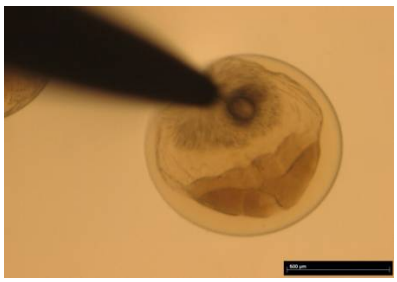
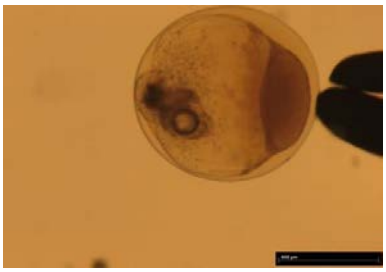
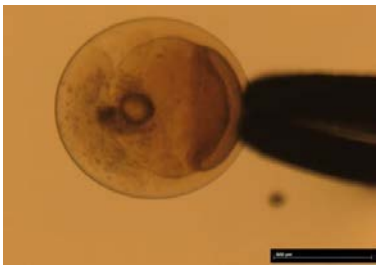
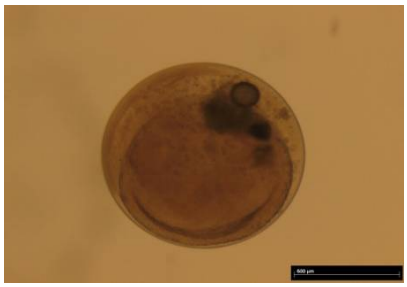
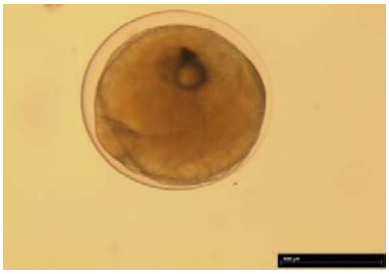
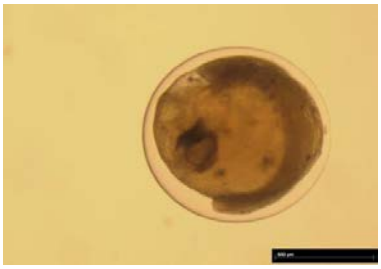
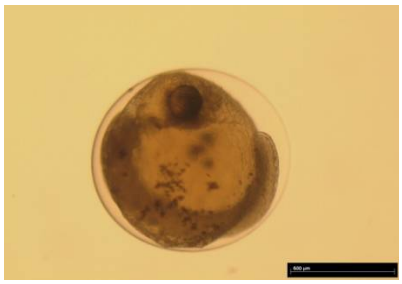
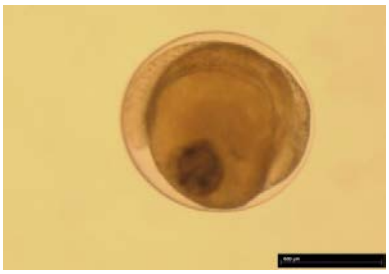
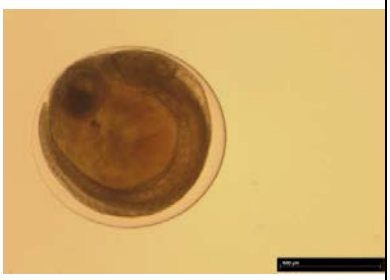
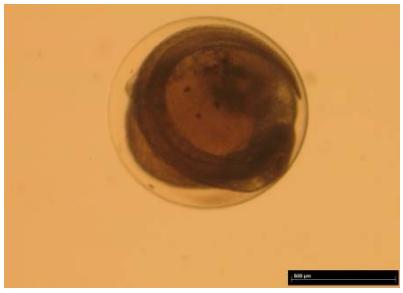
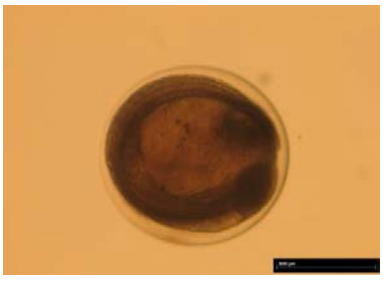
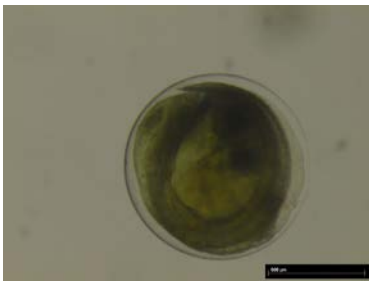
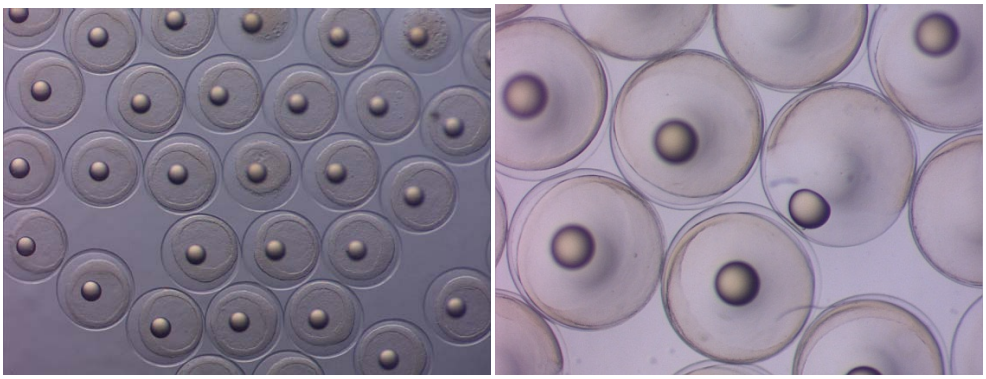
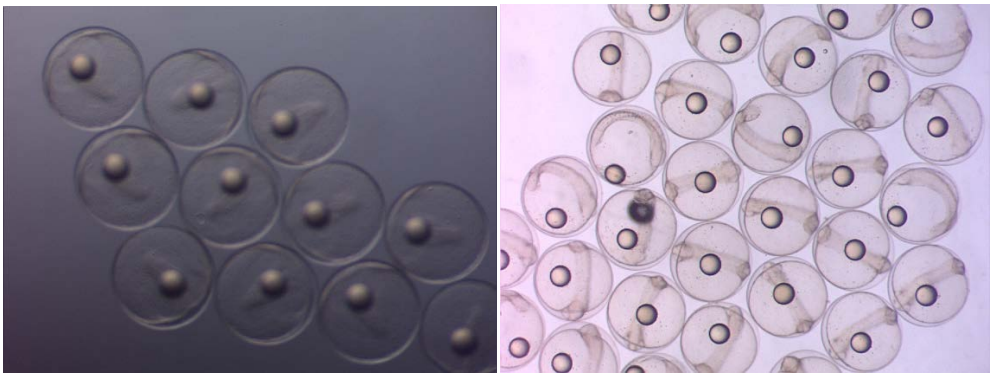
Stage IA or I	Stage IA or II	Stage IB or III
		
Stage II or IV	Stage II or V	Stage III or VI
		
Stage III or VII	Stage III or VIII	Stage IV or IX
		
Stage IV or X	Stage IV or X	Stage IV or XI
		

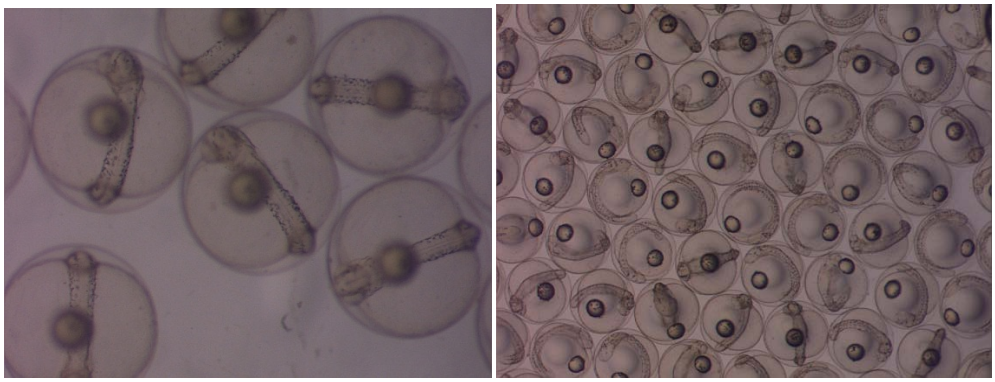
Figure 9.1.3. Development stages of horse mackerel from fertilization experiments. First stage number is the stage development used for the Western stock, second number is the stage development used for the southern stock.



Early stage 1B Late stage 1B

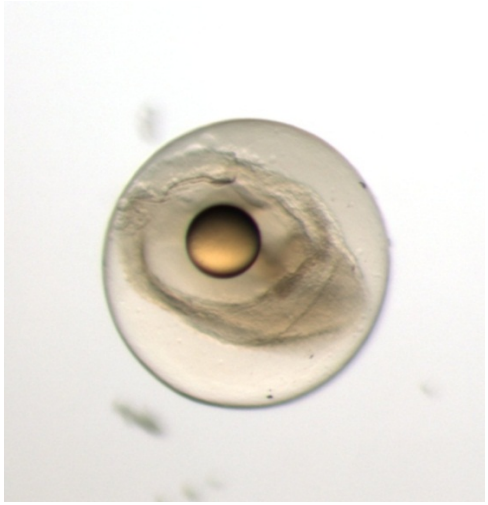


Early stage II Late stage II

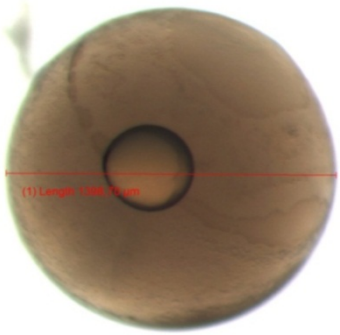


Early stage III Late stage III

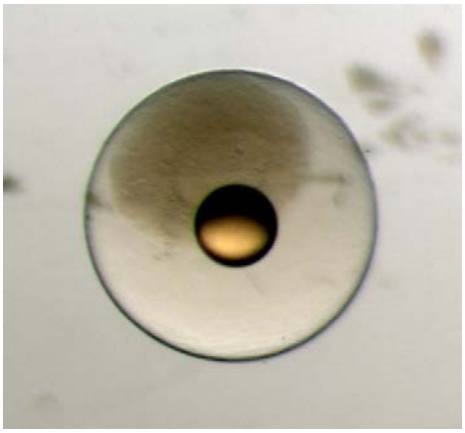
Figure 9.1.4. Development stages (1B, II and III) of horse mackerel from fertilization experiments.



Stage 1A

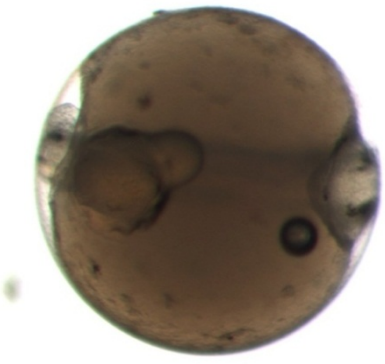


Stage 1A

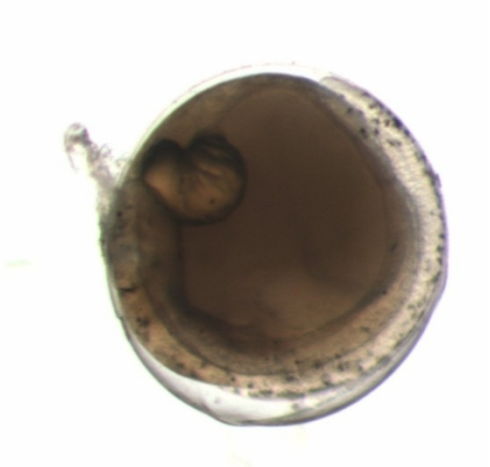


Stage 1B

Stage II



Stage III



Stage III

Figure 9.1.5. Development stages of hake eggs from fertilization experiments.

9.2 Egg identification criteria

Egg and oil globule size is the primary identification criteria used. Mackerel eggs range in size from 0.97 mm to 1.38 mm with the oil globule ranging from 0.22 to 0.38 mm.

Horse mackerel eggs range from 0.81 to 1.04 mm with an oil globule ranging from 0.19 to 0.28 mm.

Table 9.2.1 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. Eggs can also show regional variations in pigmentation and this should not be used as a primary characteristic for identification. A complete reference list is given at the end of this report. Due to this variation, egg identification should be carried out by experienced staff that have participated at the WKFATHOM egg identification and staging workshops carried out in the year prior to the survey year.

In addition to the published descriptions given in Table 9.2.1, 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.

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

- Oil globule often orientated to the top of the egg.

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

- Oil globule easily broken into several smaller pieces. This seems to be more common in eggs found in the southern area, particularly in eggs from the Portuguese coast.
- Horse mackerel yolk sacs are highly segmented, although this may not be as obvious at the southern end of the species range.
- The oil globule migrates towards the head of the embryo after stage 2.
- In stages 3 and 4 the embryos show stronger pigmentation compared to mackerel. However, the pigmentation is not as strong as in hake.

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

- Strongly pigmented oil globule. This is noted mainly from the North Sea, English Channel and the Mediterranean. It is not a characteristic found in the Celtic Sea.
- From stage III onwards embryos display strong pigmentation along the embryo. Towards the end of its development, the embryo begins to show the characteristic post-anal pigmentation of three bars.
- Positive surface adhesion test (SAT) is also used to identify hake eggs (Porebski, 1975) and (Coombs, 1994).

Megrim (*Lepidorhombus whiffiagonis*)

- Striated 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.
- Pigmentation on yolk from stage II onwards.

Longspine snipefish (*Macrorhamphosus scolopax*)

- Membrane is light amber with grainy reflections.
- Yolk with rose or violet halo depending on viewing light.

- Oil globule is amber/rose in colour.

NB

The striated appearance of megrim eggs is reasonably diagnostic in fresh specimens. However, preserved specimens of other eggs also appear to develop apparent striations on the egg membrane, which can therefore lead to misidentification of eggs which have been preserved for some time.

Table 9.2.1. Comparison of the Characteristics of Mackerel, Horse Mackerel, Blue Jack 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, 1905–1909
	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. 0.05 mm	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
	1.16	0.27			IPIMAR, fertilization experiment 2008
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, 1905–1909
	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
Blue Jack Mackerel (*Trachurus picturatus*)	0.98–1.10	0.19–0.31	Segmented yolk	W Portugal	IPIMAR, fertilization experiment 2010 (Gonçalves *et al.*, 2012)
Megrim (*Lepidorhombus whiffiagonis*)	1.02–1.22	0.25–0.30	Striated membrane. Pigment develops in the yolk, close to the caudal region and under the oil globule as embryo 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 embryo develops	North Sea	Ehrenbaum, 1905–1909
	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 preparation

Table 9.2.1 continued.

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 chromatophores on oil globule	North Sea, English Channel, Mediterranean	Ehrenbaum, 1905–1909
	0.94–1.03	~0.27		?	D'Ancona *et al.*, 1956
	1.10–1.16	0.27–0.35		Celtic Sea	Shaw, 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. US Fish and Wildlife service. FWS/OBS-78/12.

NB The information in Table 9.2.1 above is based on observations of live or recently preserved eggs. It must be noted that preservation in formaldehyde gradually destroys pigmentation and therefore observation of chromatophores may well be difficult in specimens, which have been preserved for any length of time.

9.3 Misclassification of mackerel and horse mackerel eggs in ICES Division IXa

In the southern part of the area of the triennial mackerel and horse mackerel egg survey different species of mackerel (*Scomber scombrus* and *S. colias*) and horse mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T. picturatus*) occur. The species of each genus show overlapping distributions and spawning periods and their eggs are similar in morphology. In order to help in the identification of these species, descriptions of morphometric characteristics of these eggs and the most relevant aspects for their identification are given below:

Trachurus mediterraneus

- Egg diameter: 1.00–1.04 mm
- Oil globule: 0.24 mm
- Description: Pelagic eggs, spherical, transparent. No perivitelline space. Oil globule colourless. Fine striated membrane (Padoa, 1956).
- Eggs are similar to *Trachurus trachurus*, but a bit bigger.
- Distribution of adults appears in the reports of ICES-WGACEGG.

Trachurus picturatus

Description and measurements based on eggs from a single artificial fertilization experiment carried out in 2010 by IPIMAR.

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

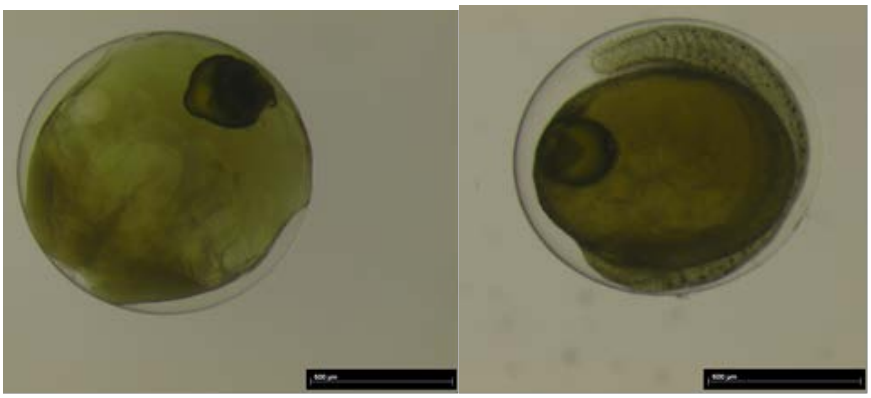


Figure 9.3.1. Eggs of *Trachurus picturatus* from a fertilization experiment (IPIMAR, 2010).

Scomber colias

- The eggs are spherical, on average ranging in diameter from 1.06–1.14 mm. Similar description was offered by Fahay (1983), with little differences in diameter range, which ranged from 1.06–1.36 mm.
- Oil globule 0.26–0.37 mm in diameter. In the Pacific oil globules diameters varies between 0.25 and 0.32 mm (Fritzsche, 1978).
- Yolk is smooth, transparent and unsegmented and under magnification (x36) can be seen to be filled with a large number of tiny vacuoles. The only difference with *S. scombrus* is that the yolk is pigmented with several melanophores, while in *S. scombrus* eggs the yolk is pigmented just before hatching, when a spot per side appears just posterior to the head.
- The perivitelline space is narrow.
- In advanced stage of development both the dorsum of the embryo and the oil globule are pigmented, the latter on the hemisphere facing the head (Kramer, 1960).
- Distribution of adults appears in the reports of ICES-WGACEGG.

Macroramphosus scolopax

- Egg diameter: 1.0 mm
- Oil globule: 0.20 mm
- Description: Pelagic eggs, spherical, transparent, single oil globule. Yolk pigmentation is described as light amber; pigmentation of oil globule is amber-rose (Spartà, 1936). Eggs are similar to those of *Trachurus trachurus* but without yolk segmentation.
- For fish distributions see for example Marques *et al.* (2005).

Boops boops

- Egg diamater: 0.93 mm (based on eggs from artificial fertilization, IPIMAR, 2008, see Figure 9.3.2).
- Oil globule: 0.18 mm (based on eggs from artificial fertilization, IPIMAR, 2008).
- Description: Pelagic eggs, spherical. Single oil globule with melanophores (Gaetani, 1937).
- Fish distribution is mapped in the reports of ICES-WGACEGG.

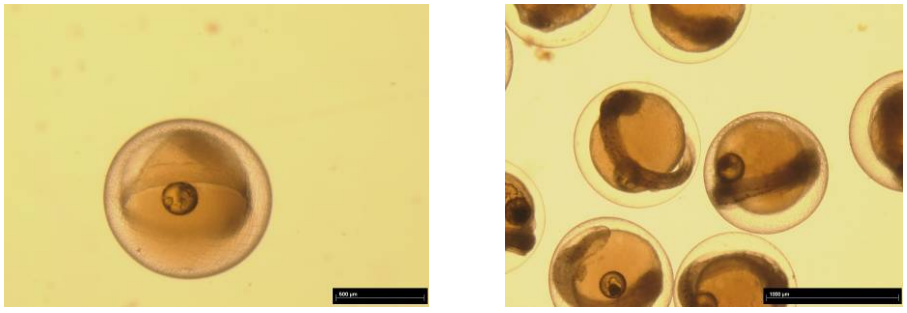


Figure 9.3.2. Eggs of *Boops boops* from fertilization experiments (IPIMAR).

10 Data analysis

10.1 Egg development of mackerel (all components) and horse mackerel (Western Stock)

The equation describing the relationship between egg development and temperature is an important parameter for the estimation of SSB, as it is directly used to calculate the daily egg production for mackerel. Lockwood *et al.* (1977; 1981), presented data on the egg development times in relation to temperature for Northeast Atlantic Mackerel, and this model has been used as the basis for calculating daily egg production of stage I eggs on all the surveys from 1977 until 2010:

Loge time (hours) = -1.61 loge (T°C) + 7.76

The formula for calculating the duration of stage I mackerel eggs from the sea temperature (T°C) was updated according to the new findings of Mendiola *et al.* (2006) and will be used to calculate the TAEP estimate in 2013:

Loge time (hours) = -1.31 loge (T°C) + 6.90

In 2013, the whole mackerel egg survey time-series will be recalculated with the new egg development equation.

For horse mackerel, similar egg development data are given by Pipe and Walker (1987) which have been used for the calculation of stage I egg production since 1977. For calculating the duration of stage I horse mackerel eggs the formula is:

Loge time (hours) = -1.608 loge (T°C) + 7.713

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

10.2 Daily egg production estimation for mackerel (all components) and horse mackerel (Western Stock)

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, MSS, Aberdeen will collate and manage the results for the entire 2013 survey (see also Section 4.3). 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 m2, 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 m2 per day production (EP) using development equation for both species in the following way:

For stage I **mackerel** eggs:

$$EP = \frac{24 \cdot A_e}{e^{-1.31 \cdot \log(T)+6.90}}$$

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).

As only stage 1 eggs are used mortality is not accounted for.

Eggs/m^2/day is then raised to the area of the rectangle it represents. 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 AR = 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. This 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 2013 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.

10.3 Daily egg production estimation for Southern horse mackerel

Egg ageing is achieved using the results from egg development with temperature obtained during incubation experiments (Cunha *et al.,* 2008) and the methods described by Murta and Vendrell (2009) and Bernal *et al.,* 2008.

In order to estimate Daily Egg Production the exponential model: E [P] = P0 e -Z age is fitted as a Generalized Linear Model (GLM) with negative binomial distribution and log link. Weights proportional to the relative area are represented by each station. The total egg production is calculated multiplying the daily egg production ratio (eggs per m2 and day) by the positive area (in m^2). The data analyses are undertaken using open source R libraries and scripts available at http://sourceforge.net/projects/ichthyoanalysis.

10.4 Annual egg production and SSB estimation

10.4.1 Mackerel (all components) and horse mackerel (Western Stock)

All data analysis should be carried out in accordance with the procedures described in detail for the 1995 survey and 1998 surveys (ICES, 1996, 1999). The detailed steps of the data analysis were updated for the 2003 WGMEGS report (ICES, 2003), and then subsequently for the WKMHMES report (ICES, 2006b) and for the MEGS survey manual (Annex 2 of ICES, 2010). Individual countries supplied data in an electronic Excel template form to the data coordinator at the Marine Laboratory, Aberdeen. The data for each station consisted of:

- sample time, date and position,
- numbers of mackerel, horse mackerel and other eggs by stage.
- sub sample size,
- volume of seawater filtered (or flowmeter counts and calibration data)
- water depth, depth sampled, temperature and salinity profiles.

Each country was responsible for validating their own basic data and there was also some checks built into the Aberdeen database.

The procedures for estimating the total annual egg production (TAEP) and its variance are those described in detail by Fryer (ICES WGMEGS, 1996). Total egg production is a weighted sum of the mean daily production in each period, p. The weights in the TAEP sum, λ_p, arise from what is termed the histogram method for raising daily egg

production, however, these weights could also come from the under-the-curve method. Both methods provide estimates of TAEP with associated variances, but the histogram method has several advantages over the under the curve method that will be discussed in a later section (7.3). The histogram method is used to provide the revised estimates for 2007 and the final estimates for 2010.

Mean daily production is estimated by raising the observed mean production per m^2, $\bar{y}_{ps}$, for each sampled cell, s, in period p, to the total area of that cell plus any additional area due to the filling in of unsampled adjacent cells given by:

$$\tilde{\mathrm{A}}_{ps} = \mathrm{A}_s + \sum_{u \in U_s} \frac{A_u}{n_{pu}}$$

Where As is area of cell s, Us is the set of all unsampled cells adjacent to s, and n_{pu} the number of sampled cells in period p adjacent to u. Fill in rules are described in detail in ICES (1996). The equation for TAEP is:

$$\sum_p \left[\lambda_p \sum_s \left(\tilde{\mathrm{A}}_{ps} \bar{y}_{ps} \right) \right]$$

The variance of the TAEP estimate is based on assuming that the raw production data are distributed with a constant Coefficient of Variation (CV) for all locations in all periods, resulting in the estimate of the variance being:

$$\sum_p \left[\lambda_p \sum_s \left(\tilde{A}_{ps}^2 \frac{\bar{y}_{ps}^2}{h_{ps}} \right) \right] CV^2$$

where CV is the CV of the raw data and h_{ps} is the number of observations (hauls) in cell s in period p. The CV of the data can be estimated by assuming a lognormal distribution for the positive egg production observations and estimating the residual variance about the expected values of log egg production. The CV of the lognormal distribution is related to its variance on the log scale, σ^2, by:

$$\mathrm{CV} = \sqrt{e^{\sigma^2} - 1}$$

In the current approach, σ is estimated by taking cells in each period that have at least two hauls of non-zero observations and using the standard deviation of the residuals about the cell means on the log scale. Effectively taking the residual standard deviation from the normal linear model:

$$\log(\text{production}) \sim \text{square:period}$$

However, as the survey is spreading out in space there are fewer and fewer cells with multiple observations. An alternative method investigated at the working group estimates the expected value in each cell from a generalized additive model using a 3 dimensional thin plate regression spline to model a smoothly changing sea surface egg production through time, with each sampling square modelled as an uncorrelated random effect.

FIXED: $\log(\text{production}) \sim s(\text{period}, \text{latitude}, \text{longitude})$

RANDOM: ~ square

This allows more data to be used in the estimate of σ, for example for western-mackerel in 2010, the alternative method uses 1024 data points as opposed to 30 when duplicates are required, this has obvious implications on the precision of the CV estimate. This is a potentially useful approach but there was not enough time to fully develop and evaluate it so the resulting data CVs are presented here for interest and as a suggestion for future research, along with the residual degrees of freedom from each model.

		Current	alternative	df current	df alternative
Southern mackerel	2007	3.63	4.03	51	123
	2010	2.16	2.98	62	114
Western mackerel	2007	1.65	1.84	61	868
	2010	1.22	2.03	15	958
Combined mackerel	2007	2.42	2.10	112	868
	2010	1.96	2.17	77	958
Western horse mackerel	2007	3.17	2.83	74	585
	2010	1.84	2.95	47	402

Spawning-stock biomass is estimated from TAEP, relative potential fecundity (RFp) and atretic loss (Ar). First relative realized fecundity (Fr) is estimated using RFp – Ar (measured in eggs per gram), then SSB (in grams) is estimated using:

$$TAEP / F_r \times 2 \times 1.08$$

where 2 is used to raise from the mass of females to the stock (assuming equal weight for males and a sex ratio of 1:1) and 1.08 is a correction factor to adjust prespawning to average spawning fish weight. A simple way to estimate the variance of the SSB estimate is to assume that TAEP and Fr are distributed with constant CV, and then the CV of the SSB estimate is:

$$CV_{SSB} = CV_{TAEP} + CV_{Fr}$$

This comes from the application of the delta method (itself based on a Taylor expansion of TAEP/Fr). The CV is estimated from an estimate and its associated variance by

$$CV = \frac{\sqrt{Var}}{estimate} = \frac{SE}{estimate}$$

Finally, the variance of Fr is estimated by assuming that RFp and Ar are independent and so the variance of RFp – Ar is the sum of their variances, Var(RFp) + Var(Ar).

10.4.2 Southern Horse Mackerel

For the southern horse mackerel a DEPM approach is implemented. The spawning biomass (SSB) is estimated according to the following expression:

$$SSB = \frac{APW}{RSF}$$

(CVs for SSB as described above)

A: spawning area

P: daily egg production density

W: female weight

R: sex-ratio

S: daily spawning fraction

F: batch fecundity

Spawning area is calculated as the sum of the area represented by each station in the positive stratum, the area is delimited by the outer zero egg stations. It may sometimes contain a few inner zero egg stations embedded in it.

Daily egg production (P0) and mortality (z) rate are estimated by fitting an exponential mortality model described above.

$E[P] = P0\ e^{-Z\ age}$

The estimation of the adult parameters, sex ratio, the mean female weight, the mean female expected batch fecundity and spawning fraction is based on the biological data collected from both survey and commercial samples.

The gonads preserved are used to measure the individual batch fecundity, to assess the mature/immature condition of females and to estimate the daily spawning fraction. Before the estimation of the mean female weight per haul (W), the individual total weight of the hydrated females is corrected by a linear regression between the total weight of non-hydrated females and their corresponding gonad-free weight. The sex ratio (R) in weight per haul is obtained as the quotient between the total weight of the females on the total weight of males and females. The expected individual batch fecundity (F) for all mature females (hydrated and non-hydrated) is estimated by the hydrated egg method (Hunter *et al.*, 1989), i.e. by modelling the individual batch fecundity observed in the sample of hydrated females and their gonad-free weight by a GLM and applying this subsequently to all mature females.

11 Standardization of adult sampling

A detailed description of shipboard methods for fecundity sampling is also given in the WGMEGS Fecundity Manual, Version 10.10.

At the WKMSPA (ICES, 2012) it was decided that during the 2013 survey potential fecundity and atresia samples of mackerel will be collected during the whole survey as was also done in previous surveys. In the period of peak spawning (period 3) enhanced sampling effort will be directed at collecting mackerel samples to estimate DEPM adult parameters.

For western horse mackerel the results of the fecundity samples have not been incorporated in the SSB estimate since 2001. WKMSPA recommends directing the effort of horse mackerel fecundity sampling and analysis to collect samples to estimate DEPM adult parameters (ICES, 2012). During the 2013, survey for horse mackerel adult samples will be collected during the peak spawning period (period 5).

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

Following WGMEGS decision to use only formaldehyde fixative (ICES, 2003) it will be possible to provide a unified sampling scheme for fecundity and atresia for use in the 2013 survey. Following the experience of the 2010 survey, the following changes have been recommended for the 2013 survey (Table 11.1.1). The fecundity manual keeps a record of all the changes in earlier surveys.

Samples for estimation of mackerel potential fecundity, atresia, batch fecundity, spawning fraction and spawning frequency 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 for the potential fecundity and atresia across the survey area aiming to complete a wide spread sampling regime for adults shown in Tables 11.1.2 a-c. 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. Maturity of fish should be determined according to the Walsh-Scale (Table 11.1.3).

In period 3, the period of peak spawning, mackerel potential fecundity and atresia samples as well as batch fecundity, spawning fraction and spawning frequency (DEPM adult parameter) samples will be taken. Each transect at the station with highest stage 1 mackerel egg production a trawl haul will be carried out (Table 11.2). It is recommended that trawling be preferably carried out at dusk or during the night.

Table 11.1.1. Changes for 2013 compared to earlier survey years.

2007	2010	2013
Mackerel		
On board ovaries are weighed and pipette subsamples of known volume and weight taken and fixed in formaldehyde solution		Samples are taken for screening for spawning markers and atresia. The results from the histology are used to decide which samples will be analysed for fecundity and which for atresia. Only samples that contain spawning markers and/or early alpha atresia will be embedded from the cassettes for further atresia analyses.
Gravimetric fecundity estimation Sub samples preserved in 3.6% buffered formaldehyde. F = O * C * S (F = fecundity, O = Ovary weight, C = count follicles > 185 µm in subsample, S = subsample weight; Hunter *et al.*, 1989)		Each cruise will collect 10 samples of one fish (stages 3 to 6) for the fecundity ring test.
Stereometric method		Ovary lobes need to be pierced with a fine needle before fixation in formaldehyde.
H&E -PAS – Toluidine blue		
Mackerel and Horse mackerel		
Fecundity samples: In 2007 count all oocytes >185 um and measure 1/3 of the oocytes.	Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis. Count all the oocytes >185 µm in the sample that are not automatically detected.	
	ImageJ and macros will be made available during the wk to all participants and they should use this for analysis of the samples.	
	Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.	
	For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.	
Spawning markers: hydrated, >5 POF's	Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample	

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 to spread the sampling trawling should not only be concentrated on 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. 11.1.2a: Desired temporal and spatial distribution of the mackerel fecundity sampling in the Southern Area

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

MACKEREL

Week	Date	Period*	Lon ° 11W	10	9	8	7	6	5	4	3	2	1	Lat ° 36N	37	38	39	40	41	42	Total
1	21-jan-13	1	100 (prespawning (purseine/trawl)											10							10
2	28-jan-13	1														10					30
3	4-feb-13	1																			20
4	11-feb-13	1														10					30
5	18-feb-13	2																	10		30
6	25-feb-13	2																			20
7	3-Mar-13	2																			0
8	11-Mar-13	2	20																		20
9	18-Mar-13	2	20																		20
10	25-Mar-13	3																			0
11	1-apr-13	3																			0
12	8-apr-13	3	10																		10
13	15-apr-13	3	10																		10
14	22-Ap-13	3	10																		10
15	29-apr-13	3																			0
16	6-May-13	4							10												10
17	13-May-13	4																			0
18	20-May-13	4																			0
19	27-May-13	4							10												10
20	3-jun-13	5																			0
21	10-jun-13	5																			0
22	17-jun-13	5																			0
23	24-jun-13	5																			0
24	1-jul-13	6																			0
25	8-jul-13	6																			0
26	15-jul-13	6																			0
27	22-jul-13	6																			0
			* Note that period **1**/**2** is dominated by prespawning fish; in periods **3** to **6** = atresia sampling																		230

	per period			
	1	2	3	4
IEO	100	40	30	
AZTI				20
IPIMAR	30	10		
Total:	130	50	30	20

Tab. 11.1.2b: 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	62	63	Total
1	21-jan-13	1																					0
2	28-jan-13	1																					0
3	4-feb-13	1																					0
4	11-feb-13	1																					0
5	18-feb-13	2							35								25						60
6	25-feb-13	2			35										25								60
7	3-Mar-13	2	10				35										25						70
8	11-Mar-13	2	10										20										30
9	18-Mar-13	2	20								20												40
10	25-Mar-13	3	See Table 12.1.2 c For the DEPM sampling in this period																				0
11	1-apr-13	3																					0
12	8-apr-13	3																					0
13	15-apr-13	3																					0
14	22-Ap-13	3																					0
15	29-apr-13	3																					0
16	6-May-13	4						15															15
17	13-May-13	4	10			15									15		15						55
18	20-May-13	4	10					15		15		15								10			65
19	27-May-13	4													15		15			10			40
20	3-jun-13	5						15															15
21	10-jun-13	5			20									10			15			10			55
22	17-jun-13	5						15		15		10								10			50
23	24-jun-13	5												15			10						25
24	1-jul-13	6																					0
25	8-jul-13	6									10			10									20
26	15-jul-13	6							20				10				10						40
27	22-jul-13	6				10		10															20
* Note that period **1**/**2** is dominated by prespawning fish; in periods **3** to 6 = atresia sampling																							660

	per period					
	1	2	3	4	5	6
AZTI				20		
vTI						
MI		105				80
SCO		115			75	
IMARES				45	50	
IMR				90		
IEO		40				
FAR				20		
ICE					20	
Total:	0	260	0	175	145	80

Tab. 11.1.2.c: Desired mackerel adult sampling in the area selected for the 2013 DEPM sampling

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

MACKEREL Lat °

Week	Date	Period	43.25N	43.35	43.45	43.55	44.05	44.15	44.45	45.15	45.45	46.15	45.45	47.15	47.45	48.15	48.45	49.15	49.45	50.15	50.45	51.15	51.45	52.15	52.45	53.15	53.45	54.15	54.45	55.15	55.45	56.15	56.45	57.15	57.45	58.15	Total
1	21-jan-13	1																																			0
2	28-jan-13	1																																			0
3	4-feb-13	1																																			0
4	11-feb-13	1																																			0
5	18-feb-13	2																																			0
6	25-feb-13	2																																			0
7	3-Mar-13	2																																			0
8	11-Mar-13	2																																			0
9	18-Mar-13	2																																			0
10	25-Mar-13	3								15				30			30		30		30																135
11	1-apr-13	3	30				30		15		15		30			30							30		30		30										240
12	8-apr-13	3			30			15				30			30													30		30				30		195
13	15-apr-13	3		30		30		15		15														30		30		30				30		30		30	270
14	22-Ap-13	3							15		15							30		30		30								30			30				180
15	29-apr-13	3																																			0
16	6-May-13	4																																			0
17	13-May-13	4																																			0
18	20-May-13	4																																			0
19	27-May-13	4																																			0
20	3-jun-13	5																																			0
21	10-jun-13	5																																			0
22	17-jun-13	5																																			0
23	24-jun-13	5																																			0
24	1-jul-13	6																																			0
25	8-jul-13	6																																			0
26	15-jul-13	6																																			0
27	22-jul-13	6																																			0
																																					1020

	per period					
	1	2	3	4	5	6
AZTI			180			
vTI			360			
MI						
SCO			270			
IMARES						
IMR						
IEO			210			
FAR						
ICE						
total:	0	0	1020	0	0	0

Table 11.1.3. 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 assign tube reference numbers to cruise leaders for labelling the Nunc tubes used on their cruises.

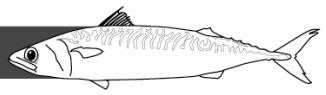
Table 11.1.4 a and b shows the procedures to follow for the collection of samples at sea, and for sample analysis in the laboratory. Provisional estimates of potential fecundity and atresia are required for the 2013 WGWIDE group in late August and final results are required for WGMEGS in the spring of 2014. If the participants or coordinator are unsure of the data quality they should pass on their concerns to the Survey Coordinator (Finlay Burns MSS).

Each country carrying out the various cruises listed in Table 11.1.2.a-c is responsible for distributing the samples collected to the countries carrying out the fecundity analysis.

Table 11.1.4.a. Adult mackerel sampling program – AEPM sampling.

Mackerel and Horse Mackerel Egg Survey 2013

MACKEREL SAMPLING AEPM



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	30	10					40	
	ESP/IEO	100*	40	30				190	
	ESP/AZTI				20			20	**230**
Western	ESP/AZTI				20			20	
	IRL/MI		105	see DEPM sampling plan			80	185	
	ESP/IEO		40					40	
	FAR/FFS				20			20	
	SCO/MSS		115			75		190	
	NED/IMARES				45	50		95	
	NOR/IMR				90			90	
	ICE/HAFRO					20		20	**660**

* Samples will be obtained by market and/or onboard sampling

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

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), ⟶ lenght, weight (total), sex, maturity,

c remove ovaries ***undamaged!***, weigh ovaries
take **2 parallel pipette samples of 25 µl** and **2 parallel pipette samples of 100µl** of one ovary in pre-filled individually coded Nunc tubes. ⟶ ovary weight, sampling for histology
Take a 2-3g (table spoon) sample of the same ovary and put the other ovary in formalin jars (ovary should be punctured before putting it into the jar).

d send the 2-3g samples immediately after the survey according to the instructions given before the 2013 survey to either Vigo, Bergen, San Sebastian, IJmuiden, (after the screening each institute will get instructions on distributing the pipette and atresia samples)

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

e Additionally per each cruise, take of 1 large stage 3 female 10 **parallel pipette samples of 25 µl** in pre-filled individually coded Nunc tubes.

send 5 samples to IMR Bergen and one each of the other parallel samples to either Vigo, San Sebastian, IJmuiden, Aberdeen, Galway ⟶ Ring test

Please refer to the sampling protocolls which will be send to all particpants before the 2013 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Table 12.1.2; for further instructions please refer to section 12.

Table 11.1.4.b. Adult mackerel and horse mackerel sampling program – DEPM sampling.

Mackerel and Horse Mackerel Egg Survey 2013

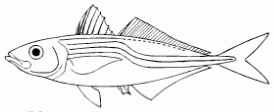
AEPM & DEPM SAMPLING

MACKEREL: Estimation of batch fecundity, potential fecundity in pre-spawning fish and the estimation of atresia for realised fecundity

Sampling at Sea in Period 3 (for details on cruises see WGMEGS report)

PERIOD 3:

Sampling by	AZTI, Spain	vTI, Germany	MSML, Scotland	IEO, Spain	Total
No of samples	180	360	270	180	990

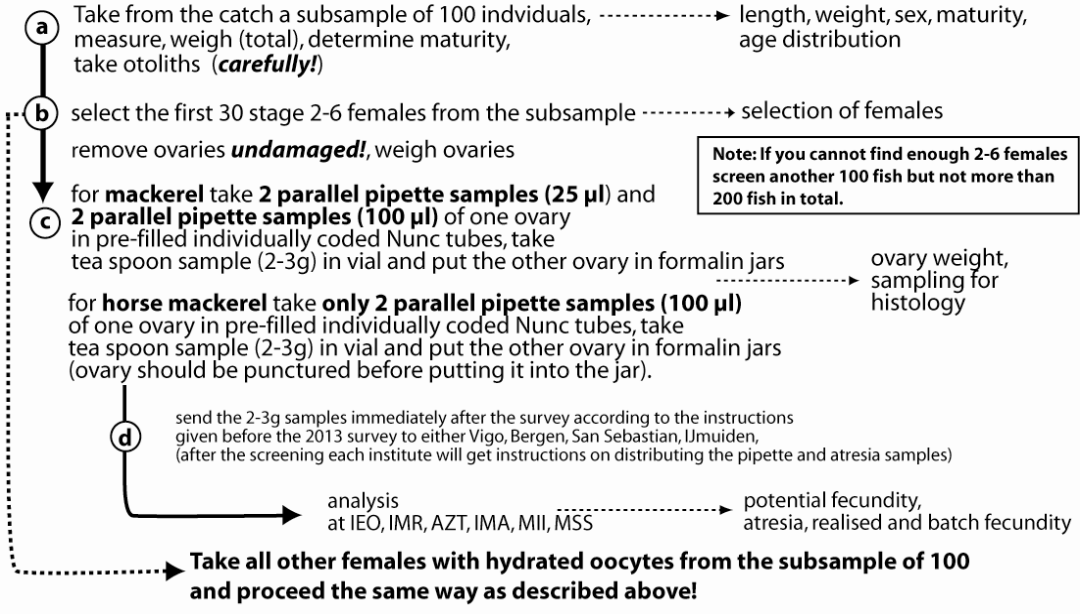


HORSE MACKEREL: Estimation of batch fecundity

Sampling at Sea in Period 5 (for details on cruises see WGMEGS report)

PERIOD 5:

Sampling by	MSML, Scotland	IMARES, Netherlands	Total
No of samples	270	180	450



Please refer to the sampling protocolls which will be send to all particpants before the 2013 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Tables 12.1.2c and 12.2.2; for further instructions please refer to section 12.

11.2 Sampling for horse mackerel fecundity in the Western area

Following the experience of the 2010 survey and WKMSPA, the following changes have been recommended for the 2013 survey (Table 11.2.1). The fecundity manual keeps a record of all the changes in earlier surveys.

Table 11.2.1. Changes for 2013 compared to earlier survey years.

2007	2010	2013
Mackerel and Horse mackerel		
Fecundity samples: In 2007 count all oocytes >185 um and measure 1/3 of the oocytes.	Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis. Count all the oocytes >185 μm in the sample that are not automatically detected.	
	ImageJ and macros will be made available during the wk to all participants and they should use this for analysis of the samples.	
	Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.	
	For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.	
Spawning markers: hydrated, >5 POF's	Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample	
Horse mackerel		
Gravimetric fecundity estimation Sub samples preserved in 3.6% buffered formaldehyde. F = O * C * S (F = fecundity, O = Ovary weight, C = count follicles > 185 µm in subsample, S = subsample weight; Hunter *et al.*, 1989)		From 2013 and onwards no samples for potential fecundity are collected. Only DEPM adult parameter samples will be collected.
On board ovaries are weighed and pipette subsamples of known volume and weight taken and fixed in formaldehyde solution		
	IPIMAR will perform a DEPM survey for horse mackerel. Batch fecundity: Gravimetric method. Take whole fixed ovary to the lab, take 3 subsamples, weigh and count all the hydrated oocytes in subsample. Spawning fraction: migratory nucleus, hydrated, POF's	

During the 2013 survey for horse mackerel adult samples will be collected during the peak spawning period (period 5). During the 2013 survey horse mackerel will be collected from trawl hauls on the Western spawning component selecting fish of maturity stages 2–6 (Walsh scale) as shown in Table 11.1.3. **Each transect at the station with highest stage 1 mackerel egg production a trawl haul will be carried out (Table 11.2.2). It is recommended that trawling be preferably carried out at dusk or during the night.**

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 11.2.3) and in Figure 11.2.1.

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 11.3.**

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. 11.2.2: Desired horse mackerel adult sampling in the area selected for the 2013 DEPM sampling

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

HORSE MACKEREL — Lat °

Week	Date	Period	47.15N	47.45	48.15	48.45	49.15	49.45	50.15	50.45	51.15	51.45	52.15	52.45	53.15	53.45	54.15	Total
1	21-jan-13	1																0
2	28-jan-13	1																0
3	4-feb-13	1																0
4	11-feb-13	1																0
5	18-feb-13	2																0
6	25-feb-13	2																0
7	3-Mar-13	2																0
8	11-Mar-13	2																0
9	18-Mar-13	2																0
10	25-Mar-13	3																0
11	1-apr-13	3																0
12	8-apr-13	3																0
13	15-apr-13	3																0
14	22-Ap-13	3																0
15	29-apr-13	3																0
16	6-May-13	4																0
17	13-May-13	4																0
18	20-May-13	4																0
19	27-May-13	4																0
20	3-jun-13	5	30		30								30		30		30	150
21	10-jun-13	5					30	30	30	30	30							150
22	17-jun-13	5		30		30						30		30		30		150
23	24-jun-13	5																0
24	1-jul-13	6																0
25	8-jul-13	6																0
26	15-jul-13	6																0
27	22-jul-13	6																0

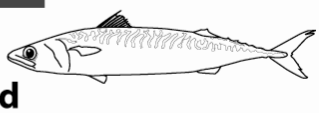
	per period						
	1	2	3	4	5	6
AZTI						
vTI						
MI						
SCO					270	
IMARES					180	
IMR						
IEO						
FAR						
ICE						
total:	0	0	0	0	450	0

Table 11.2.3. Adult mackerel and horse mackerel sampling program – DEPM sampling.

Mackerel and Horse Mackerel Egg Survey 2013

AEPM & DEPM SAMPLING

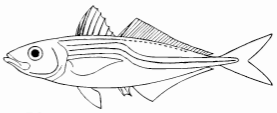
MACKEREL: Estimation of batch fecundity, potential fecundity in pre-spawning fish and the estimation of atresia for realised fecundity



Sampling at Sea in Period 3 (for details on cruises see WGMEGS report)

PERIOD 3:

Sampling by	AZTI, Spain	vTI, Germany	MSML, Scotland	IEO, Spain	Total
No of samples	180	360	270	180	990



HORSE MACKEREL: Estimation of batch fecundity

Sampling at Sea in Period 5 (for details on cruises see WGMEGS report)

PERIOD 5:

Sampling by	MSML, Scotland	IMARES, Netherlands	Total
No of samples	270	180	450

(a) Take from the catch a subsample of 100 indviduals, measure, weigh (total), determine maturity, take otoliths (***carefully!***) ⟶ length, weight, sex, maturity, age distribution

(b) select the first 30 stage 2-6 females from the subsample ⟶ selection of females

remove ovaries ***undamaged!***, weigh ovaries

Note: If you cannot find enough 2-6 females screen another 100 fish but not more than 200 fish in total.

(c) for **mackerel** take **2 parallel pipette samples (25 µl)** and **2 parallel pipette samples (100 µl)** of one ovary in pre-filled individually coded Nunc tubes, take tea spoon sample (2-3g) in vial and put the other ovary in formalin jars ⟶ ovary weight, sampling for histology

for **horse mackerel** take **only 2 parallel pipette samples (100 µl)** of one ovary in pre-filled individually coded Nunc tubes, take tea spoon sample (2-3g) in vial and put the other ovary in formalin jars (ovary should be punctured before putting it into the jar).

(d) send the 2-3g samples immediately after the survey according to the instructions given before the 2013 survey to either Vigo, Bergen, San Sebastian, IJmuiden, (after the screening each institute will get instructions on distributing the pipette and atresia samples)

analysis at IEO, IMR, AZT, IMA, MII, MSS ⟶ potential fecundity, atresia, realised and batch fecundity

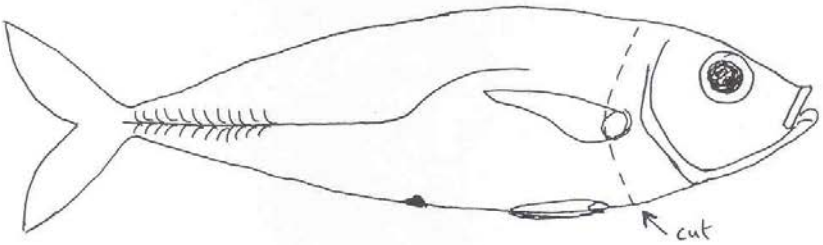
Take all other females with hydrated oocytes from the subsample of 100 and proceed the same way as described above!

Please refer to the sampling protocolls which will be send to all particpants before the 2013 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Tables 12.1.2c and 12.2.2; for further instructions please refer to section 12.

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

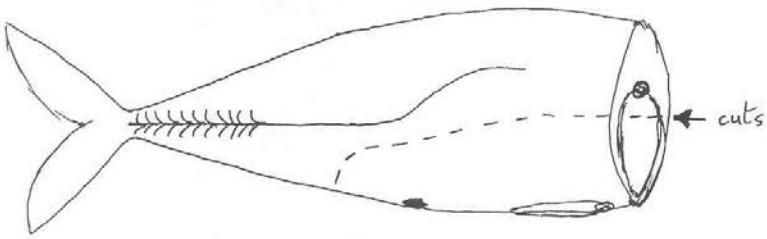
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral 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 11.2.1. Procedure for collecting ovaries from horse mackerel.

11.3 Formaldehyde solution for histological samples

All fecundity samples shall be fixed and preserved in a 3.6% 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-Hydrogen-Phosphate Hydrate (NaH_2PO_4-H_2O), the other is Di-Sodium-Hydrogen-Phosphate-Di-Hydrate (Na_2HPO_4-$2H_2O$). Two obtain 1L of fixative solution the following recipe as applied:

4.0 g NaH_2PO_4-H_2O, 7.5 g Na_2HPO_4-$2H_2O$ and 100 mL Formaldehyde 37% are filled up to 1 L with distilled or de-ionised water and thoroughly mixed.

12 References

Bernal, M., Ibaibarriaga, L., Lago de Lanzos, A., Lonergan, M. E., Hernandez, C., Franco, C., Rasines, I., Valdes, L., and Borchers, D. L. 2008. Using multinomial models to analyse data from Iberian sardine egg incubation experiments: a comparison with traditional techniques. ICES Journal of Marine Science, 65: 51–59.

Coombs, S. H. 1994. Identification of eggs of hake, *Merluccius merluccius*. Journal of the Marine Biological Association of the United Kingdom, (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.

Cunha, M. E., Vendrell, C., and Gonçalves, P. 2008. Experimental study of the dependence of embryonic development of *Trachurus trachurus* eggs on temperature. ICES Journal of Marine Science, 65: 17–24.

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. *Nordischen Plankton*, 1, 413 pp. Lipsius & Tischer, Leipzig.

Eltink, A. 2007. The spray technique: a new method for an efficient separation of fish eggs from plankton. Journal of Plankton Research, 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: 423 pp.

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

Gonçalves, P., Henriques, E., and Angélico, M. M. 2012. Co-occurrence of *Trachurus trachurus* and *Trachurus picturatus* spawners in Atlantic Iberian waters and the ability to distinguish their eggs in plankton samples. Fisheries Research, Vol. 138: 139–145.

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, Series 2, 5: 121 pp.

Holt, E. W. L. 1898. Notes on the reproduction of teleostean fishes in the south-western district. Journal of the Marine Biological Association of the United Kingdom, 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. Californian Cooperative Oceanic Fisheries Investigations (CalCOFI), **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/H:4.

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. Report of the Workshop on Identification and Staging of Mackerel and Horse Mackerel Egg. 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. Report of the Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES). ICES CM 2004/G:13.

ICES. 2006. Report of the Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES). 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, 111 pp.

ICES. 2009. Report of the Workshop on Mackerel and Horse Mackerel Egg Staging and Identification (WKMHMES). ICES CM 2009/LRC:13.

King, D. P. F., O'Toole, M. J., and Robertson, A. A. 1977. Early development of the South African maasbanker *Trachurus trachurus* at controlled temperatures. Fisheries Bulletin South Africa, 9: 16–22.

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, 8 pp.

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. Journal of Plankton Research, 3: 217–233.

Mendiola, D., Alvarez, P., Cotano, U., Etxebeste, E., and Martinez de Murguia, A. 2006. Effects of temperature on development and mortality of Atlantic mackerel fish eggs. Fisheries Research, 80: 158–168.

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

Murta, A. G., and Vendrell, C. 2009. Using the EM algorithm to age fish eggs. ICES Journal of Marine Science, 66: 607–616.

Nash, R. D. M., Dicky-Collas, M., and Milligan, S. P. 1998. Descriptions of the Gulf VII/PRO-NET and MAFF/Guildline unencased high-speed plankton samplers. Journal of Plankton Research, Vol. 20 no 10: 1915–1926.

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

Porebski, J. 1975. Application of the surface adhesion test to identify the eggs of the hake *Merluccius* spp. Collection of Scientific Papers of the Int. Commission for Southeast 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. 524 pp.

Simpson, A. C. 1959. The spawning of plaice (*Pleuronectes platessa*) in the North Sea. Fishery Investigations, London, (Series II), 22, No 7, 111 pp.

Smith, P. E., Richardson, S. L. 1977. Standard Techniques for Pelagic Fish Egg and Larvae Surveys. FAO Fisheries Technical Paper. No. 175. 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.

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Version history

Date	Version	Change	by Whom
April 2012	1	1st version for the 2013 survey; a description of the Portuguese DEPM survey on horse mackerel eggs was added; the survey core areas were updated and amended; the table of survey gears used was updated by the survey participants; the formula for calculating the duration of stage I mackerel eggs from the sea temperature (T°C) was updated according to the new findings of Mendiola *et al.* (2006)	WGMEGS
November 2012	1.1	Section on ship planning for 2013 included, sampling schemes adapted, procedures for Southern horse mackerel added	WKFATHOM
April 2014	1.3	Reviewers suggestions worked in for SISP publication	WGMEGS