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

20–24 April 2015

ICES Headquarters, Copenhagen



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Executive summary

The ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS), chaired by Cindy van Damme (IMARES, The Netherlands) and Finlay Burns (MSS, UK), met at ICES Headquarters, Copenhagen from 20–24 April 2015, to plan the Mackerel and Horse Mackerel Egg Survey in 2016. The nations participating in the 2016 Northeast Atlantic MEGS survey will be Portugal, Spain, Scotland, Ireland, The Netherlands, Germany, Iceland and the Faroe Islands. The main aim of the survey is to relate the number of freshly spawned eggs found in the water to the number of females having produced these eggs. Knowing the fecundity of the females and sex ratio provides an estimate for the spawning-stock biomass.

The 2016 survey will be based on seven regular sampling periods (Section 6) and an additional eighth period that will be tasked with recording any horse mackerel spawning taking place beyond the finish date of the regular survey. Norway has withdrawn their participation from first the North Sea MEGS survey and now also from the Northeast Atlantic (NEA) survey, at a time when the continued expansion of the NEA mackerel stock has resulted in a spatial and temporal broadening of the mackerel spawning area and season. Additional information collated from winter surveys undertaken in 2014/2015 (Section 12) point toward a continuation of the early peak spawning as observed during the 2010 and the 2013 MEGS surveys. The impact of which will be that surveying in the western area will commence during the first week of February 2016, which is two weeks earlier compared to 2013.

The net result of all these factors is a further increase of potential survey input from the current participants within the defined spawning season. Only the participation of more states that currently exploit mackerel and horse mackerel will enable continued sufficient coverage of the spawning area over all the spawning periods of both species. In the event that additional nations are not forthcoming then for 2016 the additional surveys where there are currently no confirmed participants (TBA) will be absorbed by the existing nations within the WGMEGS community. Details of this together with the finalized survey plan will be reported to WGWIDE in August 2015.

In 2016, the MEGS survey will continue as an AEPM survey; however, as with the survey in 2013 the intention will be to also carry out intensive DEPM sampling in the peak spawning periods of both species in an attempt to calculate a DEPM SSB estimate. The periods highlighted as being the likely peak spawning periods are periods 2 + 3 for mackerel and period 6 + 7 for western horse mackerel. Fecundity analysis will be conducted by Norway (IMR), the Netherlands (IMARES), Scotland (MSS), Ireland (MI) and Spain (IEO and AZTI).

The WKFATHOM staging and fecundity workshops will take place during October in Hamburg (staging) and then in November in Bergen (fecundity). These workshops are essential for maintaining the quality assurance ahead of the mackerel and horse mackerel egg surveys and it is strongly advised that participating analysts attend these workshops where necessary. Final amendments to the egg survey /fecundity sampling planning schedules will be made during these workshops and uploaded to the respective manuals thereafter.

With Norway withdrawing their participation from the 2014 Mackerel Egg Survey in the North Sea, the Netherlands were left as its sole participant. Unfortunately, serious vessel problems encountered at the start of the survey resulted in its termination and eventual abandonment. IMARES are scheduled to repeat the survey in May/June 2015.

1 Administrative details

Working Group name

Working Group on Mackerel and Horse mackerel Egg Surveys (WGMEGS)

Year of Appointment

2015

Reporting year within current cycle (1, 2 or 3)

1

Chair(s)

Cindy van Damme, Netherlands

Finlay Burns, UK (Scotland)

Meeting venue

ICES Headquarters - Copenhagen, Denmark

Meeting dates

20–24 April 2015

2 Terms of Reference a) – z)

- a) Coordinate the timing and planning of the 2016 Mackerel/Horse Mackerel Egg Survey in the ICES Sub-areas VI to IX.
- b) Coordinate the planning of the sampling programme for mackerel/horse mackerel fecundity and atresia.
- c) Review and report on procedures for egg sample sorting, species identification and staging.
- d) Review and report on procedures for fecundity and atresia estimation.
- e) Update the survey manual and make recommendations for the standardization of all sampling tools, survey gears and procedures.
- f) Analyse and evaluate the results of the 2014 mackerel egg survey in the North Sea.
- g) Analyse and evaluate the suitability of the spawning fraction and batch fecundity data collected during the 2013 enhanced DEPM sampling programme within periods 3 and 5 for both species.

3 Summary of Work plan

Year 1	Planning of the egg survey in 2016 and reporting on the North Sea egg survey of 2014.
Year 2	Survey year, the Atlantic survey is conducted in 2016, no meeting takes place in year 2. A report, by correspondence, with the updated planning and manuals is published.
Year 3	Reporting and finalizing of the results of the 2016 egg survey. Planning of the 2017 North Sea egg survey.

4 List of Outcomes and Achievements of the WG in this delivery period

- Planning for the 2016 Atlantic mackerel and horse mackerel egg survey
- Finalize historic mackerel and horse mackerel egg dataset for inclusion in the ICES Egg and Larvae database
- Prepare input format for the ICES database for the mackerel and horse mackerel fecundity and atresia data
- CV estimates of egg production
- Development of Environmental Niche Model (ENM) to improve the egg survey. A distribution forecast system for the spawning of mackerel in the Northeast Atlantic.

5 Progress report on ToRs and workplan

- ToR a) the planning process for the 2016 MEGS survey has been completed and is reported in Section 6. Fine-tuning of the adopted plan and any subsequent amendments will take place intersessionally and will be reported in the WGMEGS correspondence report in April 2016. The final settled survey plan will be inserted into the WGMEGS Manual for the Mackerel and Horse Mackerel Egg Surveys (SISP ICES 6, 2014).
- ToR b) the planning for the adult mackerel/horse mackerel fecundity and atresia sampling has been completed and is reported in Section 7. The final adult sampling scheme will be inserted into the WGMEGS Manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel (SISP ICES 5, 2014). This will be subsequent to any amendments in the overall survey plan (Section 6) for the 2016 MEGS survey as any survey changes will invariably affect the adult sampling plan.
- ToR's c and e) The Manual for the Mackerel and Horse Mackerel Egg Surveys (SISP ICES 6, 2014) was reviewed and the findings reported in Section 8. A further review of the manual will be undertaken subsequent to the WKFATHOM staging workshop in October 2015.
- ToR d) The procedures for egg sampling sorting, species ID, staging, data submission and subsampling as detailed in the WGMEGS Manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel (SISP ICES 5, 2014) were reviewed and are reported in Section 9. A further

review of the manual will be undertaken subsequent to the WKFATHOM fecundity workshop in November 2015.

- ToR f) the mackerel survey in the North Sea did not take place in 2014. The reasons for this are fully explained in Section 10.
- ToR g) a presentation was delivered on the results from the enhanced DEPM sampling programme in 2013 for both mackerel and western horse mackerel. An abstract from this presentation can be located in Annex 3 – WD6. WGMEGS has agreed to continue with the DEPM sampling for both species during the 2016 survey and the plan for the DEPM sampling can be found in Section 7 of this report.
- **WGMEGS response to recommendations from other groups**
- In response to the recommendations from WGISDAA on the alternate transect design and its implications on the SSB estimate WGMEGS has agreed to relax the rules compelling survey participants to continue sampling along a transect until 2 consecutive samples of zero mackerel/horse mackerel eggs are found. Similarly, the requirement to comprehensively sample the north-west survey areas where the egg abundance was low and stable has also been relaxed. More survey effort needs to be diverted to the core spawning areas located further south where higher variability is likely to be encountered. These two recommendations will be implemented for the 2016 survey and will take place alongside a longer-term statistical analysis of the daily egg production variability of space and time, which it is anticipated will eventually result in a modification of the MEGS survey design. A full description of the recommendations are presented in Section 13.
- Together with the ICES data centre an input format for the fecundity and atresia data from the egg survey has been prepared. The historic database will be checked and prepared in the correct format and uploaded to the ICES database in future. WKPELA recommended that the total annual egg abundance (TAEP) and spawning-stock biomass (SSB) estimates be recalculated for the historic MEGS dataset from 1992 – 2013 using the Mendiola development equation which was adopted by WGMEGS in 2012. These were presented to WGWIDE in 2014. In addition, the newly created and quality checked historic MEGS egg data was uploaded to the ICES egg and larval database in 2014. These issues are addressed in Sections 11 and 14 respectively within the report. The winter sampling for mackerel eggs and adults that was also a recommendation from WKPELA was also completed in winter 2014/2015 resulting in four additional surveys that covered the area of peak abundance as reported in the 2013 MEGS survey. The results provided valuable information ahead of the 2016 MEGS survey and have aided the survey coordinator in planning for the triennial survey. This is detailed in Section 12 of the report.
- In 2014 WGWIDE recommended that WGMEGS discuss the possibility of identifying and counting blue whiting larvae during the triennial MEGS survey in 2016 with a view to better understanding the stock structure of this species. Whereas there appears to be no issue in identifying these larvae from the MEGS samples it was decided that due to the potentially very large numbers of larvae that are likely to be encountered during the surveys that quantitative analysis would not be possible for this species. WGMEGS however has agreed to collect qualitative presence/absence information for blue

whiting larvae during the 2016 MEGS survey. This issue is discussed further in Section 15 of the report.

- WGALES recommended an extension to the WKFATHOM identification and staging workshop to deal with additional species outwith the target species of mackerel and horse mackerel. WGMEGS endorses this recommendation and an additional day will be allocated to the WKFATHOM identification and staging workshop which will be held in Hamburg in October 2015.

6 Planning of the 2016 mackerel and horse mackerel egg survey in the western and southern areas (ToR a)

6.1 Countries and Ships Participating

Germany, Ireland, Netherlands, Scotland, Portugal, Spain, Spain/Basque Country, Iceland and the Faroe Islands will participate in the mackerel and horse mackerel egg surveys in the western and southern area in 2016. Provisional dates (where available), as well as vessel details, for the forthcoming surveys can be found in Table 6.1. The 2013 survey results highlighted several challenges; 1) the ongoing shift in mackerel peak spawning from April – June to February – March, 2) the significant expansion in the western mackerel spawning area from April to June, and 3) the horse mackerel egg production did not decline in July 2013. This has resulted in an inability to fully survey the whole spawning period (start of the mackerel spawning was missed and it is unclear if the end of the horse mackerel spawning season was covered) and the spawning area in April-July in 2013. Even with the implementation of an alternate, transect sampling strategy several boundaries remained unsecured. With a net reduction in available survey days for 2016, due to the withdrawal of the Norwegian survey, this situation is set to continue. Survey coverage of the western and southern areas is given by area and period in Table 6.2. Detailed maps of survey coverage by period are given in Figures 6.2.1 – 6.2.8. Both vessel availability and area assignments are provisional and will be finalized by the survey coordinator at the appropriate time.

Table 6.1 highlights four surveys as TBA. These refer to surveys where at the time of writing the survey participant was not yet confirmed. Efforts are continuing in attempting to attract additional nations to participate in the 2016 survey, however failing this then these additional survey commitments will be covered by nations from within the existing WGMEGS community. Details of this together with the finalized plan will be reported to WGWIDE in August 2015.

Survey participants are asked to supply their **provisional egg data weekly during their survey**, and the final data to the survey coordinator within one month of the end of their respective surveys. Survey leaders of period 6 and 7 surveys will supply their data as soon as possible after the end of these surveys

The survey coordinator for the 2016 survey will be Brendan O’ Hea, Marine Institute, Galway, Ireland.

Table 6.1. Countries, vessels, areas assigned, dates and sampling periods for the 2016 surveys.

Country	Vessel	Areas	Dates	Period
Portugal	Noruega	Cadiz, Portugal and Galicia	19th Jan – 22nd Feb	1
Spain (IEO)	TBA	Cantabrian Sea and Biscay	9th Mar – 1st Apr	2, 3
		Biscay and Cantabrian Sea	9th Apr – 1st May	4
Germany	W. Herwig III	West Ireland and Celtic Sea	21st Mar – 10th Apr	3
			11th Apr – 26th Apr	4
Netherlands	Tridens	Biscay and Celtic sea	13th April – 4th May	4
		Celtic Sea and Biscay	1st June – 22nd June	6
Spain (AZTI)	Ramón Margalef	Biscay	17th Mar – 9th Apr	3
	Emma Bardán	Biscay and Cantabrian Sea	4th May – 31st May	5
Ireland	Celtic Explorer	Celtic Sea and Biscay	4th Feb – 25th Feb	2
	Charter	West of Ireland and West of Scotland	1st June – 25th June	6
Scotland	Charter	West of Ireland and West of Scotland	February (two weeks)	2
	Scotia	West of Ireland and West of Scotland	10th May – 31st May	5
	Charter	Celtic sea, West of Ireland and West of Scotland	July (3 weeks)	7
Faroe Islands	Magnus Heinason	Faroes and Shetland	9th May – 23rd May	5
Iceland	Bjarni Saemundsson	Faroes and Shetland	9th June – 23rd June	6
TBA	Charter	West of Scotland	March (two weeks)	3
	Charter	West of Scotland	April (two weeks)	4
	Charter	Celtic Sea	May (two weeks)	5
	Charter	West of Ireland and west of Scotland	August (two weeks)	8

6.2 Survey Design

The AEPM survey design for mackerel and horse mackerel for 2016 will not change, however another attempt will be made to estimate DEPM adult parameters for both species. This will require additional (adult) sampling during the perceived peak spawning periods for both species. For the 2016 survey, this sampling will take place in periods 2 and 3 for mackerel, and periods 6 and 7 for horse mackerel (see Section 7). Due to the earlier start of the survey, in 2016 the survey will be split into seven sampling periods. Another sampling period (8, August) has been proposed to investigate whether the MEGS survey is capturing the end of horse mackerel spawning. The deployment of vessels to areas and periods is summarized in Table 6.2.

In 2013, the peak of mackerel spawning occurred in period 2. Moving the timing of period 2 forward and adding an extra sampling period and concentrating sampling effort in periods 2 and 3 is an attempt to ensure that the spawning peak is adequately sampled in 2016. Cruise leaders will be asked to adopt the adaptive survey design and 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 as having the highest densities of egg abundance. Particular points to note are:

Period 1

Only the southern area will be surveyed in period 1. This will be the Portuguese DEPM survey. (Figure 6.2.1).

Period 2

Period 2 marks the commencement of the western area surveys. The Irish survey will commence at the beginning of period 2 covering Biscay and the Celtic sea, sampling alternate transects initially. The first Scottish survey will commence in the middle of the period and will survey to the west of Ireland and Scotland. Spain (IEO) will start surveying in the Cantabrian Sea at the end of the period. (Figure 6.2.2)

Survey participants are requested to collect additional adult DEPM samples in periods 2 and 3 for mackerel (Section 7). It is also especially desirable that as far as is possible comprehensive survey coverage is achieved within the enhanced area (Figure 6.3.1) and this should be the prime consideration when completing the second sweep of the survey area during these periods.

Period 3

During period 3, surveys will be carried out by Spain (IEO), Spain (AZTI), Germany, and by another as yet unconfirmed nation (TBA). IEO will continue their survey, started at the end of period 2, in the Cantabrian Sea and extend it into southern Biscay. AZTI will survey the parts of Biscay not covered by IEO. Germany will cover the Celtic Sea and the west of Ireland. Another nation (TBA) will provide a vessel to survey the area west of Scotland as well as northwest Ireland (Figure 6.2.3).

Period 4

During period 4 sampling will be carried out by four vessels. IEO will carry out their survey in the Cantabrian Sea and south of Biscay. The Netherlands will sample in the north of Biscay and part of the Celtic Sea, with Germany conducting their second survey in the north of the Celtic Sea and west of Ireland. Another nation (TBA) will conduct a survey to the west of Scotland. AZTI will start a targeted DEPM survey for anchovy in the Cantabrian Sea during the last week of the period. Although this AZTI survey provides mackerel and horse mackerel egg samples as well, the design of this survey is constrained in that purpose (Figure 6.2.4). In an attempt to complete the additional MEGS stations allocated to them within the western area AZTI will apply for additional survey days to cover this additional area.

Period 5

In period 5 AZTI will continue with their targeted DEPM survey for anchovy in the Cantabrian Sea and will also sample in Biscay. Two vessels have to cover the entire area of spawning from northern Biscay to the north of Scotland. An as yet unconfirmed

nation will cover the Celtic Sea. Scotland will survey to the west of Ireland and Scotland. In addition Faroes will provide a 2 week survey which will cover the area to the north of 58° 30'N. This will expand the survey range and attempt to secure a northern boundary within this period. See Figure 6.2.5 for survey areas, however these are provisional and definitive survey areas as well as starting positions will be provided by the survey coordinator closer to the survey start, and will largely be dependent on what is observed in period 4. Providing adequate survey coverage during this period will be challenging.

Period 6

In period 6 three vessels will survey the area between Biscay and the Northern area. The Netherlands will survey in Biscay and the Celtic sea with Ireland surveying west of Ireland and west of Scotland. Iceland will survey north of 58° 30'N. As in period 5 this will expand the survey range and attempt to secure a northern boundary within this period (Figure 6.2.6). The Dutch vessel will commence the survey along the southern boundary of the designated area although its exact latitude will depend on the results from period 5.

In 2016, survey participants are requested to collect additional adult horse mackerel samples during periods 6 and 7 ((detailed instructions on this can be located in Section 7). However, as with periods 2 and 3 every effort should be made to achieve as comprehensive coverage as is possible within the enhanced area (Figure 6.3.2).

Period 7

In period 7, only one vessel will be available, and will have to cover the entire spawning area. This assignment will be undertaken by Scotland. As with period 6 the southern boundary (starting location) will be dictated by the results of the previous period. Irrespective of this an alternate transect design will be necessary (Figure 6.2.7).

Period 8

Concern has been expressed as to whether the MEGS surveys are capturing the end of horse mackerel spawning sufficiently (see Section 12). It has been proposed to carry out an additional survey period covering the areas west of Ireland and Scotland to investigate the possibility of horse mackerel spawning continuing into August (Figure 6.2.8). This extra period will be carried out by an as yet unconfirmed nation and vessel (TBA).

Table 6.2. Periods and area assignments for vessels by week for the 2016 survey. Area assignments and dates are provisional.

week	Starts	Area							Period
		Portugal, Cadiz & Galicia	Cantabrian Sea	Biscay	Celtic Sea	Northwest Ireland	West of Scotland	Northern Area	
1	18-Jan-16	PO1(DEPM)							1
2	25-Jan-16	PO1(DEPM)							1
3	1-Feb-16	PO1(DEPM)		IRL1	IRL1				2
4	8-Feb-16	PO1(DEPM)		IRL1	IRL1				2
5	15-Feb-16	PO1(DEPM)		IRL1	IRL1				2
6	22-Feb-16	PO1(DEPM)		IRL1	IRL1	SCO1	SCO1		2
7	29-Feb-16					SCO1	SCO1		2
8	7-Mar-16		IEO1						2
9	14-Mar-16		IEO1	IEO1/ AZTI1					3
10	21-Mar-16		IEO1	IEO1/ AZTI1	AZTI1/ GER	GER	TBA		3
11	28-Mar-16		IEO1	AZTI1	AZTI1/ GER	GER	TBA		3
12	4-Apr-16			AZTI1	GER	GER			3
16	11-Apr-16		IEO2		GER	GER			4
14	18-Apr-16		IEO2	IEO2/ NED1	NED1/ GER	GER	TBA		4
15	25-Apr-16		IEO2	IEO2/NED1	NED1/GER	GER	TBA		4
16	2-May-16		IEO2/AZTI2(DEPM)	NED1	NED1				4
17	9-May-16			AZTI2(DEPM)	TBA	SCO2	SCO2	FAR	5
18	16-May-16			AZTI2(DEPM)	TBA	SCO2	SCO2	FAR	5
19	23-May-16		AZTI2(DEPM)	AZTI2(DEPM)	TBA	SCO2	SCO2	FAR	5
20	30-May-16			NED2	NED2	IRL2	IRL2		6
21	6-Jun-16			NED2	NED2	IRL2	IRL2	ICE	6
22	16-Jun-16			NED2	NED2	IRL2	IRL2	ICE	6
23	20-Jun-16					IRL2	IRL2		6
24	27-Jun-16								7
25	4-Jul-16				SCO3	SCO3	SCO3		7
26	11-Jul-16				SCO3	SCO3	SCO3		7
27	18-Jul-16				SCO3	SCO3	SCO3		7
28	25-Jul-16								7
29	1-Aug-16								8
30	8-Aug-16					TBA	TBA		8
31	15-Aug-16					TBA	TBA		8
32	22-Aug-16								8

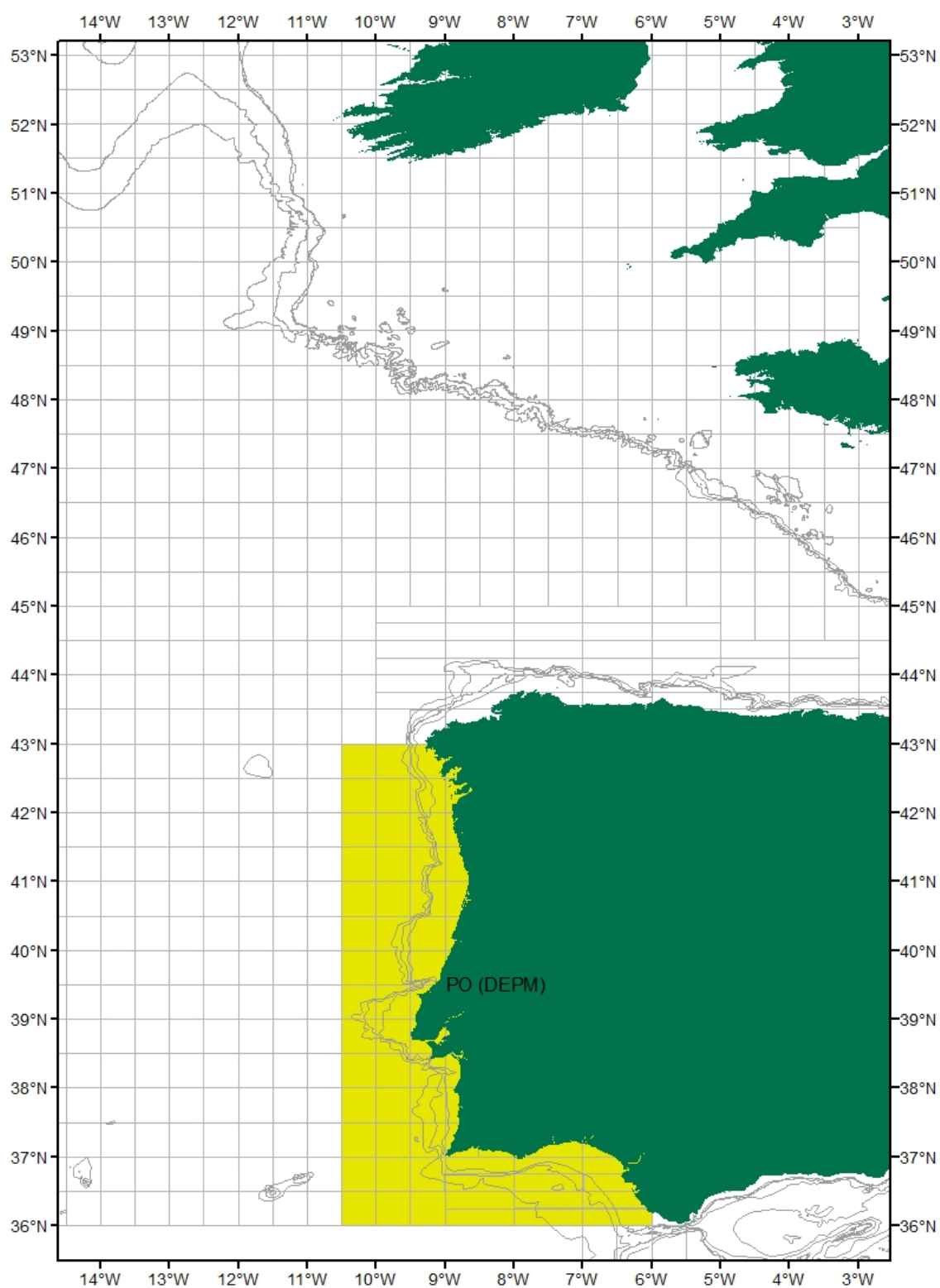


Figure 6.2.1. Survey plan for Period 1.

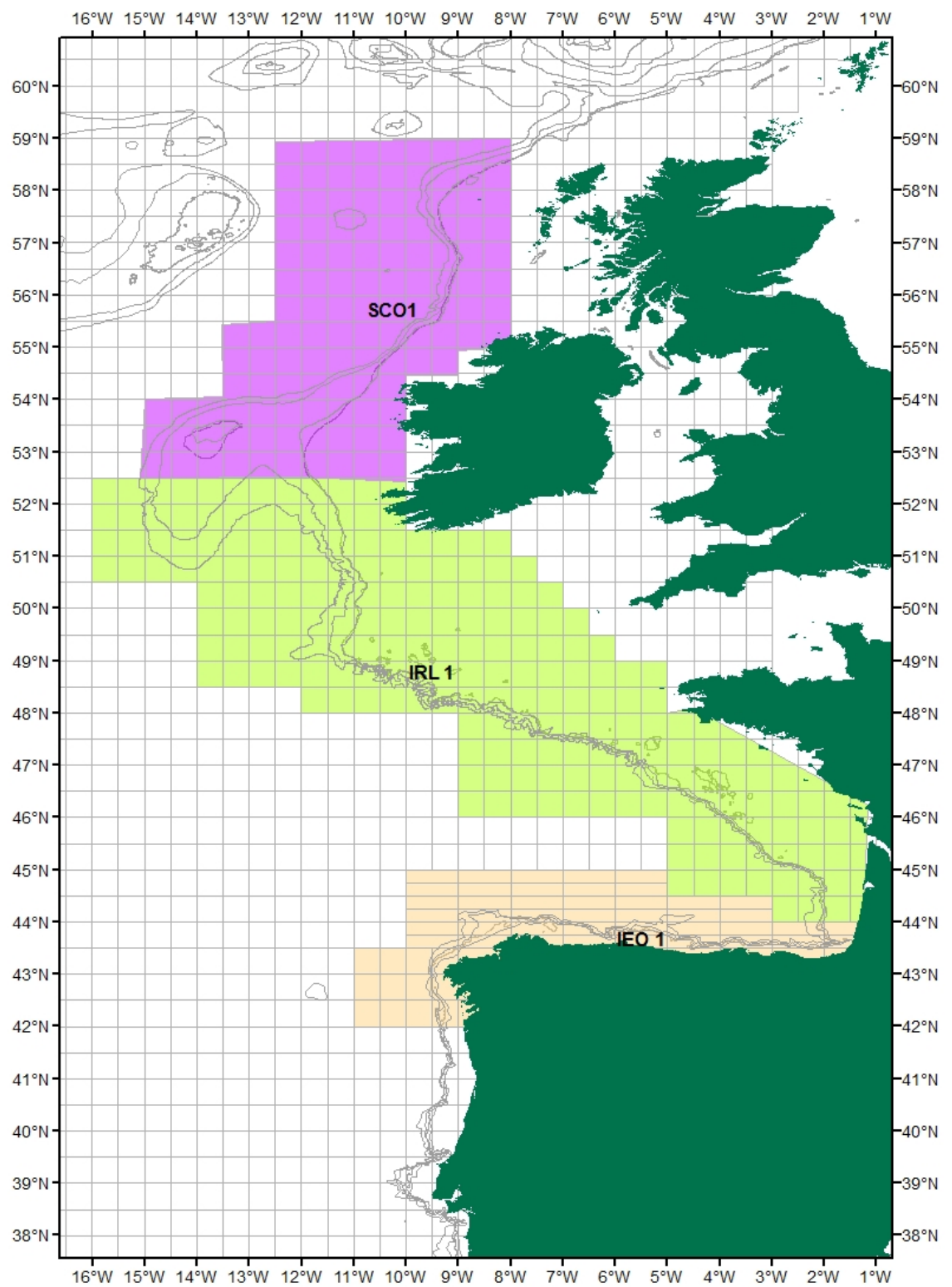


Figure 6.2.2. Survey plan for Period 2.

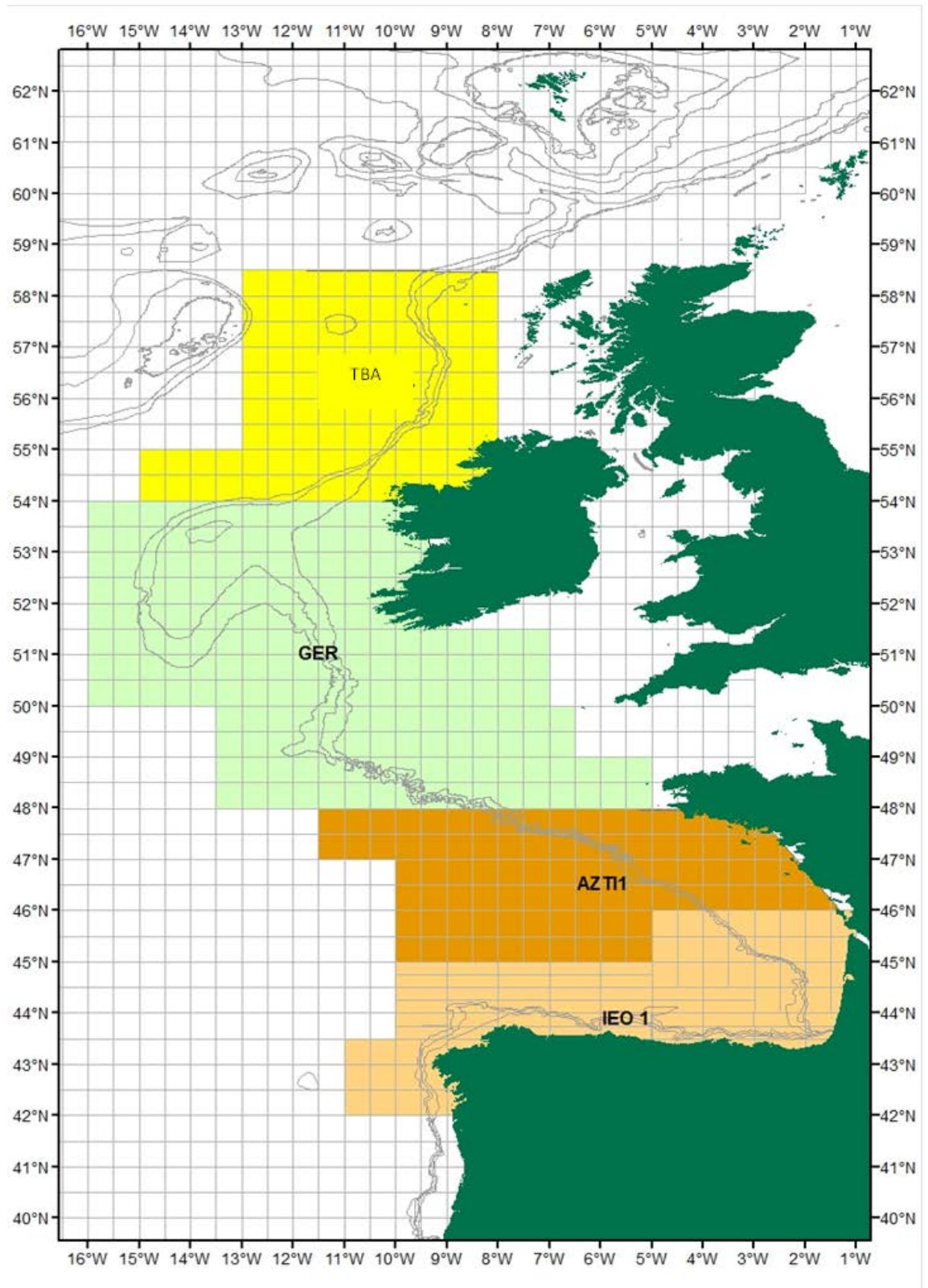


Figure 6.2.3. Survey plan for Period 3.

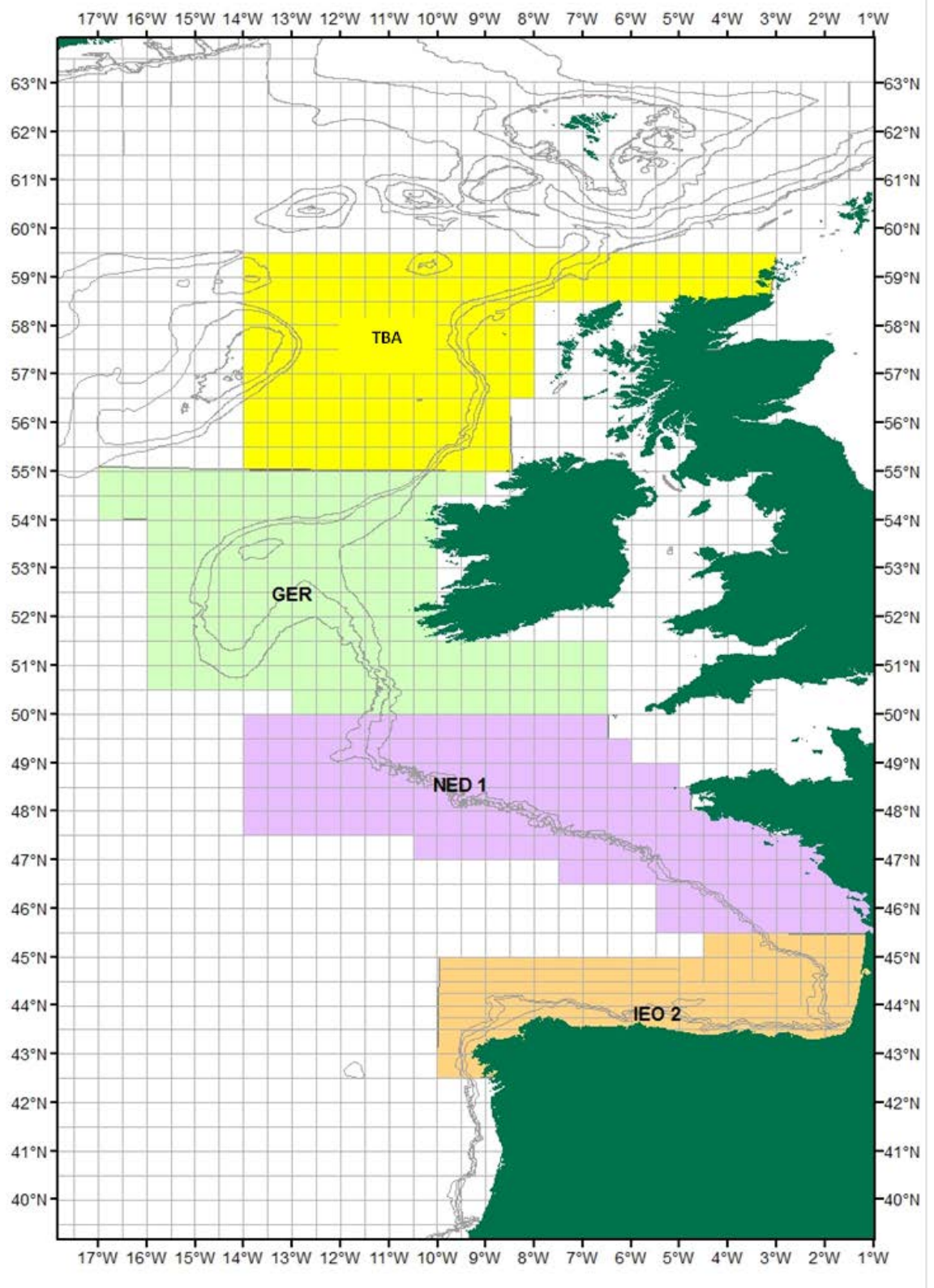


Figure 6.2.4. Survey plan for Period 4.

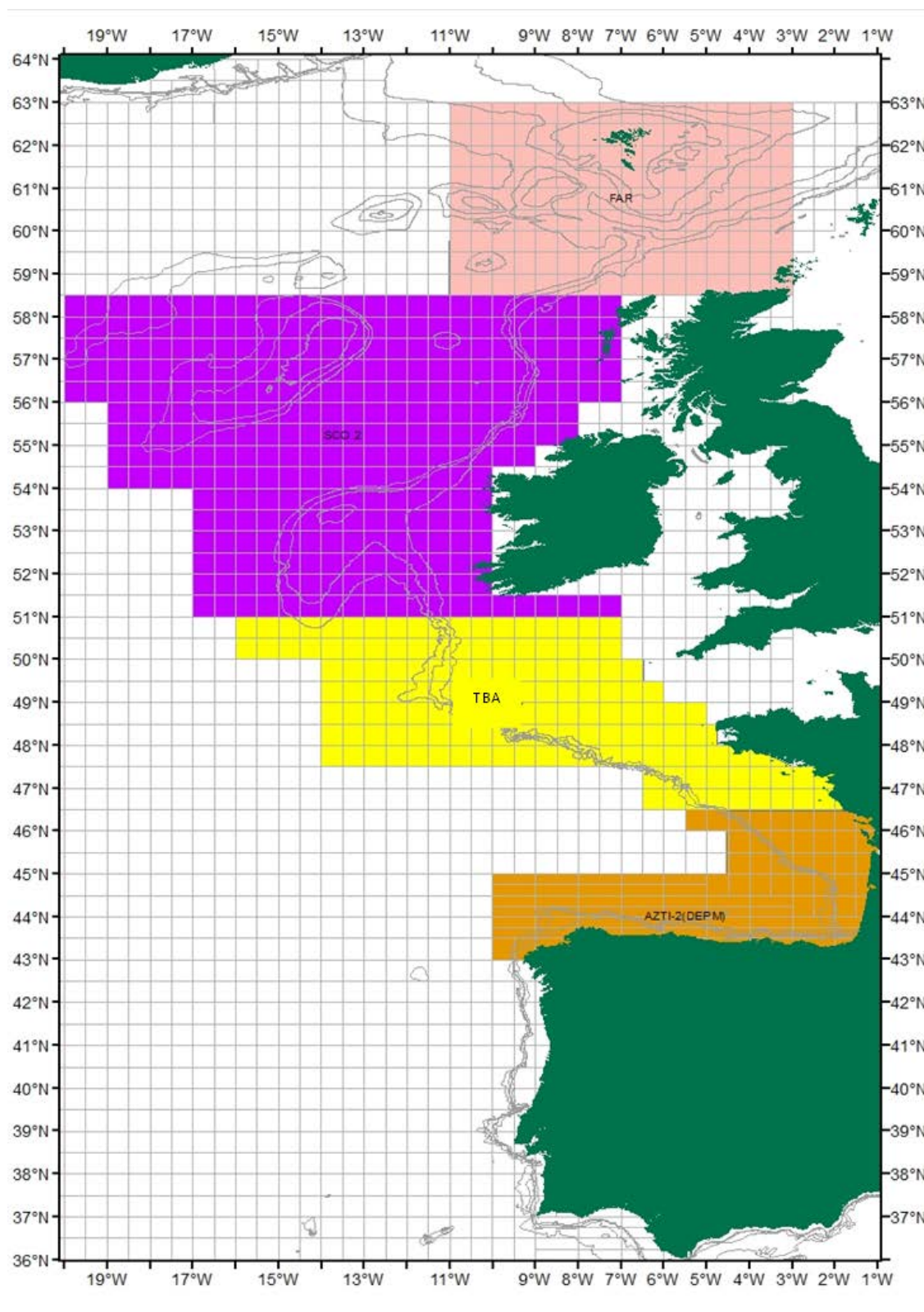


Figure 6.2.5. Survey plan for Period 5.

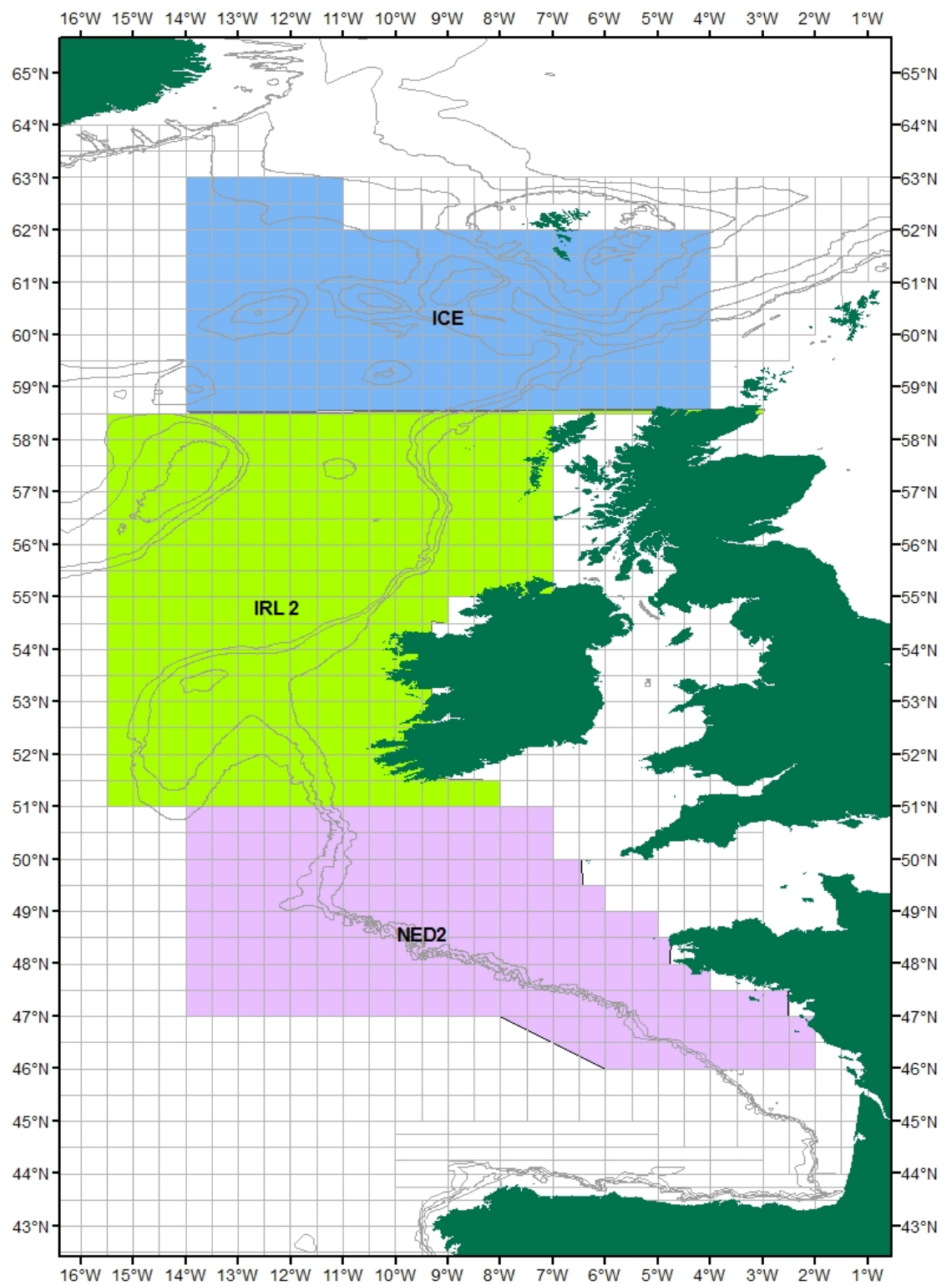


Figure 6.2.6. Survey plan for Period 6.

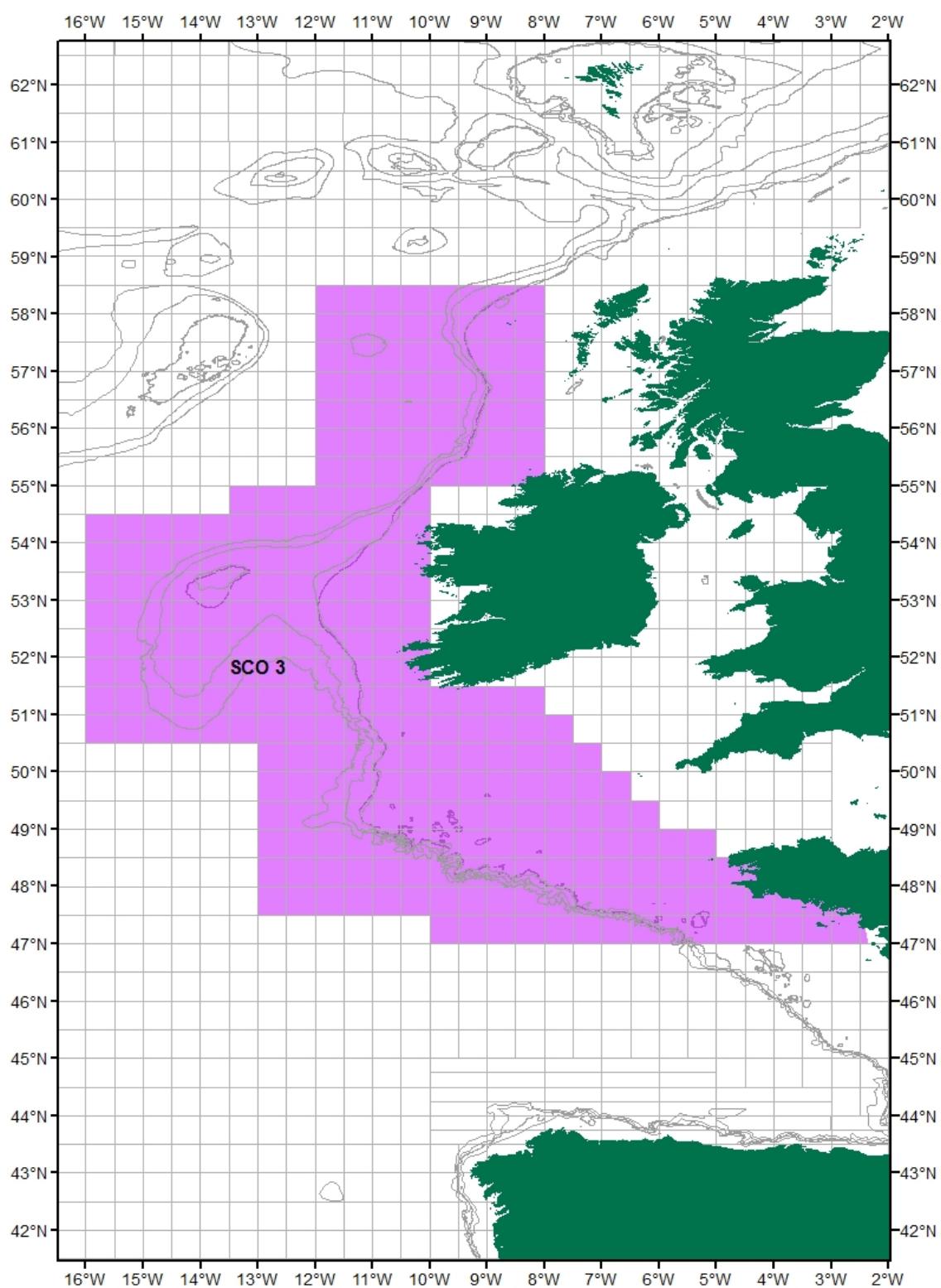


Figure 6.2.7. Survey plan for Period 7.

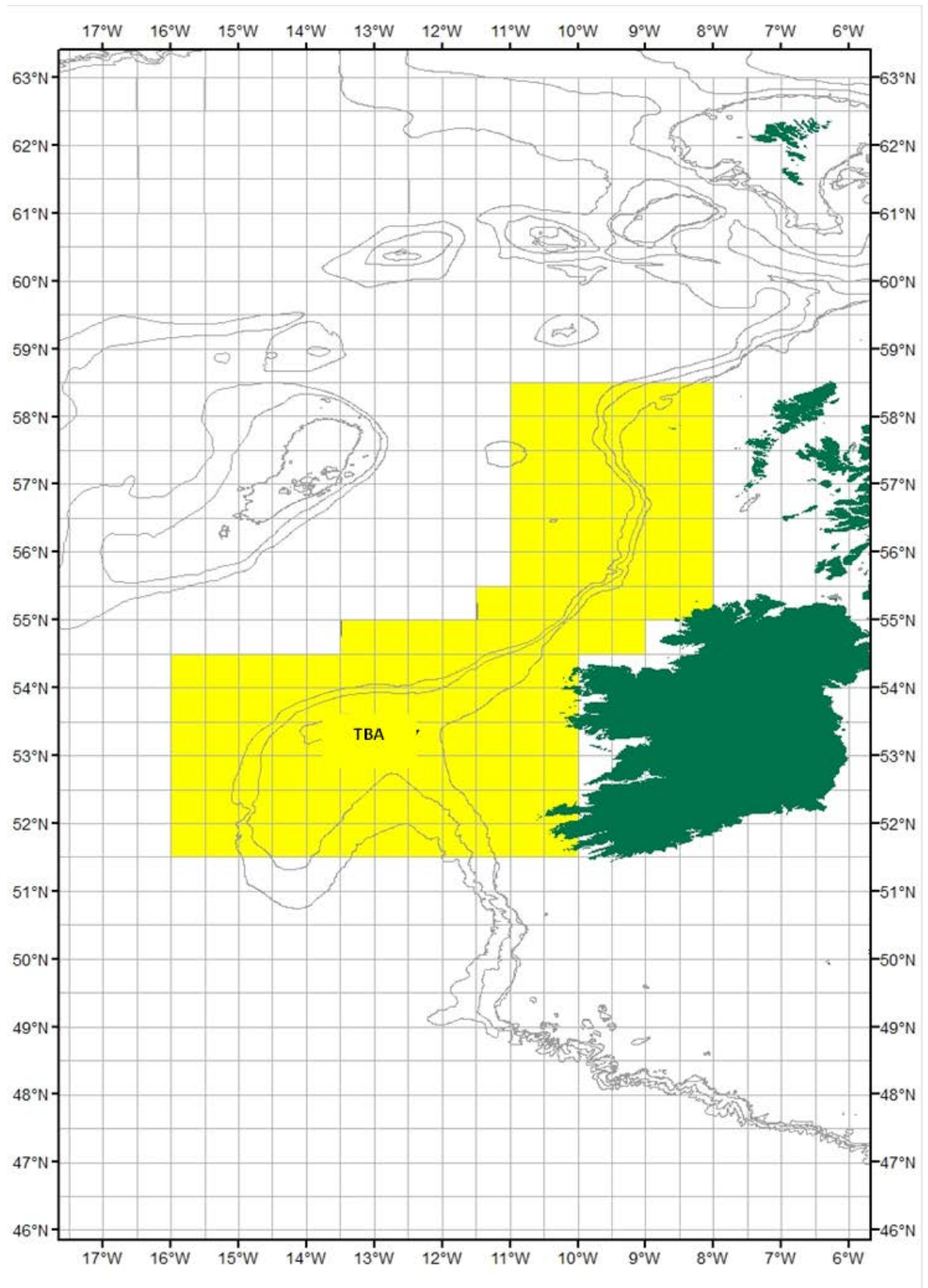


Figure 6.2.8. Survey plan for Period 8.

6.3 Sampling Areas and Sampling Effort

As in 2013, it was decided that the spatial and temporal distribution of sampling would be designed to ensure maximum coverage of both mackerel and horse mackerel spawning and that estimates of stage 1 annual egg production will be made for both species. This will be continued in 2016.

Since the first survey in 1977, considerable changes have been made to the standard sampling area (see Section 8.4 of ICES, 1994). Based on the steady expansion of the “standard area” with every subsequent survey, WGMEGS reconsidered its use. The “standard area” should be retained **only as a guide** to the core survey area for cruise leaders, and the extent of coverage should be decided based on the delineation of the edges of the egg distribution only, i.e. boundaries should be set based on the adaptive sampling guidelines (SISP 6, ICES, 2014). The core areas for the western and southern surveys for both species are presented in Figures 6.3.1 and 6.3.2. A more detailed survey map of the Iberian areas as surveyed by IEO and IPMA can be found in Figures 6.3.3 and 6.4.1. Section 6.4 also provides a description of the Portuguese DEPM survey.

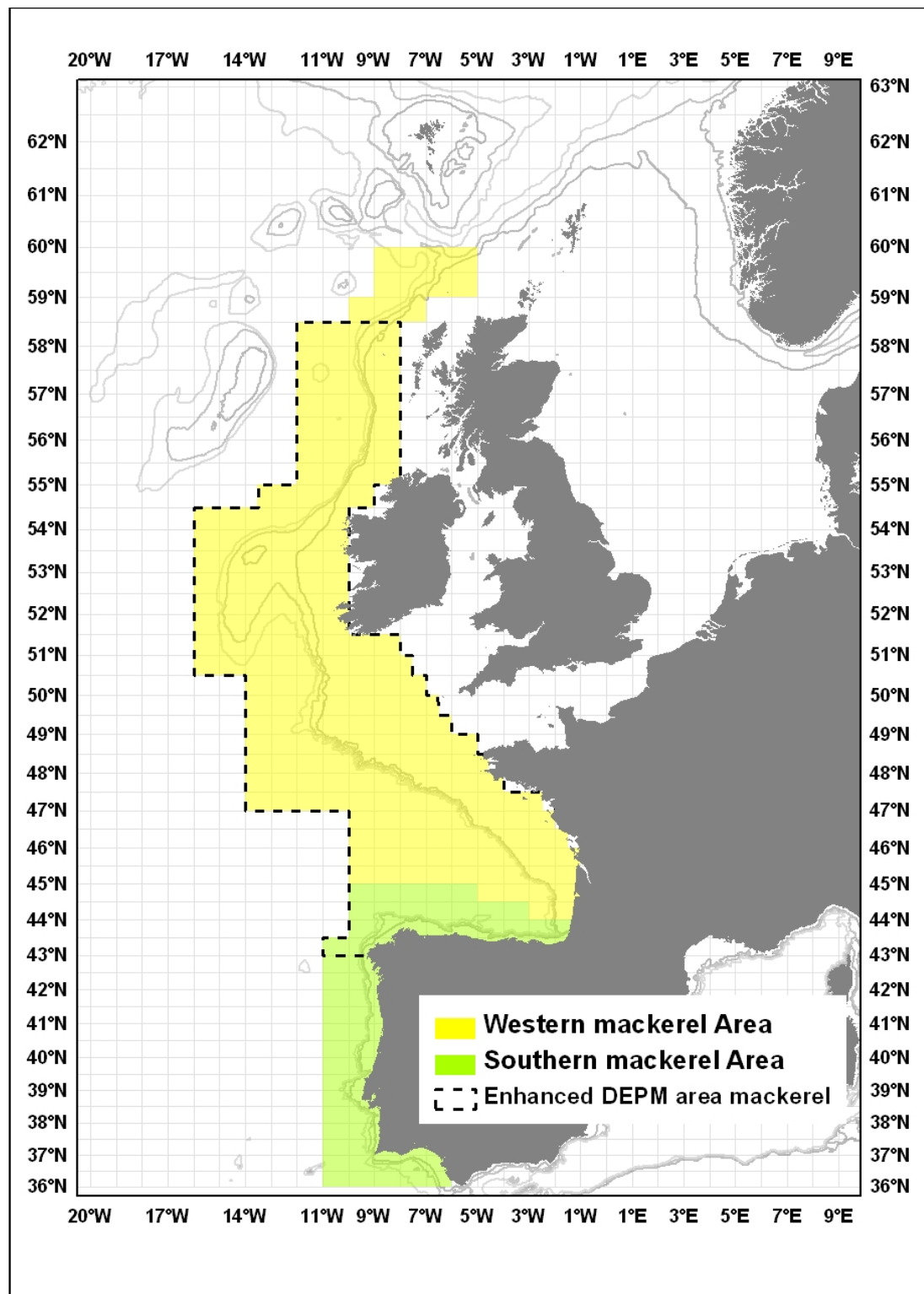


Figure 6.3.1. Core sampling areas for mackerel eggs in the western and southern areas for 2016. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines.

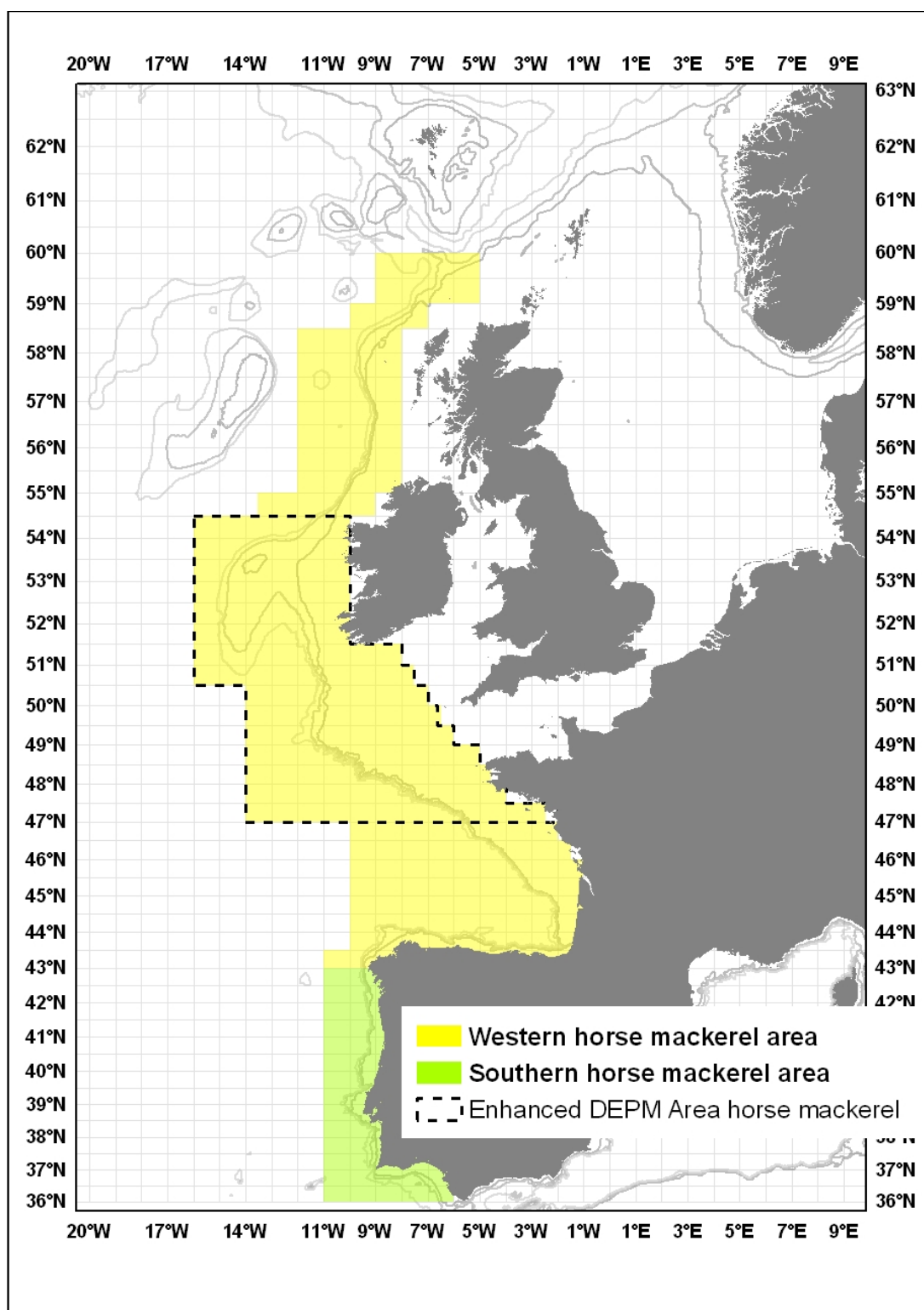


Figure 6.3.2. Core sampling areas for horse mackerel eggs in the western areas for 2016. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines.

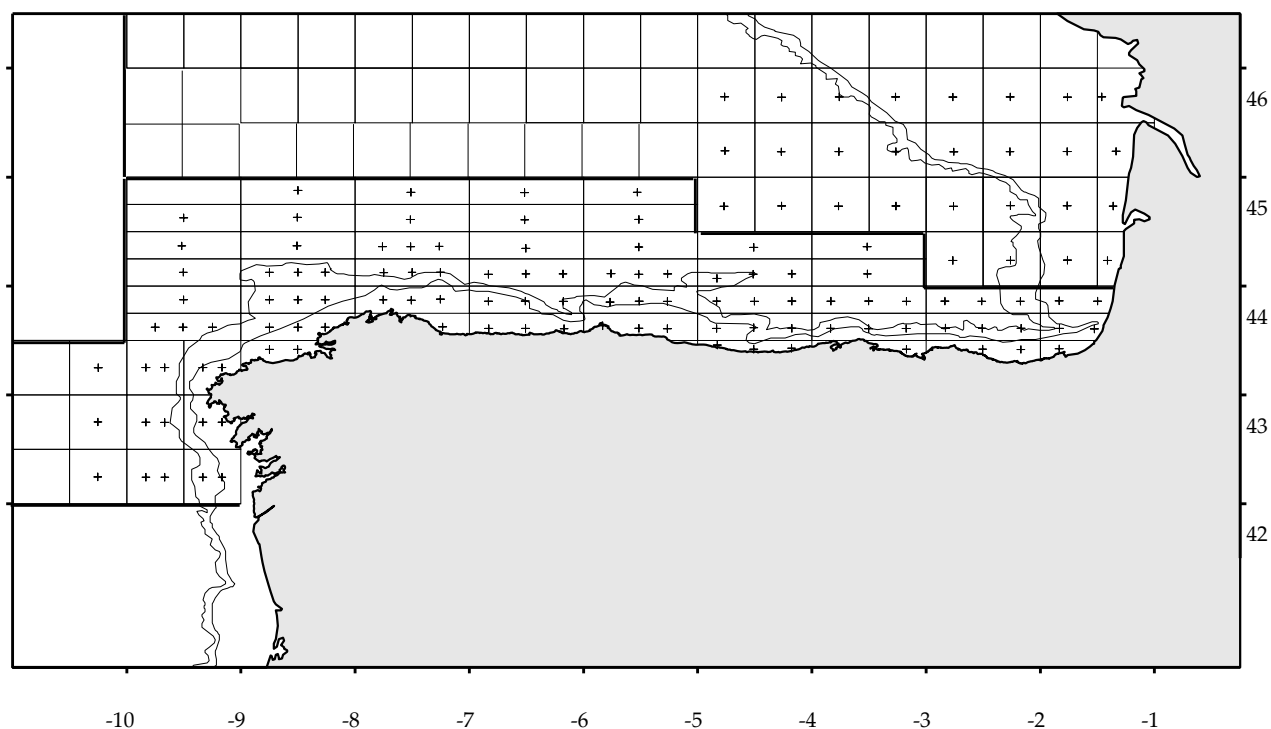


Figure 6.3.3. IEO sample locations for Galicia and the Cantabrian Sea.

6.4 DEPM survey for the southern stock horse-mackerel

Background

The horse mackerel of the southern stock ((ICES IXa - Gibraltar-Finisterre) is sampled by Portugal (IPMA) using the Daily Egg Production Method (DEPM) approach. Several improvements in the survey design, sampling gear, laboratory and data processing procedures have been introduced since the DEPM application started in 2007. Developments carried out include: (i) the new sampler, the CalVET system with double rings with 40cm mouth aperture; (ii) the setting up of a genetics analysis procedure for assessing potential egg misidentification with the eggs of other *Trachurus* species; (iii) implementation of a semi-automated method using image analysis to count the oocytes of the spawning batch for fecundity estimation; (iv) histomorphological and size description of the post-ovulatory follicle (POFs) degeneration, POFs being used as spawning markers for spawning fraction estimation; and (v) studies to address the daily spawning period definition.

6.4.1 Surveying

The DEPM survey will take place during January-February 2016, onboard RV 'No-ruega', covering the area from Gibraltar to Finisterre (Figure 6.4.1).

6.4.2 Plankton Surveying

Equipment

- adapted CalVET structure: 2 nets (Ø 40 cm), mesh 150 µm + CTDF + flow-meters
- Bongo structure: net (Ø 40 cm), mesh 250 µm + flowmeters

Sampling

- main sampler: plankton vertical hauls at grid points (200 m depth, or 3–5 m from bottom to surface; hauling 1m/s; to be repeated if angle deviates from vertical more than 30°)
- auxiliary sampler: plankton double oblique hauls (at 2 knots, from surface to 200 metres depth, or 3–5 m from bottom to surface)
- auxiliary sampler: underway CUFES samples (every 3 nmiles, between CalVET stations)

Sampling design

Plankton surveying will be undertaken following a predefined grid of sampling stations along transects perpendicular to the coast and separated by 12 nmiles (Figure 6.4.1). About 560 CalVET stations (3 nmiles apart) are planned along the 48 transects. The grid of stations is designed in an attempt to cover the whole potential spawning area with good spatial resolution within the surveying time available. However, it is not possible to anticipate if the offshore limit of the spawning grounds will be adequately surveyed. In past campaigns, some eggs were collected at stations on the edges of the survey area, over great depths (ICES, 2008). Identification of the eggs on collection is not feasible since it is a time consuming task needing experienced analysts.

Double oblique tows using a Bongo net (standard gear for the AEPM) will be carried out for selectivity comparisons between Bongo and CalVET hauls. Bongo samples will be taken opportunistically and will be dependent on time availability (~ 2 per transect).

Sample processing

The nets will be rinsed from the outside with seawater and the samples from the twin nets stored in separate containers, one preserved in formalin (at 4% in distilled water, buffered with sodium borate), the other one in ethanol (to allow the use of the eggs for genetic studies).

In the laboratory, all horse mackerel eggs will be counted and staged according to the 11 stage of development scale of classification (Cunha *et al.*, 2008). *Scomber* spp. eggs will be sorted and counted.

6.4.3 Environmental Surveying

- temperature, salinity, fluorescence – vertical profiles (CTDF)
- temperature, salinity, fluorescence – 3 m depth, underway (CTDF associated with CUFES)
- water filtrate (nitrate cellulose filters) for chlorophyll calibration (frozen filters for lab processing)

Temperature data from the CTD casts will be used for the egg ageing procedure. Other environmental variables will be used for hydrographic and spawning area characterization.

6.4.4 Adult Surveying

Surveying for adult horse mackerel fish will take place simultaneously with the ichthyoplankton sampling. An average of 2 fishing hauls, with bottom trawl, will be conducted per day, along the whole survey area. Good spatial and temporal coverage is essential for avoiding bias on fecundity and spawning fraction estimations. In order to

complement sampling, fish from commercial vessels will be obtained at 4 or 5 ports along the coast during the period of the campaign. Methodological and technical issues are detailed in the WGMEGS manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel.

Fish sampling

From each trawl, a sample of 60 fish will be randomly selected and sampled biologically onboard. The biological data will be used to estimate the mature fraction of the population and to estimate the sex ratio and female mean weight for each haul. Total length, total weight, sex, maturity stage, level of fat and stomach fullness will be recorded. For the first 30 females encountered (of all macroscopic maturity stages), the gonads will be immediately collected and preserved in formaldehyde solution (4% formaldehyde, buffered with sodium phosphate in distilled water). The otoliths of these 60 fish will be removed for ageing. In case the random sample of 60 fish contains less than 30 females for histology, continue collecting and sampling fish from the catch until this number is reached (up to a total number of 100 fish, when sampling for this haul is considered finished). In addition, extra effort will be placed to obtain spawning females (macroscopic stage 4) for batch fecundity estimation. Ideally, 150 females (but no more than 30 fish per trawl) should be obtained along the coast. Standard biological sampling including gonad preservation and otoliths collection will be conducted. Hauls with less than 30 fish will only be sampled for batch fecundity and female total weight; therefore, if less than 30 fish are caught all fish will be sampled, but only gonads in stage 4 will be collected and preserved in formaldehyde solution.

The sampling procedure for the samples coming from commercial vessels will be adjusted according to the fishing operation (gear and time before reaching the port) and facilities onboard and at the ports, but in case fish collected by the fleet need to be frozen for subsequent biological sampling, gonads are previously preserved as fresh material.

Mackerel sampling will be carried out whenever possible to support the EPM estimation undertaken by the WGMEGS.

Laboratory analysis

The preserved gonads will be weighed, a tissue sample taken from one of the lobes, then dehydrated with alcohol and embedded in paraffin. The resulting blocks will be sectioned (3–5 μm thick), mounted on slides and stained according to Harris' Hematoxylin and Eosin procedure. The analysis of the slides will produce information in order to 1) confirm microscopically the maturity stage; 2) be used in the estimation of the spawning fraction; and 3) check for the presence of post-ovulatory follicles (POFs) in the hydrated ovaries (not to underestimate batch fecundity). Batch fecundity will be obtained from hydrated females, by means of the gravimetric method applied to the hydrated oocytes, and using Image J automated routines applied to digitalized images (Hunter *et al.*, 1985, Gonçalves *et al.*, 2012). In case hydrated females are scarce in the samples, the gravimetric method may also be applied to migratory nucleus stage oocytes (Ganias *et al.*, 2010; Gonçalves *et al.*, 2012). For spawning fraction estimation, the optimal methodology is still not fully established, but different spawning markers will be first considered and compared (migratory nucleus and hydrated oocytes, POFs). In case the POFs method will be used to calculate spawning fraction, though the degeneration rate of POFs is still unknown for horse mackerel, staging of POFs will be based on both histomorphological (Gonçalves *et al.*, 2005) and biometrical criteria (cross sectional area, Ganias *et al.*, 2007), those criteria being then related to the time of capture

in order to attempt assigning each POF to a daily cohort (assuming a daily spawning synchronicity, Annex 3 (WD 7). Age reading from otoliths will allow the construction of a microscopic female maturity ogive to be used in assessment estimations.

6.4.5 Data Analyses

Data analyses will be undertaken using R routines, including for eggs data adapted versions of the R packages (geofun, eggsplore and shachar) available at ichthyoanalysis (<http://sourceforge.net/projects/ichthyoanalysis>). Results will be compiled to be presented at the 2017 WGMEGS meeting.

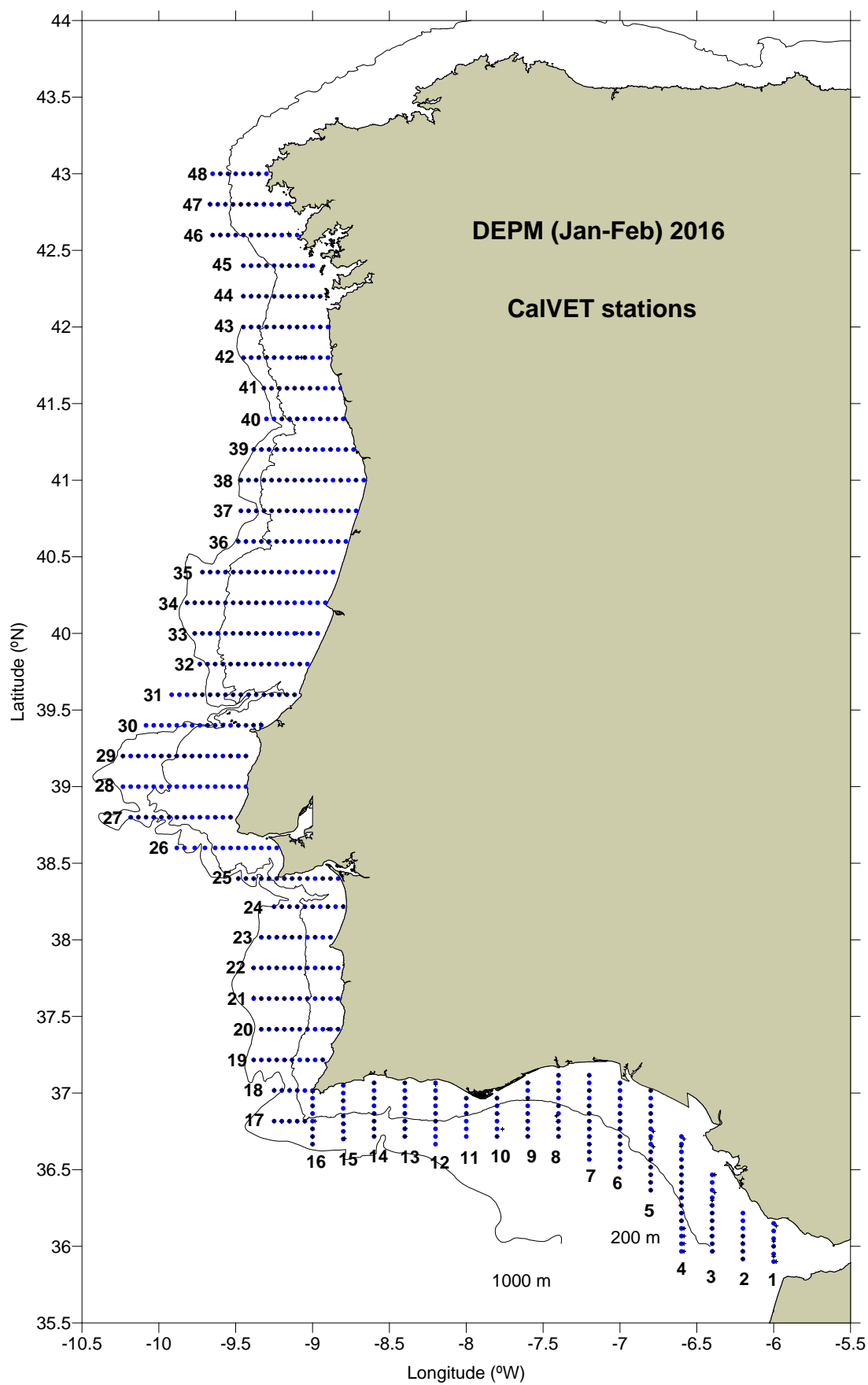


Figure 6.4.1. Sampling grid for CalVET stations.

7 Planning and sampling programme for the mackerel and horse mackerel AEPM and DEPM adult parameters (ToR b, g)

7.1 Sampling for mackerel fecundity and atresia in the Western and Southern areas

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 potential fecundity and atresia across the entire survey area aiming to complete a wide-spread sampling regime for adults shown in Tables 7.1.1 a-b. The purpose of this table is not to exactly specify the time and location of trawl hauls but to give an impression of how trawl hauls should be dispersed in time and space, and the numbers of samples required for the estimation of realized fecundity.

For a correct estimate of potential fecundity, ovary samples will be collected from prespawning fish from commercial catches in period 2. However, as mackerel spawning appears to have shifted to an even earlier date, monitoring of mackerel maturity will now commence from December 2015 so that if necessary, prespawning sampling can be brought forward to **period 1**.

In **period 2 and 3**, the period of expected 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 7.1.2). It is recommended that trawling is preferably carried out at dusk or during the night.**

Fecundity sampling (numbers of fish Southern Area (Cantabrian and Biscay))

Fecundity sampling (numbers of fish Southern Area (Cantabrian and Biscay))

MACKEREL

Lon °

Southern Area (Cadiz to Galicia)

Lat °

[illegible]

36N	37	38	39	40	41	42
10						
		10				
			10			
					10	

[illegible]

		per period			
		1	2	3	4
IEO			100		
AZTI					
IPIMAR		20	20		
	Total:	20	120	0	

Tab. 7.1.1b: Desired temporal and spatial distribution of the mackerel fecundity sampling in the Western Area																																				
Fecundity sampling			Western Area																																	
MACKEREL			Lat °																																	
Week	Date	Period*	42N	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	Total	per period										
																											1	2	3	4	5	6	7			
1	18-Jan-16	1																							0	AZTI			150		30					
2	25-Jan-16	1																							0	TI			360	50						
3	01-Feb-16	2																							0	MI		510				70				
4	08-Feb-16	2																							0	SCO		360			90	90				
5	15-Feb-16	2																							0	IMARES			50		50					
6	22-Feb-16	2																							0	TBA			270	45	45					
7	29-Feb-16	2																							0	TBA		150	210	50						
8	7-Mar-16	2																							0	FAR				30						
9	14-Mar-16	3																							0	ICE					20					
10	21-Mar-16	3																							0	Total:	0	1020	990	195	195	140	90			
11	28-Mar-16	3																							0											
12	04-Apr-16	3																							0											
13	11-Apr-16	4			15									15					15						45											
14	18-Apr-16	4	10						15		10	10			15										60											
15	25-Apr-16	4	10				20							15					15						60											
16	2-May-16	4			15				15																30											
17	9-May-16	5				10				15					15	15				10					65											
18	16-May-16	5			10			15				15	15							10					65											
19	23-May-16	5				10				15					15	15				10					65											
20	30-May-16	6							15							10	10								35											
21	06-Jun-16	6					20							15						10					45											
22	13-Jun-16	6							15				15							10					40											
23	20-Jun-16	6														10	10								20											
24	27-Jun-16	7																							0											
25	04-Jul-16	7												10		10		10							30											
26	11-Jul-16	7						10		10		10													30											
27	18-Jul-16	7											10		10		10								30											
28	25-Jul-16	7																							0											

See table 7.1.2 for the DEPM and AEPM sampling

Tab. 7.1.2: Desired mackerel adult sampling in the area selected for the 2016 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	46.45	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	18-Jan-16	1																																			0	AZTI	
2	25-Jan-16	1																																			0	VTI	
3	01-Feb-16	2														30		30		30		30		30													150	MI	
4	08-Feb-16	2						30		30		30		30																								120	SCO
5	15-Feb-16	2							30		30		30		30																							120	IMARES
6	22-Feb-16	2															30		30		30		30															120	TBA
7	29-Feb-16	2																								30		30		30		30		30		30		180	IEO
8	7-Mar-16	2	30	30	30	30	30																		30		30		30		30		30		30		330	FAR	
9	14-Mar-16	3	30		30		30				30																											120	ICE
10	21-Mar-16	3						30	30				30		30		30		30		30		30									30		30		30		330	total:
11	28-Mar-16	3		30		30								30									30		30	30	30	30	30	30							300		
12	04-Apr-16	3									30					30		30		30		30		30									30		30		240		
13	11-Apr-16	4																																				0	
14	18-Apr-16	4																																				0	
15	25-Apr-16	4																																				0	
16	2-May-16	4																																				0	
17	9-May-16	5																																				0	
18	16-May-16	5																																				0	
19	23-May-16	5																																				0	
20	30-May-16	6																																				0	
21	06-Jun-16	6																																				0	
22	13-Jun-16	6																																				0	
23	20-Jun-16	6																																				0	
24	27-Jun-16	7																																				0	
25	04-Jul-16	7																																				0	
26	11-Jul-16	7																																				0	
27	18-Jul-16	7																																				0	
28	25-Jul-16	7																																				0	

Fecundity sampling will be stratified according to weight (Table 4.1.4) except for the enhanced DEPM sampling in period 2 and 3. In period 2 and 3 the females will be taken randomly from the catch. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes in Table 7.1.3 below. In order not to concentrate the sampling on spawning fish it is preferable that trawling is not concentrated on the 200 meters depth contour but is adapted to fit in conveniently with the egg survey along the transects over the continental shelf. Details of preparation for fecundity sampling at sea are shown in Table 7.1.4.

Table 7.1.3. Weight classes for sampling females of maturity stages 2–6 (Walsh *et al.*, 1990) for potential fecundity and atresia.

Weight category [g]	<250	251 – 400	401– 550	>551	Total
Number of fish	5	5	5	5	20

Table 7.1.4 Protocol for processing and distribution of mackerel ovary subsamples for fecundity and atresia analysis.

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

The coordinator will assign unique codes to each participating cruise.

Procure Nunc type tubes and place in suitable racks.

Attach a spot label to the Nunc lid and add 1.2 ml of 3.6% formaldehyde buffered with 0.1M sodium phosphate (referred to below as 'fixative') to each tube using a dispenser. Prepare 4 tubes for each fish and label.

Procure sample bottles for the remaining ovary tissue (bottles should have parallel walls and without a restricted neck opening (otherwise we cannot extract the ovary without cutting of the jar top)). The largest ovaries will require 250 ml sample bottles but in many cases, a 100 ml or smaller capacity jar will be adequate. Label the bottle with the Nunc code and cruise.

Procure scintillation tubes for screening.

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

Procedures to follow at sea to collect samples and for sample analysis in the laboratory are shown in Tables 7.1.5, 7.1.6, 7.1.7 and the Fecundity manual (to be finalized at WKFATHOM 2015) respectively. In order to compare estimates of fecundity by each institute ring tests will be carried out. All samples will be sent around and will be analysed by different institutes. Overall targets for estimating realized fecundity are shown in Table 7.1.8. Provisional reporting of estimates for potential fecundity and atresia are required for the 2016 WGwide group in September and final results for WGMEGS in spring 2017. The results of the DEPM analyses will be reported to the WGMEGS meeting in spring 2017. If the participants or fecundity coordinators are not

certain of the quality of the data it should be passed on to the survey and data coordinator (Brendan O'Hea MI).

The mackerel biological data (all fish data, not only the fish data of the fish, which were sampled for fecundity) should be sent after the survey to the biological sampling coordinator (Jens Ulleweit, TI) to be reported to the 2016 WGWIDE group.

Table 7.1.5a. Adult mackerel sampling program AEPM - Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2016

MACKEREL SAMPLING AEPM



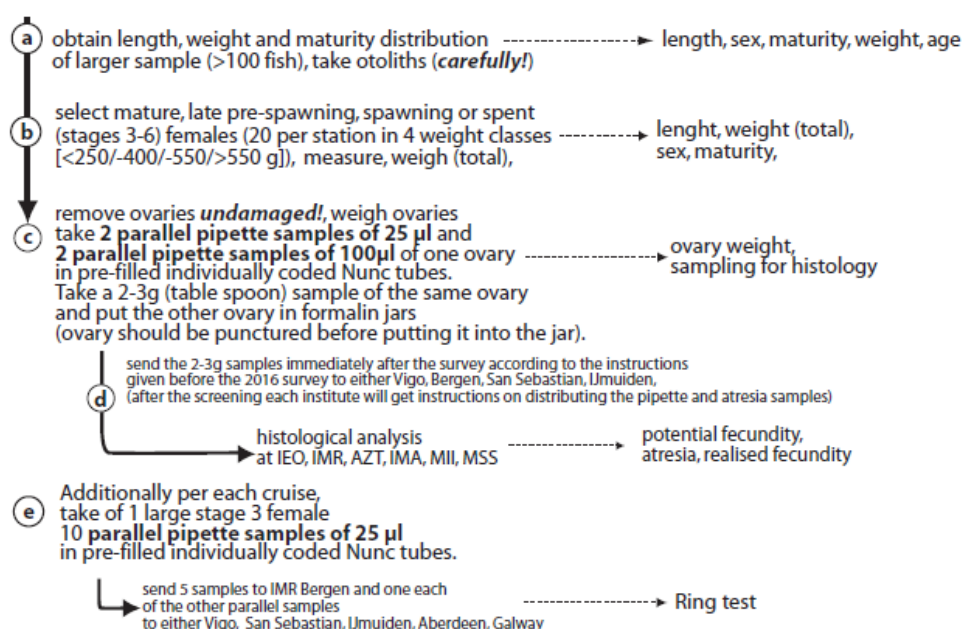
Estimation of potential fecundity in pre-spawning fish and the estimation of atresia for realised fecundity

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

Area	Sampling by	Period/samples							total no. of samples
		1	2	3	4	5	6	7	
Southern	POR/IPIMAR ESP/IEO	20	20						40
		100*							100 140
Western	ESP/AZTI					30			30
	DEU/SF				50				50
	IRL/MI						70		70
	ESP/IEO				50				50
	FAR/FFS					30			30
	SCO/MSS					90		90	180
	NED/IMARES				50		50		100
	ICE/HAFRO						20		20
	TBA**			45	45			90	720

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

** to be announced



Please refer to the sampling protocols which will be send to all participants before the 2016 survey!
For the desired temporal and spatial distribution of the fecundity samples please refer to
Table 7.1.1 a+b; for further instructions please refer to section 7.
Each institute should additionally collect ringtest samples for fecundity or batch fecundity!
See report and sampling protocols for details.

Table 7.1.5b. Adult mackerel sampling program DEPM - Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2016

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** (for details on cruises see WGMEGS report)

Number of samples by period and institute:

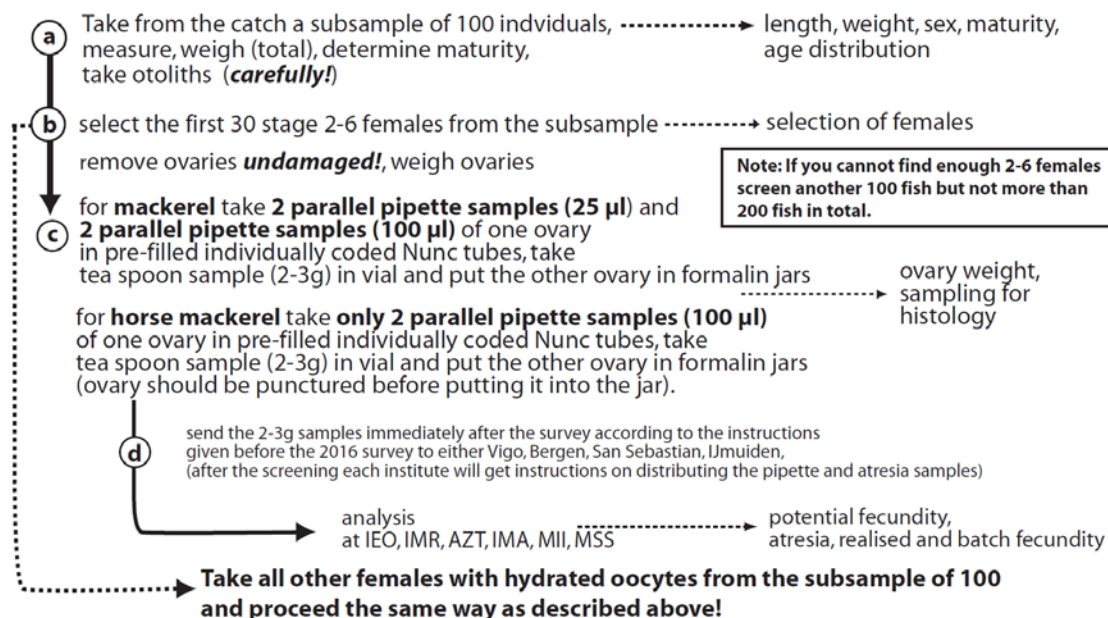
Sampling by	AZTI, ESP	TI-SF, DEU	MSML, SCO	IEO, ESP	MI, IRE	TBA *	Total
Period 2			360	150	510		1020
Period 3	150	360				270	990

* to be announced

**HORSE MACKEREL: Estimation of batch fecundity****Sampling at Sea** (for details on cruises see WGMEGS report)

Number of samples by period and institute:

Sampling by	MI, IRE	MSML, SCO	IMARES, NED	Total
Period 6	210		240	450
Period 7		450		450



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

Please refer to the sampling protocols which will be send to all participants before the 2016 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Tables 7.1.2 & 7.2.1; for further instructions please refer to section 7.

Each institute should additionally collect ringtest samples for fecundity or batch fecundity! See report and sampling protocols for details.

Adult fecundity and atresia sample analysis will be carried out by Ireland, Norway, Scotland, Spain (IEO and AZTI) and the Netherlands. Each country carrying out the various cruises listed in Tables 7.1.1.a-b and 7.1.2 is responsible for distributing their sample collection alternately to the countries carrying out the fecundity analysis. It is important that immediately upon return from each cruise samples be sent to the analysing institutes. Norway will coordinate mackerel fecundity sample analysis.

Table 7.1.6. Processing ovary and pipette samples upon return from each cruise.

Upon return from the survey, the pipette samples should immediately be sent to the analysing institutes.

After a minimum of 2 weeks fixation cut cross sections 4 mm thick from the ovary not previously sampled and place them in a labelled histological cassette. The cassettes should be engraved or labelled with an indelible label corresponding to each replicate set of Nunc tubes.

Cover the cassettes with 70% ethanol and pack them in a leak proof bottle. If it is not possible (or too expensive) to send the samples in ethanol, you can send it in 3.6% buffered formaldehyde, but remember to label the container with ethanol or formaldehyde. Pack the consignments for each country with a maximum volume of 1000 ml solution in each package. On the outer cover of the package indicate the volume of fixative and that it is within the limits for unclassified transport.

Table 7.1.7. Protocol for laboratory analysis of mackerel fecundity samples.

Task	Countries	Timing for work completion
Training	Ireland, Norway, Scotland, Netherlands and Spain (IEO and AZTI)	November workshop WKFATHOM
<ul style="list-style-type: none"> Screen samples to identify and select prespawning fish based on the presence of spawning markers and atretic oocytes. The fecundity manual will be finalized during the 2015 workshop. Ovaries that have either commenced the annual spawning or are recently spent should be processed to estimate atresia. Prepare resin sections from all mature fish identified as either in spawning or spent to determine the intensity 	Norway, Netherlands and Spain (IEO and AZTI)	Provisional results completed for 2016 WGWIDE meeting in September. Completed results for WGMEGS 2017.

Task	Countries	Timing for work completion
and prevalence of atresia.		
Apply image analysis protocol based on the fecundity manual to determine fecundity using the gravimetric method.	Ireland, Norway, Scotland, Netherlands and Spain (IEO and AZTI)	Provisional results completed for 2016 WGWIDE meeting in September. Completed results for WGMEGS 2017.
Determine atresia in mature fish identified as either spawning or spent above by stereometric analysis using the protocol in the fecundity manual.	Norway, Scotland, Netherlands and Spain (IEO and AZTI)	Provisional results completed for 2016 WGWIDE meeting in September. Completed results for WGMEGS 2017.

Table 7.1.8. Sampling targets for western and southern mackerel spawning components.

Spawning component	Targets for potential fecundity analysis	Targets for atresia analysis*
Southern	200	200
Western	660	660
DEPM enhanced sampling**	1000	1000
Total	1700	1700

* The samples above suitable for atresia analysis will be selected from a much larger collection from the surveys detailed in the cruise sampling Table 7.1.1a-b and 7.1.2.

** Based on the results of the egg sampling it will be decided which period DEPM samples will be analysed.

7.2 Western horse mackerel DEPM adult parameter sampling

During the 2016 survey for horse mackerel adult samples will be collected during the expected peak spawning period (**period 6 and 7**). During the 2016 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 7.2.1. **Each transect at the station with highest stage 1 mackerel egg production a trawl haul will be carried out (Table 7.2.1). It is recommended that trawling is 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 7.2.2).

Tab. 7.2.1: Desired horse mackerel adult sampling in the area selected for the 2016 DEPM sampling

Fecundity sampling

Biscay, Celtic Sea, North West Ireland, West of Scotland

HORSE MACKEREL

Lat °

per period																										
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	2	3	4	5	6	7	
1	18/jan/16	1																0	AZTI							
2	25/jan/16	1																0	VTI							
3	1/feb/16	2																0	MI					210		
4	8/feb/16	2																0	SCO						450	
5	15/feb/16	2																0	IMARES					240		
6	22/feb/16	2																0	IMR							
7	29/feb/16	2																0	IEO							
8	7-Mar-16	2																0	FAR							
9	14-Mar-16	3																0	ICE							
10	21-Mar-16	3																0	total:	0	0	0	0	0	450	450
11	28/mrt/16	3																0								
12	4/apr/16	3																0								
13	11/apr/16	4																0								
14	18/apr/16	4																0								
15	25/apr/16	4																0								
16	2-May-16	4																0								
17	9-May-16	5																0								
18	16-May-16	5																0								
19	23-May-16	5																0								
20	30/mei/16	6						30		30	30		30					120								
21	6/jun/16	6	30	30		30									30		30	150								
22	13/jun/16	6			30		30		30					30		30		150								
23	20/jun/16	6										30						30								
24	27/jun/16	7																0								
25	4/jul/16	7									30		30		30		30	120								
26	11/jul/16	7	30		30		30		30									120								
27	18/jul/16	7		30		30		30		30		30		30		30		210								
28	25/jul/16	7																0								

Table 7.2.2. Adult horse mackerel sampling program - Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2016

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** (for details on cruises see WGMEGS report)

Number of samples by period and institute:

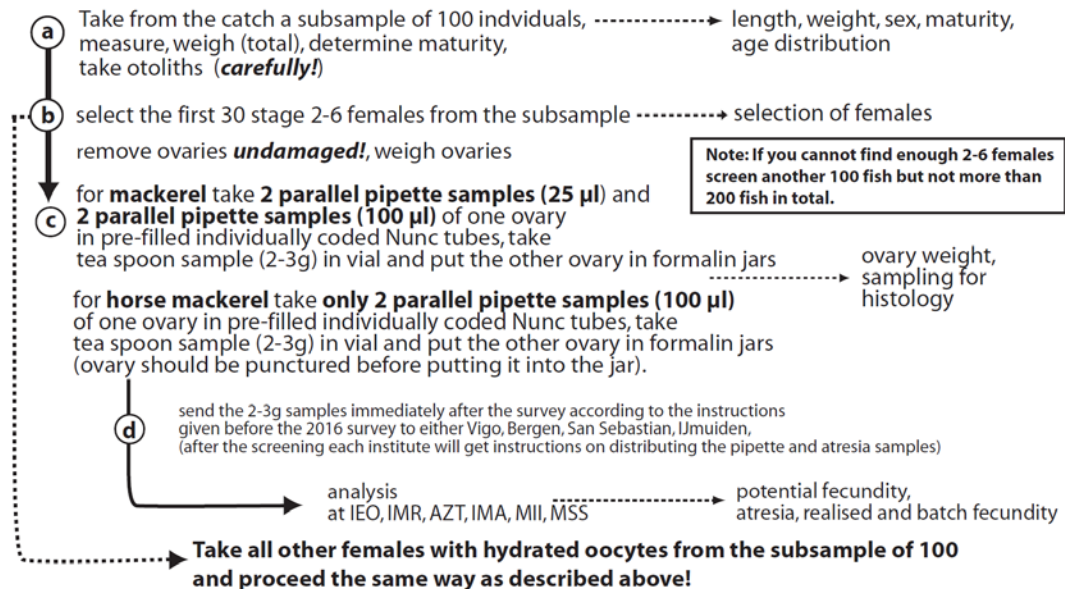
Sampling by	AZTI, ESP	TI-SF, DEU	MSML, SCO	IEO, ESP	MI, IRE	TBA *	Total
Period 2			360	150	510		1020
Period 3	150	360				270	990

* to be announced

**HORSE MACKEREL: Estimation of batch fecundity****Sampling at Sea** (for details on cruises see WGMEGS report)

Number of samples by period and institute:

Sampling by	MI, IRE	MSML, SCO	IMARES, NED	Total
Period 6	210		240	450
Period 7		450		450



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

Please refer to the sampling protocols which will be send to all participants before the 2016 survey!

For the desired temporal and spatial distribution of the fecundity samples please refer to Tables 7.1.2 & 7.2.1; for further instructions please refer to section 7.

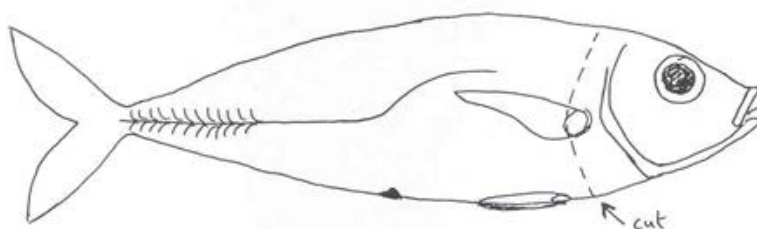
Each institute should additionally collect ringtest samples for fecundity or batch fecundity!

See report and sampling protocols for details.

Removal of horse mackerel (*Trachurus trachurus*) ovaries

(A technique that was found to work well during Ciro 2/00)

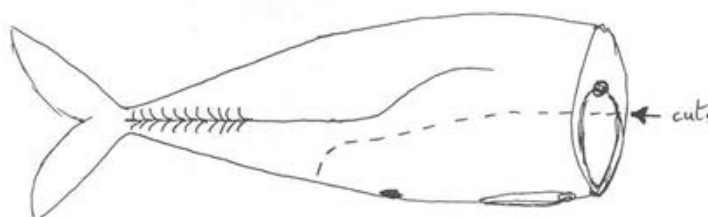
- 1) Measure and weigh the fish and make a temporary note of the information.
- 2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral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

Protocols for horse mackerel sampling preparations, sampling at sea and analysis in the laboratory are the same as for mackerel (Section 7.1).

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

The horse mackerel DEPM sampling results will be presented at the WGMEGS meeting in spring 2017.

The horse mackerel biological data (all fish data, not only the fish data of the fish which were sampled for fecundity) should be sent after the survey to the biological sampling coordinator (Jens Ulleweit, TI) to be reported to the 2016 WGWide group.

8 Review procedures for egg sample sorting, species ID, staging, data submission and subsampling (ToR c, e) including update of manual

The procedures for egg sampling sorting, species ID, staging, data submission and subsampling were reviewed and published in 2014 as SISP 6 (Series of ICES Survey Protocols) Manual for the Mackerel and Horse Mackerel Egg Surveys (MEGS): sampling at sea. At the time of this report only the table containing the Walsh scale on mackerel maturity staging had to be corrected for a minor error. A small paragraph on a ring test for fecundity analysis was also added to the manual. The ring test will be utilized to monitor the potential changes of analyst perception in fecundity analysis. Otherwise, no changes were noted by the working group. The manual will be updated after the egg identification and staging workshop in autumn 2015 when the final planning of the 2016 mackerel and horse mackerel egg survey in the western and southern areas should be available and will be inserted, as well as possible amendments identified during WKFATHOM.

WGMEGS decided to carry out qualitative checks on the presence or absence of blue whiting larvae in the 2016 egg survey samples. A chapter on methods for this activity will be added to the survey manual at WKFATHOM in October 2015.

9 Review procedures for fecundity, batch fecundity, spawning fraction and atresia estimation (ToR d)

The WGMEGS Manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel (SISP ICES 5, 2014) was reviewed in advance of the 2016 survey. Most of the changes considered are minor, and concern in particular:

- the gonads sampling flow chart
- the table with the countries codes for sampling and analysis
- a more detailed clarification of when the manual refers to ovary stages based on the Walsh scale or to the most advanced batch oocyte stages.
- the ring tests
- the whole mounts evaluation
- the image analysis manual
- the inclusion of the calculation of the atresia incidence (apart from the atresia intensity)
- all the excel templates.

The amendments to the manual will effectively be carried during the WKFATHOM meeting in November.

Regarding the experimental application of the DEPM to the mackerel stock and to western and southern horse-mackerel stocks, work is currently in progress, concerning the issues listed below, and if required, those aspects will be discussed during the WKFATHOM meeting in November and included in the updated version of the manual. Those issues are:

- the enhanced adult sampling during the AEPM surveys for the DEPM, in relation for instance to the often limited number of available hydrated females used for batch fecundity estimation.

- the identification and staging/ageing of POFs for spawning fraction estimation.

10 Analysis and evaluation of the results of the 2014 mackerel egg survey in the North Sea (ToR f)

After originally agreeing that both Netherlands and Norway would participate in the North Sea mackerel egg survey in 2014, Norway withdrew their participation in March 2014. At this short notice, it was not possible to find another institute to replace them. IMARES was the only institute available to carry out the North Sea mackerel egg survey and planned to cover the North Sea mackerel spawning area and season in four survey weeks. The mackerel egg survey started on 26 May. After one successful plankton sampling survey week, RV 'Tridens' encountered serious engine problems and could not continue the sampling. The second week of the survey was spent trying to fix the engine problems. On the 6 June it became clear that the engine problems of RV 'Tridens' could not be solved and no other vessel was available to continue the egg survey. After consulting various experts, the 2014 North Sea mackerel egg survey was terminated and postponed to 2015.

11 Revised Mackerel TAEP recalculation estimates (WKPELA 1)

11.1 TAEP Recalculation 1992–2013

Following the recommendation of the 2014 WKPELA benchmark workshop (ICES, 2014a), WGMEGS carried out a revision of the mackerel egg survey historical database and a recalculation of the whole time-series of the TAEP (Total Annual Egg Production) and SSB (Spawning-stock biomass) in 2014. The historical time-series was recalculated by applying the Mendiola mackerel egg development equation (Mendiola *et al.*, 2006) instead of the Lockwood equation (Lockwood *et al.*, 1977). The decision to use the Mendiola mackerel egg development equation instead of Lockwood's was adopted by WGMEGS in 2012 (ICES, 2012). In general, the Mendiola equation gives slightly higher egg productions due to a shorter observed egg development time compared with Lockwood's equation. In addition, the TAEP estimates for the whole time-series were calculated using new and updated code in R that has been developed in recent years. This resulted in a new time-series of TAEP and SSB estimates that show an increase of around 25% for the TAEP and SSB compared to previously reported estimates. (Table 11.1.1. and 11.1.2)

Differences in the TAEP and SSB in the time-series between reported values and the new update of the egg development equation over the revised egg production database from 1992 to 2013 are shown in Figures 11.1.1 and 11.1.2 and Tables 11.1.1 and 11.1.2. In the updated time-series the 1992 and 1995 estimations were revised substantially. The reasons for this are described below.

- The reported 1992 estimate had not included the egg production from the southern area of the survey so it was corrected to include those data. In addition, the 1992 survey did not cover the entire distribution of the mackerel eggs because the survey design just covered a denoted "standard area" that was defined in previous reports (ICES, 1993).
- In the original calculation of the 1995 reported estimate only the data from the "standard area" corresponding to that used in 1992 (ICES, 1996) were used. The revised estimate in 2014 includes all the data collected from the

entire surveyed area, thus providing more complete coverage of the spawning distribution in the western area.

11.2 Revision of TAEP Estimates

A re-examination of the R script in April 2015 revealed a bug in the interpolation algorithm used in the recalculation of the TAEP index (WD_Abstract). This resulted in an overall underestimation of the egg production for interpolated rectangles. Consequently, the revised time-series estimates provided within this working document in Figures 11.1.1 and 11.1.2 and Tables 11.1.1 and 11.1.2 do not correspond to and supersede those TAEP and SSB estimates presented to WGWIDE in 2014 (ICES, 2014c). In light of the discovery of the bug in the interpolation algorithm, it will also be necessary to recalculate the annual egg abundance estimates for the horse mackerel as well over the same period (2004 – 2013). This work will be completed and the results presented to WGWIDE in August 2015.

11.3 TAEP Variance Estimates, 1992–2013

The estimate of TAEP variance was also calculated over the same period and is presented using the Mendiola equation (Table 11.3). These variances were calculated using 2 methods. The variance of the TAEP estimate is based on assuming that the raw production data are distributed with a constant Coefficient of Variation (CV). The CV of the data can be estimated by assuming a lognormal distribution for the positive egg production observations. The CV by traditional methodology is calculated by an ANOVA of log daily production on replicate rectangles that have at least two hauls of non-zero observations in each period. An alternative methodology is to estimate the CV by a GAM using interaction latitude, longitude and period to model the log egg production. This alternative methodology permits the use of more data points as opposed to the traditional methodology and is therefore more applicable to the MEGS dataset.

Table 11.1.1. NE Atlantic Mackerel TAEP estimates (stage I egg production) derived from the mackerel egg surveys for the Southern, Western and combined survey area. The reported Annual egg Production data for the mackerel were estimated using Lockwood egg development equation up to 2010, but the estimate reported for 2013 given by WGMEGS in 2014 report the Mendiola equation was used.

	1992	1995	1998	2001	2004	2007	2010	2013
Mendiola_eq Southern	4.45 *e14	3.02 *e14	6.43 *e14	3.66 *e14	1.65 *e14	4.42 *e14	5.72 *e14	7.79 *e14
Mendiola_eq Western	2.82 *e15	2.35 *e15	1.65 *e15	1.48 *e15	1.51 *e15	1.63 *e15	2.12 *e15	2.37 *e15
Mendiola_eq combined	3.27 *e15	2.66 *e15	2.29 *e15	1.85 *e15	1.68 *e15	2.07 *e15	2.70 *e15	3.15 *e15
Reported Southern	-	1.69 *e14	4.34 *e14	2.83 *e14	1.20 *e14	3.27 *e14	4.25 *e14	7.16 *e14
Reported Western	1.94 *e15	1.49 *e15	1.37 *e15	1.21 *e15	1.20 *e15	1.21 *e15	1.70 *e15	2.40 *e15
Reported combined	-	1.66 *e15	1.80 *e15	1.49 *e15	1.32 *e15	1.54 *e15	2.13 *e15	3.12 *e15
Variation from Reported est.	-	160%	127%	124%	127%	121%	127%	101%

Table 11.1.2. NE Atlantic Mackerel SSB estimates (t) derived from the mackerel egg surveys for the Southern, Western and combined survey area. The reported SSB data for the mackerel were calculated using the Lockwood egg development equation up to 2010, the reported estimate for 2013, the Mendiola equation was used.

	1992	1995	1998	2001	2004	2007	2010	2013
Mendiola_eq Southern	6.72 *e05	6.02 *e05	1.19 *e06	4.80 *e05	3.70 *e05	9.46 *e05	1.15 *e06	1.39 *e06
Mendiola_eq Western	4.26 *e06	3.90 *e06	3.56 *e06	3.09 *e06	3.10 *e06	3.49 *e06	4.28 *e06	4.23 *e06
Mendiola_eq combined	4.94 *e06	4.50 *e06	4.74 *e06	3.57 *e06	3.47 *e06	4.44 *e06	5.43 *e06	5.63 *e06
Reported Southern	-	3.09 *e05	8.00 *e05	3.70 *e05	2.80 *e05	7.01 *e05	8.58 *e05	1.28 *e06
Reported Western	2.93 *e06	2.47 *e06	2.95 *e06	2.53 *e06	2.47 *e06	2.95 *e06	3.43 *e06	4.29 *e06
Reported combined	2.93 *e06	2.78 *e06	3.75 *e06	2.90 *e06	2.75 *e06	3.65 *e06	4.29 *e06	5.57 *e06
Variation from reported est.	-	162%	126%	123%	126%	122%	127%	101%

Table 11.3. Total Egg Production (TAEP) for NE Atlantic Mackerel by year and component. var. Trad means Variance calculated by traditional methodology and var. GAM means variance using GAMs. CV means coefficient of variation.

Component	year	TAEP	sd trad	sd GAM	var. Trad.	var. GAM	CV Trad.	CV GAM
Southern	1992	4.45 *e14	1.31 *e13	1.26 *e13	1.71 e*26	1.59 e*26	3%	3%
Western	1992	2.82 *e15	1.88 *e14	1.67 *e14	3.53 e*28	2.78 e*28	7%	6%
Combined	1992	3.27 *e15	1.88 *e14	1.67 *e14	3.55 e*28	2.80 e*28	6%	5%
Southern	1995	3.02 *e14	2.43 *e14	6.10 *e13	5.90 e*28	3.72 e*27	81%	20%
Western	1995	2.35 *e15	6.14 *e14	6.24 *e14	3.77 e*29	3.90 e*29	26%	27%
Combined	1995	2.66 *e15	6.60 *e14	6.27 *e14	4.36 e*29	3.94 e*29	25%	24%
Southern	1998	6.43 *e14	1.57 *e15	6.33 *e14	2.47 e*30	4.01 *e29	244%	98%
Western	1998	1.65 *e15	2.62 *e14	2.38 *e14	6.88 e*28	5.65 e*28	16%	14%
Combined	1998	2.29 *e15	1.59 *e15	2.38 *e14	2.53 e*30	5.65 e*28	69%	10%
Southern	2001	3.66 *e14	1.90 *e14	1.17 *e14	3.60 e*28	1.37 e*28	52%	32%
Western	2001	1.48 *e15	3.42 *e14	2.47 *e14	1.17 e*29	6.12 e*28	23%	17%
Combined	2001	1.85 *e15	3.91 *e14	2.74 *e14	1.53 e*29	7.49 e*28	21%	15%
Southern	2004	1.65 *e14	6.69 *e13	3.05 *e13	4.48 e*27	9.28 e*26	41%	18%
Western	2004	1.51 *e15	3.12 *e14	1.89 *e14	9.72 e*28	3.57 e*28	21%	12%
Combined	2004	1.68 *e15	3.19 *e14	1.91 *e14	1.02 e*29	3.66 e*28	19%	11%
Southern	2007	4.42 *e14	2.64 *e14	1.40 *e14	6.97 e*28	1.97 e*28	60%	32%
Western	2007	1.63 *e15	2.84 *e14	1.76 *e14	8.09 e*28	3.10 e*28	17%	11%
Combined	2007	2.07 *e15	3.88 *e14	2.25 *e14	1.51 e*29	5.07 e*28	19%	11%
Southern	2010	5.72 *e14	3.85 *e14	2.08 *e14	1.48 e*29	4.34 e*28	67%	36%
Western	2010	2.12 *e15	3.29 *e14	2.79 *e14	1.08 e*29	7.80 e*28	16%	13%
Combined	2010	2.70 *e15	5.07 *e14	3.48 *e14	2.57 e*29	1.21 e*29	19%	13%
Southern	2013	7.79 *e14	8.77 *e14	3.53 *e14	7.69 e*29	1.25 e*29	113%	45%
Western	2013	2.37 *e15	1.83 *e15	5.52 *e14	3.34 e*30	3.05 e*29	77%	23%
Combined	2013	3.15 *e15	2.03 *e15	6.56 *e14	4.11 e*30	4.30 e*29	64%	21%

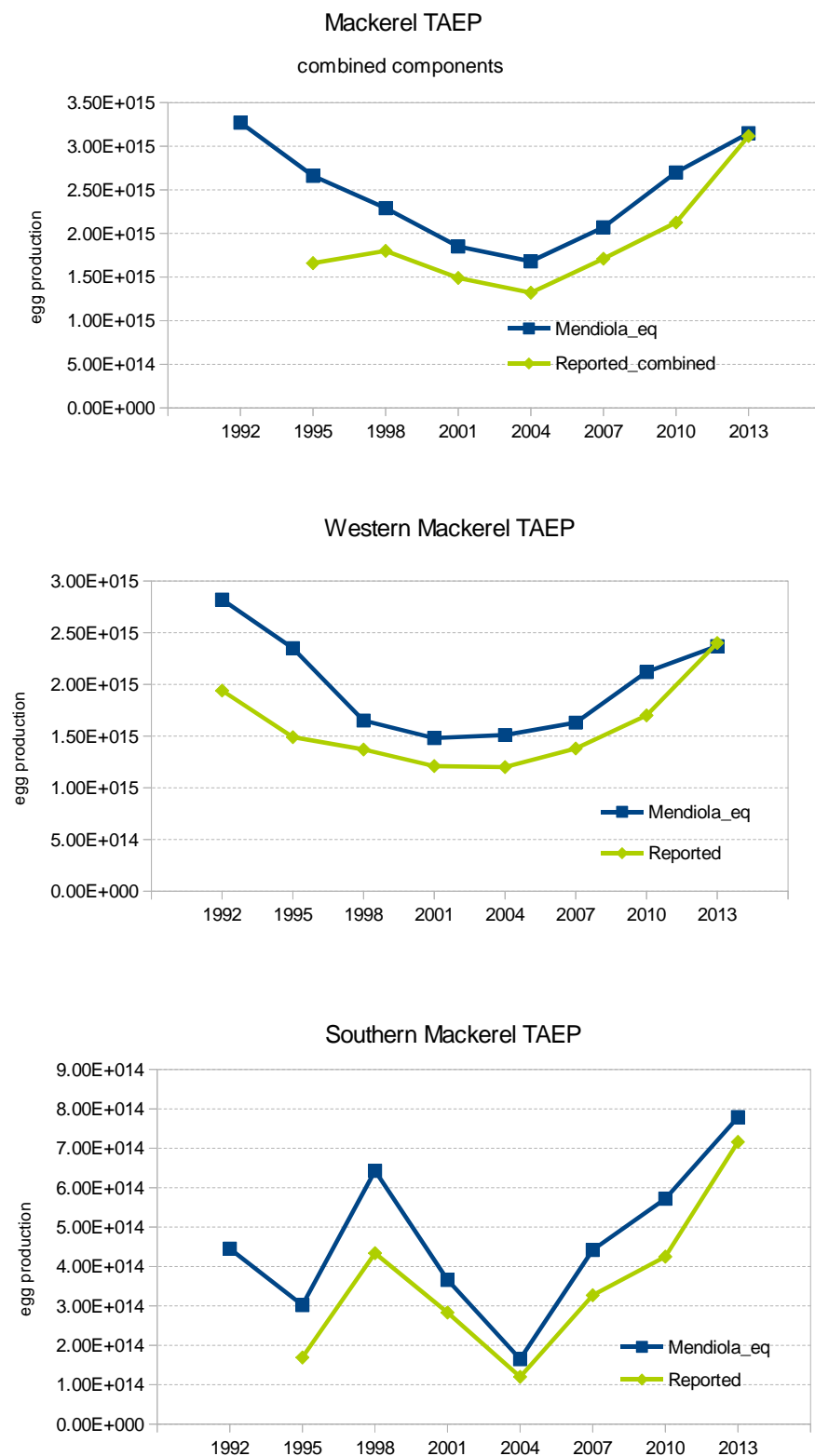


Figure 11.1.1. NE Atlantic Mackerel TAEP estimates derived from the mackerel egg surveys for the Southern, Western and combined survey area. The green line represents the reported Annual egg Production (stage I egg production) data for the mackerel using Lockwood egg development equation. The estimate given by WGMEGS in 2014 report Mendiola eq. was used. The blue line represents the agreed TAEP data using Mendiola egg development equation.

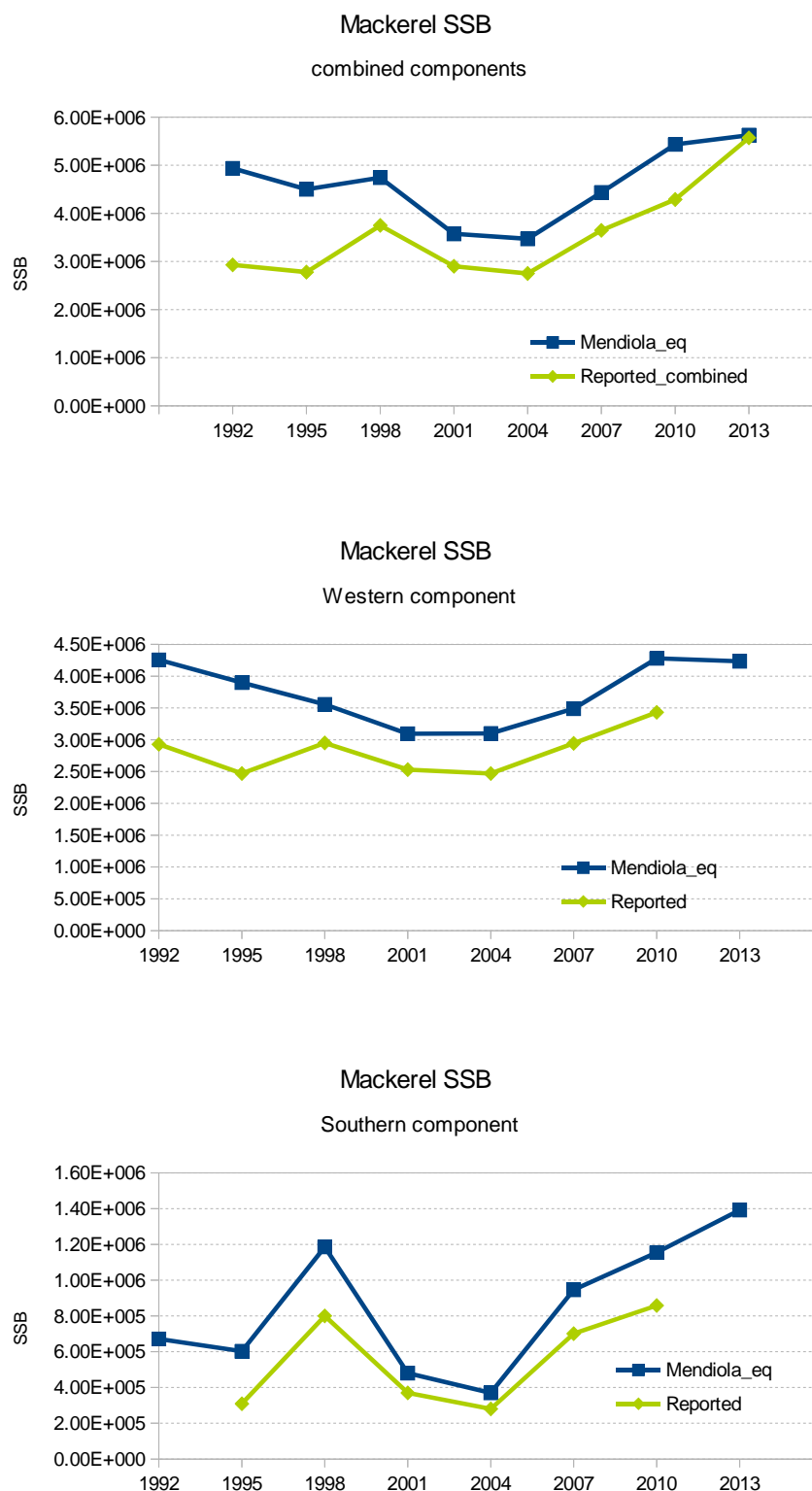


Figure 11.1.2. NE Atlantic Mackerel SSB estimates derived from the mackerel egg surveys for the Southern Western and combined survey area. The green line represents the reported SSB data for the mackerel using Lockwood egg development equation. The estimate given by WGMEGS in 2014 report was used Mendiola eq. The blue line represents the agreed SSB data using Mendiola egg development equation.

12 Results of the winter surveys (2014–2015; WKPELA 2)

Four monthly ichthyoplankton surveys were completed during the winter period in 2014 – 2015, with surveying taking place between December and March. This was a joint industry – science project that was coordinated by Dave Reid from the Marine Institute in Galway. The surveys were undertaken on commercial fishing vessels with a survey duration of approximately 12 days. The justification for this project was to provide additional information on the start date of spawning ahead of the 2016 MEGS survey. It was designed to address specifically the concern that in 2010 and especially in 2013, peak mackerel spawning in the western area as was observed by the MEGS survey to have taken place very close to the nominal start date of mackerel spawning. The 2013 mackerel egg survey results were used to provide the standard area used during the surveys. Participating nations were Ireland, Scotland, Netherlands and Denmark.

A joint industry – science project meeting was held in London on the 9 – 10 April to discuss the surveys and report and finalize the results. The results of the survey together with the main discussion points were presented to WGMEGS (Annex 3 – WD 8). The meeting in London highlighted a number of useful developments that could be made in industry – science collaboration. These recommendations are listed in the final project report and will be taken forward by WGMEGS and reported in the 2016 WGMEGS correspondence report.

13 WGISDAA

During the 2014 WGMEGS meeting, it was recommended that the effect of the alternate transect survey design on the SSB estimation should be thoroughly investigated in collaboration with the Working Group on Improving use of Survey Data for Assessment and Advice (WGISDAA). The mackerel egg survey and its associated problems were presented at the January 2015 WGISDAA meeting showing that while mackerel spawning area has increased considerably and the onset of and peak spawning is earlier, WGMEGS tried to adapt by recruiting new survey participants (Iceland and Faroese), enabling the coverage of a larger area by omitting transects and interpolating the missing rectangles as well as starting the survey earlier. The latter two adaptations, however, bear the risk that interpolation may lead to biased egg production estimates and that the survey may lose information of horse mackerel spawning, which in the recent survey continued to spawn into the final survey period with no signs of cessation. These issues were discussed at WGISDAA and lead to the following conclusions/recommendations:

- In order to minimize any detrimental effects of the increased monitoring effort requirements associated with a temporal and spatial expansion of the stocks and their relative spawning seasons under constant or decreasing resources, an analysis of the importance and variability of daily egg production in space and time is essential.
- The distribution maps indicated that in the extended northwestern survey areas mackerel egg production was small and stable, while the core area further south appeared to be more temporally variable and larger in absolute terms. This suggests that reducing sampling in the core area is likely to increase variability of the index for the limited benefit of small reduction in bias of the estimate. Although undesirable in an absolute index, a slight bias

reduction should be much less significant in a relative index such as the MEGS TAEP index than an increase of its variability.

- Similarly, the current requirement to continue sampling along a transect until 2 consecutive samples contain no mackerel eggs is less important for an index used relatively since the variability is low in this area and a higher cut-off is likely to produce a more efficient sampling design.

The two latter WGSDAA recommendations are currently being followed by WGMEGS whilst planning the next survey. A statistical analysis of daily egg production variability of space and time has been initiated and will be continued in future. The outcomes of these activities will be presented and discussed with respect to the results of the 2016 survey at the next WGSDAA meetings. It is anticipated that initiating this process will result in the modifications of the MEGS design necessary to cope with the current changes in mackerel and horse mackerel spawning phenology.

14 Database (WKPELA 1)

The benchmark for pelagic assessments (ICES WKPELA, 2014) recommended the retrospective recalculation of the entire mackerel TAEP and SSB index from 1992 to 2013 using the Mendiola development equation. In order for this to be possible a significant overhaul of the existing dataset was required that resulted in the creation of a new quality checked database with a coherent and standardized format. This dataset was subsequently uploaded to the ICES egg and larval database. These new mackerel TAEP and SSB time-series were presented at WGALES in December 2014.

Together with the MEGS egg dataseries in the ICES egg and larval database there is also a corresponding metadata file in excel format. This provides additional information on the surveys included within the database. This is a living document and will be updated subsequent to each new MEGS survey data submission.

During the meeting, it was agreed with the ICES Data centre to add to the ICES Eggs and Larvae database required fields to allow for the upload and storage of the fecundity and atresia data.

15 Blue whiting larvae

The ICES Stock Identification Methods Working Group (SIMWG) reviewed evidence on stock discreteness in blue whiting in 2014 (ICES SIMWG 2014) and concluded that the perception of that species in the NE Atlantic as a single-stock unit is not supported by the available science. SIMWG recommended that the blue whiting stock should be considered as two units. However, there is currently no information available that can be used as the basis for generating advice on the status of the individual stocks. The Working Group on Widely Distributed Stocks (WGWIDE) therefore concluded that there is a need to begin collating information on these stocks and recommended (among others) that WGMEGS checks the possibility of identifying and counting blue whiting larvae in the samples of the 2016 survey. This recommendation is based on the fact that the mackerel egg survey has previously been shown to provide valuable information on spawning distributions on species other than those targeted on the survey, including blue whiting (Horstmann and Fives 1994, O'Brian and Fives 1995, Fives *et al.*, 2001, Ibaibarriaga *et al.*, 2007).

WGMEGS investigated and discussed possibilities to sort blue whiting larvae from the plankton samples taken during the 2016 survey. Background information such as the

description of blue whiting eggs and larvae as well as their temporal, horizontal and vertical distribution along the European shelf edge was presented to aid the discussion. During the prospective survey time in 2016 blue whiting could potentially occur in the entire survey area over most of the survey time at high to very high abundances, particularly in the major spawning areas west of the British Isles but probably also in the Bay of Biscay. Principally, only larvae ≥ 3 mm can be expected in the samples, because eggs and recently hatched larvae < 3 mm occur at greater depths than the maximum sampling depth during the survey. However, in areas with upwelling, eggs and small larvae may also be found in the near-surface layers. For an unbiased estimate of larval abundance, only large larvae should be considered for further analyses. Thus, if quantitative information is needed, length measurements are required.

Identification of the larvae was considered as fairly easy. The group concluded, that sorting blue whiting larvae from the samples and performing the necessary length measurements, additional staff and, hence, funding would be necessary. Altogether roughly 1.5 man-years (about 100,000 €) would be required if quantitative data are requested. As an alternative, the group suggested to collect qualitative presence/absence information on blue whiting larvae. That could be achieved at no additional cost. Each survey participant would be requested to note presence of blue whiting larvae while sorting the samples for mackerel and horse mackerel eggs. To achieve this, a chapter on blue whiting larvae description to aid quick identification will be added to the MEGS manual. Also extra data fields will be added to the egg data template to allow for reporting of the presence of blue whiting larvae.

Following the INDICES (see Ibaibarriaga *et al.*, 2007) project, AZTI has continued identifying the fish larvae in their egg survey samples and will continue to do so with their 2016 samples. Consequently, AZTI holds triennial blue whiting larvae data of March and April for the Bay of Biscay and the Celtic Sea from 1998 onwards. Data from their 2016 participation should become available one year after the survey.

16 Deficiencies

The expansion of the mackerel spawning area and season and the possible necessity to carry the egg survey into August to sufficiently cover horse mackerel spawning, resulted in an increase of potential survey effort for all participants. Despite this Norway has decided to withdraw from the survey in 2016 and thereafter. With the current survey design and available ship time, the survey with the remaining participants cannot effectively monitor the complete spawning area and throughout the entire season.

In 2016, the mackerel and horse mackerel egg survey will therefore be reliant on attracting additional nations either to participate in the survey or on the existing nations to fill in remaining gaps (see Section 6).

It was originally planned to carry out the North Sea mackerel egg survey in 2014. Due to technical problems encountered by their research vessel, the Netherlands (after withdrawal of Norway, the sole participant in the North Sea egg survey) had to terminate the survey a few days after commencing. It is planned to conduct the North Sea mackerel egg survey in May/June 2015.

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18 Next meetings (Interim reports only)

The 2016 report (year 2) will be via correspondence as it falls within the year of the triennial MEGS survey. The next meeting of WGMEGS will be in April 2017 and the destination for that meeting will be decided then and reported in the 2016 correspondence report.

Annex 1: List of participants

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Annex 2: Recommendations

Recommendation	Adressed to
1. WGMEGS is extremely concerned about the limited resources that are available to complete the 2016 egg survey. Norway's decision to withdraw from both the North Sea survey and also the western survey has resulted in additional concerns regarding the ability to adequately cover the entire spawning area during all of the survey periods. In addition, the information collated from the winter surveys undertaken in 2014–2015 (Section 12) point towards a continuation of early peak spawning as corroborated in the 2010 and 2013 surveys. Coupled with the expansion of the spawning area for mackerel it will be impossible to cover the whole of the spawning area during all of the sampling periods. WGMEGS encourages the coastal states to discuss whether fishing rights might be coupled with an obligation to participate in the triennial egg surveys and in the work analysing egg and fecundity data after the surveys.	EU/RCM North Atlantic/Pelagic RAC's
2. WGMEGS is extremely concerned about the limited resources that are available to complete the 2016 egg survey. Norway's decision to withdraw from both the North Sea survey and also the western survey has resulted in additional concerns regarding the ability to adequately cover the entire spawning area during all of the survey periods. WGMEGS encourages EU mackerel fishing countries which are not yet involved in the survey to investigate the possibility to participate in the mackerel and horse mackerel egg survey in the NEAtlantic and North Sea and in the work analysing egg and fecundity data after the surveys.	National Delegate of France, National Delegate of Denmark
3. The group reiterates the need to continue with the egg identification/staging and fecundity workshops prior to the egg surveys. WKFATHOM are crucial refreshers for scientists and technicians who participate in the triennial egg surveys. Therefore, WGMEGS recommends that all survey participants and/or sample analysts are participating in the workshops. The group recommends investigating the possibility of securing DCF funding to assist with the cost of these workshops.	WGMEGS participants, WGBIOP
4. WGMEGS recommends that the mackerel and horse mackerel egg data from the IEO continuous transect dataset should be made available to WGMEGS. And these data are incorporated within a local model. This will enhance the mackerel predictor model which has already been developed.	IEO/DTU Aqua
5. WGMEGS recommends the creation of a general egg survey training course, using mackerel as an example. This would aim to comprehensively cover all aspects of the ichthyoplankton survey methodology.	TI-SF/IMARES

Recommendation	Adressed to
6. WGMEGS recommends that the following work is conducted during the next survey in 2016:	WGMEGS Survey participants
i) Participants are if possible urged to recreate the mackerel egg development experiments as undertaken by both Lockwood and Mendiola with the results being presented to WGMEGS.	
ii) Participants should attempt to compile information on spawning periods from other sources of data for all the target species, including all species of mackerel and horse mackerel.	
iii) Participants should attempt to compile photos and obtain egg samples from fertilization experiments and produce comparative egg descriptions for presentation at the next WKFATHOM meetings and use in the egg survey manuals.	
iv) Participants should look into the feasibility of conducting genetic analyses on egg samples from the whole survey area to assess degree of misidentification between species with very similar eggs.	

Annex 3: Working documents presented to the working group

1) Mackerel Egg Survey Design. WGISDAA Conclusions

Matthias Kloppmann

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Abstract

At the recent (2015) WGISDAA meeting, objectives and methods of the mackerel egg survey together with associated problems were presented. These are in particular the risks of obtaining biased egg production results due to the increasing amount of interpolated values in egg production and of losing the horse mackerel while shifting the main effort of the survey earlier to map the preterm spawning of mackerel. The major conclusions of WGISDAA were that it will be necessary to analyse the importance and variability of mackerel spawning in space and time in order to efficiently adapt the survey design, and that effort of the survey should focus on the core area of mackerel spawning in order to correctly map the variability of egg production. Also, the rule of sampling along a transect until 2 consecutive samples contain no mackerel eggs should be replaced by a more meaningful one.

2) Blue Whiting Eggs and Larvae. Description and Distribution

Matthias Kloppmann

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Abstract

In order to aid the discussion on the possibilities of collecting data on blue whiting larvae appearance in the plankton samples of the coming 2016 mackerel egg survey basic information on egg and larvae description and distribution are given. Eggs are in the same size range as mackerel eggs but lack the oil globules while larvae show characteristic and unique pigmentations patterns. During the blue whiting-spawning season, eggs and larvae occur along the entire European shelf edge between Portugal and north of Scotland. In the major spawning areas, which are located west of the British Isle, Eggs and larvae can become quite numerous. Eggs and recently hatched larvae principally occur at greater depths, deeper than the maximum sampling depth during the survey. Only feeding larvae ≥ 3 mm can be found in reliably quantifiable numbers in the top 200 m.

3) Population structure of Atlantic mackerel (*Scomber scombrus*) within the Northeast Atlantic; analysis based on genetic markers

Paula Alvarez

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Abstract

Northeast Atlantic mackerel (*Scomber scombrus* L.) is widely a distributed fish that performs extensive migrations from feeding to spawning grounds. Determining population structure of this species as consequence of a hypothetical homing behaviour

requires the study of genetic structure at different life stages. In order to do that, we sampled mackerels from Canada, Mediterranean waters and NEA waters (adults, juveniles and larvae) and analysed thousands of Single Nucleotide Polymorphisms (SNPs) discovered and genotyped through restriction site associated DNA sequencing (RAD-seq). Our results show that i) there is genetic differentiation between mackerels from Canada, NEA and Mediterranean waters; ii) there is some degree of genetic differentiation within the Mediterranean (Adriatic vs. Tyrrhenian and Western Mediterranean); iii) there is no significant genetic differentiation between individuals of the different spawning component inside the Northeast Atlantic; therefore, no homing behaviour between Southern and Western component was proved.

4) Review of the Mackerel egg survey data series

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Abstract

In 2014 WGMEGS carried out a revision of the triennial Egg survey historical database and a recalculation of TAEP and SSB for mackerel using the update mackerel egg development equation as was recommended by WKPELA. The updated mackerel egg development equation (Mendiola *et al.*, 2006) was adopted in 2012 WGMEGS replacing the previous egg development equation developed by Lockwood (Lockwood *et al.*, 1977). As a result, a new time-series of TAEP and SSB was produced resulting in an increase of around 25% for TAEP and SSB compared with the previously reported estimations. It should be noted that the 1992 and 1995 estimations were revised substantially. The original reported 1992 estimate had not included the eggs from the Southern area of the survey so that was corrected to include those. In addition, the 1992 survey did not cover the entire distribution of the mackerel eggs, as survey area just covered a denoted “standard area”. The 1995 survey had covered the whole distribution of the mackerel eggs but in the calculation of the reported 1995 estimate only data from within the “standard area” used in 1992 were included. The updated estimate includes data from the entire survey area.

The review of the TAEP estimates for the whole time-series were calculated using a new updated code in R that has been developed in recent years. Until 2007, a FORTRAN code was used to estimate TAEP for mackerel. From 2010 onwards, a new code in R was used to estimate mackerel and horse mackerel TAEP. This has been updated and developed further in 2015 to include quality-checking routines which consequently detected some bugs in the existing script and which have now been corrected. The most important bug detected was in the interpolation algorithm. This bug resulted in the algorithm not correctly integrating the unsampled neighbouring rectangle area to the sampled rectangle. This resulted in an underestimation of the interpolated area and by consequence an underestimation of the TAEP.

5) "Oh wouldn't it be lovely..." Using Environmental Niche Modelling to improve the Mackerel Egg Survey

Mark R. Payne

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Abstract

Ecosystem monitoring programmes and environmental niche modelling (ENM, also known as species distribution models) have traditionally had an asymmetrical relationship data generated by surveys is used to parameterize such models and forms the basis for high-impact publications, but there is rarely feedback in the other direction. However, knowledge of the factors driving the distribution of a species that ENMs encompass can potentially be of great value to improve the design of surveys, especially when combined with modern advances in oceanographic observations and modelling, and especially the emerging predictability of some marine systems. Here I describe the development of a distribution forecast system for the spawning of Mackerel (*Scomber scombrus*) in the Northeast Atlantic. While the model is in the initial stages of development, significant forecast and hindcast skill of the spatial distribution of this species is already apparent. There therefore exists the potential to use these outputs in real-time (nowcasts) to modify the design of the survey dynamically. Future modifications to the model should also allow prediction of the onset of spawning, thereby allowing the start of the survey to be set to ensure full temporal coverage. Coupling of the model to oceanographic forecast models can also potentially generate forecasts with lead-times of months to years. The application of this approach to the upcoming 2016 Mackerel Egg Survey (MEGS) will be discussed.

6) Comparing mackerel AEPM and DEPM results and horse mackerel DEPM results from the 2013 survey

Cindy J.G. van Damme

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Abstract

In 2012, it was agreed that during the 2013 survey sampling for the Daily Egg Production Method (DEPM) would be carried out. During the 2013 survey, enhanced sampling for the mackerel DEPM was planned in period 3. However, it turned out that the peak of spawning occurred already in period 2. Both samples from period 2 and 3 were analysed for batch fecundity and spawning fraction estimation. For horse mackerel DEPM sampling was planned in period 5. No other adult sampling was carried out for horse mackerel during the 2013 survey.

Batch fecundity was estimated counting all oocytes > 500 µm. Average batch fecundity for mackerel and horse mackerel was low and varied between periods for mackerel. Spawning fraction was estimated using the POF method. POF's presence/absence was noted for 7 POF stages. Spawning fraction was calculated as the numbers of spawning females with POFs in stage 1–3 divided by the total number of females. Spawning fraction for mackerel was similar in both periods and was high. For horse mackerel spawning fraction was low.

Sex ratio of 0.5 was used for both mackerel and horse mackerel. The SSB estimation for mackerel varied between periods. For period 2 SSB was 4.95 million tonnes, in period 3 2.14 million tonnes. The SSB estimated using the AEPM was 3.79 million tonnes. For horse mackerel estimated SSB using the DEPM method was 0.74 million tonnes. The assessment of WGWIDE was that horse mackerel SSB was 0.61 million tonnes.

7) Developments regarding daily spawning pattern of the southern stock horse mackerel, *Trachurus trachurus*.

Maria Manuel Angélico¹, Ana Maria Costa¹, Kostas Ganas², Elisabete Henriques¹, Foivos Alexandros Mouchlianitis², Cristina Nunes¹

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Summary of results

The initial efforts towards the application of the DEPM for the southern stock horse mackerel (*Trachurus trachurus*) were implemented during the 2007 survey. Since then several developments were introduced: (i) the new sampler, the CalVET system with double rings with 40cm mouth aperture plus a CTDF; (ii) the setting up of a genetics analysis procedure for assessing potential egg misidentification with the eggs of other *Trachurus* species; (iii) implementation of a semi-automated method using image analysis to count the oocytes of the spawning batch for fecundity estimation; (iv) histomorphological and size description of the post-ovulatory follicles (POFs) degeneration, POFs being used as spawning markers for spawning fraction estimation; and (v) studies to address the daily spawning period definition.

The present work shows the highlights of the ongoing work, which has been conducted in order to address matters, related to the daily spawning pattern of the southern stock horse mackerel. Several lines of investigation were pursued: (a) time distribution of the stage I eggs; (b) time evolutionary pattern of the diameter of the oocyte advanced batch; (c) time variation of the relative batch fecundity (Rfb) of the hydrated individuals with/without post-ovulatory follicles (POFs); (d) time distribution of POFs size (cross sectional area).

The time distribution of stage I (11 stage scale) egg abundance is a very valuable information from which to draw the daily spawning pattern. At water temperatures around 15°C, this initial planktonic phase lasts for less than 10 hours, on average only 3–4 hours, and therefore key for spawning time definition. However, also due to a general patchy distribution, stage I eggs are scarce in the plankton samples and for that reason the approach was to re-analyse the eggs from all possible plankton samples collected from 1998 to 2014. Data from 14 surveys (AEPM and DEPM surveys directed at horse-mackerel and sardine) were gathered and analysed. The stage I abundances distribution obtained throughout the 24h of the day is shown in Figure 1. A total of 587 eggs (from 112 samples out of the total 4257 plankton samples) were identified. The individual and the frequency time distributions clearly show a daily pattern of spawning centered around 20–22 (+/- 6) hours; 65% of the stage I eggs collected fell within this period. These results together with information from females spawning markers will be combined to define the spawning period.

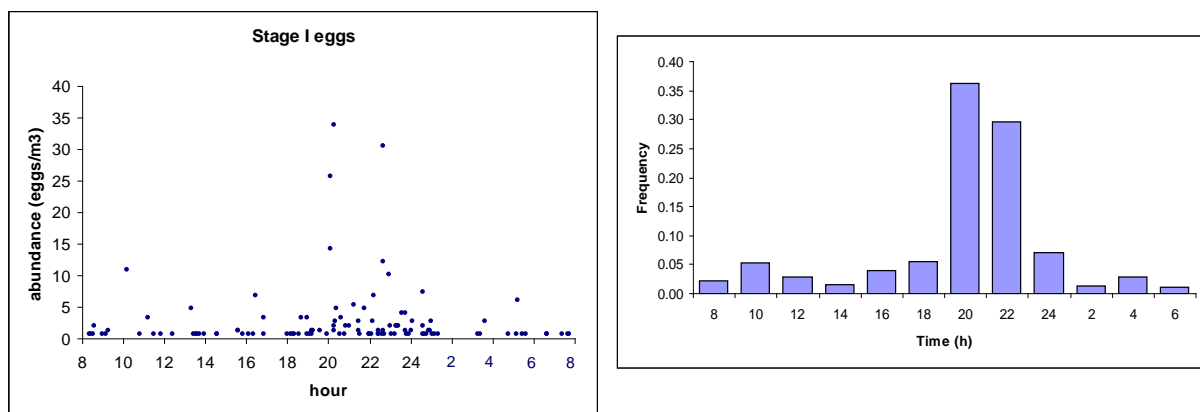


Figure 1. *Trachurus trachurus* stage I egg time distribution. Data from 14 surveys (1998–2014). Left panel individual sample distribution; right panel frequency distribution using 2h intervals.

The variation of the mean diameter of the oocytes advanced batch (AB) of 223 females from the 2010 horse-mackerel DEPM survey was examined in relationship with the time of capture (hauls time range: 7:00 – 17:00). The results revealed a statistically significant positive relationship ($P < 0.01$) only for the individuals with hydrated oocytes (Figure 2), suggesting a synchronization in the development of the hydrated oocytes.

As for the time variation of the relative batch fecundity (RFb) of the hydrated individuals (2010 DEPM survey, $n = 39$), the relationship with the time of capture was not significant ($P > 0.1$), and RFb did not differ significantly ($P > 0.05$) between individuals with POFs and those without POFs (Figure 2). This result, together with additional information confirming the absence of newly formed POFs amongst the individuals examined, suggest there were no running females up to 17:00, and thus that most spawning would occur later than 17:00.

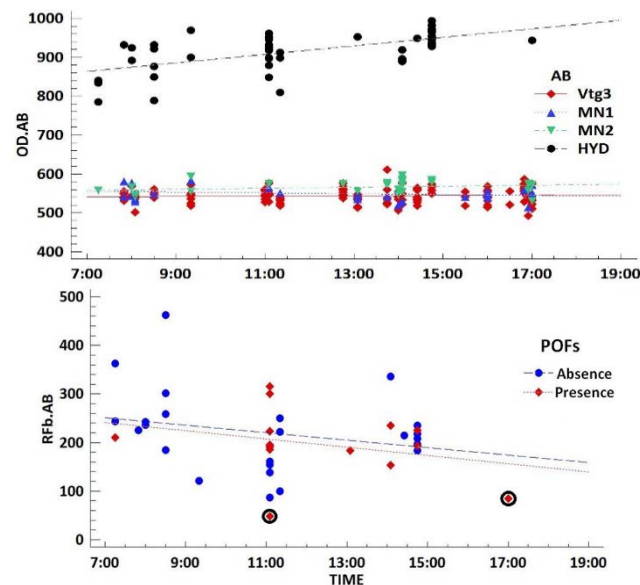


Figure 2. *Trachurus trachurus* variation of oocyte diameter of the advanced batch (OD.AB; left panel), and of the relative batch fecundity (RfB.AB) of the hydrated individuals (right panel) in relation to the time of capture (TIME). Abbreviations: AB=advanced batch, Vtg3=tertiary stage, MN1=beginning of nucleus migration stage, MN2=completion of nucleus migration stage, HYD=hydration stage, POFs=post ovulatory follicles.

The cross sectional area of the largest POF observed in the histological slide was measured for all females containing POFs (2013 DEPM survey, $n = 197$, hauls time range: 9:00 – 17h30), using digitalized images. The frequency size (area) distribution of the POFs at two periods of the day (9:00, 17:00) show multimodal distributions (especially at 17:00, Figure 3), an additional argument in favour of a daily synchronicity in spawning. Further investigation of the histomorphological characteristics of these POFs is important to understand how these size modes fit within a 24h-cycle, with the aim of eventually assign POFs to daily cohorts, for spawning fraction estimation.

Running females (with newly formed POFs and hydrated oocytes) collected during the 2013 DEPM survey were observed not earlier than 16:00 (with the exception of a female at 15:00).

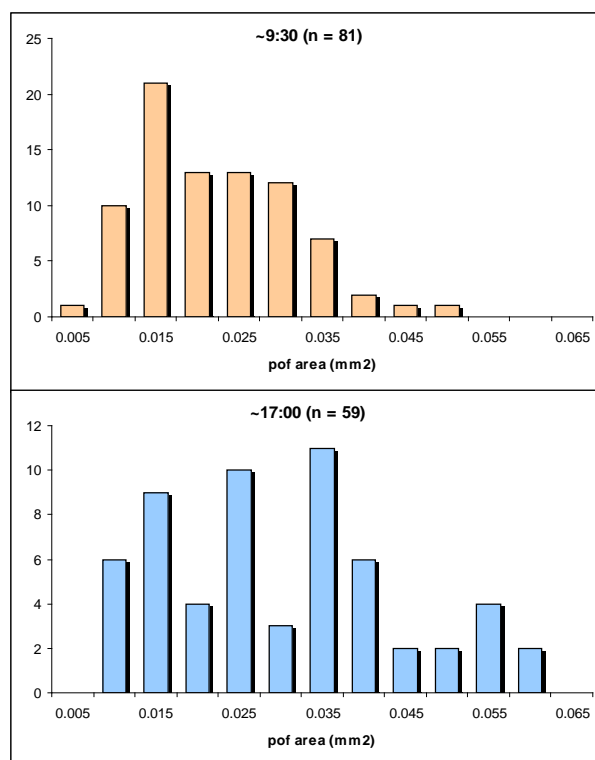


Figure 3. *Trachurus trachurus* variation of frequency size (area, mm²) distribution of the POFs at two periods of the day (left panel: 9:00, right panel: 17:00).

All the above-mentioned results argue strongly in favour of the existence of a daily spawning pattern for the southern stock horse mackerel. Spawning seems to take place mostly towards the end of the day (20:00–22:00 from eggs data, after 16:00 from adults data), though the exact period of spawning is still not fully specified. In order to verify the time frame during which the spawning occurs, the sampling period should cover the whole day (and in particular, samples from the night period are lacking), and POFs information from all available DEPM surveys be included in the analysis.

Acknowledgments: PNAB/DCF - National Biological Sampling Programme (EU DCF), Carmo Nunes, Catarina Vendrell, Patrícia Gonçalves, all people involved in the surveys and laboratory work over the years of the surveys series.

8) Industry–Science collaborative survey – Final meeting Report – London, 9–10 April 2015

Dave Reid – Marine Institute, Galway, Ireland.

Introduction

The aim of this project was to determine the start time for mackerel spawning in the western spawning component area in 2015, preparatory for the full DCF (Data Collection Framework) funded egg survey in 2016. Recent full DCF funded surveys in 2010 and 2013 have clearly shown that spawning is starting earlier and that the spawning peak occurs earlier than has been observed previously. The net result being that potentially, an unknown part of the spawning early in the season was missed in 2010 and 2013. The DCF funded ICES (International Council for the Exploration of the Seas) surveys are designed to cover the whole spawning area and period. Therefore, this missed

spawning makes the egg production method and hence management of this stock less accurate, and potentially biased. If there is spawning in the western area prior to the normal start of the survey in March, this will probably produce a relatively lower estimate than previous surveys which successfully covered the whole spawning season and hence be biased. The survey design in 2015 is intended to quantify mackerel egg production at the start of the spawning season, to provide more information for the design of the DCF funded ICES Triennial mackerel egg survey to be conducted in 2016. The 2015 surveys are therefore an important contributor to the design and conduct of the 2016 stock assessment survey carried out under the DCF. The 2015 surveys are funded under the 2% quota available for new scientific work linked to fisheries. This funding has been agreed by the national authorities in the Netherlands, Denmark, Ireland and Scotland, with each country carrying out one of four surveys covering the period from December 2014 to March 2015.

Methods

The four surveys were allocated as follows. Netherlands in December 2014, Denmark in January 2015, Ireland in February 2015 and Scotland in March 2015

The aim of these surveys was to determine the start date and location of the southern boundary of mackerel spawning in the western area. Each survey was planned to track southeasterly along the 200m contour south from SW of Ireland and collect standard egg survey ichthyoplankton samples every 0.5 degree. (Figure1). This was planned to continued until the inner corner of the Bay of Biscay. The vessel was then planned return 0.5 degrees west (or east) along the same track again taking ichthyoplankton samples each 0.5 degree.

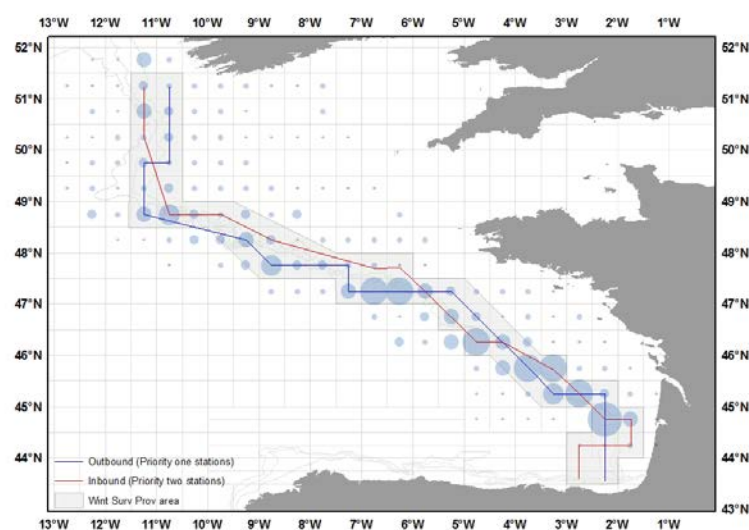


Figure 1. Egg survey design for winter surveys in 2014/2015.

Ichthyoplankton samples

At each station a high speed sampler (GULF VII type) was to be deployed. The sample tows were carried out at 4 knots through the water, up to 200m depth maximum and took approximately one hour each. All deployment and conduct protocols followed the survey manual from the ICES WG on Mackerel Egg Surveys. Deployment of the

sampling gear varied between vessels. Ichthyoplankton samples were preserved in 4% buffered formaldehyde and analysed on return to the laboratory. A small number of 10-minute calibration tows close to the surface were also to be carried out.

Adult fish samples

The survey design also included the collection of adult fish to examine fecundity and maturity state to further inform on the spawning state of the population.

Results

All four surveys were carried out to some extent and detailed cruise reports are attached. Several important points should be noted.

The first survey on *Nida* in December 2014 was intended to test the assumption that no spawning had occurred at this time. 24 stations were occupied between 43 and 48 deg N, and in the planned locations along the shelf edge. No mackerel eggs were found. No adult sampling was carried out due to equipment failure on the vessel.

The second survey was carried out on the *Ceton* in January 2015. It was hypothesized that some spawning may have been underway by this time. This survey was seriously compromised by weather, and was able to carry out one egg sampling tow, with no eggs found, and four pelagic trawl stations with only two mackerel caught.

The third survey was carried out on the *Atlantic Challenge* in February 2015. Spawning was expected to have started at this time. 45 plankton tows were carried out, 356 mackerel eggs were identified, 276 at stage 1 and the stage 1 egg densities are presented in Figure 2. No adult samples were taken.

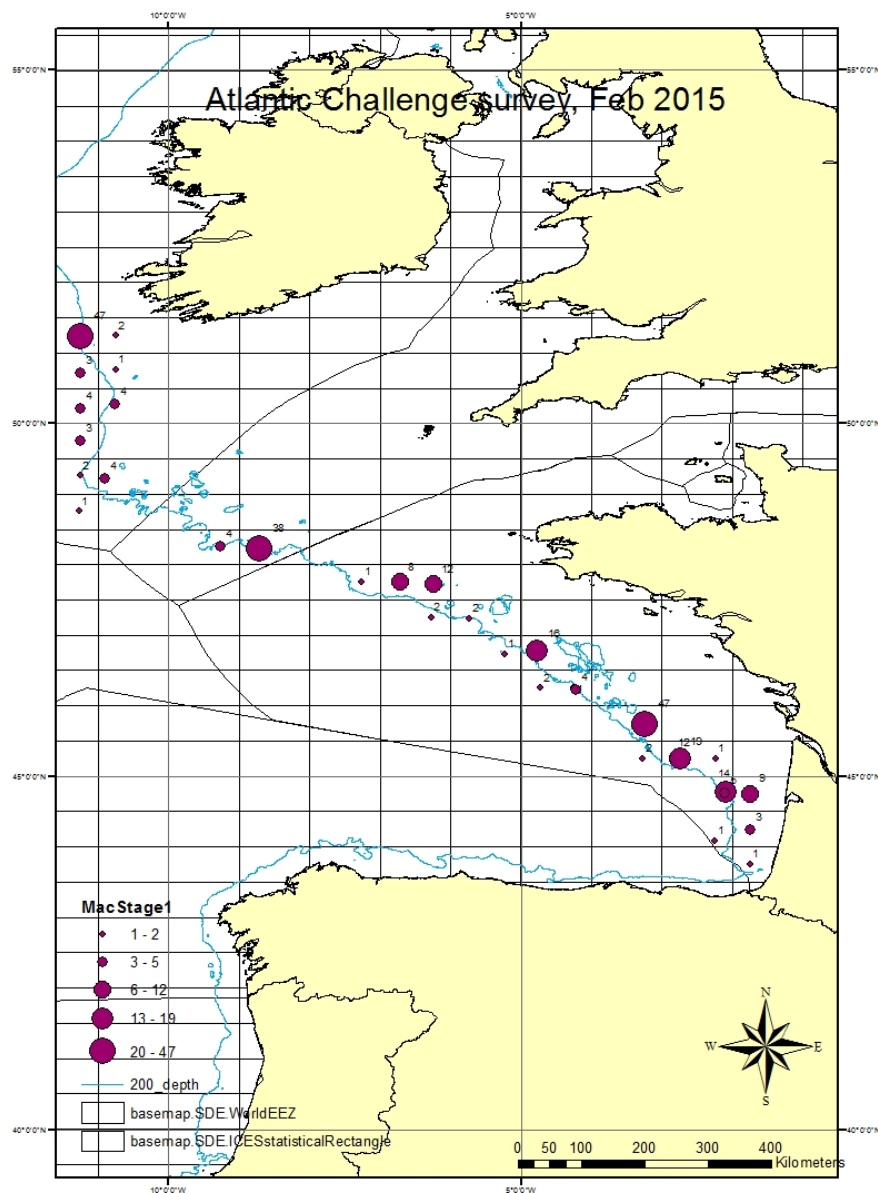


Figure 2. Map of survey stations and egg densities for *Atlantic Challenge* February 2015.

The fourth survey was carried out on the *Altaire*, from 2 March, when spawning was expected to be well underway. 41 plankton stations and 4 calibration hauls were carried out and 4536 mackerel eggs were identified, 2875 at stage 1 and the stage 1 egg densities are presented in Figure 3. French diplomatic clearance was refused preventing coverage in Biscay. There were two pelagic trawls carried out, and three mackerel caught.

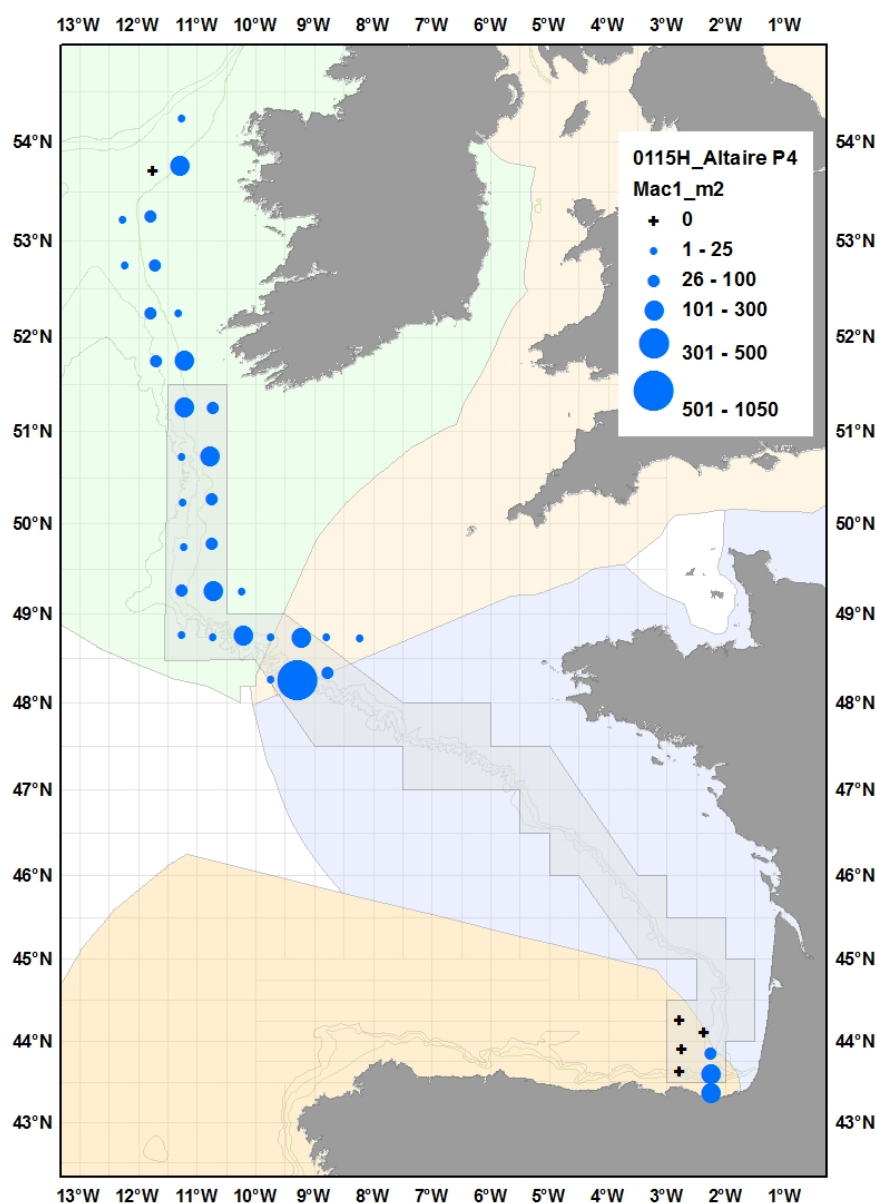


Figure 3. Map of survey stations and egg densities for *Altaire*, March 2015.

Discussion

The surveys were not designed to be directly comparable with the standard Triennial design. They were principally intended to demonstrate the presence/absence of spawning in each month from December 2014 onwards, and its spatial scale and amplitude when observed. The first clear observation was that no mackerel eggs were found at all in December. This was expected but represents valuable confirmation. No useable egg samples were taken in the January survey, and so scale and amplitude are unknown. Therefore, the major conclusions need to be drawn from the final two surveys. The nominal start date for the triennial survey in the western area is currently 10 February (Day 42). Implicit in this start date would be that there was no spawning before that. The February 2015 survey started on day 47, just 5 days after the nominal start. Eggs were found, albeit at low densities across the surveyed region. The first reasonable numbers of stage 1 eggs were taken on day 49. Taken together, this would suggest

that the nominal start date, only seven days earlier, is probably too late in the current context.

The second important finding comes from comparison with the recorded egg densities in the fourth survey starting at the beginning of March. The two surveys (February and March) cannot be directly compared due to the differences in area surveyed. However, it is important to notice that the average egg densities in the March survey were an order of magnitude greater than the February survey. It is also worth noting that these egg densities were considerably less than those observed in the first western survey period in 2013. This period in 2013 started on day 51, with a mid-point at day 69. The 2015 March survey started on day 62 and was concluded by day 71, close to the mid-point of the first survey period in 2013.

The conclusion from the last two surveys in particular is probably that spawning was still occurring earlier in 2015 than in survey years prior to 2010, but may have been slightly later than that seen in 2013. The February survey shows low but consistent spawning underway by the middle of February, suggesting that the quite long 2013 survey period starting on day 62 was combining lower spawning activity in late February with much higher activity in the early part of March. Based on this we would recommend first that the first western period for the 2016 survey should start much earlier than in 2013, ideally no later than the start of February. There should also be a second survey period starting in early March. Combined with an earlier nominal start date this should provide a more robust sampling of the start of spawning and of the Total Annual Egg Production from the survey. A precise definition of the new nominal start date is impossible from this series of surveys as we were unable to sample in January. If possible, we would recommend that a pilot survey of a similar design to the present surveys should be carried out in January 2017, to solidly confirm a reasonable start date.

Several other key recommendations can be made on the basis of these surveys. First, the basic model for a fully collaborative industry and science survey proved workable, albeit with some issues, discussed later. The surveys included substantial exchanges of staff, with the December survey being managed by the Dutch and operated by Irish scientists, the January survey managed by the Danish and operated by a Danish/Dutch team, the February survey managed by the Irish and operated by an Irish/Dutch team. Only the March survey was a single nation, executed by Scotland. A key issue for all the surveys was the short lead-time for the actual surveys. This was mainly explained by the considerable time needed to set up the national arrangements for the use of the 2% scientific quota. However, the result was that a lot of the operational planning had to be rushed. A critical outcome was that diplomatic clearances for the surveys had to be sought at less than the stipulated 6 months' notice. This proved surmountable for all but the March survey where French clearance was not granted. Clearly, a longer planning lead-time would be needed for any future work along these lines.

It should be noted that all the commercial vessels obtained for the surveys worked very well with the scientists on board, and should be commended for this. The use of a Lithuanian vessel for the December survey was not ideal. This caused some problems as the vessel had no experience of this type of work, and was not linked directly to the Dutch industry who chartered her. One definite benefit of the whole exercise was that for the other surveys the scientists were able to work with vessels and crews who also worked within the studied fishery.

Finally, the wrap up meeting in London in April highlighted a number of other useful developments that could be made in industry science collaboration in this fishery.

These are presented here in bullet form and will be expanded for presentation to the industry partners.

- 1) Industry representatives reported that there had been considerable changes in the timing of the start of the migration in recent years, and indeed in the pattern of that migration. This had gone from a sequence of movements with different large aggregations starting at different times, to one single massive aggregation all-starting together. It was agreed that it would be fruitful to look at vessel logs and determine where and when mackerel catches were taken in the last ten or so years, to see what changes had occurred and how these might link to the changes in the egg production curves from the surveys. The Northern Pelagic group will look at using a student to compile these data.
- 2) The industry also felt that the commercial vessels would be useful to collect more biological data to back up the surveys. This would include in particular material for fecundity and maturity testing and particularly in the years between the triennial surveys. This would require some training of crew, and provision of appropriate facilities for handling and chemical and sample storage on board. However, it was agreed that this was achievable, and would be valuable.
- 3) It was also agreed that the development of a small number of key transects across the survey area would be useful, and could be occupied using commercial vessels in non-survey years. Similar approaches have been taken in the Cantabrian Sea, and could be developed for the western waters. WGMEGS was asked to make recommendations for the location of such transects, and for the timing and frequency of their occupation. These could be used for both egg sampling and for zooplankton in relation to feeding linked to spawning of both mackerel and horse mackerel
- 4) WGMEGS was also asked to make recommendations of any other valuable survey supplements that the industry could consider fulfilling either in the survey years or in to non-survey years, that could enhance the reliability or value of the triennial survey sequence. WGMEGS should plan the 2016 survey using available research vessels. A second survey plan should also be prepared indicating areas where industry may be able to participate to improve the outcome of the survey.
- 5) The industry were keen for WGMEGS through ICES to develop training courses for industry skippers, crew and representatives to learn how these surveys work, and to also allow them to make informed suggestions themselves for how they could contribute.
- 6) Industry representatives were very interested in the conclusions of WGIS-DAA on the surveys and their conduct and requested that WGMEGS provide a laymans conversion of the conclusions for the industry to use.

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