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Manual for mackerel and horse mackerel egg surveys, sampling at sea

Version 2.2

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

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Contents

1 Introduction

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

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

This manual incorporates the current protocols (together with recent changes) for the collection and analysis of adult fish parameters required for the AEPM method. It is recommended that this manual is updated on a regular basis and is distributed for use by all participants during the triennial surveys.

2 Sampling areas and sampling effort

The spatial and temporal distribution of sampling is designed to ensure an adequate coverage of both mackerel (*Scomber scombrus* L.) and horse mackerel (*Trachurus trachurus* L.) spawning areas. Sampling effort is targeted at producing estimates of stage 1 egg production for both species, except for the southern stock of horse mackerel where eleven egg stages are used to produce an estimate of numbers of eggs spawned.

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

The 'western' area for mackerel is from 44º N (45º N in the west) to 63º N (Figure 2.1). It includes Biscay, the Celtic Sea and the shelf edge to the northwest of Scotland. Sampling is focussed along the shelf edge (200m isobaths) but also occurs from the French and Irish coasts out to Rockall and Hatton Bank. Sampling in this area usually begins in February and continues to the end of July.

The western area for horse mackerel includes the Cantabrian Sea and is from 43º N to 60º N with same western boundary as for mackerel (Figure 2.2).

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

Since the surveys began in 1977 considerable changes have been made to the 'standard' sampling area and some of these were described in Section 8.4 (ICES, 1994). Based on the expansion of the "standard area" since 1977, it was agreed (ICES, 2002) to reconsider its use. It was agreed that the "standard area" should no longer be used but that an adaptive sampling strategy be employed based on the distribution of eggs found in the previous survey.

In 1992 and 1995 the standard sampling area was used for the calculation of the survey index. During the 2017 benchmark (WKWIDE) a comprehensive re-analysis of the survey time-series was carried out utilizing all the station data available going beyond the boundaries of the old standard area.

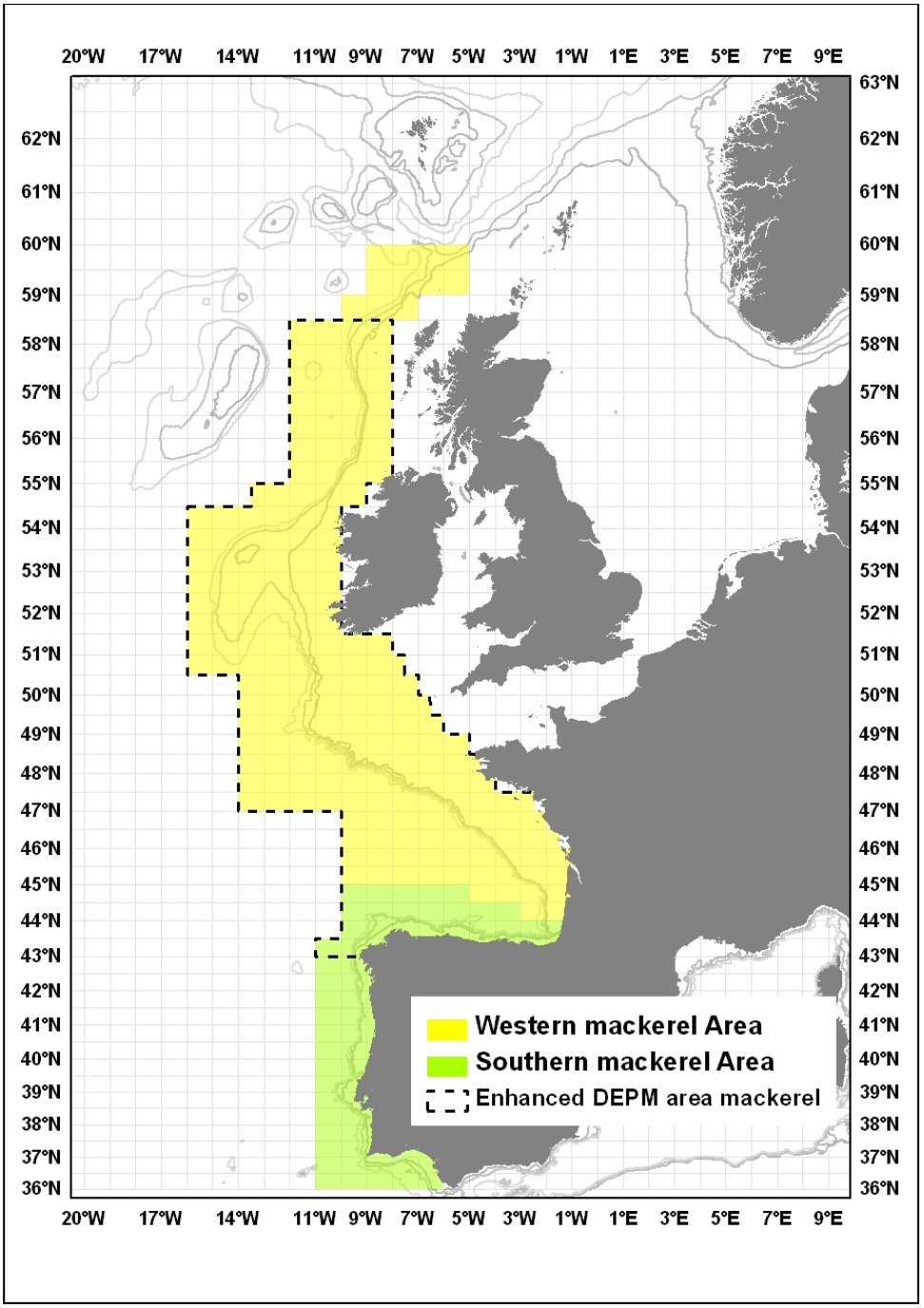


Figure 2.1. The priority areas for the sampling of mackerel eggs in both the western and southern areas. The dashed line delineates the 'enhanced' area for the sampling of adults for DEPM.

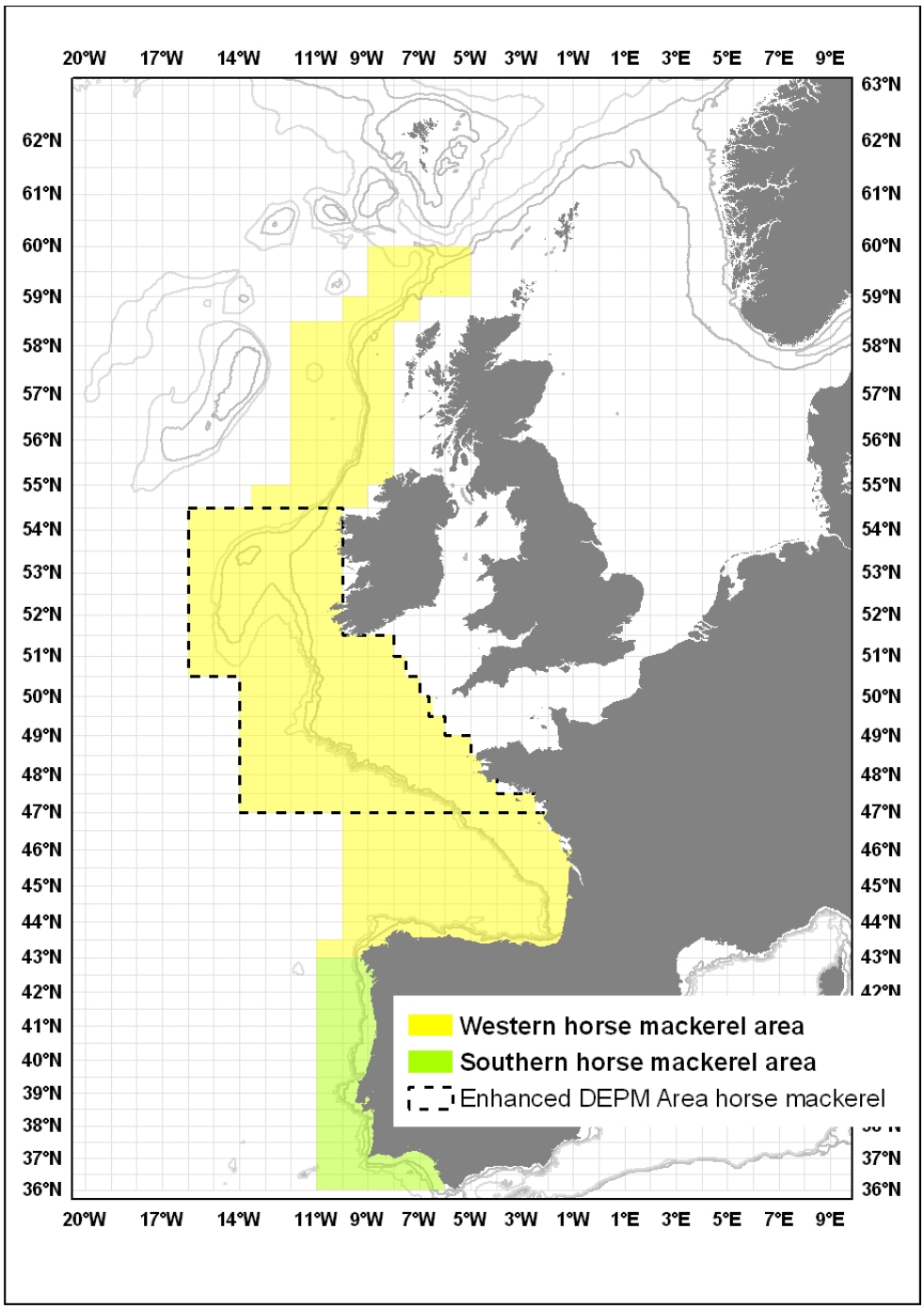


Figure 2.2. The priority areas for the sampling of horse mackerel eggs in both the western and southern areas. The dashed line delineates the 'enhanced' area for the sampling of adults for DEPM.

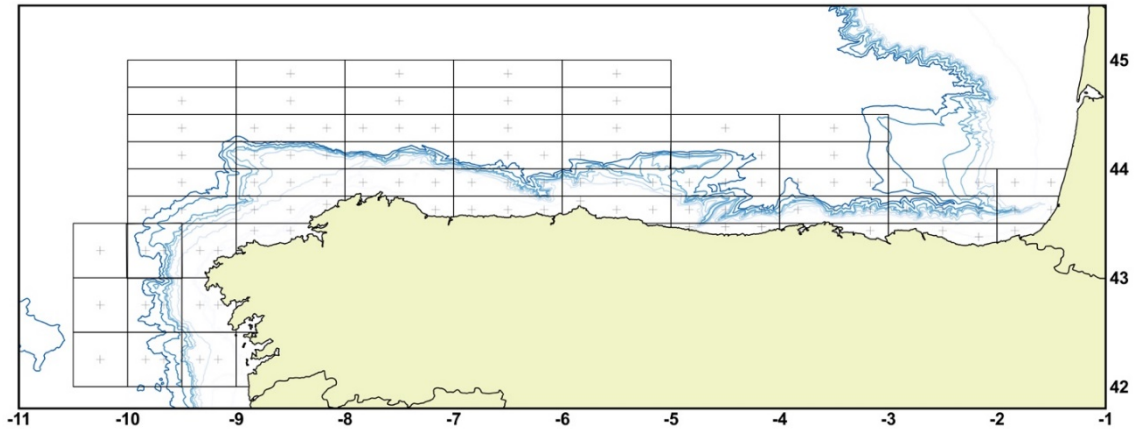


Figure 2.3: IEO sample locations for Galicia and the Cantabrian Sea.

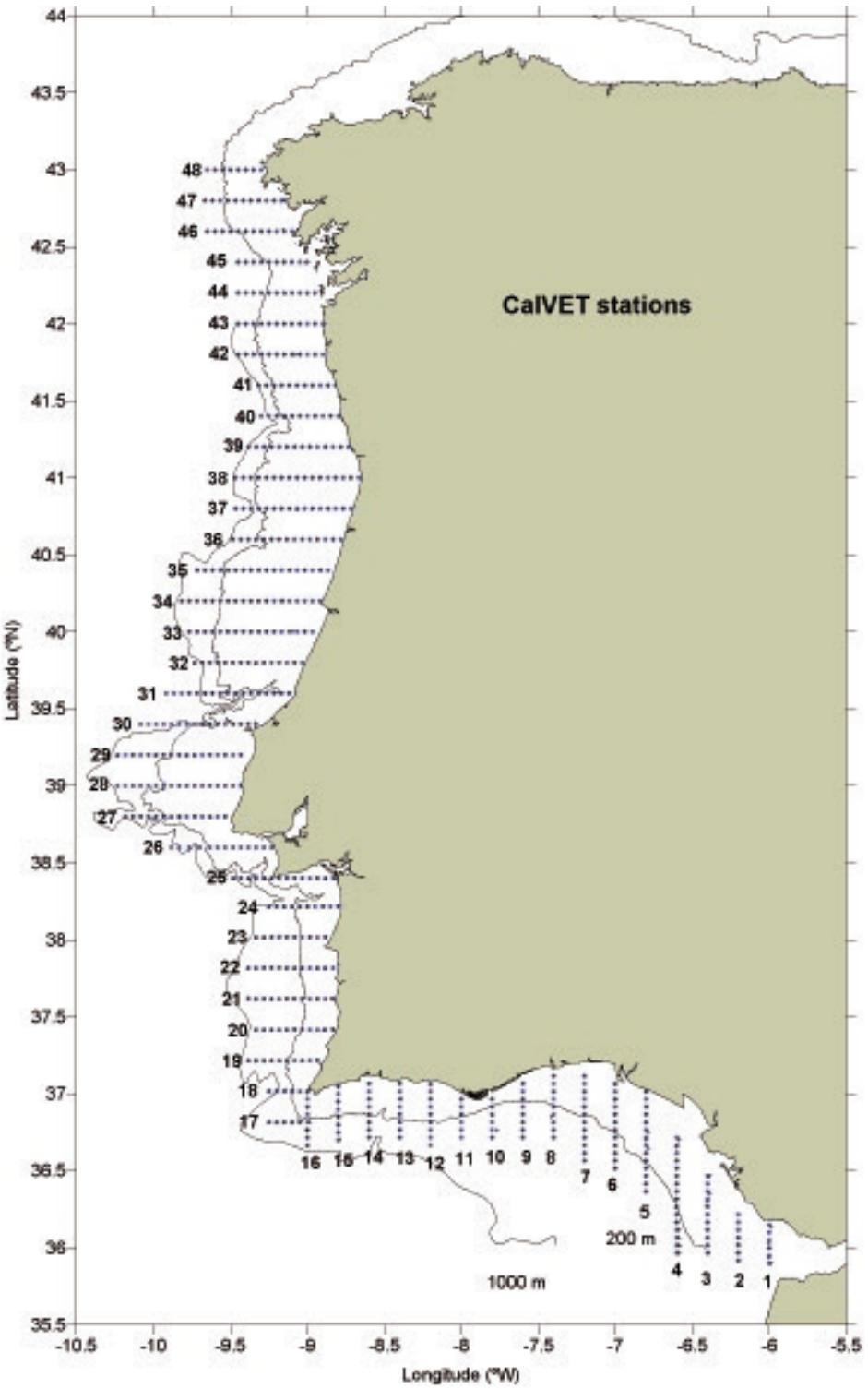


Figure 2.4. Distribution of planned plankton stations for the sampling of southern horse mackerel eggs.

3 Sampling strategy and survey design

Two important factors need to be considered when planning the survey strategy. Firstly, a set of rules must be established in order to decide when to stop sampling along a given transect, in order to ensure that the entire spawning area is sampled with no effort wasted outside the spawning area. Secondly, some guide-lines need to be provided to cruise leaders on the number and spacing of transects which may be omitted in order to best match available effort to the size of the area to be surveyed. As a first guide to planning the distribution of sampling effort, historic egg distributions should be reviewed with particular reference to the latest WGMEGS reports. The main areas of egg abundance, identified for each of the different sampling periods, should always be sampled to the north/south and east/west limits although alternate transects may be omitted. When sampling along transects, shipboard enumeration of results should be undertaken several stations before the limit of the core area is reached. The introduction of the 'Spray technique' (Eltink, 2007) should allow a rapid assessment of the numbers of eggs present in each station. Sampling shall concentrate on the principal spawning areas of mackerel and horse mackerel as laid out in Figures 2.1 and 2.2 and sampling shall continue along a transect ideally until either two consecutive stations containing zeros are recorded or low numbers of mackerel or horse mackerel. Caution however should be used when employing this rationale and particularly in the North and Northwest, as it may often be necessary to sample beyond those priority areas. Cruise leaders should always consult the survey coordinator with regards to providing a provisional survey plan prior to departing on survey.

The entire spawning time of mackerel and horse mackerel is divided into different sampling periods. The amount of ship time available and the size of the area to be covered will determine the spacing and omission of sampling transects within the sampling periods. During periods when several ships are available it should be possible to sample all transects, while at other times it may be necessary to omit several, at least during the first pass over the designated sampling area. **No more than one consecutive transect should ever be omitted.** Given that the area to be covered is more or less known, as is ship time, cruise leaders should be able to estimate fairly accurately the number of the full transects they will be able to make. It is strongly recommended that, where practical, and even where total coverage is expected, a first pass over the area be made on alternate transects. The intervening transect should be sampled on the return leg. If time is limited on the return leg, sampling should be concentrated in areas where high egg densities were observed in the first pass. The cruise leader should be aware of edge definition problems where the contours run east-west. In this way, weather problems, equipment failure and vessel breakdown need not seriously prejudice results. Furthermore, such a strategy enables better evaluation of distributional change with time, which is likely to be important in modelling the results. This procedure does not apply to the area surveyed only by Portugal.

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

4 Standardisation of survey gears

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

Nash *et al*., 1998, provides a comprehensive description for a Gulf type sampler, which they call a Gulf VII. The Bongo net is sufficiently described in Smith and Richardson (1977) while a useful review of Bongo designs and a suggested standard is given by Coombs *et al*. (1996) in an annex to the final report of EU AIR project AIR3 CT94 1911.

The estimation of volume of water filtered by each sampler is critical in the calculation of egg abundance. Again, the suggestions provided by Nash *et al*. (1998), and Smith and Richardson (1977) provide an acceptable standard. These standards should be followed as closely as possible**.** It is also critical that the importance of calibrating flowmeters, and changes in flowmeter performance, when they are mounted in the apertures of plankton samplers is understood (EU AIR3 CT94 1911). It is recommended that the flowmeters and sampling devices **are calibrated prior to the survey**, in terms of the volume of water filtered. There are two aspects to calibration. The first requirement is to know and understand the relationship between flowmeter revolutions and distance travelled through the water. The second is to relate flowmeter revolutions, (whilst mounted *in-situ* in the aperture of a plankton sampler), to volume filtered by the sampler. The only way in which the second aspect can be accurately determined is to calibrate the sampler fitted with its flowmeter(s) under controlled conditions in a circulating water channel or in a large towing tank. These facilities provide independent measures of water or towing speed and also enable water velocity to be measured extremely accurately at numerous positions across the sampler aperture (EU AIR CT94 1911). Such facilities are extremely expensive and alternative methods to calibrate flowmeters in-situ have been employed by various participants. This usually involves calibration at sea using a reference flowmeter mounted on the outside of the sampler and two tows in opposite directions to overcome the effects of tides or currents on ship and sampler speed through the water. Such calibrations will provide a crude estimate of volume filtered (under non-clogged net conditions) but it must be remembered that there are differences in water velocity across the aperture of any sampler and that this water velocity profile may change as clogging of the net progresses. However, it is recommended that participants conduct calibrations of their flowmeters *in-situ* over a range of towing speeds at least at the beginning and end of each survey.

It is recommended that all participants review the performance of their flowmeters and regularly check their calibration in-situ (i.e. within the sampling device). The current flowmeters used in the survey are largely considered as state-of-the-art; however, new developments are being made in non-intrusive flow meters. It is recommended that participants investigate the utility and cost-benefits of these and report back to WGMEGS as appropriate.

Although a mesh size of 500 micron is adequate for sampling mackerel and horse mackerel eggs, a monofilament mesh between 250 and 280 microns is the recommended size for these surveys. This allows the plankton samples to be more widely used for

investigations on other species and taxa. In the North Sea surveys, where clogging is a problem, a 500 micron mesh is used

The aperture on the Gulf type sampler is 20 cm in diameter in order to ensure that an adequate volume of water is filtered. The aperture of the Bongo samplers is either 40 cm or 60 cm diameter. It is recommended that no ad hoc changes take place. The Cal-VET-net deployed by Portugal currently has a double mouth aperture both of 25 cm. The mesh size of the CalVET-nets are 150 microns. Different mouth openings for Bongos do not seem to make a difference in sampling efficiency or performance, although 60 cm nets (vs. 40 cm) are apparently more prone to clogging **if the filtering area of the net is not adjusted adequately**.

Since the 2004 surveys a high level of standardization of sampling equipment has been achieved for the mackerel and horse mackerel egg surveys (Table 5.1). According to the table presented below all Gulf VII type samplers used by the respective participants are more or less comparable with respect to their dimensions and therefore also their sampling performance. Provided that calibration of flowmeters is carried out carefully and the sampling manual is strictly followed it can be assumed that there is no sampler related bias.

Table 5.1: Gulf type ¨high-speed¨ plankton sampler designs as used by WGMEGS survey participants.

Institute	Wageningen Marine Research		TI-SF	MI	CEFAS	MSS		IMR	HAFRO	DTU
Country	Netherlands	Netherlands	Germany	Ireland	England	Scotland	Scotland	Norway	Iceland	Denmark
Torpedo type	Gulf III	Gulf VII	Nackthai*	Gulf VII	Gulf VII	Gulf III	Gulf VII	Gulf VII	Gulf VII	Nackthai
Years in use	Before 2004	After 2004	Since 2004	Pre 2004	Since 1995	before 2007	2007	until 2013	After 2004	
Frame	Encased	Open	Open	Open	Open	Encased	Open	Open	Open	Open
Total length (cm)	224	275	275	272	278	230	273	273	275	247
Length frame (without nosecone) (cm)	199	215	221	214	215	199	213	213	215	195
Length nosecone (cm)	35	60	54	59	63	31	60	60	60	52
Length of streched planktonnet (cm)	165	180	173	177	193	177	177	180	180	139
Diameter frame (cm)	50	50	43	53	53	50	53	50	50	43
Diameter planktonnet (cm)	41	40	38	50	45	46	46	38	40	38
Diameter codend (mm)	80	70	92	95	80	75	75	80	70	110
Diameter nosecone (cm)	19	20	20	20	20	19	20	20	20	20
Mesh size µm	280	280	280	280	280	250	250	500	280	280
Flowmeter position	internal	internal and external	internal and external	internal and external	internal and external	internal and external	internal and external	internal	internal and external	internal
Flowmeter brand/type		Valeport	Hydro-Bioss	Hydro-Bioss	Valeport	In-house design	Valeport-replica	Valeport	Valeport	General Oceanics
Flowmeter blade diameter (cm)			7.5	7.5	12.5			5		
Mechanical/electronic	Mechanical	Electronic	Electronic	Electronic	Electronic	Mechanical	Electronic	Electronic	Electronic	Mechanical

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

Portugal (IPMA) used a vertically deployed CalVET-net. Spain (AZTI and IEO) use 40 cm Bongo nets (Table 5.2). Also, the Faroese use Bongo nets. All specifications are listed in the table below. As with the Gulf VII samplers it can be assumed that no sampler related bias is present provided that the WGMEGS manual is strictly followed.

Table 5.2: Other plankton sampler designs as used by WGMEGS survey participants in the southern area.

Country	Net	Diameter (cm)	Shape	Mesh size (µm)	Total length (cm)	years in use	Depressor weight (kg)	Flowmeter
Spain (IEO)	Bongo	40	Cylinder-cone	250	250	since 1995	35	Mechanical
Spain AZTI	Bongo	40	Cylinder-cone	250	250	since 1995	45	Mechanical
Portugal (IPMA)	CalVET	25	Cylinder-cone	150	150	no info	no info	Mechanical
Faroes	Bongo	60	Cylinder-cone	280	250	since 2010	22	Mechanical
Iceland	Bongo	60	Cylinder-cone	no info	no info	only 2010	no info	Mechanical

5 Plankton sampler deployment and recording of haul data

Gulf type samplers are deployed on a double oblique tow, at 4 knots through the water, from the surface to maximum sampling depth (see below) and return. The Bongo samplers are deployed at 2–3 knots through the water on similar double oblique tows. The aim is for an even (not stepped) 'V' shaped dive profile, filtering the same volume of water from each depth band. The aim is to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band. At shallow stations, multiple double-oblique dives may be necessary to enable a sufficient volume of water to be filtered. A minimum sampler deployment time of 15 minutes is recommended. If possible the external flow meter should be used to monitor the correct speed of the Gulf type sampler, approximately $2ms^{-1}$.

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

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

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

Clogging of plankton samplers

Clogging of plankton nets occurs particularly in areas with high phytoplankton production or high abundance of gelatinous zooplankton, and can lead to erroneous estimates of zooplankton abundance. Even though in previous years clogging occurred in some stations during the mackerel egg surveys, the recording of it was omitted from the procedure of calculating egg abundances and production. Instead, it was assumed that the use of flowmeters would readily compensate for decreasing volume of filtered water. However, since sampling integrates across a large vertical depth range, clogging can result in significant bias if it impedes the ability of the sampler to operate efficiently and consistently throughout the entire sampled water column.

In order to identify possible clogging in the samples, survey participants are required to collect additional data during the hauls that should be reported with the principal survey data. The following data shall be recorded and reported:

From sampling gears with external flowmeter:

- either flowmeter counts together with calibration values (revs/m) or towed distance in m from the external flowmeter.

From the vessel:

- Towed distance over ground from GPS data between sampler deployment and retrieval. If this is not possible it is to ensure that the vessel aim to keep as straight a course as possible and that the heading at the start and end is recorded;
- Towed distance through the water between sampler deployment and retrieval if available;
- End of haul in hours and minutes;

- Haul duration in minutes and seconds;
- Speed through the water in knots averaged over the whole haul time between sampler deployment and retrieval recorded at 20 sec intervals if available;
- Speed over ground in knots averaged over the whole haul time between sampler deployment and retrieval recorded at 20 sec intervals. If not available the start and finish time of the haul;
- Heading averaged over the whole haul time between sampler deployment and retrieval recorded at 20 sec intervals;
- End position of haul;
- Wind speed in m/sec and direction in ° both averaged over the whole haul time between sampler deployment and retrieval recorded at 20 sec intervals. (nice to have but not mandatory).

The data shall be analysed after the survey to define thresholds for the clogging stages of the ICES fish egg and larvae database.

6 Plankton sample collection and fixation

It is recommended that the standard plankton samples collected for the SSB estimates will be handled carefully and preserved as soon as practicable. The recommended procedure will be as follows:

a) Remove the end bag used on the station before washing down the net.

b) Attach a clean end bag and gently wash down the net from both ends of the sampler, taking care to wash the lower surface of the net just in front of the end bucket.

c) Always wash down from the nosecone.

d) Make sure the net is clean, using more than one end bag if necessary.

e) Make doubly sure that a clean end bag is left on the sampler ready for the next station.

f) Wash the plankton from the end bags into a jar with the 4% formaldehyde solution in a wash bottle.

g) Top up the jar with 4% formaldehyde, making sure that the volume of plankton does not exceed 50% of the volume of the jar.

h) Any excess sample should be fixed separately in additional jars.

i) Label jars with station details and put labels containing same details in pencil into all jars.

The standard fixative for use on these surveys will be a 4% solution of buffered (pH 7 - 8) formaldehyde in either distilled or fresh water. (420g of sodium acetate trihydrate is dissolved in 10 litres of 4% formaldehyde, ICES, 2001). **This solution is only slightly hyper-osmotic to seawater but much less than formaldehyde-seawater solutions and will, therefore,** minimise damage and distortion of the eggs. The sample should be directly fixed with the addition of the 4% formaldehyde solution and should not come into contact with formaldehyde strength in excess of 4%.

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

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

It is recommended, that where possible, the spray technique be used at sea to quickly remove the majority of fish eggs from plankton samples. This will allow a rapid decision to be made on whether to continue sampling along a transect or to move to the next transect line.

The eggs removed by the 'spray technique' can be stored in separate vials within the plankton sample jar. However, it is imperative that every sample is subjected to a manual sorting and removal of any remaining eggs, to ensure that all eggs are removed from each sample. The SAT test, (surface adhesion test), should be performed on eggs removed from the manually sorted sample to check for the presence of hake eggs. The use

of the spray technique will remove the need for any sub-sampling of the plankton samples collected.

Immediately before the manual sorting, it is recommended that the 4% formalin is drained from the sample and the sample washed gently with seawater. The sample can then be placed in a sorting/observation fluid (Steedman, 1976), which also acts as a preservative **once the eggs are sufficiently fixed with formaldehyde (normally after 48 hours in formaldehyde)**. The observation fluid stock solution is made with 50ml of propylene phenoxetol mixed with 450ml of propylene glycol (propane-1,2-diol). Before use, 5ml of the stock solution is diluted with 95ml of distilled water to produce a sorting fluid which is non-toxic and pleasant to use (odourless).

Based on the experiences of the WKFATHOM workshops the binocular microsopes used for the egg sampling should have the following features:

- • Options for a black or white stage plate for use with incident (top) light.
- • A transparent stage plate for transmitted (bottom) light.
- • Dark field illumination for contrast.
- • Adjustable brightness.
- • Magnification with click stops.
- • Magnification should be at least 1.6x.
- • A choice of 10x and 20x eyepieces.
- • Adjustable binocular head and ergonomic design to allow flexibility of movement.
- • Adjustable focus on all eyepieces.
- • Calibrated eyepiece graticules.
- • Double (fibre optic) cold light source, with adjustable focus, to avoid shadows.
- • Mechanical stages to position samples easily in the field of view and to hold the samples firmly.

The whole sample should be sorted in order to remove all the eggs of non-target species such as hake, megrim, pearlside (*Maurolicus muelleri*) and sardine. In some areas these may be present in numbers that are significantly higher than those of the target species. All sorted eggs should be kept in tubes in 4% buffered formaldehyde, inside the sample container for future reference and use. Usually only th eggs of mackerel and horse mackerel need to be identified to species and staged. However, ling and hake eggs should also be staged as visual identification of these species depends on identifying stage specific characteristics. **Where large numbers of eggs have been removed from a plankton sample, a minimum 100 eggs of each of the target species must be identified and staged from the sorted sample. The rest of the eggs must then be apportioned across the appropriate species and stages. If 100 eggs of one of the target species are NOT found in 25% of the sample, then the whole sample will have to be sorted.**

7 Egg identification and staging

The eggs and larvae of most of the species found in the area are well described by Russell, 1976. His book is well known and used by all the participants of the ICES triennial surveys. It is generally regarded as the definitive work on the subject in this area. Descriptions of the eggs of mackerel, horse mackerel and species with similarly sized eggs can also be found in Munk and Nielsen (2005), Rodriguez, Alemany and Garcia (2017) and Re and Menses (2009).

Some difficulties do occur, particularly with the identification of eggs from species that do not show great differences in their morphological features. In some instances, it is even difficult to recognize differences between mackerel and horse mackerel eggs when the segmentation of the yolk is not distinct in the latter.

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

Spraying of the samples also gives an indication of the species composition of the sample. Hake eggs, and eggs such as *Maurolicus*, with its corrugated chorion, attract and retain microbubbles of air and are subsequently lifted upwards during the spraying procedure, tending to float at the surface. This is in contrast to mackerel and horse mackerel eggs, which drop downwards and can be drained.

Within WGMEGS the eggs of mackerel are classified into one of six morphological stages (Ia, Ib, II, III, IV and V; Lockwood et al., 1981; Figure 7.1.1), following the development criteria described for plaice (Simpson, 1959). For horse mackerel, and hake the description of stages is the same with the exception of stage V, which does not exist for these species (Figure 7.1.2). Horse mackerel and hake larvae hatch at the end of egg stage IV (Pipe and Walker, 1987; Coombs and Mitchell, 1982).

Egg staging criteria

The primary characteristics are based on those presented in Lockwood et al. (1977) for mackerel (Figures 7.1-1 and 7.1-2), but include some other (secondary) characteristics, which are thought to be crucial in determining egg stage. Figures 7.1-3 and 7.1-4 shows the development stages for horse mackerel and figure 7.1-5 provides some development stages for hake eggs.

Stage IA

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

Secondary characteristics: There are no signs of a thickening of cells around the edge of the cell bundle.

NB. In preserved eggs, the edge of the cell bundle can sometimes fold over giving the appearance of a 'signet ring' seen in a stage Ib.

Stage IB

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

Secondary characteristics: The cell bundle has thickened around the edge giving a distinct ring appearance. Cells in the centre of the ring form a progressively thinner layer and eventually disappear.

NB. At the end of this stage, the ring can become very indistinct as it spreads towards the circumference of the egg.

Stage II

Primary characteristics: From the first sign of the primitive streak, which begins as a cleft in the cell bundle, until closure of the blastopore. Towards the end of this stage the tail tapers and is flattened against the yolk. Also at the end of this stage, the embryo should be half way around the circumference of the egg.

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

Stage III

Primary characteristics: The end of the tail has thickened, becoming bulbous in appearance, and may have lifted clear of the yolk sac. Growth of the embryo is from half way to three-quarters of the way around the circumference of the egg.

Secondary characteristics: Widening of the head and development of the eyes. Pigment spots develop on the embryo.

Stage IV

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

Secondary characteristics: Eyes continue to develop and the lenses become visible. Development of the marginal fin and the tail separates from the yolk. Pigmentation on the embryo increases compared to stage 3.

Stage V

Primary characteristics: The tail of the embryo is touching the nose or beyond and circumnavigates the egg following the inner margin of the membrane.

Secondary characteristics: Pigmentation develops in the eye.

NB

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

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

Figure 7.1-1. Mackerel eggs at the beginning and end of the six development stages.

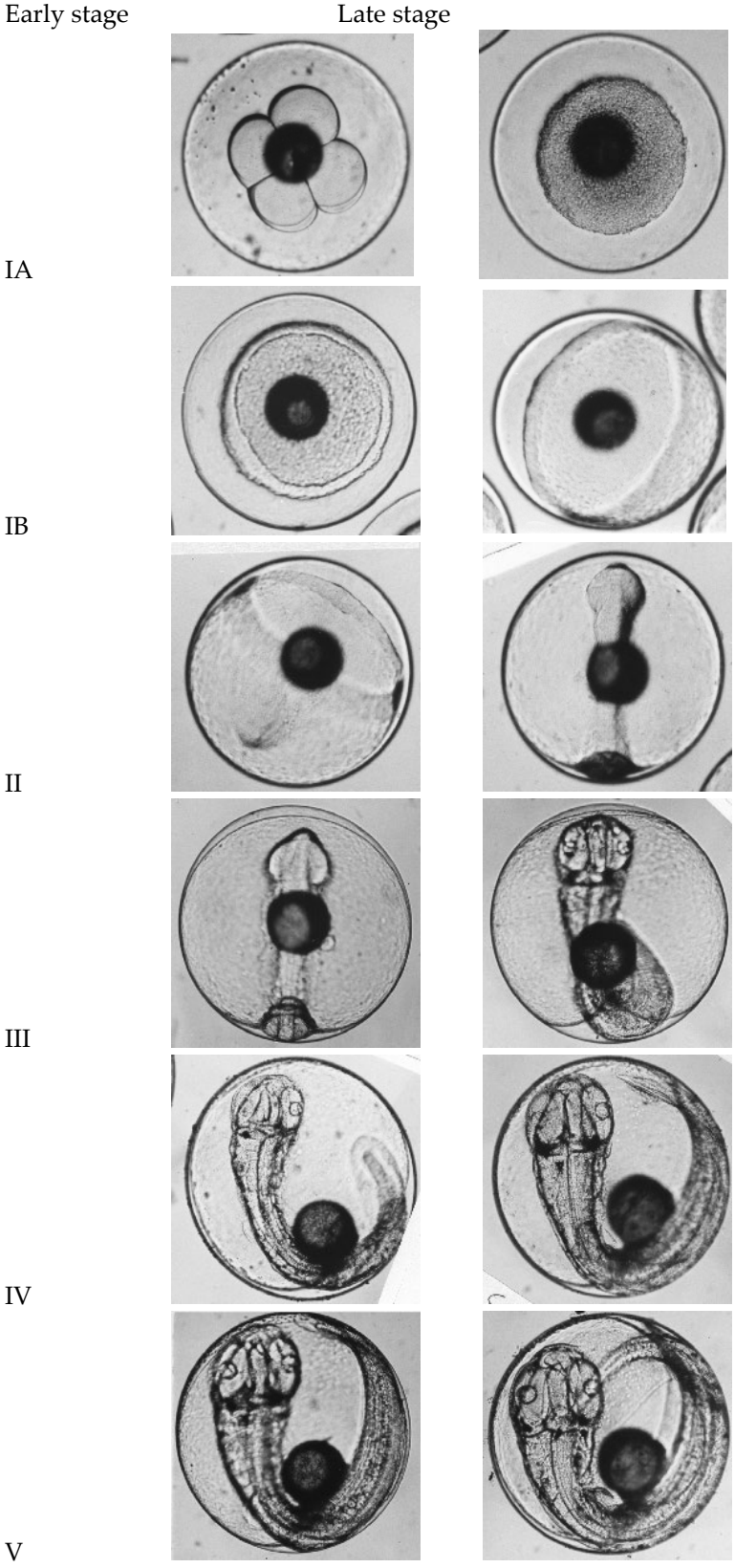
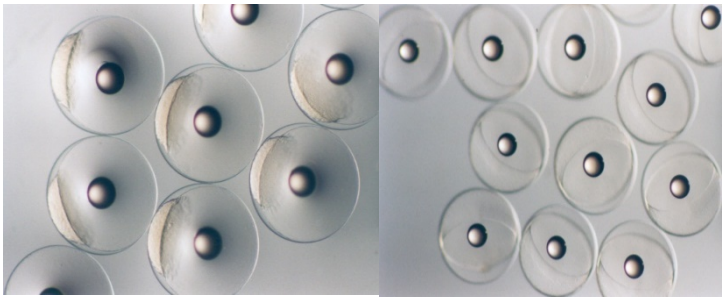
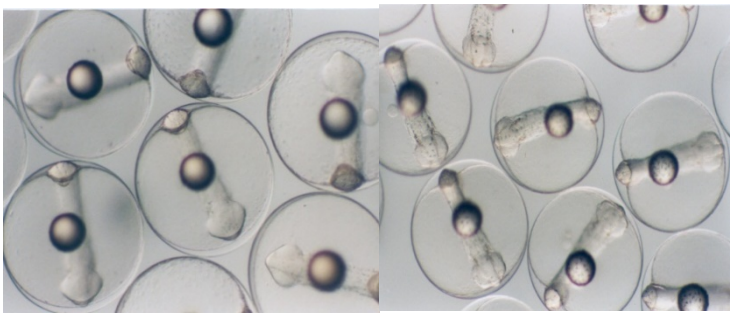


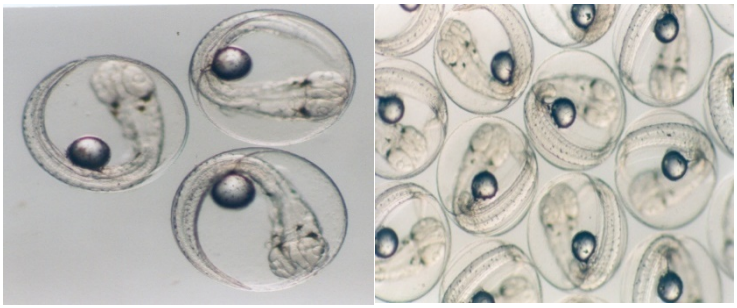
Figure 7.1-2. Development stages of mackerel from fertilization experiments.



Stage 1A Stage 1B

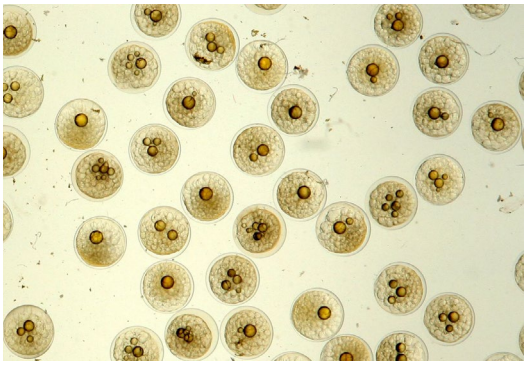


Stage II Stage III

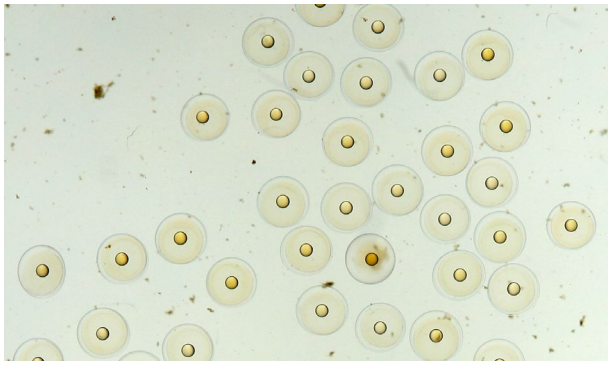


Stage IV Stage V

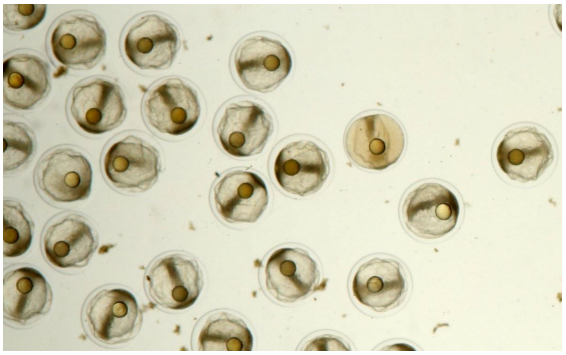
Figure 7.1-4. Development stages of horse mackerel from fertilization experiments



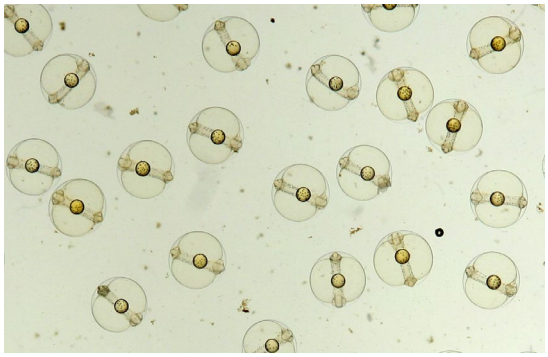
Stage IA



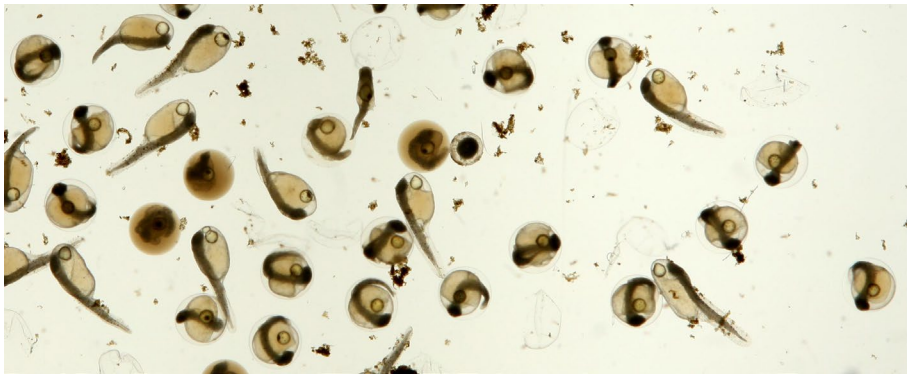
Stage IB



Late stage II

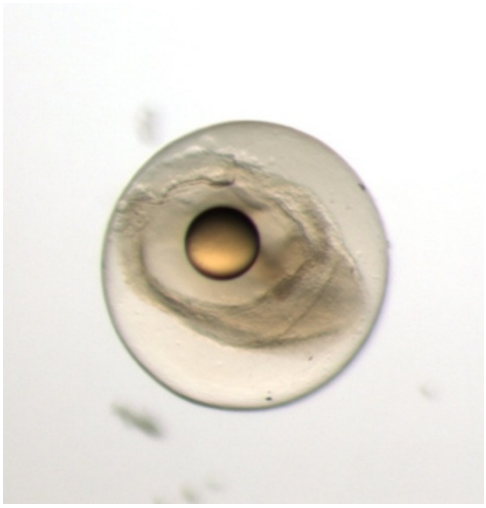


Early stage III

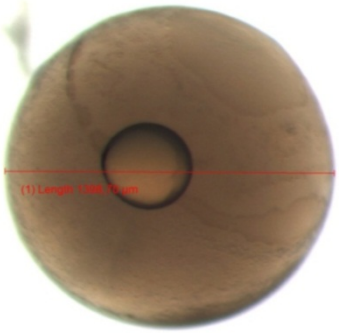


Late stage IV and hatching

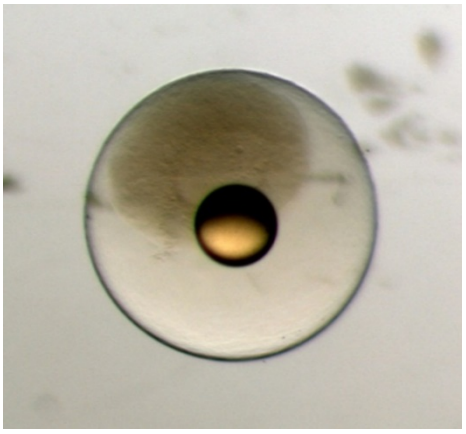
Figure 7.1-5. Development stages of hake eggs from fertilization experiments.



Stage 1A

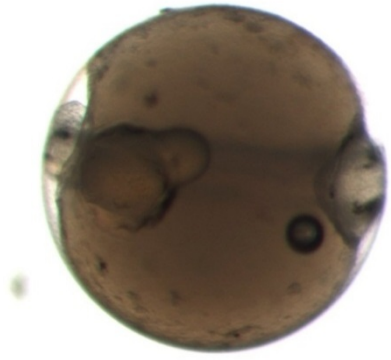


Stage 1A

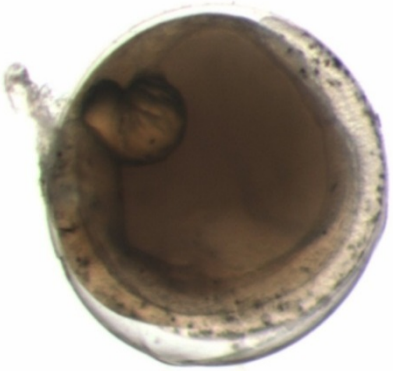


Stage 1B

Stage II



Stage III



Stage III

Egg identification criteria

Egg and oil globule size are the primary criteria used to identify eggs. Mackerel eggs range in size from 0.97 mm to 1.38 mm with the oil globule ranging from 0.22 to 0.38 mm. According to literature horse mackerel eggs range from 0.81 to 1.04 mm with an oil globule ranging from 0.19 to 0.28 mm.

Table 7.2.1 summarizes published descriptions of mackerel, horse mackerel and other species of eggs that contain similar morphological features. It provides validated observed egg and oil globule diameters for each species as well as the diagnostic features and criteria used by the participants to help with egg identification. It should be noted that the diameter of the egg and oil globule within a species can and may vary through the spawning season and also from area to area. Variation in egg size for the same species can also be observed within the same sample

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

Table 7.2.1: Comparison of the Characteristics of Mackerel, Horse Mackerel, Blue Jack Mackerel, Megrim, Hake and Snipefish Eggs (Details of fixative and concentration unknown).

Species	Diameter (mm) Egg	Oil Globule	Reference	Area	Diagnostic Features
Mackerel (*Scomber scombrus*) (See Lockwood *et al.*, 1977)	1.0-1.38	0.28-0.35	Russell, 1976	North Sea, English Channel	• Unsegmented/ Homogenous yolk • Perivitelline space approx 0.05mm • Not typically found where water temperature at 20m is less than 8.5 degrees Celsius.
	1.09-1.36	0.26-0.37	Fahay, 1983	N.W. Atlantic	
	0.97-1.38	0.25-0.35	Ehrenbaum, 1905-09	Irish Sea, North Sea	
	1.24	?	Mendiola et al, 2006	Biscay	
	0.97-1.38	0.22-0.38	Fritzsche, 1978	Mid-Atlantic Bight	
	1.0-1.38			North Atlantic	
	0.97-1.38	?	Johnstone, Scott and Chadwick, 1934	Isle of Man	
	1.21-1.33	~0.32	Holt, 1893	West of Ireland	
	1.16	0.27	IPMA, fertilization experiment 2008		
Horse Mackerel (*Trachurus trachurus*) (See Pipe and Walker, 1987)	0.81-1.04	0.19-0.28	Russell, 1976	North Sea, English Channel	• Granular / segmented yolk, although this may not be as obvious at the southern end of the species range. • The oil globule migrates towards the head of the embryo after stage 2.
	1.03-1.09	0.26-0.27	Holt, 1898	North Sea	
	0.81-0.93	0.22-0.23		Plymouth	
	0.84-1.04	0.19-0.24	Ehrenbaum, 1905-09	North Sea, English Channel	
	Max. 0.84	0.24-0.26	Holt, 1893	English Channel	

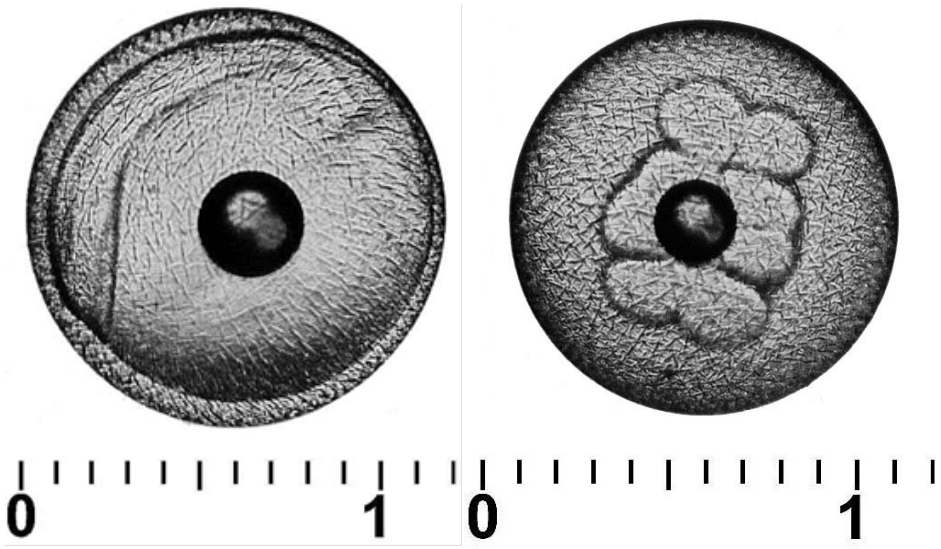
Species	Diameter (mm) Egg	Oil Globule	Reference	Area	Diagnostic Features
	0.90–1.00	0.18–0.28	Cunha *et al.*, 2008, Gonçalves *et al.* 2013	Atlantic – Iberian waters	• In stages 3 and 4 the embryos show stronger pigmentation compared to mackerel. However, the pigmentation is not as strong as in hake. • Oil globule easily broken into several smaller pieces. This seems to be more common in eggs found in the southern area, particularly in eggs from the Portuguese coast.
Blue Jack Mackerel (*Trachurus picturatus*)	0.98-1.10	0.19-0.31	IPMA, fertilization experiment 2010 (Gonçalves *et al.*, 2013)	W Portugal	• Segmented yolk
Megrim (*Lepidorhombus whiffiagonis*)	1.02-1.22	0.25-0.30	Russell, 1976	North Sea, Irish Sea	• Striated appearance of egg membrane*. (See below)
	1.07-1.22	0.25-0.30	Ehrenbaum, 1905-09	North Sea	
	1.07-1.13	0.30	Holt, 1893	West of Ireland	

Species	Diameter (mm) Egg	Oil Globule	Reference	Area	Diagnostic Features
	1.08-1.30	0.29-0.34	Milligan *et al.*, In prep.	Celtic Sea	• Oil globule is closer to egg membrane than in mackerel. • Embryo thinner than a mackerel embryo. • Yolk unsegmented and the egg has a small perivitelline space. • Pigmentation on yolk from stage II onwards. • Pigment on oil globule as embryo develops ***Striations can be observed on the membranes of preserved eggs of other species. This can lead to misidentification of eggs which have been preserved for some time.**
Hake (*Merluccius merluccius*) (See Coombs, 1982)	0.94-1.03	0.25-0.28	Russell, 1976	North Sea, English Channel, Mediterranean	• Positive surface adhesion test (SAT) is used to identify hake eggs (Porebski, 1975) and (Coombs, 1994). • From stage III onwards embryos display strong pigmentation along the embryo. Towards the end of its
	0.94-1.03	~0.27	Ehrenbaum, 1905-09	North Sea, English Channel, Mediterranean	
	0.94-1.03	~0.27	D'Ancona *et al.*, 1956	?	

Species	Diameter (mm) Egg	Oil Globule	Reference	Area	Diagnostic Features
	1.10-1.16	0.27-0.35	Shaw, 2003	Celtic Sea	development, the embryo begins to show the characteristic post-anal pigmentation of three bars. • Tend to be retained on the surface/in plankton when sample is sprayed.
	1.063 ± 0.021	0.26 ± 0.012	Guevara-Fletcher et al. 2016.	Galician waters	
Longspine Snipefish *(Macrorhamphosus scolopax)*	1.00	0.2	Development of Fishes of the Mid-Atlantic Bight, 1978. U.S. Fish and Wildlife service. FWS/OBS-78/12.	Europe	• Membrane is light amber with grainy reflections • Yolk with rose or violet halo depending on viewing light. • Oil globule is amber/rose in colour
Lings (Molva spp.)	0.97 – 1.13	0.28 – 0.31	Russell, 1976	North Sea	• Unsegmented yolk • Pigmented oil globule • Pigmentation in later stage embryo is concentrated into 2 distinct lines that run all the way along the back. • Includes eggs of *Molva molva* that are typically found in temperatures less than 8.5 degrees Celsius. Otherwise eggs very similar to *S.scombrus*.

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

Figure 7.1-6. Eggs of megrim, showing the striations on the membrane.



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

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

Scomber colias

The distributions of *S. scombrus* (Atlantic mackerel) and *S. colias* (chub mackerel) coincide in the northeast Atlantic, i.e., both species coexist in the Iberian Peninsula waters. In fact, both species are jointly exploited in this area, although chub mackerel is not a target species. However, biomass and abundance are much lower compared to the target congener.

Egg description: Morphological characteristics of chub mackerel eggs are given below:

- The eggs are spherical, on average ranging in diameter from 0.8 to 1.35 mm (Ré and Meneses, 2009). Similar descriptions were provided by Fahay (1983) Rodriguez et al. (2017) with diameter measurements ranging from 1.06 to1.36 mm and 1.04 to1.14 mm respectively.
- Oil globule 0.22-0.31 mm in diameter (Ré and Meneses, 2009). In the Mediterranean oil globules diameters varies between 0.22-0.27 mm (Rodriguez et al., 2017) while in the Pacific ranges 0.25-0.32 mm (Fritzsche, 1978).
- Yolk is smooth, transparent and unsegmented and under magnification (x36) can be seen to be filled with many tiny vacuoles. The only difference with S. scombrus is that the yolk is pigmented with several melanophores, while in S. scombrus eggs the yolk is pigmented just before hatching, when a spot per side appears just posterior to the head.
- The perivitelline space is narrow.

- In advanced stage of development both the dorsum of the embryo and the oil globule are pigmented, the latter on the hemisphere facing the head (Kramer, 1960).

Spawning time: Spawning time varies according to its location. In general, the spawning seems to be limited to the first half of the year, that is, from March to July in the Bay of Biscay and Cantabrian Sea (Lucio, 1997; Villamor et al., 2017) and from February to June in Portuguese waters (Martins et al., 1983; Martins and Serrano, 1984).

Trachurus mediterraneus

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

Trachurus picturatus

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

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

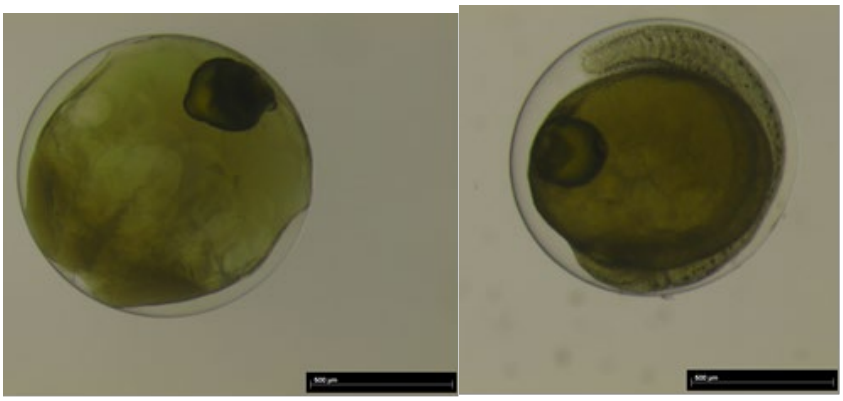


Figure 7.3-1. Eggs of *Trachurus picturatus* from a fertilization experiment (IPMA, 2010).

Macroramphosus scolopax

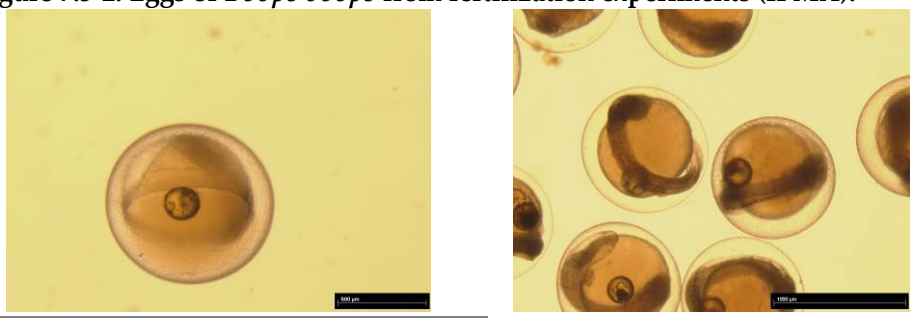
- Egg diameter: 1.0 mm
- Oil globule: 0.20 mm

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

Boops boops

- Egg diameter: 0.93 mm (based on eggs from artificial fertilization, IPMA, 2008, see Figure 9.3-2)
- Oil globule: 0.18 mm (based on eggs from artificial fertilization, IPMA, 2008)
- Description: Pelagic eggs, spherical. Single oil globule with melanophores (Gaetani, 1937).
- Fish distribution is mapped in the reports of ICES-WGACEGG.

Figure 7.3-2. Eggs of *Boops boops* from fertilization experiments (IPMA).



8 Data submission

The results of the egg analysis should be submitted to the survey data coordinator, using the updated excel spread sheets, within a month of the end of each cruise, but at the latest the 31st June for survey periods earlier than period 5. All later surveys report by 31st July.

All participants should attempt to meet the deadline for the submission of survey results. The processing of sub-sets of samples should be avoided in order to provide a reliable preliminary estimate of the SSB index. If it becomes obvious that a participating institute will fail to provide their survey results on time, then the survey coordinator and the WGMEGS chairs should be notified as soon as possible. The survey coordinator and the WGMEGS chairs will then liaise with the participant about the selection of a representative subset of samples that can be processed as a priority.

An excel template for the data entry of the plankton data will be distributed by the survey coordinator prior to the surveys commencing. Since the last survey in 2016 the data table has been modified, and extended, to collect additional information, both to fulfil requirements for the ICES egg and larval database, and also modifications recommended by WGMEGS at the 2018 meeting in Dublin.

All participants must use the template provided to avoid time-consuming conversions from varying formats.

9 Data analysis

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

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

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

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

Log_e time (hours) = -1.31 $\log_e$ (T°C) + 6.90

The TAEP time series from 1992 has also now been updated using the Mendiola equation.

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

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

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

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

Detailed procedures for the post analysis of egg abundance data to produce daily and, finally, annual egg production estimates are given below. **A designated data co-ordinator** will collate and manage the results for the entire survey (see also section 4.3). This analysis is subject to examination and approval by the full working group and ensures a standard approach and methodology. Participant must supply their plankton data in a standard MS Excel spreadsheet (see Annex), to be distributed by the data co-ordinator.

The stages in the estimation of annual egg production are:

- Estimating the daily egg production per rectangle
- Estimating the period egg production for each survey period
- Integrating the daily egg production using the histogram method, to estimate the total annual egg production (TAEP)
- Calculating the variance of the estimate of TAEP

The method was modified for use in the analysis of the 1995 survey data. It is fully described in section 5.3.3 of the report of those surveys (ICES, 1996b). The same methods will be used for the analysis of the survey data.

Daily egg production per rectangle

To convert the number of eggs in each sample to the number of eggs per m², the following calculations are made. Firstly the volume of sea water filtered by the sampler during the haul is calculated.

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

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

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

Where:

- V = Volume filtered in m³
- r = Number of revolutions of the flow meter during tow
- a = Aperture: The area of the mouth opening of the sampler in m²
- cal = The number of flow meter revolutions per metre towed, obtained from the flume or sea calibration in free flow
- A_e = Egg abundance in eggs m^{-2}
- C_e = Number of eggs in sub-sample
- S = Raising factor from the sub-sample to the whole sample
- D = The maximum depth of the sampler during the tow in metres
- F = The sampler efficiency from flume or towing tank calibration (ideally 1)

Numbers of eggs per m² are raised to number of eggs per m² per day production (D_{sh}) using development equation for both species in the following way:

For stage I **mackerel** eggs:

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

For stage I **horse mackerel** eggs:

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

Where D_{sh}= egg production in eggs m^{-2} day^{-1} and T = temperature in °C at 20 m depth (5 m in the North Sea, or sea surface temperature if 20m is not available).

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

When there was more than one observation per rectangle within a sampling period, the arithmetic mean of the observed values (h) were used to estimate the Mean Daily egg production/m² in rectangle s ().

Where:

- n_s = number of hauls in rectangle s
- D_{sh} = number of stage I egg/m²/day in haul h in rectangle s
- A_s = area of sampled rectangle s in m²

The mean egg/m²/day is then raised by the area of the rectangle it represents, to give the daily egg production on rectangle *s*:

$$A_s \cdot \overline{D}_s$$

Rectangle areas are calculated by each ½° row of latitude using the formula:

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

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

Some rectangles are reduced in size because they border land. Only the sea surface area of these rectangles is considered in the calculation, with the area covered by land being removed.

For unsampled rectangles within the designated survey areas there is a well-defined protocol to interpolate daily egg production for unsampled rectangles which fulfil the following criteria. In order to qualify for an interpolated value an unsampled rectangle must have a minimum of two sampled rectangles immediately adjacent to it. Once qualified, the mean daily egg production values of all surrounding rectangles, both immediately adjacent and diagonally adjacent are used to calculate the interpolated value. The interpolated value is the arithmetic mean of all those surrounding rectangles including zeros.

Let u denote such an unsampled rectangle, and let $\overline{D}$ if rectangle s is adjacent (immediately or diagonally) to rectangle unsampled and 0 otherwise. Daily egg production/m² in rectangle unsampled is then estimated to be:

$$\overline{D}_u = \frac{1}{\delta_u} \sum_s \delta_{us} \cdot \overline{D}_s$$

Where $\delta_{u\cdot}$ is the number of sampled rectangles adjacent to u.

$$\delta_{u\cdot} = \sum_s \delta_{us}$$

Raising by the area of the rectangle unsampled then gives the daily egg production in rectangle u:

$$A_u \cdot \overline{D}_u$$

Once calculated, interpolated values are not used in order to calculate values for other unsampled rectangles, or to qualify those rectangles for interpolation. No values are to be extrapolated outside the sampled area. As a general recommendation, cruise leaders should try to avoid situations where interpolation is going to be problematic.

Daily egg production in each period survey

Daily egg production in each period (D_P) is then estimated as a sum of the daily egg production on sampled and interpolated rectangles:

$$D_p = \sum_s A_s \cdot \bar{D}_s + \sum_s A_u \cdot \bar{D}_u$$

Annual egg production

Finally, total annual egg production, (D_A) is a weighted sum of the mean daily production in each period. There have been two approaches used in the past to raise mean daily egg production from each period to the total annual egg production: the under-the-curve method and the histogram method. The weights in the TAEP sum, length in days, arise from what is termed the histogram method for raising daily egg production, however, these weights could also come from the under-the-curve method.

The under-the-curve method takes the estimate of TAEP on the mid-points of the sampled periods to be the area under the egg production curve. The under-the-curve method only depends on the midpoints of the sampled period so implicitly doesn't recognize unsampled periods. The under-the-curve method also has associated weights, but these do not depend on the length of the sampled periods, only the mid points of the sampled periods and the start and end times of spawning.

The histogram method involves calculating the egg production in each sampling period p and then interpolating or extrapolating the unsampled periods to calculate the egg production in these. This is equivalent to sum of the daily egg production estimates in each sampling period p, multiplied by its length in days, λ, and the sum of the D estimates in each interpolated unsampled period p:

$$D_A = \frac{D_{p=1}}{2}\left(d_{p=1,s} - d_0\right) + \sum_{p=1}^{P}\left[D_p\left(d_{p,e} - d_{p,s}\right) + \frac{D_p + D_{p+1}}{2}\left(d_{p+1,s} - d_{p,e}\right)\right]$$

Where $d_{p,s}$ and $d_{p,e}$ are the start and end dates (in Julian days) of period p, and d_0 is the start date of the spawning season. For the last sampling period (when $p=P$), Dp_{+1} will equal 0, and $d_{p+1,s} = d_P$, the end date of the spawning season.

The histogram method has several advantages over the under the curve method that is discussed in 2011 WGMEGS Report. The histogram method is used to provide the revised estimates for 2007 and the final estimates since 2010.

Variance estimation

The variance of the TAEP estimate is based on assuming that the raw mean daily production on rectangle in each period is distributed with a constant Coefficient of Variation (CV).

Resulting in the estimate of the variance of Mean daily egg production on a single rectangle as:

$$\sigma^2(\overline{D}_s) = \frac{(A_s \cdot \overline{D}_s \cdot CV)^2}{n_s}$$

The CV of the data can be estimated by assuming a log normal distribution for the positive egg production observations and estimating the residual variance about the expected values of log egg production. The CV by traditional methodology is estimated by the residual standard deviation from an analysis of variance of log daily production on replicate rectangles for each period. To avoid the influence of zero egg counts, any rectangles with any zero counts are excluded. In practice, there are too few rectangles with replicate hauls to estimate a reliably for each period, so the estimates for each period are pooled.

An alternative methodology investigated at the working group estimates the CV by a Generalised Additive Model using interaction latitude, longitude and period to model the log egg production through time, with each sampling rectangle modelled as an uncorrelated random effect.

FIXED: $log(egg\ production) \sim s(period, lat, long)$

RANDOM: $\sim rectangle$

This alternative methodology permits the use of more data points as opposed to the traditional methodology and is therefore more suited to the MEGS dataset

Variance of Daily egg production for each sampled period is given by the sum of variances for the individual sampled rectangles in period:

$$\sigma^2(D_p) = CV_p^2 \cdot \sum_s \left(A_s + \sum_u \frac{\delta_{us}}{\delta_{u\cdot}} \cdot A_u \right)^2 \cdot \frac{\overline{D}_s^2}{n_s}$$

Where:

- s = Sampled rectangle
- p = Period
- $\delta_{u\cdot}$ = Number of sampled rectangles adjacent to u.
- Ns = Number of hauls in rectangle s
- u = Unsampled rectangle

This is based on an estimate of the variance in Daily egg production for each sampling rectangle (s) calculated as variance of sampled rectangle plus any additional variance of area due to the filling in of unsampled adjacent rectangles. The added unsampled rectangle area should be calculated adding the unsampled rectangle area dividing by number adjacent sampled rectangle used at filling in the unsampled rectangle.

The procedures for estimating the Total Annual Egg Production (TAEP) and its variance are those described in detail by Fryer (ICES WGMEGS, 1996). The variance of the TAEP is assumed to be the weighted sum of the variances of the total daily egg production in each sampling periods (ICES, 1993, 1996; 2003).

$$\sigma_A^2 = \sum_{p=1}^{P} \lambda_p^2 \cdot \sigma^2(D_p)$$

Where:

- λ_p = the length in days by period.

Annual egg production and SSB estimation

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

All data analysis should be carried out in accordance with the procedures described in detail for the 1995 and 1998 surveys (ICES, 1996, 1999). The detailed steps of the data analysis were updated for the 2003 WGMEGS report (ICES 2003), and then subsequently for the WKMHMES report (ICES 2006b) and for the MEGS survey manual (Annex 2 of ICES 2010). Individual countries supplied data in an electronic Excel template form to the data coordinator. The data for each station consisted of:
sample time, date and position,
numbers of mackerel, horse mackerel and other eggs by stage.
sub sample size,
volume of sea water filtered (or flowmeter counts and calibration data)
water depth, depth sampled, temperature and salinity profiles.

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

Spawning Stock Biomass estimation

Spawning Stock Biomass (SSB) is estimated as

$$SSB = \frac{TAEP}{Realized\ Fecundity} \cdot \frac{1}{sex\ ratio} \cdot (correction\ factor)$$

Where

- correction factor = 1.08. Value used to adjust pre-spawning to average spawning fish weight.
- sex ratio = assuming equal weight for males and a sex ratio of 1:1

The variance of the SSB estimate is based on both variance estimates, Realized fecundity and Total Annual Egg Production scaled by all the remaining factors.

$$\sigma^2(SSB) = SSB^2 \cdot \left(\frac{\sigma^2(F_r)}{(F_r)^2} + \frac{\sigma^2(TAEP)}{(TAEP)^2} \right)$$

Where

- Fr: Realized fecundity
- TAEP: Total Annual Egg Production

This comes from the application of the delta method (itself based on a Taylor expansion of $TAEP/F_r$).

10 Standardization of adults sampling

A detailed description of ship board methods for fecundity sampling is also given in the WGMEGS Fecundity Manual (SISP 5).

For western horse mackerel the results of the fecundity samples have not been incorporated in the SSB estimate since 2001. The effort of horse mackerel fecundity sampling and analysis is directed to collect samples to estimate DEPM adult parameters (ICES 2012). During the survey for horse mackerel adult samples will be collected during the peak spawning period (**period 6 & 7**).

Before the cruise

Procure **25-50 µl capillary pipettes** (Wiretrol II 25-50µl, Cat. Number 5-000-2050 (VWR). Extra plungers can be ordered from the same supplier; be sure to order the long plungers). Test performance of the pipette by practice, taking 25 µl fresh gonad or water samples.

Buffered formaldehyde : 3.6% buffered ($NaH_2PO_4*H_2O$: 29.48 mM, $Na_2HPO_4*2H_2O$: 46.01 mM) formaldehyde (see also excel-file on the IMR ftp-sever: "Buffered formaldehyde").

Labels: IMR and Wageningen Marine Research will send around labels to all the institutes participating in the survey to use on tubes and vials. Each institute will get its own code (Table 10.2.1). Each institute has a Rlabo (sampling institute) code on the labels to show who has collected the samples and a Alabo (analysing institute) to indicate who the sample should be sent to.

Nunc and tubes: fill the labelled 2.5 ml Nunc tubes (with screw on lids) with 1.2 ml of 3.6% buffered formaldehyde. Also fill the labelled 50 ml tube with 45 ml of 3.6% buffered formaldehyde or use the BiopSafe containers.

Bottles: these will be labelled and filled with formaldehyde during the cruise, or use the BiopSafe containers.

IMR ftp-server: make sure each institute has access to the ftp-server in order to upload/download information.

Table 10.2.1. Sampling and analysing code for each institute used on the fecundity and atresia samples

RLABO	ALABO	Sample type	Country	Institute and address	Responsible person
E, F	E, F	a,b,c,d,e	Norway	IMR, Nordnesgaten 50, 5005 Bergen, Norway	Merete Fonn / Anders Thorsen
A, B	A, B	a,b,c,d	Ireland	MI, Rinville, Oranmore, Co. Galway, Ireland	Brendan O`Hea
C, D	C, D	a,b,c,d	Scotland	Marine Scotland Science, Marine Laboratory, Victoria Road, Torry, Aberdeen, AB11 9DB, Scotland	Finlay Burns / Hannah Holah
M, N	M, NM	a,b,c,d,e	Spain	IEO, Subida A Radio Faro 50-52, 36390 Vigo, Spain	Antonio Solla
K, L	K, L	a,b,c,d,e	Spain	AZTI, Herrera Kaia, Portualdez/ g 20110 Pasaia, Basque Country, Spain	Paula Alvarez / Maria Korta
I, J	I, J	a,b,c,d,e	Netherlands	IMARES, Haringkade 1, 1976 CP IJmuiden, Netherlands	Cindy van Damme / Hanz Wiegerinck

G, H	-	-	Germany
O, P	-	-	Portugal
Q, R	-	-	Faroe
S, T	-	-	Iceland
U, V	-	-	Denmark
W, X	-	-	England

During the cruise (Mackerel AEPM and DEPM)

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

Measure total length, weight, assess maturity (Walsh scale and WKMATCH 2012 maturity scale revised) and sex of each fish in the subsample.

Select females in maturity stages 3-6 (Walsh scale) (stages Bb-Db) from the subsample of 100 (if less than 100 fish are in the catch, sample all the mackerel) for fecundity and atresia analysis. If possible divide the total quota of females equally into the 4 weight categories: < 250g, 251-400g, 401-550g and >550g. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes.

Measurements:

Total length (nearest mm)

Total weight (nearest gram)

Sex

Maturity (Walsh scale and WKMATCH 2012 maturity scale revised)

Otoliths

Weight of ovary (nearest 0.1 gram). (If it is not possible to measure the ovary weight at sea, take out the ovary and weigh the fish without the ovary. Then take the pipette and atresia samples and fix the remainder of the ovary and weigh the ovary in the lab. The fixed and frozen weights should be corrected to fresh weights.) Note if fresh, fixed or frozen ovary weight is taken in the datasheet.

Screening and atresia sampling (e, f):

From one ovary lobe cut a 0,5 cm thick section (Fig.10.2.1), with a scalpel through the whole ovary in the middle of the lobe. Immediately put this sample into a histology cassette and put the cassette in a prefilled individually coded 50 ml tube. Make sure

the sample is covered with 3.6% buffered formaldehyde solution (1 part ovary and 9 parts formaldehyde).

Place the remaining parts of the ovary lobe in a bottle (100-250 ml with wide opening) and fill it with 3.6% buffered formaldehyde (Fig. 10.2.2). Label (f) the bottle with coded label with the sample reference number. Make sure that the bottle is completely filled with formaldehyde and that the tissue is not more than 50% of the volume of the formaldehyde.

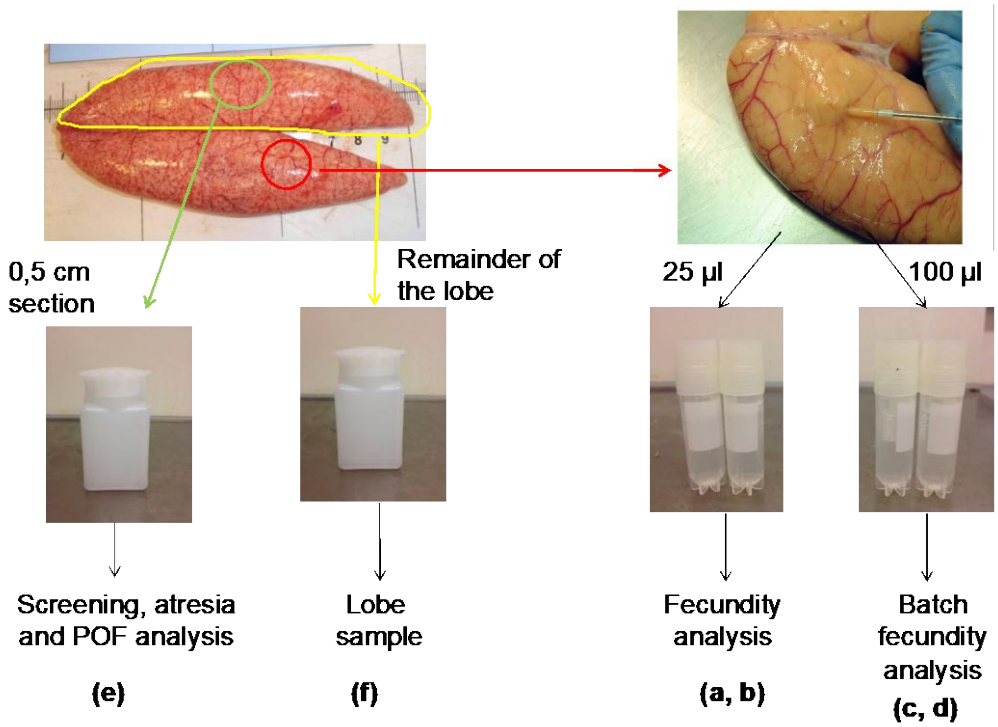


Fig. 10.2.1. Subsampling of mature females (stages 3-6, Walsh and stages Bb-Db in the WKMATCH 2012 maturity scale revised scales) ovaries at sea.

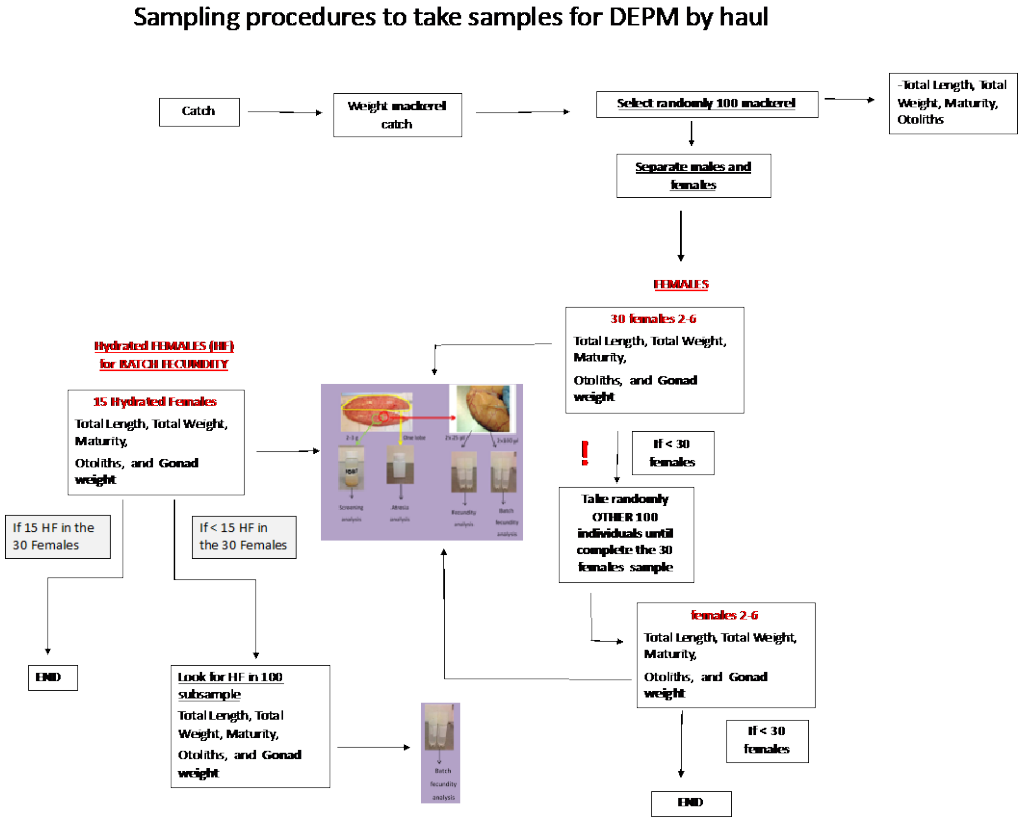


Fig. 10.2.2. Sampling procedures to take samples for DEPM by haul.

Fecundity sampling (a,b,c,d):

- From the other lobe of the ovary, take 2 samples of 25µl (a, b) and 2 samples of 100µl (c, d) with a pipette (Fig. 10.2.1 & 2) and immediately put each sample in its own individually coded tube. Take in a bit more sample than you need and press the plunger until it reach the line (25 or 50µl) and blot of any oocyte that is outside the tip on your hand or a piece of paper. Ensure all oocytes are immersed in 3.6% buffered formaldehyde solution. For the 100µl samples take two times 50µl with the pipette. Rinse the pipette with water and dry it with a paper towel prior to sampling another fish. The reason to obtain 2 samples of 25 µl and 100 µl respectively is to guarantee samples in the case a sample is lost during the processing. Send out the samples coded as (a,b) and (c,d) to the analysing institutes following the Alabo code as indicated on the label.

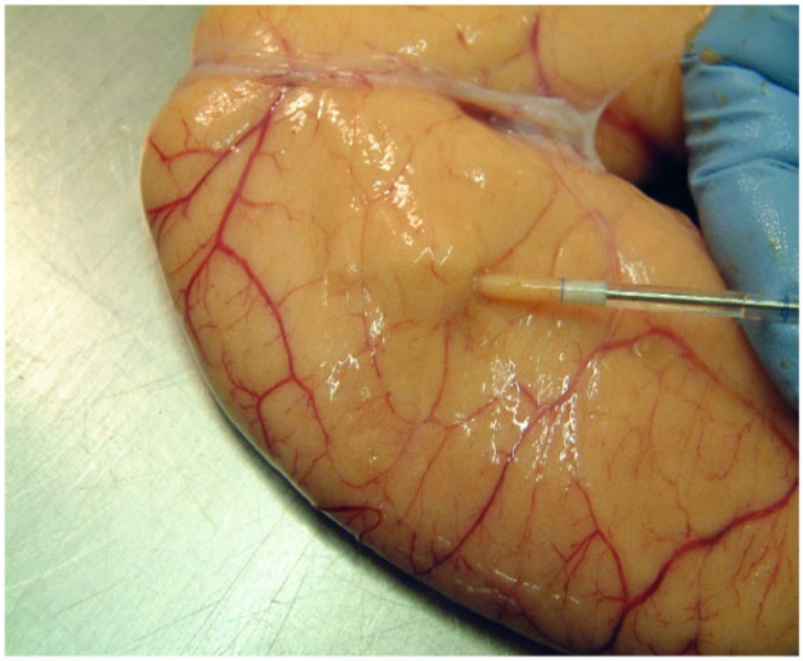


Fig. 10.2.2. Method to use a capillary pipette to remove an ovary sample.

Ring test sampling:

Each institute should additionally collect ring test samples for fecundity. Take 10 samples from one suitable fish (not used for AEPM or DEPM) and mark them with the extra labels numbered from X901_X.

Send out 1 sample to each institute involved in the fecundity analysis.

After the cruise

Immediately after the cruise:

- Screening samples in the 50 ml tubes should be sent to the analysing institutes (AZTI, IEO, Wageningen Marine Research and IMR, Table 10.2.1);
- Also send out Nunc tubes for the fecundity and batch fecundity samples (AZTI, IEO, Wageningen Marine Research, IMR, MI, and MSS).

On the outer cover of the package indicate the volume of fixative and that it is within the limits for unclassified transport. Add safety sheets to the package.

Once results of the screening are obtained, the adult sampling coordinators will divide the samples between the analysing institutes.

Formaldehyde solution for histological samples

All fecundity samples shall be fixed and preserved in a 3.6% buffered formaldehyde solution suitable for later histological examination. Two types of phosphate buffers are utilized in order to obtain a stable pH. One agent is Sodium-Di-Hydrogen-Phosphate Hydrate (NaH_2PO_4-H_2O), the other is Di-Sodium-Hydrogen-Phosphate-Di-Hydrate (Na_2HPO_4-$2H_2O$). Two obtain 1L of fixative solution the following recipe as applied:

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

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Annex 1: Planning of the 2019 Mackerel and Horse Mackerel Egg Survey in the Western and Southern Areas

Countries and Ships Participating

Germany, Ireland, Netherlands, Scotland, Portugal, Spain (IEO), Spain (AZTI), Iceland, Faroe Islands, and Norway will participate in the mackerel and horse mackerel egg surveys in the western and southern area in 2019. Provisional dates (where possible), as well as vessel details, for the forthcoming surveys can be found below in table 1.1. The return of Norway to the survey will provide additional coverage in the northern area compared to 2016. During their survey they will also survey along the coast of Norway helping to close off the northern boundary in this area. Their inclusion in indeed timely as the 2016 results provided evidence of further significant challenges facing the survey, with both a spatial as well as a temporal shift in peak spawning of mackerel with a move away from February/March to May, and from the Bay of Biscay to a large swathe of open Ocean to the west of Scotland. This resulted in an inability once again to delineate fully the spawning boundaries in the North and West and of particular concern during 2016 was how close these were to the area of peak spawning. Subsequent interim monitoring work provide some evidence suggesting that 2016 might well have been anomalous, however the proposed survey plan for 2019 has been devised to try and manage either scenario with additional effort being devoted to the Northern areas whilst also retaining sufficient survey effort at the start of the spawning season. Survey coverage of the western and southern areas is given by area and period in table 1.2. Detailed maps of survey coverage by period are given in figures 1.1 –1.7. Both vessel availability and area assignments are provisional and will be finalised by the survey coordinator at the appropriate times.

The survey coordinator for the 2019 survey will be Brendan O' Hea, Marine Institute, Galway, Ireland.

For 2019 survey cruise leaders are asked to submit their station plan to the survey coordinator before heading to sea. The survey coordinator, on the advice of Gersom Costas, may select a number of survey rectangles in each survey area, and time period, where participants will be asked to collect two plankton samples in quick succession. This will assist in the calculation of variance estimates for the survey.

Table 1.1 Countries, vessels, areas assigned, dates and sampling periods for the 2019 surveys.

Country	Vessel	Areas	Dates	Period
Portugal	Noruega	Cadiz, Portugal & Galicia	17th Jan – 20th Feb	1,2
Spain (IEO)	Vizconde de Eza	Cantabrian Sea & Biscay	14th Mar – 6th Apr	3
		Biscay & Cantabrian Sea	11th Apr – 4th May	4
Germany	W. Herwig III	West Ireland & Celtic Sea	21st Mar – 26th Apr	3,4
Netherlands	Tridens	Biscay & Celtic sea	6th May – 24th May	5

		Celtic Sea & Biscay	3rd June – 21st June	6
Spain (AZTI)	Ramon Margalef	Biscay	19th Mar – 9th Apr	3
		Biscay & Cantabrian Sea	6th May – 31st May	5
Ireland	Celtic Explorer	Celtic Sea & Biscay	8th Feb – 28th Feb	2
	Charter	West of Ireland & west of Scotland	11th Apr – 25th Apr	4
	Charter	West of Ireland & west of Scotland	9th June – 30th June	6
Scotland	Scotia	West of Ireland & west of Scotland	IBTS	2
	Charter	West of Ireland & west of Scotland	17th Mar – 30th Mar	3
	Scotia	West of Ireland & west of Scotland	5th May – 25th May	5
	Charter	Celtic sea, West of Ireland & West of Scotland	7th July – 27th July	7
Faroe Islands	Magnus Henderson	Faroes & Shetland	22nd May – 5th June	5
Iceland	Bjarni Saemundsson	Iceland	5th May – 18th May	5
Norway	Johann Hjort	Faroes, west of Norway	9th June – 29th June	6

Survey Design

The AEPM survey design for mackerel and horse mackerel for 2019 will not change, however another attempt will be made to estimate DEPM adult parameters for both species. This will require additional sampling during the perceived peak spawning periods for both species, as identified from the 2010 surveys during WKMSPA 2012. For the 2019 survey this sampling will take place during periods 3 and 4 for mackerel, and periods 6 and 7 for horse mackerel.

In 2019 the survey will be split into seven sampling periods, and the design and survey deployment plan will be very similar to that employed in 2016. Once again the Faroe Islands and Iceland will participate in the survey during May, which will expand the geographic range of the survey in the North during that period. In 2019 Norway will once again participate in the survey, during period 6.

Period 1 (mid to end of January) will involve a survey in ICES area IXa only, with more extensive coverage starting in period 2. In 2019 the survey effort in area IXa will again be targeted on a single extended DEPM survey. No sampling in area IXa will take place after the end of period 2. Period 2 will commence at the start of February.

Sampling in the western area will commence in period 2. During period 2 the survey will concentrate on Biscay, the Celtic Sea, West of Ireland and West of Scotland. Periods 3 and 4 will see sampling begin in the Cantabrian Sea and continue north to the northwest of Scotland. No sampling will take place in the Cantabrian Sea, or southern

Biscay, after period 5. Periods 5 and 6 will also see the survey area extend north into Faroese and Icelandic waters. In periods 6 and 7 the surveys are designed to identify a southern boundary of spawning and to survey all areas north of this. The deployment of vessels to areas and periods is summarised in Table 1.2.

In 2013 the peak of mackerel spawning occurred in period 2, in Biscay, however in 2016 it occurred in May, to the west of Scotland. Due to the expansion of the spawning area that has been taking place since 2010 the emphasis in 2019 will once again be focussed on maximising area coverage. Cruise leaders will be asked to cover their **entire** assigned area using alternate transects and then use any remaining time to fill in the missed transects. If time is short this should be concentrated in those areas identified as having the highest densities of egg abundance. Particular points to note are:

Period 1

Only the southern area, area IX to the west of Portugal, will be surveyed in period 1. This will be the Portuguese DEPM survey, which will also extend into period 2, (Figure 1.1).

Period 2

Portugal will continue their DEPM survey in area IX.

Period 2 marks the commencement of the western area surveys. As a result of the early spawning encountered in 2013 the timing of this period in 2016 was moved earlier in the year to commence at the beginning of February. It was hoped that this would help capture the start of mackerel spawning in the western area. As it turned out spawning in 2016 was later than in 2013 with peak spawning also taking place much further north. For 2019 it has been decided to retain the same start dates as for the 2016 survey. The Irish survey therefore will commence at the beginning of period 2 covering Biscay, the Celtic sea, and to the west of Ireland, initially sampling on alternate transects. Scotland will be undertaking their IBTS survey off the west coast of Scotland and has allocated some effort to MEGS sampling, (figure 1.2).

Period 3

Period 3 surveys will be carried out by Spain (IEO), Spain (AZTI), Germany, and Scotland. IEO will survey in the Cantabrian Sea and southern Biscay. AZTI will survey the northern part of Biscay not covered by IEO. Germany will cover the Celtic Sea and the west of Ireland. Scotland will survey the area west of Scotland, as well as northwest Ireland, (figure 1.3).

WGMEGS have undertaken to collect additional adult DEPM samples in periods 3 and 4 for mackerel, and instructions for collection of these samples can be found in section 4.3. It is also especially desirable that as far as is possible comprehensive survey coverage is achieved within this enhanced area and this should be the prime consideration when completing the second sweep of the survey area during this period.

Period 4

During period 4 sampling will be carried out by three vessels. IEO will carry out their second survey in the Cantabrian Sea and south of Biscay. This survey will extend its range further north than normal by three transects. Germany will sample in the north

of Biscay, the Celtic sea, west of Ireland and west of Scotland. As this area is quite large the area can only be sampled on alternate transects. Ireland will conduct a survey on a commercial vessel to the west of Scotland and northwest of Ireland, and northwards towards the Faroes, (figure 1.4).

Mackerel DEPM sampling will continue within period 4.

Period 5

In period 5 AZTI will conduct a targeted DEPM survey for anchovy in the Cantabrian Sea and the design of the survey is therefore constrained by that purpose. This survey does however provide data on mackerel and horse mackerel egg numbers. Netherlands will sample in the northern part of Biscay and the Celtic sea to the southwest of Ireland. Scotland will survey to the west of Ireland and Scotland. In addition the Faroe Islands and Iceland will each provide a 2 week survey which will cover the area to the north of 58° 30'N. Iceland will survey the area west of 11° 30'W whilst the Faroese will survey eastwards of that line, (figure 1.5). These survey areas are provisional and definitive survey areas, as well as starting positions, will be provided by the survey coordinator and will largely be dependant 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. Netherlands will survey in Biscay and the Celtic sea with Ireland surveying west of Ireland and west of Scotland. Norway will survey north of 58° 30'N, and will continue sampling through the Faroes / Shetland channel, and along the Norwegian coast. This will be an exploratory component of the survey and will test whether mackerel spawning extends up along the Norwegian coast. As in period 5 this will expand the survey range and attempt to secure a northern boundary within this period, (figure 1.6). 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 2019, as with the mackerel in periods 3 and 4, WGMEGS have undertaken to collect additional adult horse mackerel DEPM samples during periods 6 and 7, and information and instructions pertaining to the collection of these samples can be found in Annex 2. As with periods 3 and 4 however every effort should be made to achieve as comprehensive coverage as is possible within this enhanced area.

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 1.7)

Horse mackerel DEPM sampling will continue.

Table 1.2. Periods and area assignments for vessels by week for the 2019 survey. Area assignments and dates are provisional.

		Area							
week	Starts	Portugal, Cadiz & Galicia	Cantabrian Sea	Biscay	Celtic Sea	North west Ireland	West of Scotland	Northern Area	Period
3	13-Jan-19	PO1 (DEPM)							1
4	20-Jan-19	PO1 (DEPM)							1
5	27-Jan-19	PO1 (DEPM)		IRL1	IRL1				2
6	3-Feb-19	PO1 (DEPM)		IRL1	IRL1				2
7	10-Feb-19	PO1 (DEPM)		IRL1	IRL1				2
8	17-Feb-19	PO1 (DEPM)				SCO(IBTS)	SCO(IBTS)		2
9	24-Feb - 19					SCO(IBTS)	SCO(IBTS)		2
10	3-Mar-19								3
11	10-Mar-19		IEO1						3
12	17-Mar-19		IEO1	AZTI1	GER1	SCO2	SCO2		3
13	24-Mar-19		IEO1	AZTI1	GER1	SCO2	SCO2		3
14	31-Mar - 19		IEO1	AZTI1	GER1	GER1			3
15	07-Apr-19		IEO2	IEO2	GER2	GER2			4
16	14-Apr-19		IEO2	IEO2	GER2	GER2	GER2	IRL-EX	4
17	21-Apr-19		IEO2	IEO2	GER2	GER2	GER2	IRL-EX	4

18	28-Apr-19	IEO2	IEO2					4
19	5-May-19	AZTI2 (DEPM)	AZTI2 (DEPM)	NED1	SCO3	SCO3	ICE	5
20	12-May-19	AZTI2 (DEPM)	AZTI2 (DEPM)	NED1	SCO3	SCO3	ICE	5
21	19-May-19	AZTI2 (DEPM)	AZTI2 (DEPM)	NED1	SCO3	SCO3	FAR	5
22	26-May-19	AZTI2 (DEPM)	AZTI2 (DEPM)				FAR	5
23	2-Jun-19	AZTI2 (DEPM)	NED2	NED2			FAR	5
24	9-Jun-19		NED2	NED2	IRL2	IRL2	NOR	6
25	16-Jun-19		NED2	NED2	IRL2	IRL2	NOR	6
26	23-Jun-19				IRL2	IRL2	NOR	6
27	30-Jun-19							6
28	7-Jul-19		SCO4	SCO4	SCO4	SCO4		7
29	14 – Jul-19		SCO4	SCO4	SCO4	SCO4		7
30	21-Jul-19		SCO4	SCO4	SCO4	SCO4		7
31	28-Jul-19							7

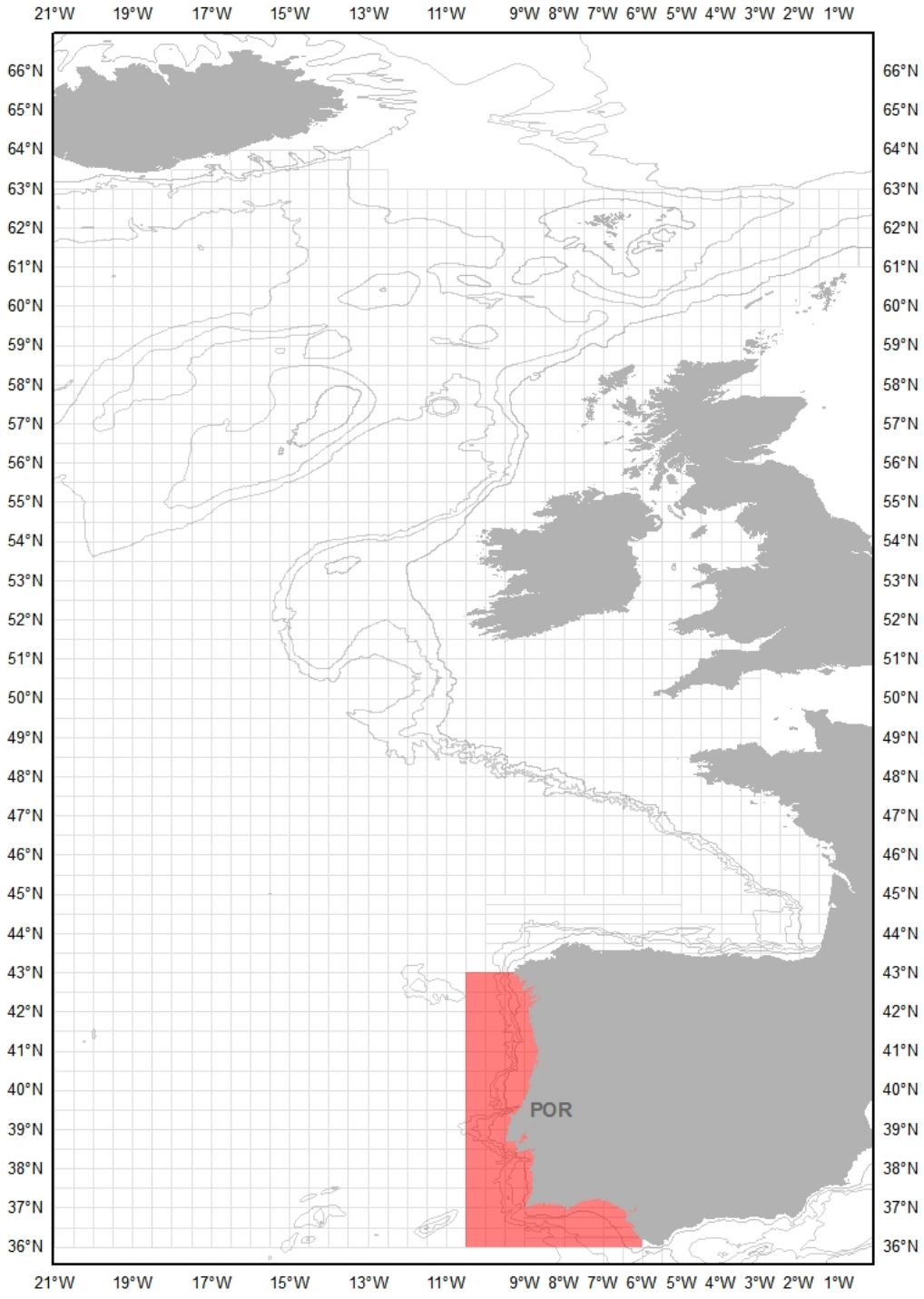


Figure 1.1 Survey plan for Period 1

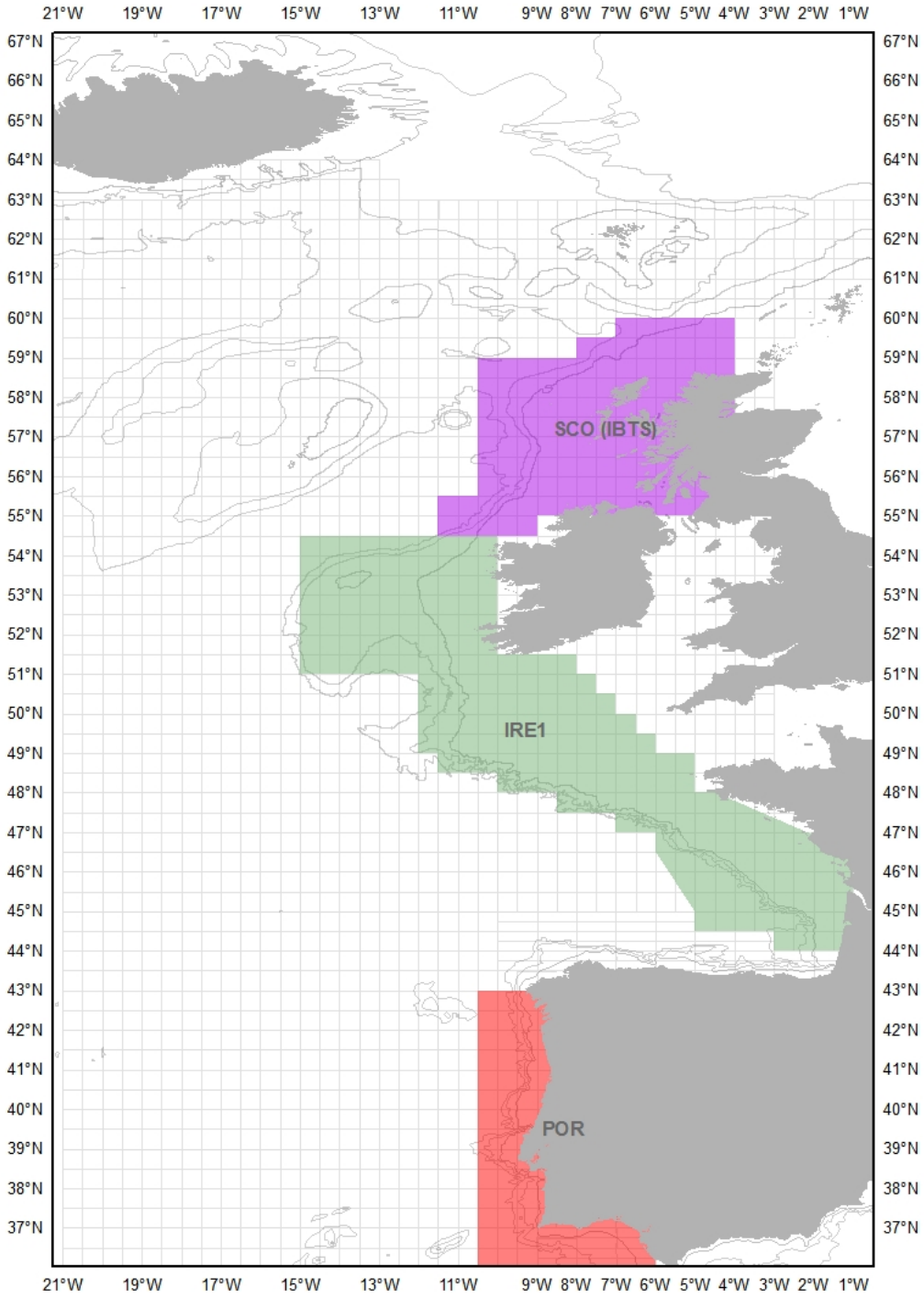


Figure 1.2 Survey plan for Period 2

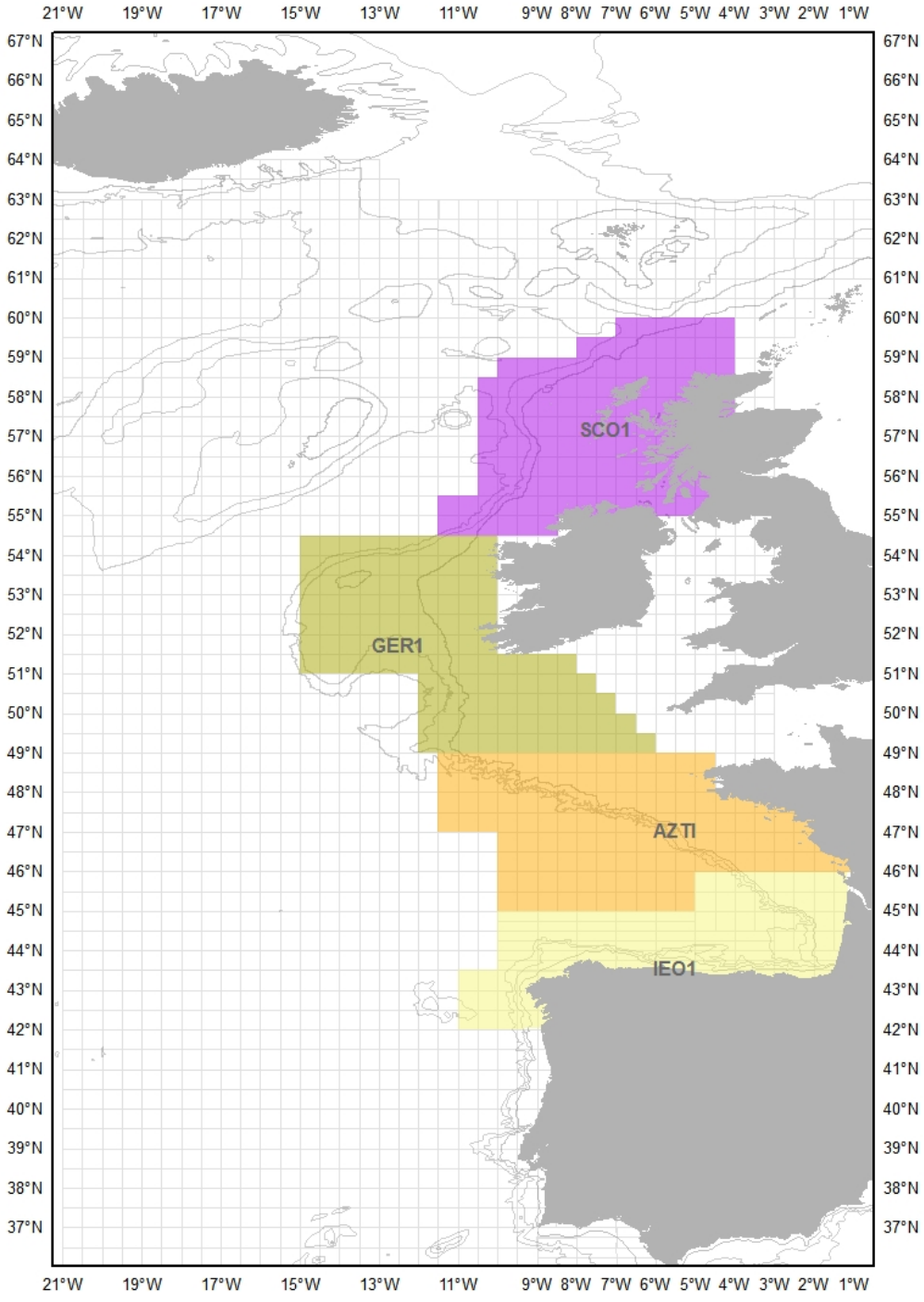


Figure 1.3 Survey plan for Period 3.

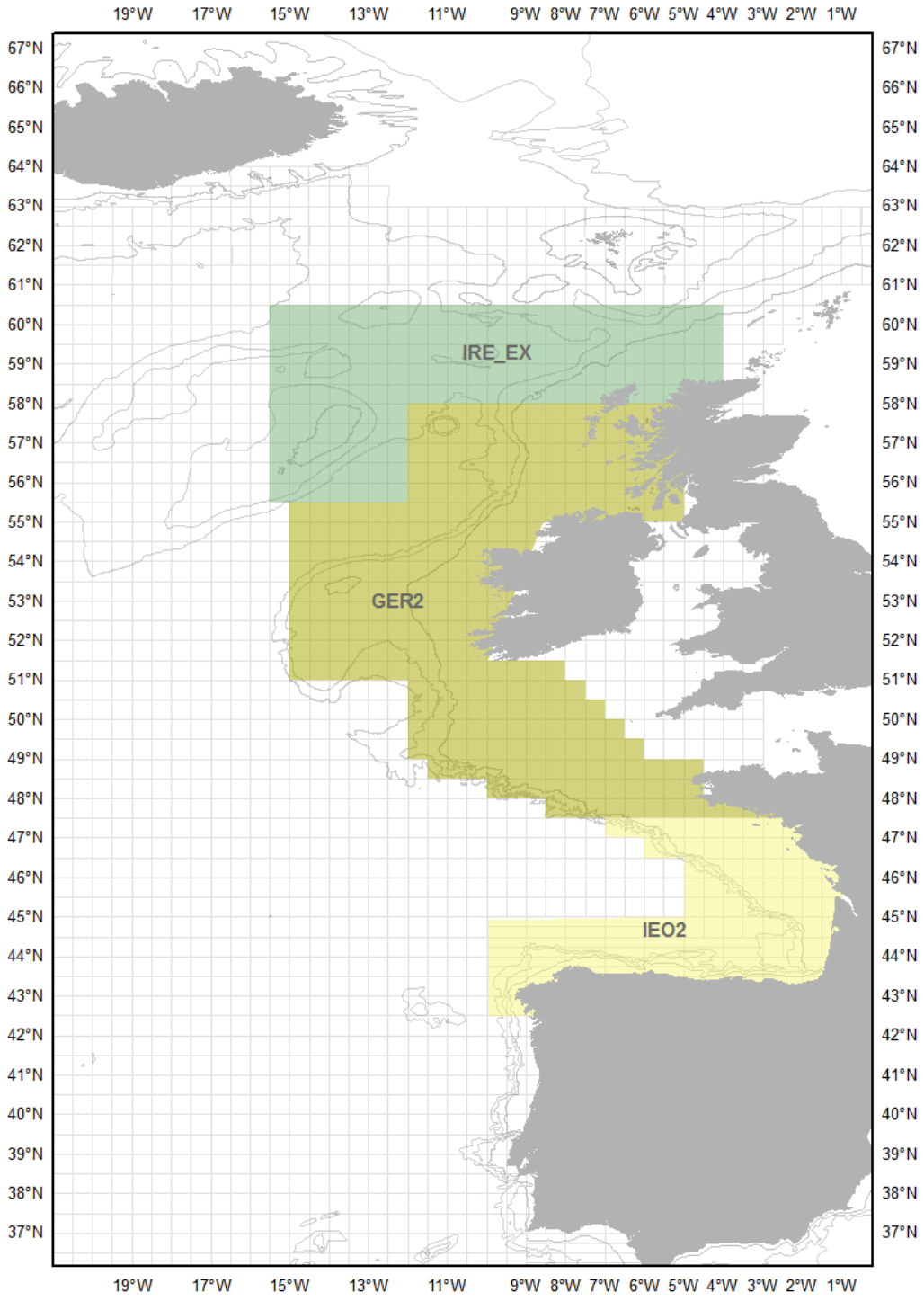


Figure 1.4 Survey plan for Period 4

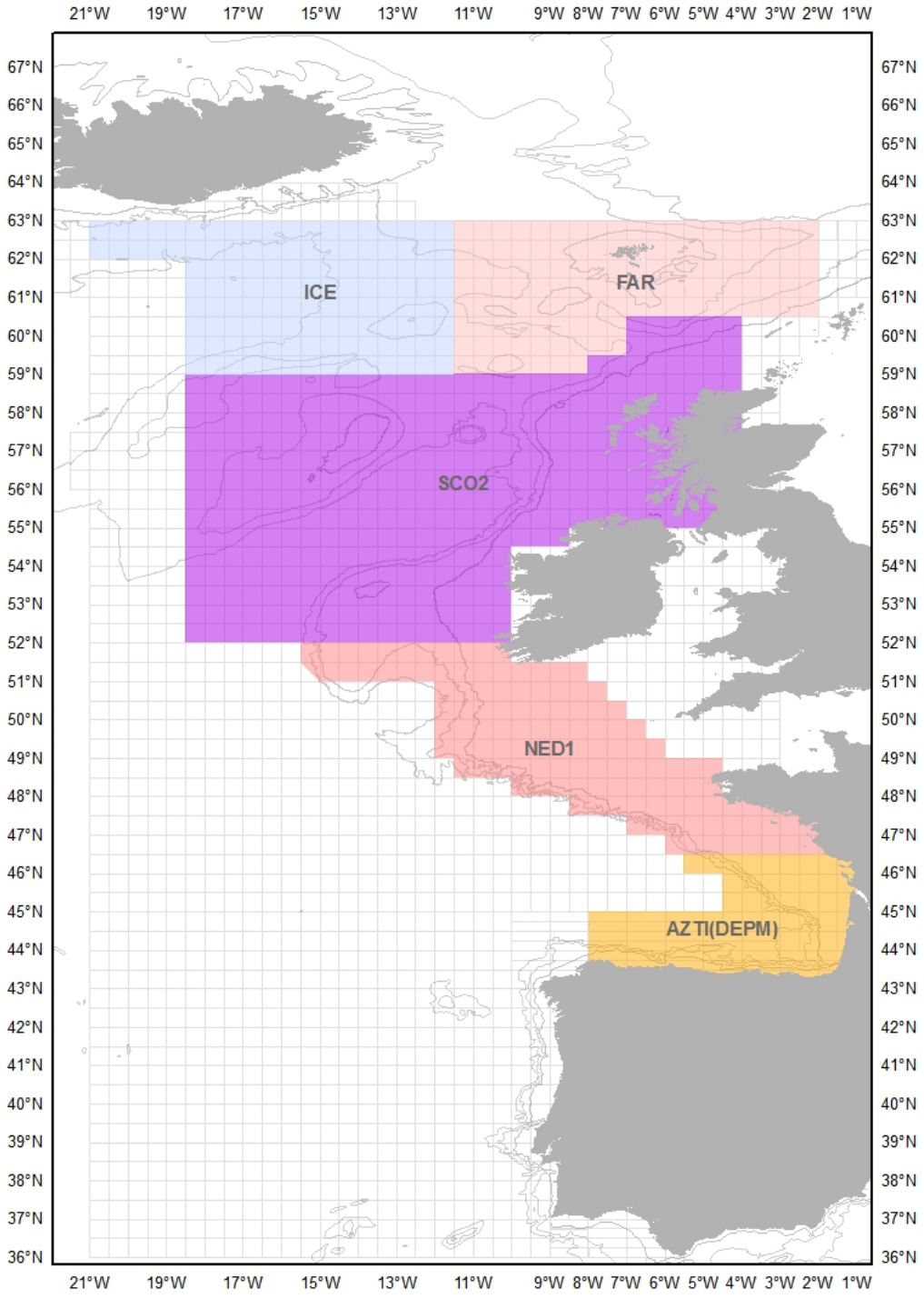


Figure 1.5 Survey plan for Period 5.

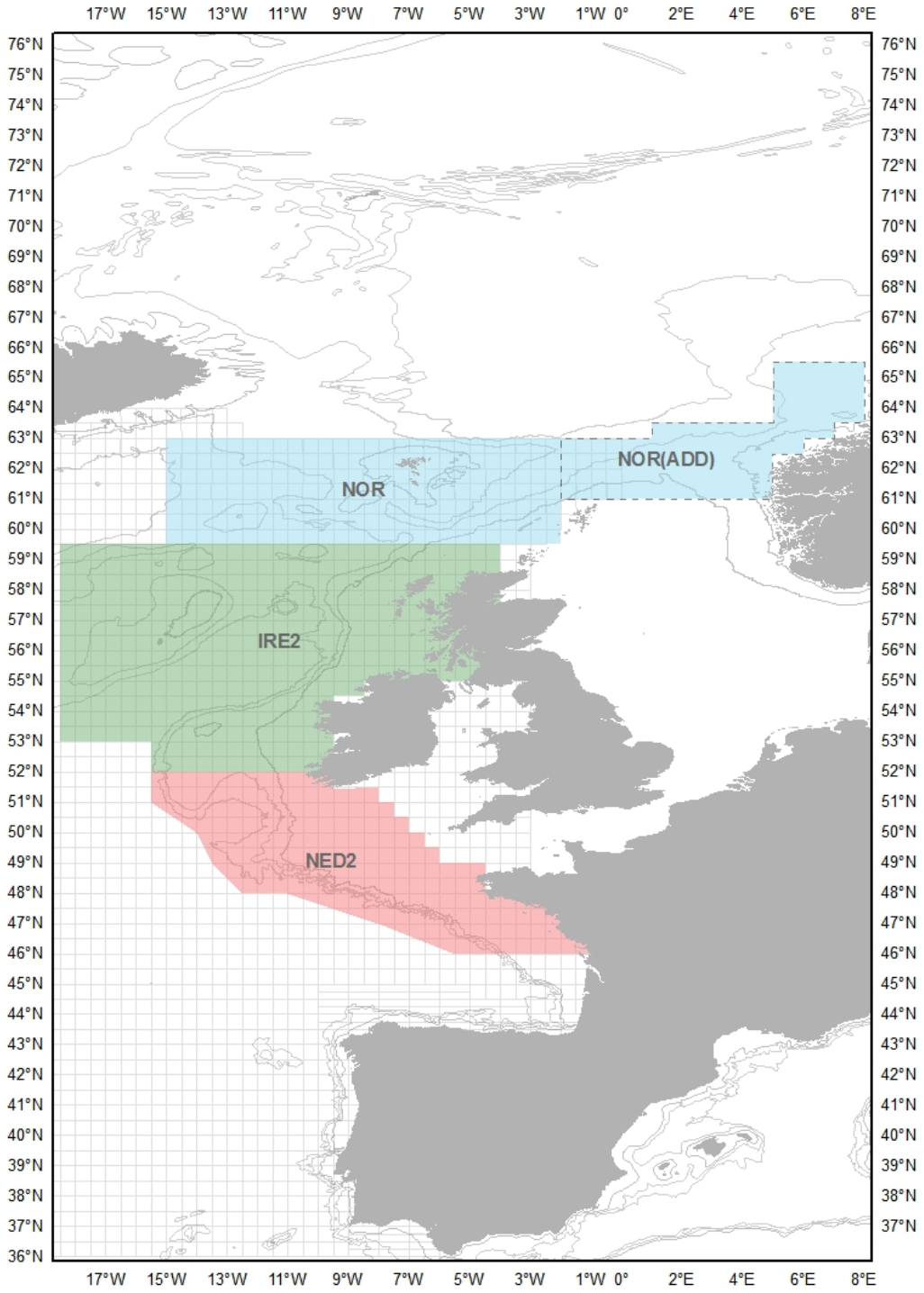


Figure 1.6 Survey plan for Period 6

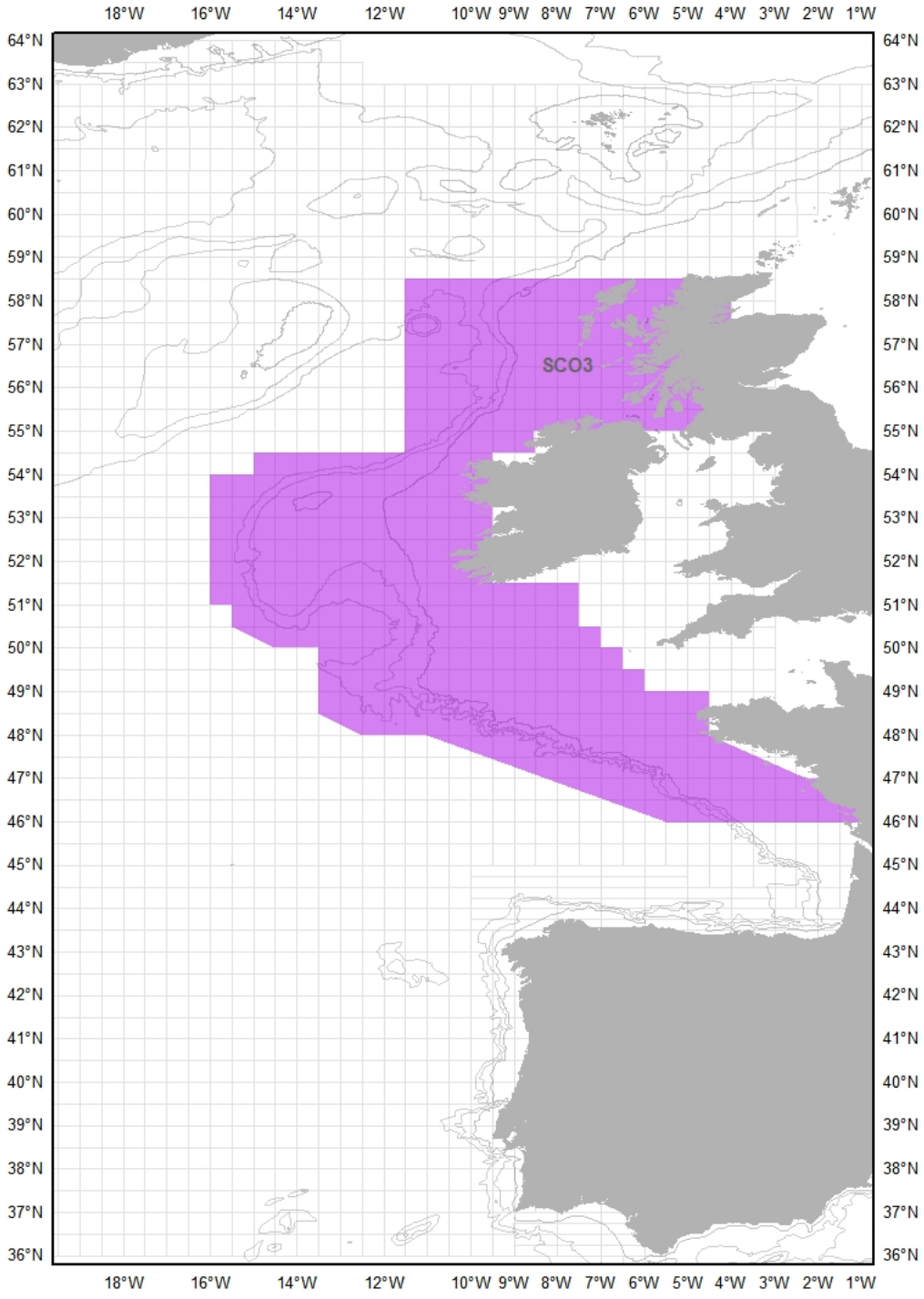


Figure 1.7 Survey plan for Period 7

6.3 Sampling Areas and Sampling Effort

As in 2016 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 would be made for both species.

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

Based on this steady growth of the "standard area" every survey, the Working Group agreed at the Dublin meeting (2002) to reconsider its use. It was agreed that the existing "standard area" should be retained **only as a guide** to the core survey area for cruise leaders, and that the extent of coverage should be decided based on finding the edges of the egg distribution only i.e. boundaries should be set based on the adaptive sampling guidelines. The core areas for the western and southern surveys for both species are presented in figures 2.1 and 2.2. A more detailed survey map of the Iberian area as surveyed by IEO and IPMA can be found in Figure 2.3 and 2.4.

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

Since 2007 IPMA have adopted the DEPM - Daily Egg Production Method, for horse-mackerel of the Southern stock (ICES division 27.9.a - Gibraltar-Finisterre) and have developed and implemented several aspects of the methodology since. Modifications introduced included the plankton sampling gear and design, and laboratory and data analyses developments for egg and adult samples.

The DEPM survey will take place during January-February 2019 on board RV Noruega covering the area from Gibraltar to Finisterre, figure 2.4.

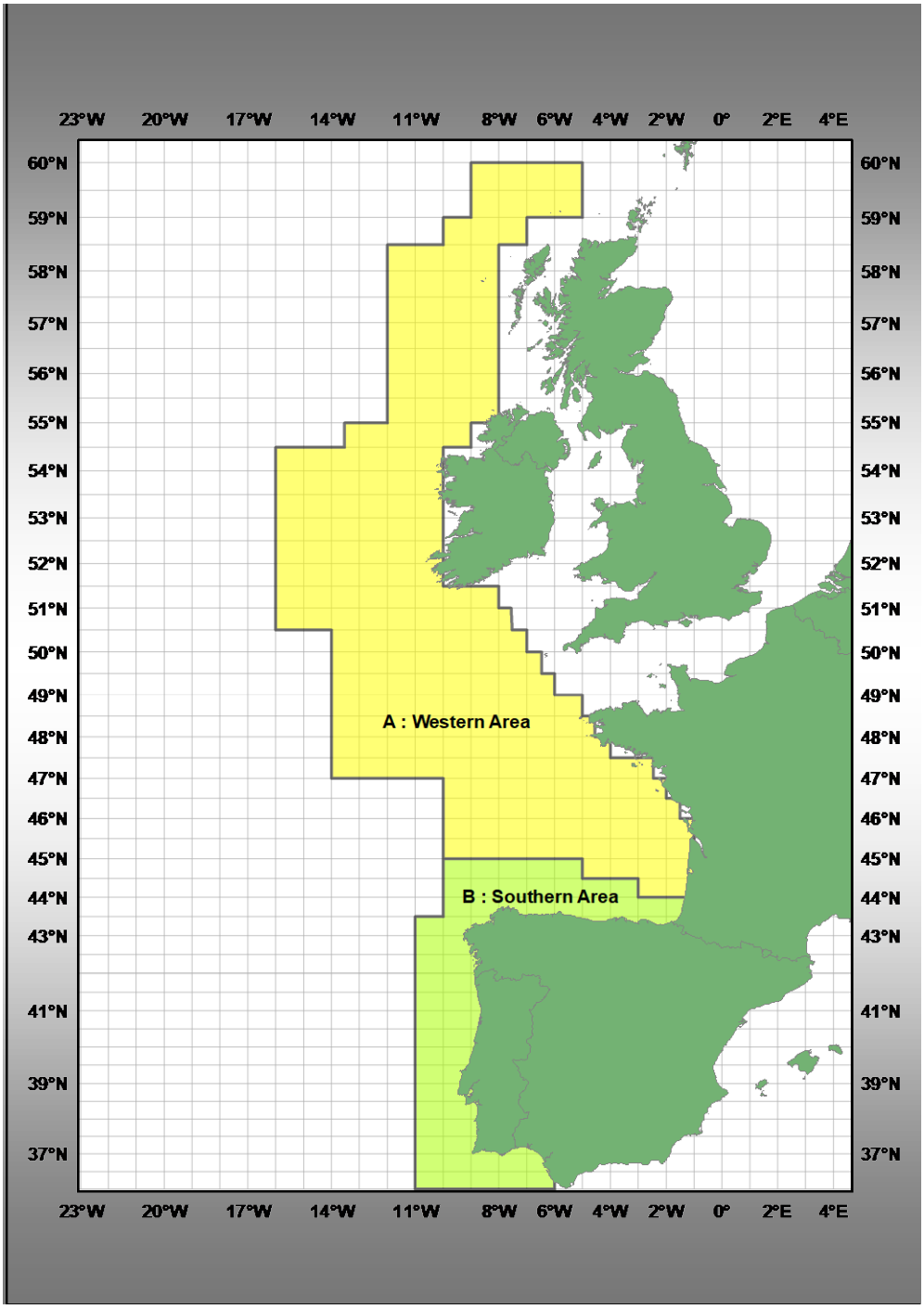


Figure 2.1: Core sampling areas for mackerel eggs in the western and southern areas for 2019. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines

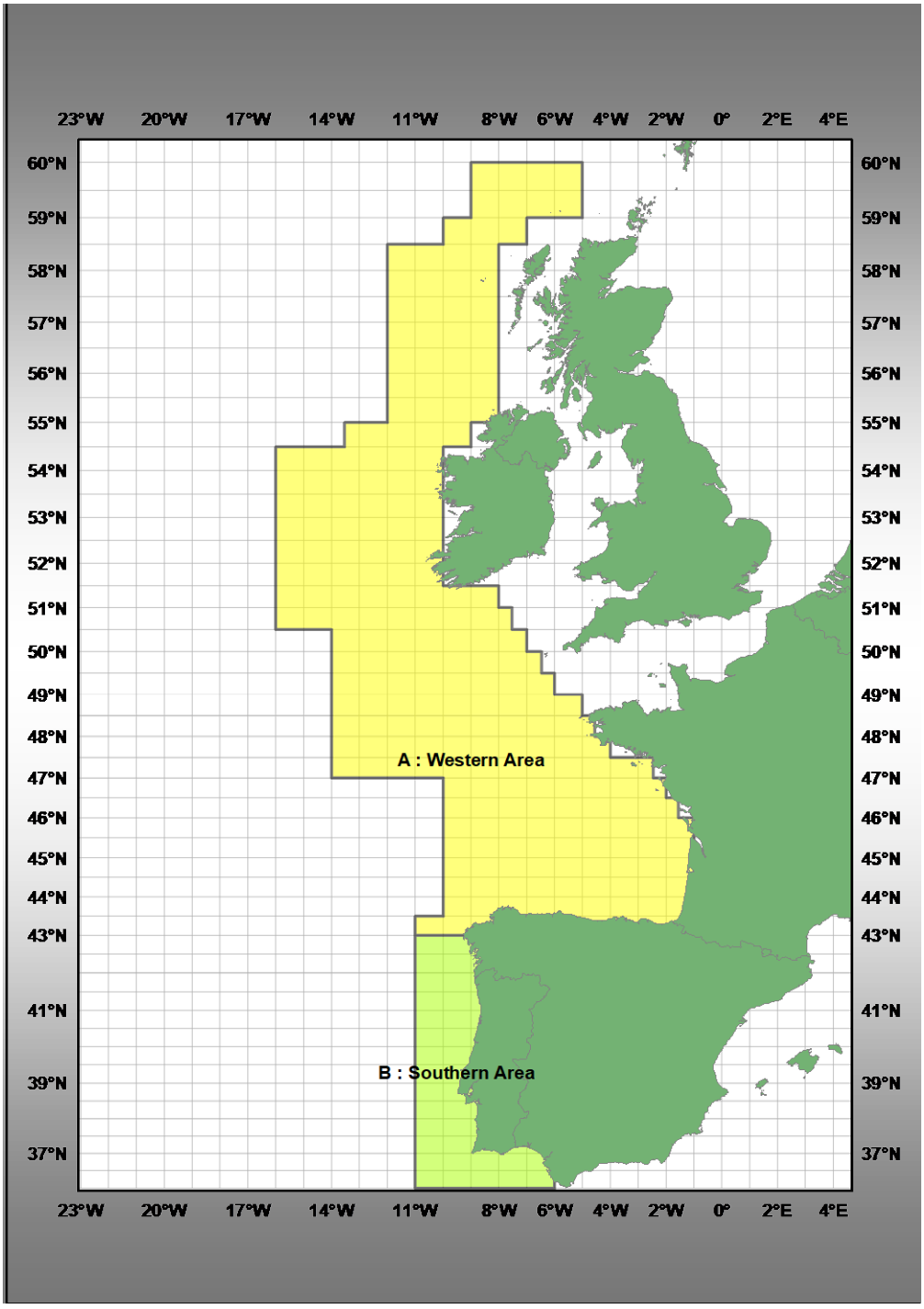


Figure 2.2: Core sampling areas for horse mackerel eggs in the western areas for 2019. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines

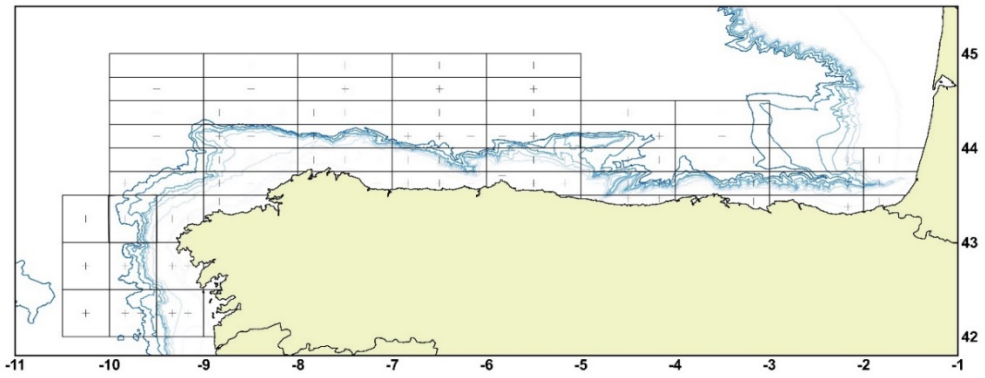


Figure 2.3: IEO sample locations for Galicia and the Cantabrian Sea.

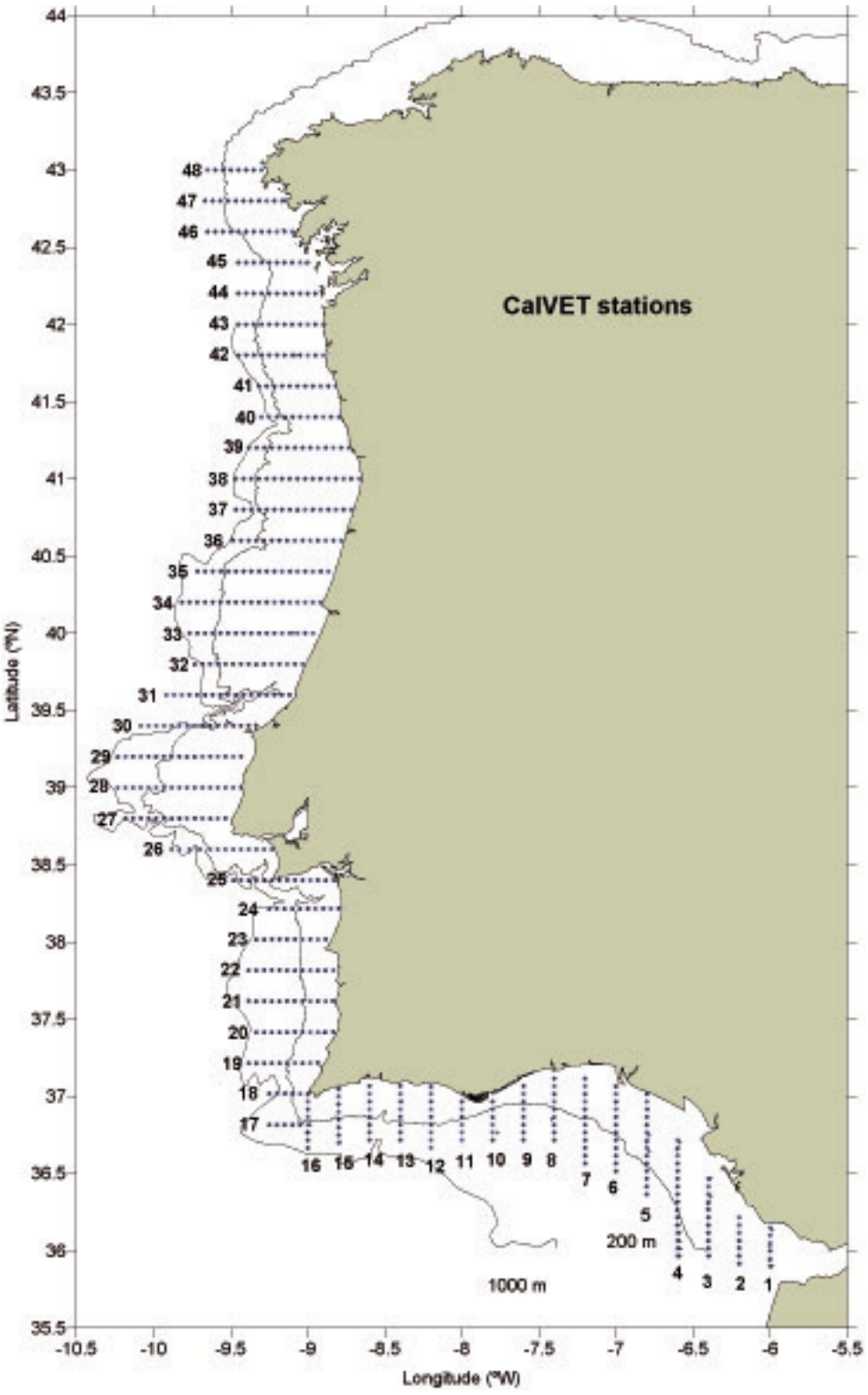


Figure 2.4. Southern Horse Mackerel. Sampling grid for CalVET stations.

Annex 2: Annex 2: 2019 Sampling for mackerel potential fecundity and atresia in the Western and Southern areas.

Following WGMEGS decision to use only formaldehyde fixative (ICES 2003) it will be possible to provide a unified sampling scheme for fecundity and atresia for use in the surveys. Following the experience of the 2016 survey the following changes have been recommended for the 2019 surveys (Table 1.1). The fecundity manual keeps a record of all the changes in earlier surveys.

Samples for estimation of mackerel potential fecundity, atresia, batch fecundity, spawning fraction and spawning frequency will be mostly taken on vessels participating in the egg survey or from commercial fishing vessels by observers. Recognising the constraints of the egg survey cruise leaders should try to distribute trawl stations for the potential fecundity and atresia across the survey area aiming to complete a wide spread sampling regime for adults shown in Appendices 1.1-1.4. The purpose of this table is not to exactly specify the time and location of trawl hauls but to give an impression of how trawl hauls should be dispersed in time and space and the numbers of required for the estimation of realised fecundity. Maturity of fish should be determined according to the Walsh Scale and the WKMATCH 2012 Maturity scale revised (Tab. 1.3).

In periods 3 & 4, 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 (Appendice 1.3). It is recommended that trawling is preferably carried out at dusk or during the night in the western area, and during the afternoon in the southern area.

Appendice 1.1: Southern mackerel adult sampling scheme for 2019

Appendice 10.1: Desired temporal and spatial distribution of the mackerel fecundity sampling in the Southern Area

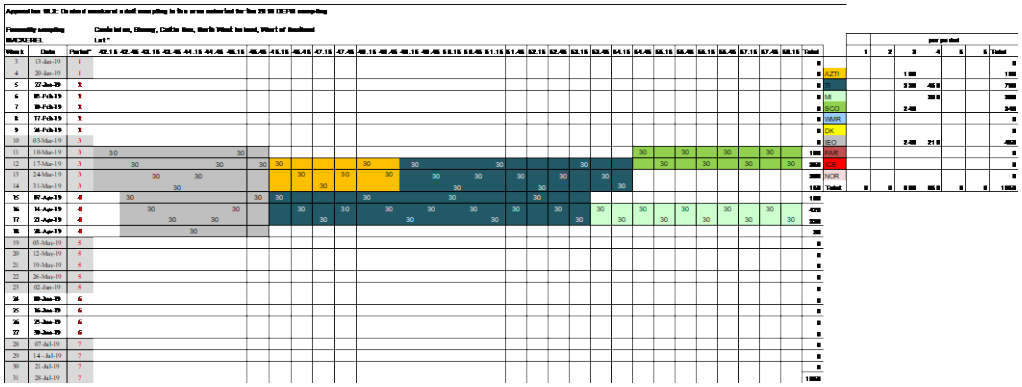
Fecundity sampling (numbers of fish) Southern Area (Cantabrian and Biscay) — **Southern Area (Cadiz to Galicia)**

MACKEREL			Lon °											Lat °							Total
Week	Date	Period*	11W	10	9	8	7	6	5	4	3	2	1	36N	37	38	39	40	41	42	
3	13-Jan-19	1												10							10
4	20-Jan-19	1														10					10
5	27-Jan-19	2	100 (prespawning (purseine/trawl)														10				10
6	03-Feb-19	2																			0
7	10-Feb-19	2																			10
8	17-Feb-19	2																	10		0
9	24-Feb-19	2																			100
10	03-Mar-19	3																			0
11	10-Mar-19	3																			0
12	17-Mar-19	3																			0
13	24-Mar-19	3																			0
14	31-Mar-19	3	See Table 7.1.2 for the AEPM and DEPM sampling																		0
15	07-Apr-19	4																			0
16	14-Apr-19	4																			0
17	21-Apr-19	4																			0
18	28-Apr-19	4																			0
19	05-May-19	5				10	15	10	15	10	15	10	15								85
20	12-May-19	5																			0
21	19-May-19	5																			0
22	26-May-19	5																			0
23	02-Jun-19	5																			0
24	09-Jun-19	6																			0
25	16-Jun-19	6																			0
26	23-Jun-19	6																			0
27	30-Jun-19	6																			0
28	07-Jul-19	7																			0
29	14 –Jul-19	7																			0
30	21-Jul-19	7																			0
31	28-Jul-19	7																			225

		per period					
		1	2	3	4	5	total
10	IEO		100				100
10	AZTI					100	100
10	IPIMAR	20	20				40
0	Total	20	120	0	0	100	240

Appendice 1.2: Western mackerel adult sampling scheme for 2019

Appendice 1.3: Mackerel DEPM adult sampling scheme for 2019



Appendice 1.4: Horse mackerel DEPM adult sampling scheme for 2019

Appendice 1.4: Desired horse mackerel adult sampling in the area selected for the 2019 DEPM sampling

Fecundity sampling: Biscay, Celtic Sea, North West Ireland, West of Scotland

HORSE MACKEREL — Lat °

Week	Date	Period*	40.15	40.45	41.15	41.45	42.15	42.45	43.15	43.45	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	Total
3	13-Jan-19	1																														0
4	20-Jan-19	1																														0
5	27-Jan-19	2																														0
6	03-Feb-19	2																														0
7	10-Feb-19	2																														0
8	17-Feb-19	2																														0
9	24-Feb-19	2																														0
10	03-Mar-19	3																														0
11	10-Mar-19	3																														0
12	17-Mar-19	3																														0
13	24-Mar-19	3																														0
14	31-Mar-19	3																														0
15	07-Apr-19	4																														0
16	14-Apr-19	4																														0
17	21-Apr-19	4																														0
18	28-Apr-19	4																														0
19	05-May-19	5																														0
20	12-May-19	5																														0
21	19-May-19	5																														0
22	26-May-19	5																														0
23	02-Jun-19	5																														0
24	09-Jun-19	6	30		30		30		30		30		30		30												30				30	210
25	16-Jun-19	6		30		30		30		30		30		30														30		30		210
26	23-Jun-19	6																											30			30
27	30-Jun-19	6																														0
28	07-Jul-19	7	30						30														30				30				30	120
29	14 –Jul-19	7		30		30				30				30														30		30		210
30	21-Jul-19	7			30						30														30				30			120
31	28-Jul-19	7																														0
																																900

	per period							
	1	2	3	4	5	6	7	Total
AZTI								0
BI								0
MI						150		150
SCO							510	510
WMR						360		360
DK								0
IEO								0
FAR								0
ICE								0
NOR								0
Total:	0	0	0	0	0	510	510	1020

Table 1.1. Changes for 2019 compared to earlier survey years

2010	2013	2016	2019
Mackerel			
	Samples are taken for screening for spawning markers and atresia. The results from the histology are used to decide which samples will be analysed for fecundity and which for atresia. Only samples that contain spawning markers and early alpha atresia will be embedded from the cassettes for further atresia analyses.		The screening samples are taken from a cut section from the middle of the ovary instead of the spoon sample. It will be checked if the atresia and POF analysis can be done from this section as well.
	Each cruise will collect 10 samples of one fish (stages 3 to 6) for the fecundity ring test.		
	Ovary lobes need to be pierced with a fine needle before fixation in formaldehyde.	Cut off both ends (1-2 cm depending on the size of the ovary) before fixation in formaldehyde.	Since the screening section is taken from this ovary lobe, the remaining parts will be fixed without extra cutting.
Mackerel and Horse mackerel			
Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis. Count all the oocytes >185µm in the sample that are not automatically detected.	Measure the oocyte diameters automatically using ImageJ software provided for the batch fecundity analysis. Count and measure all the oocytes >500◙m in the sample that are not automatically detected.		
ImageJ and macros will be made available during the wk to all participants and they should use this for analysis of the samples.			
Distribute the sample randomly in the tray. If it is not		If possible try using an ultrasound pen to	

possible to separate the oocytes, exclude the sample for fecundity analysis.	separate the oocytes in whole mounts.	
For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.	5 mackerel slides will be provided for POF staging calibration between institutes.	Images will be provided by IMR for POF staging calibration.
Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample	Examine the screening sample for the most advanced oocyte stage, POFs, hyaline eggs, early alpha atresia, massive atresia, if it is spent and if it should be discarded. Oocyte development stages is changed to stage 1-5. Hyaline eggs is taken out of the oocyte stage as well as the spent stage.	The hydrated oocytes stage has an extra column in the data template where it is divided into 3 different states: 5.1, 5.2 and 5.3
	New screening and POFs staging template	

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

Table 1.3: Walsh and 'WKMATCH 2012 maturity scale revised' maturity scales (Walsh Scale, Walsh *et al.*, 1990; WKMATCH 2012 maturity scale revised, ICES 2018)

Female	Walsh scale	WKMATCH 2012 maturity scale revised	Male
Ovaries small, wine red and clear. Torpedo shaped. No sign of development.	1 Virgin	A Immature	Testes small, pale, flattened and translucent. No sign of development
Ovaries occupying 1/4 to 3/4 body cavity. Opaque eggs visible, giving pink to yellowish colouration. Largest eggs without oil globule.	2 Early ripening	Ba Developing but functionally immature	Testes occupying 1/4 to 3/4 body cavity, off-white, no milt running.
Ovaries occupying 3/5 to almost filling body cavity. Yellow to orange in colour. Largest eggs may have oil globule.	3 Late ripening/ partly spent (early)	Bb Developing and functionally mature	Testes occupying 3/5 to almost filling body cavity. Creamy white in colour.
Ovaries size variable from a full to 1/4. Characterised by externally visible hyaline eggs, not matter how few or how early the stage of hydration. Ovaries with hyaline eggs only in the lumen are not included.	4 Ripe	Ca Actively spawning	Testes filling body cavity. Milt freely running
Ovaries occupying 3/4 to <1/4 of body cavity. Slacker than stage 3 and often blood shot.	5 Partly spent (late)	Cb Spawning capable	Testes occupying 3/4 to <1/4 body cavity, with free running milt and shrivelled at anal end.
Ovaries occupying 1/4 or less of body cavity. Reddis and often murky in appearance, sometimes with a scattering or patch of opaque eggs.	6 Spent/ Recovering spent	D Regressing/ Regenerating	Testes occupying 1/4 or less of body cavity. Opaque with brownish tint and no trace of milt.

No evidence of omitted spawning	E Omitted spawning	No evidence of omitted spawning
Hard parts (connective tissue), only one lobe developed, intersex, or similar. Fecundity at least partly reduced.	F Abnormal	Hard parts (connective tissue), only one lobe developed, intersex, or similar.

Prior to cruise departure the mackerel adult sampling coordinator will coordinate the analysis of mackerel fecundity samples and assign tube reference numbers to cruise leaders for labelling the tubes used on their cruises.

Section 10 shows the procedures to follow for the collection of samples at sea, and for sample analysis in the laboratory. Provisional estimates of potential fecundity and atresia are required for the WGWIDE group in late August and final results are required for WGMEGS in the spring of the next year. If the participants are unsure of the data quality they should pass on their concerns to the adult sampling coordinator.

Each sampling institute (Rlabo) is responsible for distributing the collected samples to the analysis institutes (Alabo).

Sampling for horse mackerel fecundity in the Western area

Following the experience of the 2016 survey and WKMSPA the following changes have been recommended for future surveys (Table 2.1). The fecundity manual keeps a record of all the changes in earlier surveys.

Table 2.1. Changes for 2019 compared to earlier survey years

2010	2013	2016	2019
	Mackerel and Horse mackerel		
Measure the oocyte diameters automatically using ImageJ software provided for the fecundity analysis. Count all the oocytes >185µm in the sample that are not automatically detected.	Measure the oocyte diameters automatically using ImageJ software provided for the batch fecundity analysis. Count and measure all the oocytes >500◎m in the sample that are not automatically detected.		
ImageJ and macros will be made available during the wk to all participants and they should use this for analysis of the samples.			

Distribute the sample randomly in the tray. If it is not possible to separate the oocytes, exclude the sample for fecundity analysis.		If possible try using an ultrasound pen to separate the oocytes in whole mounts.	
For 10 mackerel and 10 horse mackerel (2 from each survey) 6 subsamples will be taken and used for calibration between the institutes.		5 mackerel slides will be provided for POF staging calibration between institutes.	Images will be provided by IMR for POF staging calibration.
Spawning markers: hydrated (>800 um) oocytes or POFs, or all oocytes diameter < 400 um in the whole sample		Examine the screening sample for the most advanced oocyte stage, POFs, hyaline eggs, early alpha atresia, massive atresia, if it is spent and if it should be discarded. Oocyte development stages is changed to stage 1-5. Hyaline eggs is taken out of the oocyte stage as well as the spent stage.	The hydrated oocytes stage has an extra column in the data template where it is divided into 3 different states: 5.1, 5.2 and 5.3
		New screening and POFs staging template	
	Horse mackerel		
	From 2013 and onwards no samples for potential fecundity are collected. Only DEPM adults parameter samples will be collected.		
IPMA will perform a DEPM survey for horse mackerel. Batch fecundity: Gravimetric method. Take whole fixed ovary to the lab, take 3 subsamples, weigh and			

count all the hydrated oocytes in subsample.	
Spawning fraction: migratory nucleus, hydrated, POF's	

During the 2019 survey for horse mackerel adult samples will be collected during period 6 & 7. During the 2019 survey horse mackerel will be collected from trawl hauls on the Western spawning component selecting fish of maturity stages 2-6 Walsh and stages Ba-Db in the WKMATCH 2012 maturity scale revised scales as shown in table 1.3. **On each transect a trawl haul will be carried out at the station with highest stage 1 horsemackerel egg production (Appendice 10.4). 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 (Figure 2.2).

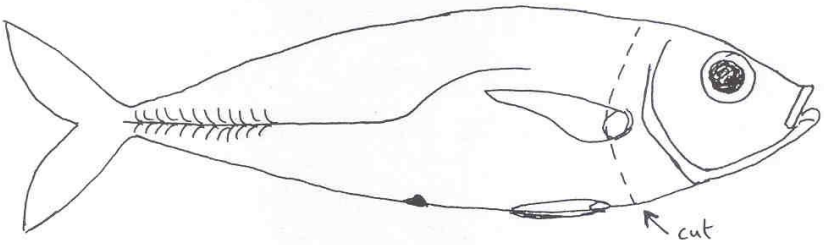
Ovaries should be weighed and subsamples taken by pipette before fixing in 3.6% buffered formaldehyde solution in sealed vials (e.g. Nunc tubes) on board. **The recipe for formaldehyde solution for both, mackerel and horse mackerel fecundity sampling is given in section 10.**

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

Prior to cruise departure the Horse mackerel adult sampling coordinator will coordinate the analysis of horse mackerel fecundity samples and provide cruise leaders with tube reference numbers for labelling the tubes used on their cruises.

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

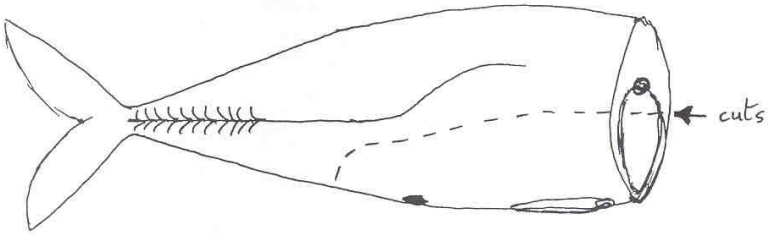
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral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



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

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

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



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

Figure 2.1 Procedure for collecting ovaries from horse mackerel

Annex 3: 2019 Requests for extra sampling during the mackerel and horse mackerel egg survey

1) Collection of immature mackerel samples for maturity staging calibration exercises.

For the maturity ogive it is necessary to collect data on the maturity stage of mackerel. ICES organises workshops to calibrate maturity staging. But the samples available for these workshops are mostly from mature individuals and very few immature fish. A request is put forward by WKMACQI (ICES, 2018) to collect immature samples through regular samplings in order to have immature samples available for the next maturity staging calibration exercise.

Each participating institute in the mackerel egg survey is requested to collect data, images and gonads of 5 immature females and 5 immature males.

Data collection

Three different data sources will need to be collected:

1. Pictures (digitally available)
2. Histological samples
3. Details on fish sampled

1 Pictures

Technical details

The bullet points describe the essential information which has to be visible on the picture.

- Pictures have to be taken on fresh fish.
- Add at least sampling time, area, unique sampling number, fish length and species in the picture.
- Take care that the samples should be clean/tidy, preferable without intestines.

- Take at least four pictures:
  1. Overview of the fish on a measuring board, with the gonads visible in the fish. The ability to look at the whole fish with the gonad intact is vital to get the ratio of gonad to body length.
     Label containing information on:
     - Date
     - Fish number
     - Measuring board on the background
     - Species (MAC)

  2. Detail of picture 1, zoomed in on the gonads. Show the pressure characteristic on the picture to see if fish is running.
     Label containing:
     - Fish number
     - Species (MAC)

  3. Picture of gonads outside the fish, placed on a measuring board, allowing to view the gonad in more detail, blood vessels etc.
     Label containing:

- Fish number
- Species (MAC)

4. Detail of picture 3, zoomed in on the gonads.

Label containing:

- Fish number
- Species (MAC)

Filenames of the pictures:
Files have to be stored as *.jpg format, in a resolution as high as possible.
Filenames have to be composed as

countryyear_species_fishnumber_number:

- Country codes as used in ICES databases
- Year in 4 digits
- Species codes MAC for mackerel
- Fishnumber: unique number for the fish in the year
- Number: referring to number 1 to 4 in the above protocol

Examples of picture filenames:
Dutch mackerel, fish number 142, overview:

NED2014_mac_142_1.jpg

Dutch mackerel, fish number 142, detail of gonad:

NED2014_mac_142_4.jpg

Belgian horse mackerel, fish number 101, detail in fish:

BEL2014_hom_101_2.jpg

Belgian horse mackerel, fish number 101, gonad outside fish:

BEL2014_hom_101_3.jpg

2 Histological samples

For histological samples one whole lobe of the gonad has to be put in 3.6% buffered formaldehyde solution. This should be done as soon as possible after catching the fish. The protocol is as following:

1. weigh the fish (g)
2. measure the fish (to the mm below)
3. open the fish carefully, not to damage the gonads
4. macroscopic stage the fish using Walsh scale or WKMATCH 2012 maturity scale revised
5. take the pictures a described above
6. take out the gonads
7. weigh the gonads (both lobes) (g, 2 digits)
8. put one whole lobe of the gonads and store in a jar with 3.6% formaldehyde, take care the gonad is completely covered in the fluid
9. put a label in the jar containing year, month, day, country, sample number, species, fish number

3. Details on fish sampled

Information for the pictures and histological in an *.xls file.

Variable	Units	Digits	Remarks
Country	ICES country code		
Species	MAC (mackerel)		
Year		4 digits	
Month			
Day			
Time		GMT	
Latitude	decimal	like 53.95	
Longitude	decimal	like 3.75; W from 0°: -3.75	
sample_number			
fish number			
picture name			
histological_sample_number			
length		cm	(total length)
weight	g	(total weight)	
gender	m=male, f=female		
maturity scale	Walsh/WKMATCH 2012 maturity scale revised		
maturity	maturity scale value		
gonad_weight	g		

After sampling mail the data to cindy.vandamme@wur.nl. Keep the histological sample in your storage. There will be a request for the histological sample to be sent when the next calibration exercise is planned.

2) CLIMRATES Adult mackerel gonads samples:

A total of 200 adult mackerel ovary samples per period (Table 1) has been requested for the project CLIMRATES (IMR – Thassya C. dos Santos Schmidt). There is no specific geographical position (preferable north of 52°N) (Figure 1), but it is suggested to collect females for at least 8 trawling stations.

Table 1. Number of adult mackerel ovary samples requested per country and period.

Period	Country	N
Period 3	Scotland	200
Period 4	Germany	100
Period 4	Ireland	100
Period 5	Iceland	100
Period 5	Faroe Islands	100
Period 5	Scotland	100
Period 6	Ireland	100
Period 6	Norway	100

Sampling procedures:

After record the basic biological parameters: total length, body weight, maturity stage, age (otolith), and gonad weight. Cut a small subsample (~5 cm) – middle part and preserve in formaldehyde 3.6%. It is important to record the gonad weight. If not possible, preserve entire ovary

(both lobes) in a bottle with 3.6% formaldehyde. Remember the ratio one-part tissue and 10 part of formaldehyde. Sexually mature females in any maturity stage can be sampled.

Label the vials with station number, sampling date, and fish number. Samples should be sent to IMR in the end of the survey. Shipping address:

Institute of Marine Research (Havforskningsinstituttet)
Att. Thassya C. dos Santos Schmidt and Vemund Mangerud
Nordnesgaten 50
5005 Bergen-NO

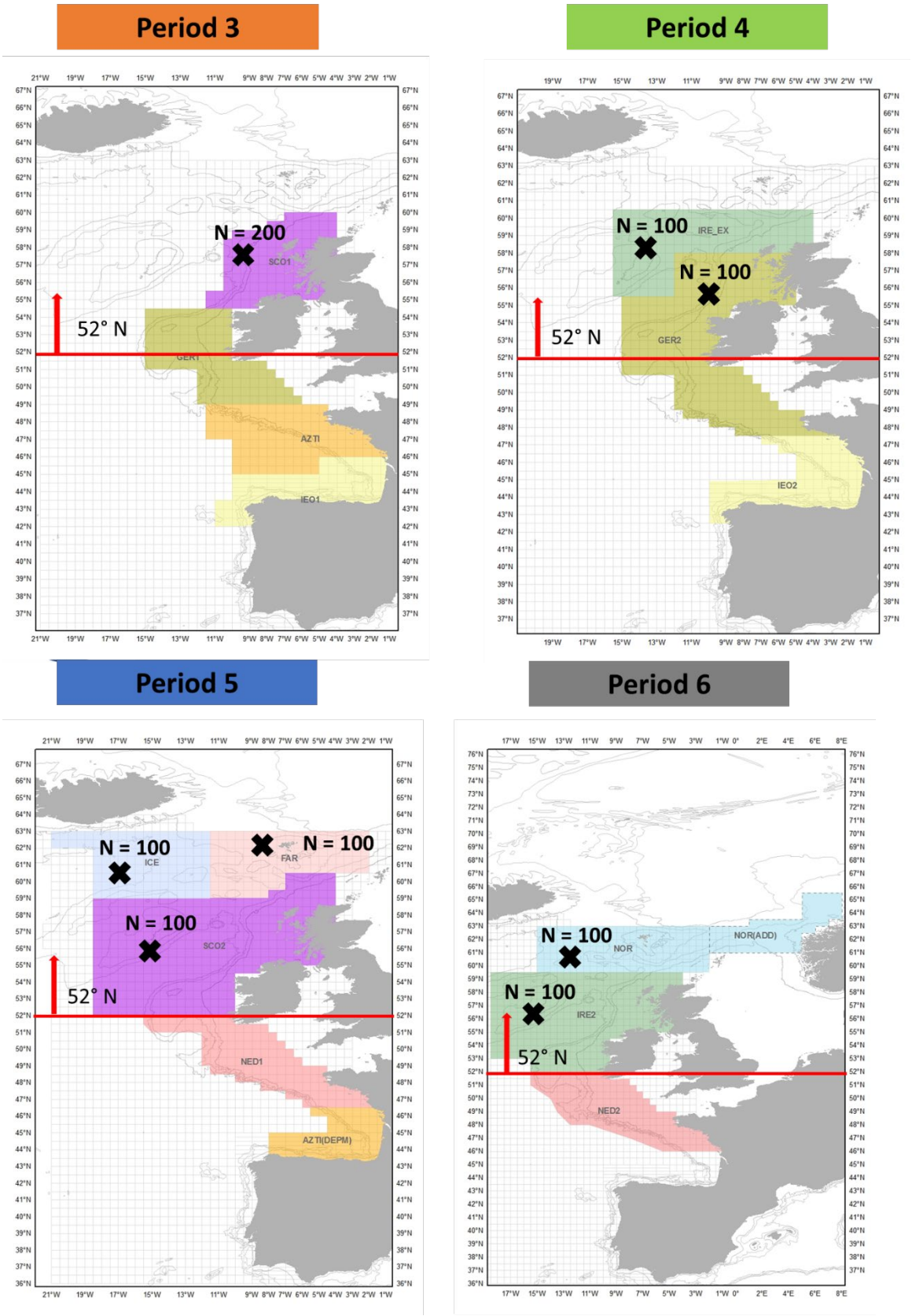


Figure 1. Area suggested, and number of adult mackerel ovary samples requested per period and country.

3) Collecting fish eggs for genetical/molecular analysis

Whenever time allows, fish eggs should be freshly sorted from the non-preserved samples and stored for genetical/molecular analysis. This is most important, where mackerel and/or horse mackerel eggs co-occur with those of other, non-target species, like e.g. hake or ling.

The following procedure is advised for collection of eggs:

10. Eggs shall be sorted freshly from the samples
11. The eggs are individually measured (both egg and oil globule diameter), staged and wherever possible visually identified.
12. Each egg is individually preserved in an Eppendorf vial in 96 % pure ethanol
13. The vials are labeled with an ID number
14. The vial ID is noted together with the information on cruise, date, station, and the egg specifications
15. All samples shall be stored in a cool and dark place until shipment (preferably a fridge or freezer)
16. All samples shall be sent to
    Matthias Kloppmann
    Thünen Institute of Sea Fisheries
    Herwigstraße 31
    27572 Bremerhaven
    Germany

Sorting of the fresh plankton samples shall be done quickly and preferably in a cool environment. The quality of the plankton sample has the highest priority. Therefore, it is not expected that a quantitative sample of eggs is sorted from the plankton. Just select and process as many eggs as you can in 10 – 15 minutes. Then, the plankton has to be preserved.

Annex 4: List of contributors

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Annex 5: Version history

DATE	VERSION	CHANGE	BY WHOM
April 2012	1	1st version for the 2013 survey; a description of the Portuguese DEPM survey on horse mackerel eggs was added; the survey core areas were updated and amended; the table of survey gears used was updated by the survey participants; the formula for calculating the duration of stage I mackerel eggs from the sea temperature (T°C) was updated according to the new findings of Mendiola *et al*. (2006)	WGMEGS
November 2012	1.1	Section on ship planning for 2013 included, sampling schemes adapted, procedures for Southern horse mackerel added	WKFATHOM
April 2014	1.3	Reviewers suggestions worked in for SISP publication	WGMEGS
April 2015		1st version for the 2016 survey;	WGMEGS
November 2015	2.0	Section on ship planning for 2016 included, sampling schemes adapted, modified double-zero-rule for transect length assignment, sections on egg staging and identification, data analyses and adult sampling amended	WKFATHOM
April 2018	2.1	Section on ship planning and adult sampling streamlined and generalized, sampler specifications updated, literature references added, information on the spraying method updated,	WGMEGS
October / November 2018	2.2	Sampling sections generalized, spraying method elaborated, section on clogging added, update on data requirements and entry formats, species identification section updated, adult sampling description updated	WKFATHOM