ICES WGMEGS REPORT 2008

ICES Living Resources Committee

ICES CM 2008/LRC:09

Ref. RMC

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

7-11 April 2008

IJmuiden, Netherlands

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

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Recommended format for purposes of citation:

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

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Executive Summary

The Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS) is primarily responsible for the planning and analysis of the ICES Triennial mackerel and horse mackerel egg surveys. As in previous years, the annual egg production method was implemented using international egg surveys conducted in 2007 between February 2 and July 16 and associated estimates of fecundity and atresia.

The sampling was completed as planned, and the Working Group concluded that in 2007 the temporal and spatial coverage of the plankton sampling was good. Also, the sampling for fecundity and atresia was completed successfully. As with previous years, several replicates from each fish were collected and then distributed equitably between analysis groups according to codes assigned by the coordinator. Unlike 2004, southern samples were included in this scheme as well.

The ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS) met in Ijmuiden on April 7-11, under the chair-ship of Dr. Paula Álvarez, to analyse the data from the 2007 Mackerel and Horse Mackerel Egg Survey. This survey takes place triennially with the participation of Portugal, Spain, Scotland, Ireland, The Netherlands, Norway and Germany. The basis of the survey is to relate the number of freshly spawned eggs found in the water with the number of females having produced these eggs. Knowing the fecundity of the females provides an estimate for the spawning stock biomass. As the large number of samples have now been analysed, the group met to evaluate the results and to assess the size of the mackerel population in the Northeast Atlantic, the southern horse mackerel stock and the egg production of horse mackerel in the western stock.

The analyses show that the **NEA mackerel stock** has increased by 504 700 t to a total of 3.25 mill. t (+18%). The spawning stock biomass estimate by components was:

i) 2,590 million tonnes (±787, 510 tonnes) for western component. This can be compared to 2,468 million tonnes in 2004;

ii) 667, 909 tonnes (±414,852 tonnes) for southern component. This can be compared to 281,427 tonnes in 2004.

The increase in the SSB for **NEA mackerel stock** was due to an increase in mackerel egg production coupled with only a small decrease in mackerel fecundity. The estimate of total egg production was 1.52*1015 which is an increase of 0.19*1015 (+14%) with respect to 2004. The total egg production estimate by component was:

1.208*1015 (se = 0.105*1015) for western component. This can be compared to 1.202*1015 in 2004;

0.3119*1015 (se = 0.1709*1015) for southern component. This can be compared to 0.126*1015 in 2004;

The analyses of potential fecundity gave a value of 1098 eggs/gr female for mackerel for the western and southern components combined. This represents a reduction of 29 eggs /g female when compared to the 2004 western component and an increase of 82 eggs /gr female when compared to the 2004 southern component. The overall prevalence of atresia as a percentage of the population was 38% and the relative intensity was 30 eggs per gram. This reduced the potential fecundity by 9% giving a realised fecundity of 1009 egg per g female.

During the last few years the WG has not provided SSB estimates for **horse mackerel** due to the problems associated with fecundity of horse mackerel, namely the debate as to whether horse mackerel are a determinate or indeterminate species. In 2004 a new definition of **horse mackerel stock** was accepted and as a result Triennial surveys were adopted to this change in 2007. For the **western horse mackerel stock** (including the ICES Division VIIIc) a meticulous adult protocol was prepared in order to address the problems with fecundity. On the other hand, the SSB was estimated for the **southern horse mackerel** (IXa ICES Division) applying the DEPM method for the first time.

The new **western horse mackerel stock** was found to have produced far more eggs in 2007 (1.427*1015; se = 0.269*1014) than in 2004 (0.889 *1015). The increase in total egg production was 61%. The results derived from the fecundity study show a high variability in fecundity over the time and latitude that together with the indeterminacy of this species resulted in a WG decision not to use fecundity data in an AEPM biomass estimate. A new study using image analyses was considered by the Group with the objective of reducing the variance of spawning fraction if this parameter is used to forecast egg production for several days instead of for one day.

The SSB of the new **southern horse mackerel** stock was estimated applying the daily egg production method for the first time. This method requires the estimate of three specific parameters: Batch fecundity, Spawning fraction and Po. Batch fecundity and spawning fraction was calculated for current samples (2007 samples) and for samples collected in previous years. The analyses of batch fecundity gave a value of 146.8 eggs/g., and the spawning fraction fluctuated greatly from 0.0977 (a batch every 5 days) to 0.2009 (a batch every 10 days) according to the criteria considered. The reduced number of females analyzed and the variability observed suggest that further analyse be carried out to improve these estimates. Po calculations from the exponential decay model gave a value of 13 eggs/square meter and Z of 0.014 hour. The SSB estimate for the new southern horse mackerel stock using the DEP method varied from 48 741 tonnes (variance 6.49*109) to 97 482 tonnes (variance 3.05*1010) depending on criterion used for estimating the spawning fraction.

In general the quality and reliability of the surveys were good. There was an increase in survey effort in 2007 compared to 2004, in spite of the lack of participation by England. This absence was mainly compensated by an additional survey carried out by Scotland and specific modifications in coverage carried out by several other countries. The adult sampling methodology was extended to all participants and the replication of the samples and its distribution between all the laboratories improved the reliability of the estimate, which was broadly similar to that obtained in 2004.

As in 2003 the WG held an egg identification and staging workshop prior to the surveys. This permitted a harmonisation of egg identification and realised fecundity in mackerel as well as spawning rate in horse mackerel across the participating institutes. Both activities led to an improvement in the quality of the estimate.

Even when the survey coverage was good the WG concluded that while the starting of the spawning event was fully covered for mackerel and horse mackerel, the surveys ended too early to adequately cover the end of spawning in the north for both mackerel and horse mackerel and in the southern area (South of 47°N) for horse mackerel.

A mackerel egg survey in the North Sea is planned for summer 2008 and it is expected that preliminary results will be reported by September 2008.

1 Introduction

1.1 Terms of Reference

At the ICES Annual Science Conference in Helsinki, Finland, September 2007 it was decided that (C.Res. 2007/2G07) the Working Group on Mackerel and Horse Mackerel Egg Surveys [WGMEGS] (Chair: P. Alvarez, SP) will meet in Ijmuiden, Netherlands, 7-11 April 2007 to:

a) Analyse and evaluate the results of the 2007 mackerel and horse mackerel egg surveys of the western and southern areas;

b) Calculate the egg production

  i) total seasonal stage 1 egg production estimates for mackerel for western and southern component together.

  ii) total seasonal stage 1 egg production estimates for horse mackerel for western stock.

  iii) Po estimates for horse mackerel for southern stock (DEPM application).

c) Analyse and evaluate the results of the mackerel and horse mackerel fecundity and atresia sampling for mackerel for western and southern component and horse-mackerel southern stock.

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

d) evaluate the results of studies on horse mackerel fecundity determination and proxies on the basis of data collected during the 2007 surveys and in other relevant work (captivity studies);

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

f) provide estimates of the spawning stock biomass of horse mackerel, using Po production estimates and the estimates of batch fecundity and spawning frequency for southern stock.

g) evaluate the quality and reliability of the 2007 survey in the light of the previous surveys.

WGMEGS will report by 1 June 2008 for the attention of the Living Resources and the Resource Management Committees.

1.2 Participants

Name	Country	Name	Country
Paula Alvarez	Spain (Basque country)	Finlay Burns	UK (Scotland)
Ana Maria Costa	Portugal	Gersom Costas	Spain
Cindy van Damme	Netherlands	Merete Fonn	Norway
Dolores Garabana	Spain	Svein A. Iversen	Norway
Matthias Kloppmann	Germany	Steve Milligan	UK (England)
Alberto Murta	Portugal	Brendan O'Hea	Ireland
José Ramón Pérez	Spain	Anders Thorsen	Norway
Dave Reid	UK (Scotland)	Jens Ulleweit	Germany
Peter Witthames	UK (England)		

Details on participants can be consulted in Annex 1.

2 General aspects

2.1 Summary of WGMEGS activities in 2006 and 2007

WGMEGS met in Vigo 2006 to plan the ICES Triennial Mackerel and Horse Mackerel Egg Survey in 2007. The report was published as ICES CM 2006/LCR:09 and presented to the joint session of LRC and RMC at the ASC in Helsinki in 2007. A Workshop on Mackerel and Horse Mackerel Egg Staging and Identification was held from23–27 October 2006 at CEFAS, Lowestoft, England. Details of the workshop are presented below in section. The report was published as ICES CM 2006/LCR:17. The surveys were carried out from February to July 2007 and are reported in detail in this report. The details of the survey conduct and vessel deployment were controlled by separate coordinators for the western (F. Burns, Scotland) and southern areas (C. Franco, Spain). WGMEGS prepared a report by correspondence summarising this process (ICES CM 2007/LCR:14). Survey data (egg abundances and ancillary data plus preliminary fecundity and atresia estimates) were collated in August 2007 and eggs production for mackerel and horse mackerel and fecundity and atresia information presented for use in the annual assessment to the September meeting of WGMHSA in Copenhagen,(ICES CM 2007/ACFM:31). This was the second time that the survey estimate was available using updated data for fecundity and atresia to WGMHSA in the same year as the survey, and led to substantial changes in the perception of the state of the stock.

2.2 Workshop on Mackerel and Horse Mackerel Egg Staging and Identification

2.2.1 Scientific justification

Identification of eggs to species and the staging of those eggs remain two of the key areas in the execution of the mackerel and horse mackerel egg surveys. As this process is carried out by a number of different analysts in many different countries, and then the data combined, it is vital that the process be standardised. WGMHMSA and WGMEGS feel strongly that this is best done through the mechanism of sample exchange programmes and regular workshops to compare results. In the context of the triennial egg surveys it would seem appropriate to hold a workshop prior to every survey to standardise approaches and methodologies in the run-up to the surveys. This will have the advantage of training new participants as well as harmonising the approach of experienced analysts. An egg-staging workshop was

held for the first time in 2000 and was very successful in achieving some of these aims. The scope of these workshops were extended in 2003 (prior to the 2004 survey) to address all aspects plankton analysis, including removal of eggs from the samples, identification as well as allocation to development stage. The 2003 workshop (ICES, 2004) was also tasked to produce a standard manual of procedures, descriptions and photographs to assist in the plankton sample handling and identification process. The latest workshop held in 2006, (ICES, 2006b) provided further enhanced descriptions and utilised some 'validated' eggs of known species.

2.2.2 Results and recommendations from WKMHMES 2006

Egg sorting

The 'spray technique' was, once again, evaluated at WKMHMES in 2006. The results were consistent, showing that the technique was very effective at removing eggs from the rest of the plankton samples. This led to a recommendation from WKMHMES that the 'spray technique' be used as the primary method for removing eggs from plankton samples during the 2007 triennial surveys.

Egg identification and staging

The majority of the time at the workshop was spent identifying and staging mackerel, horse mackerel and similar eggs. The results promoted discussion and highlighted specific problem areas. These discussions led to the further development of standard protocols, and enhancements to the species and stage descriptions. The results were very re-assuring and similar to those obtained at the 2003 workshop. There was a slight under-estimate of stage 1 mackerel eggs (stages 1a and 1b combined) during the first round of analysis (-2%) and a slight over-estimate (2%) during the second round. The results for stage 1 horse mackerel eggs were similar with under-estimates of –2% and –1% respectively. This is particularly re-assuring as it is this stage on which the egg production estimates are based.

Recommendations and terms of reference

WGMEGS recommends that the next meeting of WKMHMES (Chair, C. van Damme, Netherlands), should be split between two different locations and with different participants. The egg identification and staging workshop will take place at IMARES - Ijmuiden, Netherlands and the fecundity workshop will take place at AZTI – Basque country, Spain. Both workshops will take place in the autumn of 2009, with the following terms of reference:

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

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

c) update a set of standard pictures and descriptions for species identification and egg staging;

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

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

f) carry out inter-calibration work on fecundity determination and harmonise the analysis and interpretation of fecundity samples;

2.3 Biennial mackerel egg surveys: benefits for operations, assessment and management

Recent meetings of the Pelagic RAC have suggested that there would be advantage in carrying out the mackerel egg survey on a two-year (biennial) basis rather than a three year (triennial basis). While this issue was not raised as a formal ToR for WGMEGS, the WG agreed that it was worthy of appraisal.

There would be a number of advantages to biennial surveys; for the survey operations themselves and for assessment and management.

Operational advantages

The current triennial arrangement means that there is a three-year gap between surveys. As a result there is generally a need to "relearn" how to carry out the work each time even if the same people are available for the work. In addition personnel can change between surveys, and often without the possibility of skill transfer. A biennial structure would reduce if not eliminate this problem.

Three-year spacing means that knowledge of spawning distribution will be out of date. The planning for the triennial surveys depends to a large extent on knowing the approximate spawning distribution for mackerel. The surveys are designed to be adaptive, so the coverage can be expanded within a given survey if eggs are found outside the expected area. However, in terms of vessel logistics, this has to be limited. Each vessel in the survey is assigned an area based on the time available and the operating constraints, and on the known spawning distribution. So any major expansion of the area to be surveyed may have an impact elsewhere on the survey coverage. In the context of triennial surveys and of climate change, it is quite possible that the spawning distribution may change substantially in the intervening years. For instance there was evidence from the 2007 survey that the northern limit of egg distribution was not reached. As a result, survey areas based on a spawning distribution three years ago, may be inappropriate in the current year. Biennial surveys would help to reduce the impact of such changes

The current three-year cycle also exposes the survey to difficulties in relation to the measurement of adult parameters, particularly fecundity. For example, the 1998 survey identified a large drop in realised fecundity, which substantially changed the biomass interpretation. This was perceived as a step change from the previous survey, however, a shorter gap between surveys would help to minimise the impact of such changes, and provide more temporally resolved data.

Assessment advantages

The triennial egg surveys represent the only fishery independent data available for tuning the stock assessment. With a three year cycle, the assessment will tend to follow a trajectory based on the last two or three surveys. Each new survey will tend to result in a new stock perspective. While this is true of any assessment and its tuning indices, the situation is exacerbated by the single index and the three-year cycle in mackerel. In the intervening years between surveys, the assessment is basically an extrapolation from the last survey point. Again a biennial pattern would reduce the impact of this, and allow us to pick up changes in the stock more rapidly.

The mackerel fishery is mainly targeted on adult fish (>=3 years old). This means that it is difficult to pick up drops in recruitment and hence to SSB for up to 3 years in the assessment using landings data. Combined with a three year cycle in the egg surveys, it is possible for the assessment to fail to pick up such a recruitment based SSB

reduction. A biennial survey might reduce this weakness. At present there is no reliable estimate of juvenile mackerel, although the western bottom trawl surveys may be able to provide a juvenile index in the future.

Management advantages

The three year cycle in surveys and hence in assessment means that each time a new survey is carried out, the change in perspective of the stock can be substantial. This can lead to substantial changes in TAC with concomitant impact on the economic performance of the fishery. Substantial increases may reduce price, conversely decreases will produce low quota and loss of earnings. Fishermen's organisations stress that the best scenario would be stability or small-scale change. A shorter survey/assessment cycle could facilitate this

Logistic and organisational implication

The proposal suggested the use of commercial vessels for the additional effort required for the surveys. The WG would support this, if the additional effort could be funded from industry rather than public resources. Ideally, each biennial survey could be conducted with a combination of research and commercial vessels. The use of commercial vessels for mackerel egg surveys has been shown to be feasible following recent work using a commercial vessel in Scotland in 2007 (MFV Unity) and in Ireland in 2002 (MFV Atlantean).

The proposal would also require additional commitment of staff resources in the participating institutes. Essentially, this would represent approximately an additional 50% for staff time, and appropriate consumables and meetings. For the institutes involved, there would still be a need to put science teams aboard the commercial vessels to carry out the sampling, sorting and staging of eggs, as well as choose the survey design and station location. Lab work, for fecundity and atresia, would also be increased by 50%.

It would be important that the same institutes involved in the current triennial surveys also took part in the biennial surveys. Current institute and vessel commitment is sufficient to carry out a full survey, but could not be reduced further without potentially compromising the survey. It is recognised that this increased commitment may be difficult for some institutes and so would require support from the management in all institutes.

2.4 Added value to mackerel eggs triennial surveys

In recent years the WG has highlighted the importance of continuing to exploit the icthyoplankton samples collected from the triennial surveys for species other than mackerel and horse mackerel along the lines of the INDICES project which was funded by the EC in 1998. The results of that study were published in Ibaibarriaga *et al*. (2007). The 2008 list of work programmes for public contract (DG Fisheries and Marine Affairs, 28 January 2008) includes a call entitled "Added value to mackerel and horse mackerel triennial surveys". This call was discussed by the WG.

The main conclusion was that the budget assigned to the project was too limited to complete the objectives of this project as described. The main problem is that the survey produces approximately 2000 samples, and full analysis of these would not be possible within the proposed budget.

Some alternatives were proposed: i) Presenting the project in different phases in order to allowing funding over several years; ii) reducing the number of samples to

be analysed, e.g. every second sample; iii) Reducing the target species to be identified and focusing the project on egg identification only; and iv) Supplementing the budget with other sources of financing.

Only the first one seems to be suitable to the WG, so the WG recommends that the DG Fisheries and Marine Affairs considers to support this study with an increased budget spread over several years.

3 North Sea egg survey 2008

3.1 Countries and Ships participating

Mackerel egg surveys have been carried out in the North Sea more or less regularly since 1967. Since 1996 these surveys have been carried out triennially.

As in 1999 and 2002 the Netherlands and Norway will carry out a mackerel egg survey in the North Sea in 2008. The survey period, 9 June-4 July, will not cover the total spawning period (mid May-end July). The peak of spawning has usually been observed during the second half of June. The timing of the different coverages will therefore probably be adequate to define the main part of the egg production curve. Usually one vessel can cover the North Sea spawning area in about two weeks, and two vessels will cover the area in one week. The spawning area is planned to be surveyed four times in 2008:

Table 3.1.1. Timing and areas for North Sea mackerel egg survey in 2008.

Vessel/Coverage	1	2	3	4
RV "Tridens"	2-7 June	9-12 June	16-20 June	-
RV "Håkon Mosby"	-	9-15June	16-22 June	23 June-4 July

3.2 Sampling Area and Survey Design

The suggested sampling area for each of the four periods based on recent surveys is shown in Figure 3.2.1. During the second coverage both RV "Tridens" and RV "Håkom Mosby" will start north in their respective areas working southwards and in the third coverage they will start in the south working northwards. The first and fourth coverages will be restricted due to survey time, but are planned to cover the most important parts of the spawning area. RV "Tridens" will start and end in Ijmuiden, break for the two weekends in Aberdeen and Scheveningen respectively and RV "Håkon Mosby" will start and end in Bergen and break in Stavanger 24-25 June.

The survey grid during the second, third and fourth coverages will be adjusted according the findings during the previous coverage. The samples will be analysed onboard the vessels during the survey. The two vessels will be in daily contact to exchange data.

As usual, sections along whole or half degree latitudes will be worked, and plankton samples will be collected along these lines in the middle between whole and half degree longitudes. Both vessels will use a Gulf VII (mesh size 500 microns) towed in double oblique hauls with a towing speed of 5 knots. Mackerel will be sampled during the survey for fecundity and atresia purposes and, if possible, it will also be a basis for a DEPM estimate during period two.

3.3 Sampling and Data Analysis

The plankton samples will be placed in buffered 4% formaldehyde. The sea temperature at 5 m will be noted from each of the plankton stations and used for ageing the eggs.

The fish eggs will be sorted from the plankton samples and the mackerel eggs will be classified and the number of stage I eggs will be counted. During the survey an automatic image analysis procedure for detection and diameter measurements combined with visual identification and staging of mackerel eggs will be tried onboard both "Tridens" and "Håkon Mosby". The volume of seawater filtered on each of the plankton stations should also be recorded. Thereby the number of mackerel eggs produced per m2 sea surface per day will be calculated. A preliminary estimate of the mackerel egg production in the North Sea will probably be available for the WGWIDE meeting in September 2008. The final results will be reported to the next WGMEGS meeting in 2009.

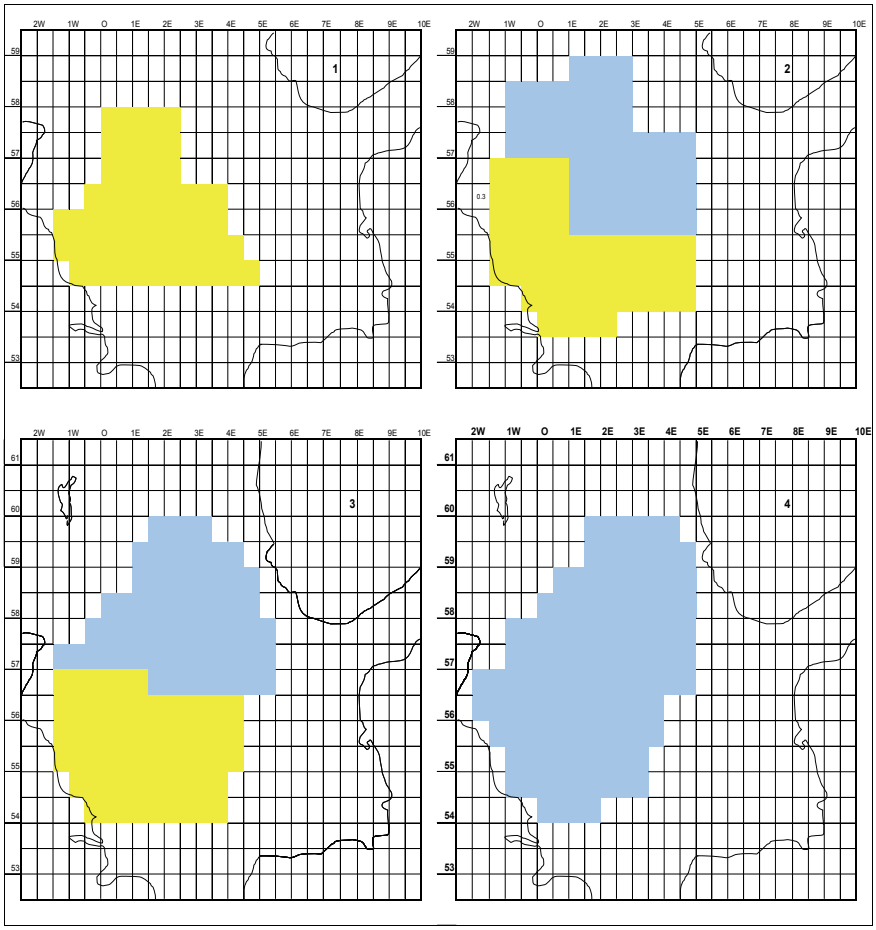


Figure 3.2.1: Suggested sampling areas for "Tridens" (orange) and "Håkon Mosby "(blue) during the four surveys in 2008.

3.4 Fecundity and Atresia

A fecundity study is planned to be carried out by Norway during this season. The intention is to investigate 100 ovaries for potential fecundity and 50 ovaries for atresia. The samples will be taken, handled and analysed as described in ICES (2006 LRC:09). Ovaries for fecundity and atresia studies will be taken from mature, late pre-spawning, spawning or spent females from the for size groups: <250g/-400g/-

550g/>550g. The ovaries have to be removed, weighed, and two parallel samples taken from one ovary (25µl) by a pipette. These samples should be put in Eppendorf tubes (4% formalin). The other ovary should be preserved in formalin jars. The liver, gut and carcass should also be weighed. The samples will be collected from trawl catches from different parts of the spawning area.

However, since there are hardly any mackerel fisheries going on in the North Sea during May it might be difficult to collect ovaries in late pre-spawning stage. If there are surveys in the east part of the North Sea in May this year the WG recommends that they should try to provide samples for potential fecundity studies of North Sea mackerel.

4 Western and southern egg surveys in 2007

4.1 Countries and ships participating

As for previous surveys, the 2007 mackerel and horse mackerel egg survey was designed to cover the whole spawning area of the two species within 6 sampling periods of differing geographical coverage (Table 2.1.2, ICES 2007a). The deployment of research vessel effort in 2007 in the combined western and southern mackerel and horse mackerel sampling area is given in Table 4.1.1. A total of 314 ship days were invested in the complete 2007 mackerel and horse mackerel egg survey, which is a slight increase (8%) on the number of ship days employed during the 2004 survey (291 days). This was despite the loss of the Cefas (England and Wales) survey, which was offset by an additional (industry funded) Scottish charter vessel survey and slightly increased effort by several participating countries.

Table 4.1.1. Deployment of research vessel effort in the 2007 combined (western and southern) mackerel and horse mackerel egg survey.

Date	Country	Vessel	Cruise Dates	Area Coverage	Ship days
2/02 – 3/03	Portugal	Noruega	2/02 – 3/03	36º00′ – 42º00′N	29
6/03 – 21/04	Spain (IEO)	Cornide	13/03 – 5/04	42º15′ – 45º45′N	22
	Spain (AZTI)	Itsaslagunak	2/04 – 21/04	46º15′ – 48º15′N	20
	Ireland	Celtic Explorer	6/03 – 26/03	48º15′ – 52º15′N	21
	Germany	Walther Herwig	20/03 – 7/04	51º15′ – 58º45′N	18
9/04 - 12/05	Spain (IEO)	Cornide	15/04 – 12/05	43º15′ – 46º45′N	27
	Germany	Walther Herwig	9/04 – 24/04	47º15′ – 50º15′N	16
	Scotland	Scotia	3/04 – 23/04	50º45′ – 59º45′N	21
7/05 - 24/05	Spain (AZTI)	Investigador	3/05 – 24/05	43º15′ – 46º15′N	22
	Netherlands	Tridens	7/05 – 23/05	46º45′ – 49º15′N	17
	Norway	Johan Hjort	14/05 – 9/06	49º45′ – 54º45′N	27
	Scotland	Unity	7/05 – 21/05	55º15′ – 59º45′N	15
4/06 - 24/06	Netherlands	Tridens	4/06 – 21/06	47º15′ – 51º15′N	18
	Scotland	Unity	4/06 – 24/06	51º45′ – 59º45′N	21
9/06- 27/06	Ireland	Celtic Explorer	26/06 – 16/07	47º45′ – 54º45′N	20
Sum of realised ship days					314

4.2 Sampling areas and sampling effort

4.2.1 Egg surveys in the western and southern areas

The number of hauls taken by sampling rectangle and by sampling period are presented in Figures 4.2.1.a - f. It should be noted that the rectangles in the western area and in Division IXa are 30′ north-south, and 30′ east-west. In area VIIIc and in the Gulf of Cadiz, IXa are 15′ north-south, and 1o east-west. The figures also include those rectangles where egg production was calculated by interpolation from neighbouring, sampled, rectangles. In contrast to 2004, the 2007 Mackerel and Horse Mackerel Egg Surveys were designed to survey the area within six sampling periods of differing geographical coverage, allowing full coverage of the expected spawning area and season. In period 1 only the western and southern seaboard of the Iberian Peninsula were surveyed. In period 2 the Galician and Cantabrian Sea areas were surveyed, plus the western area as far north as 59°N. In period 3 again the Galician and Cantabrian Sea areas were surveyed as well as the western area to 60°N. In period 4 although very limited sampling took place in the Cantabrian Sea, overall surveying was restricted to the western area from 44°N to 60°N. In period 5 surveying was restricted further, with the western area being sampled between 47°N and 60°N. In period 6 the survey was restricted to the western area between 47° 30′ and 55°N.

Within the periods surveyed, the spatial and temporal coverage was generally good, although there were some periods where additional sampling would have been helpful – particularly in the area north of 58° in the western area in period 4 as well as in the western area south of 47° in period 5. In period 6 additional sampling across the whole western area would have been desirable but especially north of 56°N. Overall surveys were completed within period however several issues arose concerning period overlaps and these along with their potential implications are discussed further in section 8. In general, sampling appeared to cover the bulk of the spatial range for both mackerel and horse mackerel spawning with the edges of spawning being well defined although in 2007 mackerel spawning took place over a much wider area and consequently the spawning boundaries were harder to delineate during peak spawning. This resulted in a significantly higher number of samples along the boundary edges containing small number of stage 1 mackerel eggs than was seen in 2004.

A detailed description of survey coverage by period is provided below:

- Period 1 – Sampling for this period was planned to cover the area from Gibraltar to 42°N on the Portuguese coast. Overall coverage was very good and there were no interpolated samples.
- Period 2 – Sampling for this period was planned to cover the area from the west Iberian coast north of Portugal all the way up the western shelf to 58°N. There were very few interpolated samples. Most rectangles on the north Spanish coast were sampled more than once, as well as good numbers of rectangles across the rest of the area.
- Period 3 – Sampling in this period was planned from 42° to 60°N and again did not include the Portuguese coast. Survey coverage was very comprehensive and there were very few interpolated samples. Again most rectangles in the Cantabrian Sea and also in the southern part of Biscay were sampled more than once. In the western area, there continued to be a good number of replicate samples taken.

- Period 4 - Sampling in this period was planned from 43° to 60°N. There was good coverage overall although the number of interpolated samples was increased notably at 53° 15N and 54° 15N. Elsewhere boundaries NW of Scotland and on the east side of the Celtic sea were not well delineated and therefore include significant interpolation. Again there was a significant level of replicate sampling.
- Period 5 - Sampling in this period was planned from 47° to 60°N and did not include the southern area or the southern part of Biscay. There was significantly more interpolation in this period especially in the Celtic Sea area but overall coverage was good and interpolation was restricted to the boundary edges. Only 5 replicate samples were undertaken during this period.
- Period 6 – Due to lack of ship time, sampling in this period was restricted to the area from 47° 45′ to 55°N, which was believed to be the main spawning area at this time. Only alternate transects were sampled with all the intervening transects being interpolated. No replicate samples were collected during this period.

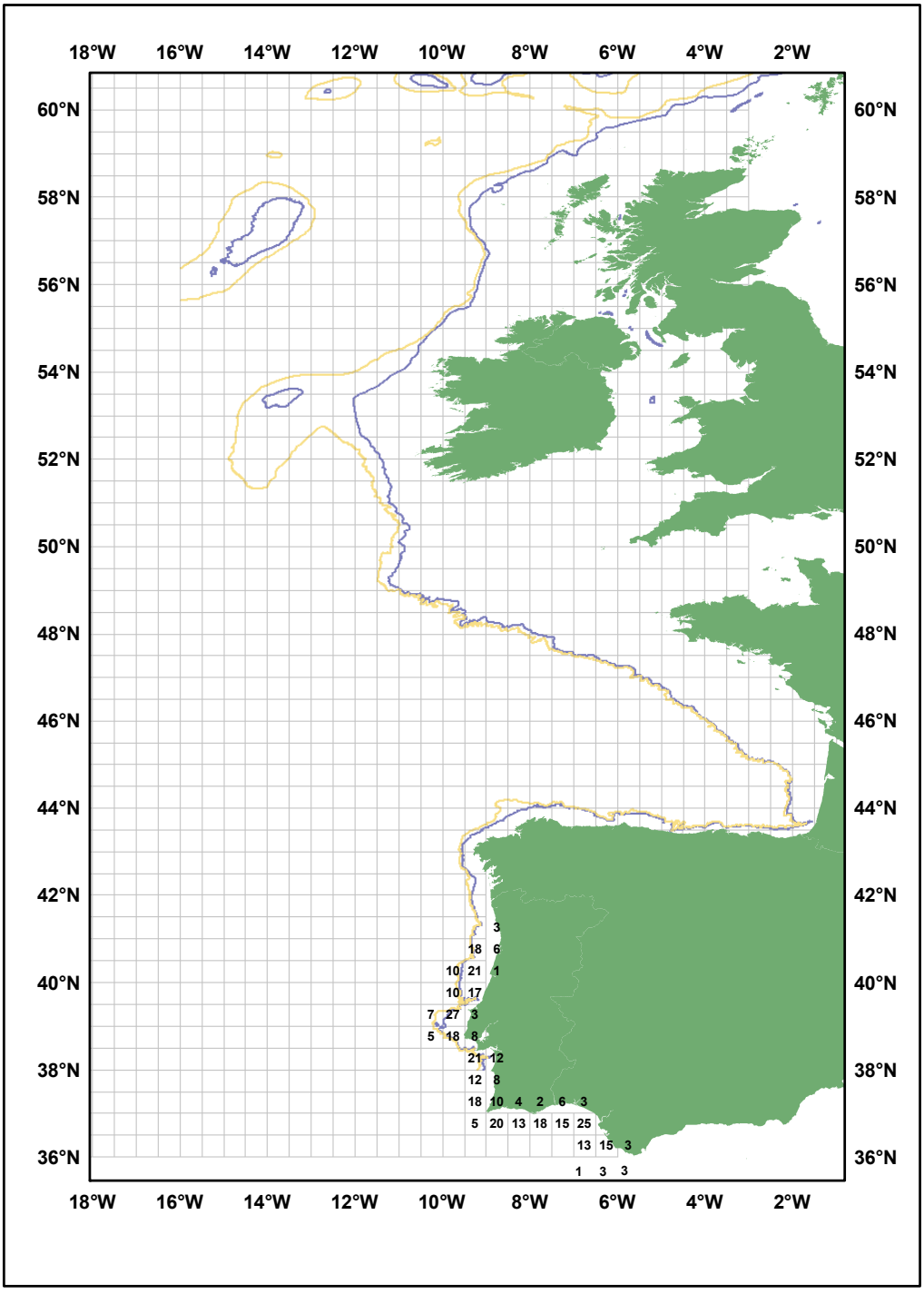


Figure 4.2.1a: Number of observations per rectangle in period 1 (3 February-2 March) – X represents interpolated rectangles.

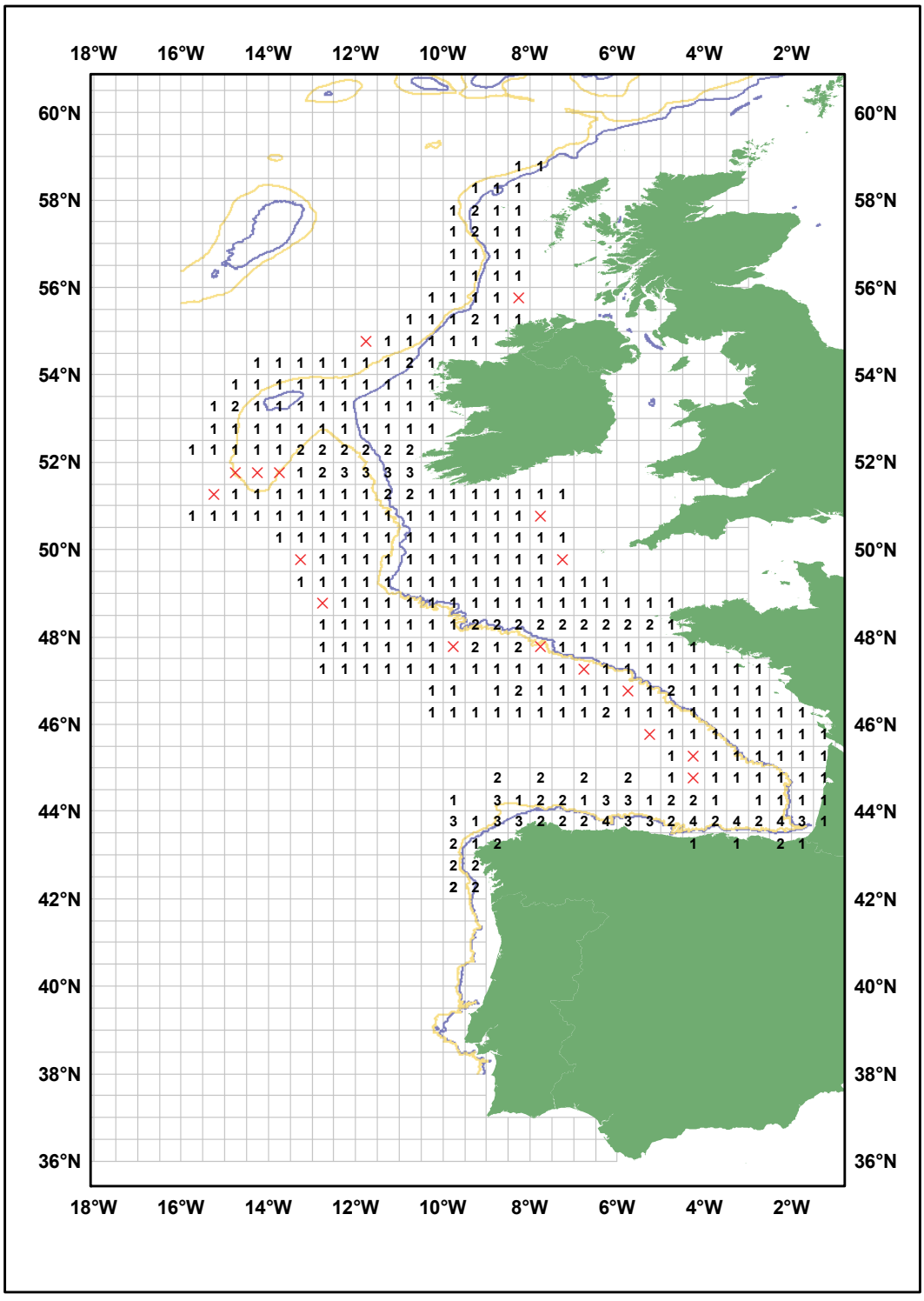


Figure 4.2.1b: Number of observations per rectangle in period 2 (7 March – 8 April) – X represents interpolated rectangles.

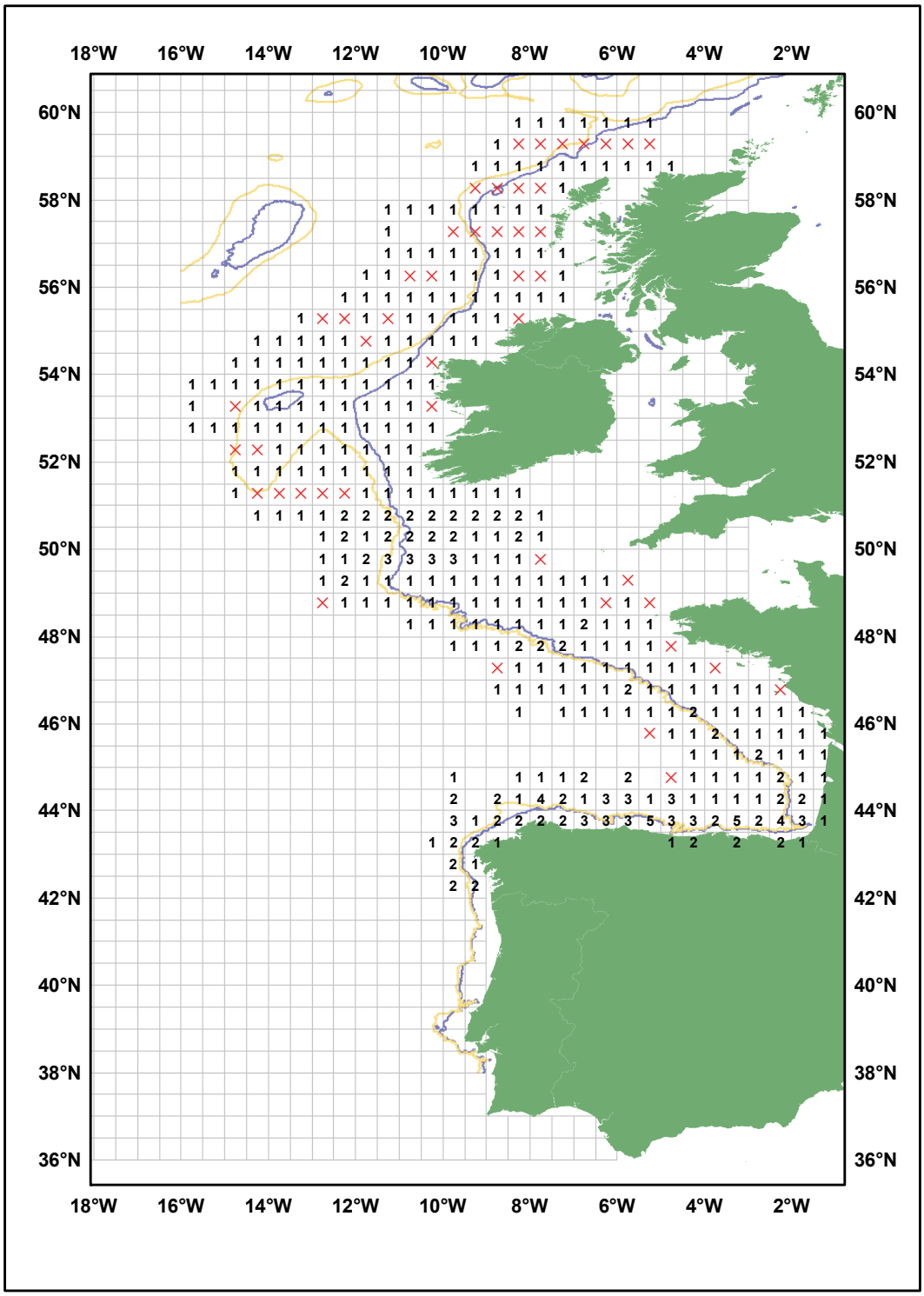


Figure 4.2.1c: Number of observations per rectangle in period 3 (9 April – 6 May) – X represents interpolated rectangles.

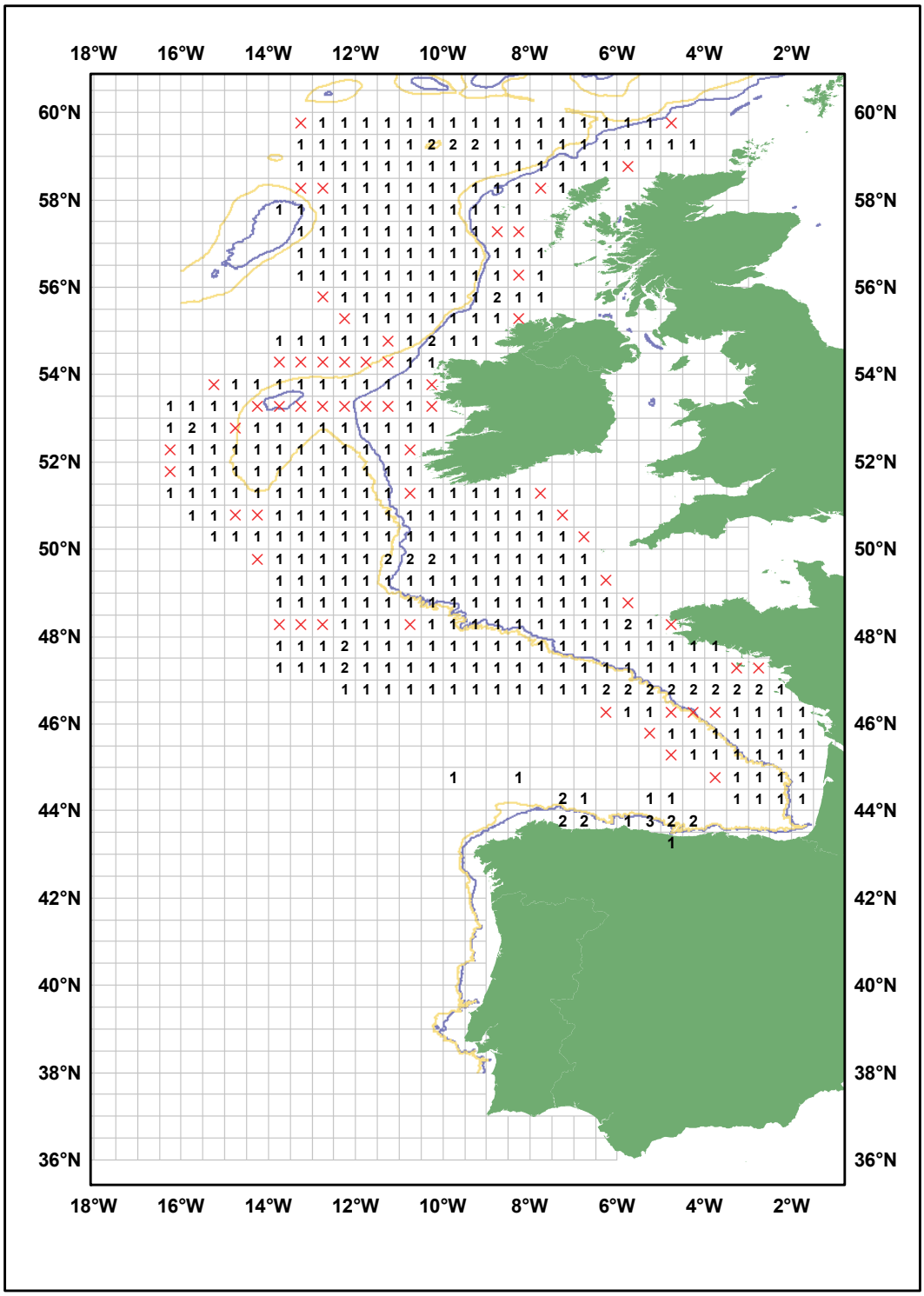


Figure 4.2.1d: Number of observations per rectangle in period 4 (7 May – 3 June) – X represents interpolated rectangles.

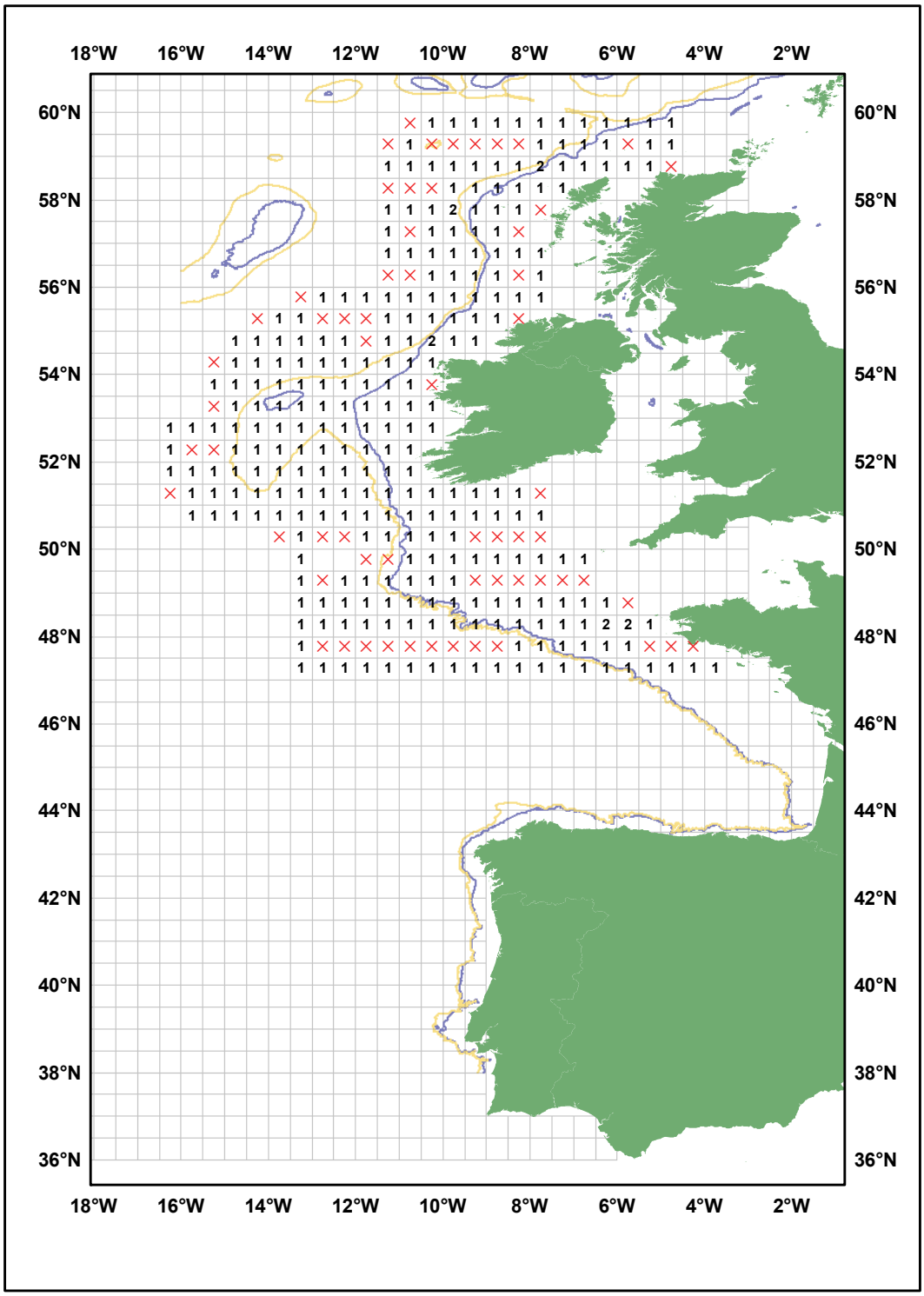


Figure 4.2.1e: Number of observations per rectangle in period 5 (4 June – 24 June) – X represents interpolated rectangles.

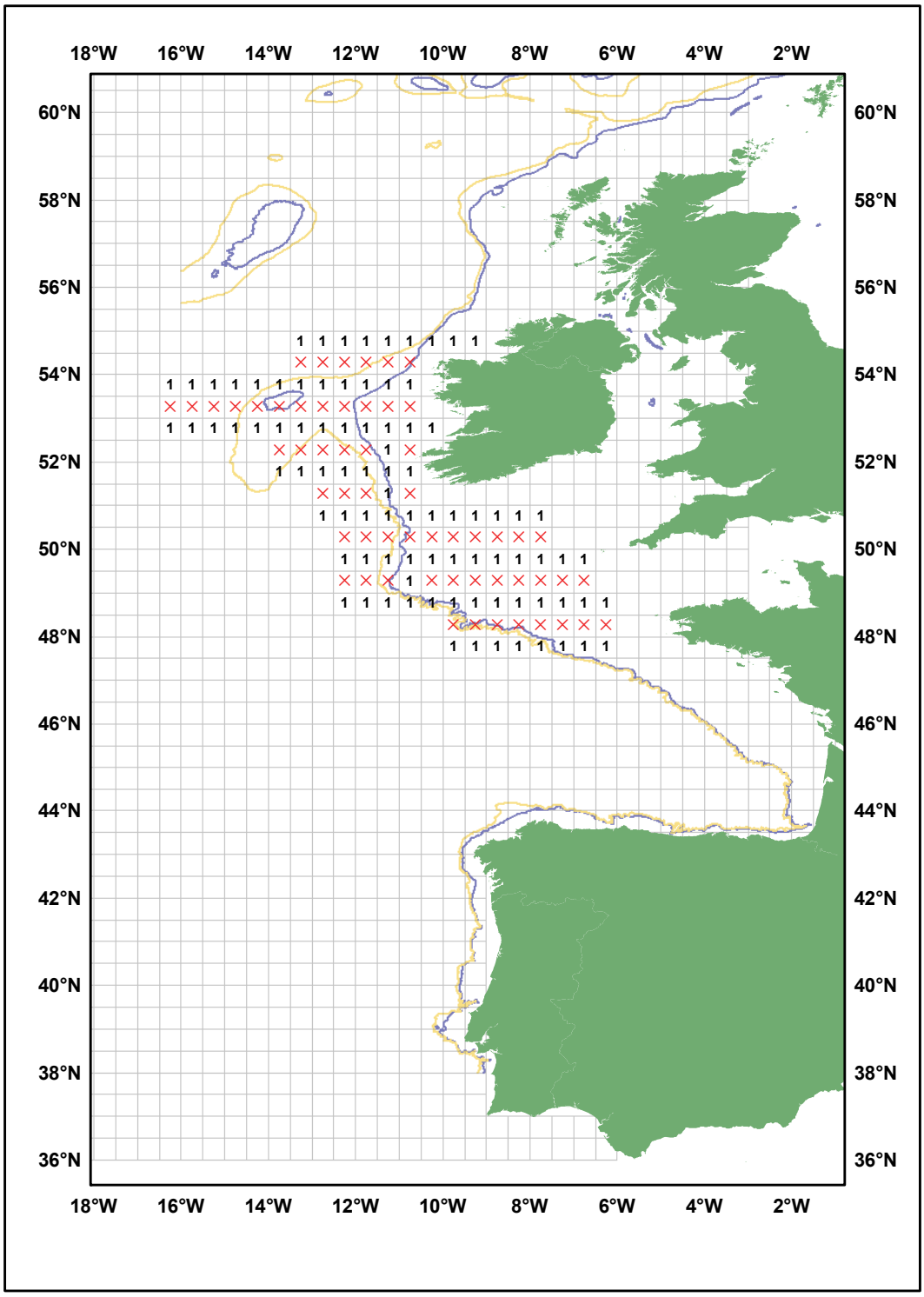


Figure 4.2.1f: Number of observations per rectangle in period 6 (25 June – 31 July) – X represents interpolated rectangles.

4.3 Sampling and data analysis

As in previous surveys, the 2007 survey was carried out in accordance with the modified sampling strategy described in detail for the 1995 survey (ICES 1996, 1997). The adaptive strategy for setting transect boundaries was used. This strategy worked well, particularly during the Scottish survey in period 4 when eggs were still being found as far west as the Rockall plateau.

4.3.1 Sampling strategy for horse mackerel in the southern area

A survey directed at the DEPM for southern horse mackerel was carried out from February 2nd to March 3rd 2007, on board research vessel "Noruega", covering the area from 35° N to 42.5° N. Egg sampling with double CalVET nets was carried out in 406 stations 3 nm apart along 46 transects perpendicular to the coast line separated 12 nm. No samples were performed from 41° N to 42° N due to bad weather conditions. The number of samples per ICES rectangle used for DEPM, is given in Figure 4.2.1a. A total of 32 Bongo net samples were also collected along the Portuguese coast, for selectivity comparisons between Bongo and CalVET nets.

Adult fish samples for batch fecundity and spawning fraction estimation were sampled in 14 hauls with a Norwegian Campelen bottom-trawl. Horse mackerel were caught in 10 of those hauls and a total of 1021 fish were sampled. All the collected gonads were preserved in 4% buffered formalin for histology. From the 63 females with hydrated oocytes only 38 were in condition for batch fecundity determination, as described by Hunter *et al*. (1985) and Watson *et al*. (1992). Spawning fraction was determined based on 602 ovaries of randomly collected mature females. Three different criteria were applied to these samples in order to obtain spawning fraction estimates: the presence of migratory nucleus (MN), hydrated oocytes (HO) and post-ovulatory follicles (POFs).

4.3.2 Sampling gears and procedure

In the western area plankton sampling was carried out using national versions of Gulf VII type samplers with the exception of Spain which used a Bongo sampler and Portugal which used a double CalVET. Gulf VII type sampler was fitted with a conical nose cone with an aperture of 20 cm diameter. The samplers were deployed to within 3 m of the bottom or to a maximum of 200 m in deeper water. A double-oblique haul was carried out at each sampling position at a ship speed of approximately 4 knots. Calibrated flowmeters mounted both inside the nose cone and externally on the body of each sampler, were used to calculate the volume of water filtered on each deployment. When a thermocline was identified, the samplers were deployed to 10m below the thermocline. In the southern area Bongo samplers with 40 cm openings were used by Spain while Portugal used double CalVET sampler. Bongo sampler was deployed on double oblique hauls to a maximum depth of 200 m or to within 3 m of the bottom in shallower water. They were towed at a ship speed of 2–3 knots and calibrated flowmeters mounted in the aperture were used to calculate the volume of water filtered. In all the surveys a full temperature/depth profile was recorded. The temperature at 20 m on each deployment was used as a parameter in the calculation of the production of eggs per day in each rectangle. Pairovet sampler used by Portugal was deployed on vertical hauls to a maximum depth of 200 m or to within 3 m of the bottom in shallower water. Table 4.3.2.1 shows the main characteristics of high speed plankton samplers used by WGMEGS participants.

Table 4.3.2.1. Gulf type ¨high-speed¨ plankton sampler designs as used by WEMEGS survey participants.

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

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

4.3.3 Data Analysis

All data analysis was carried out in accordance with the procedures described in detail for the 1995 survey and 1998 surveys (ICES, 1996, 1999). The detailed steps of the data analysis were updated for the 2003 WGMEGS report (ICES 2003), and then subsequently for the WKMHMES report form the workshop in 2006 (ICES 2006b). For all sampling in the western area, individual countries supplied data in an electronic Excel template form to the data coordinator at the Marine Laboratory, Aberdeen. For sampling in the southern area data were supplied in Excel spreadsheet format to the data coordinator in Madrid. 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 Aberdeen database. The estimation of uncertainty (variances) for the Total Annual Egg Production (TAEP) for Mackerel and Horse mackerel are performed following the procedures developed by Fryer (ICES, 1996). The variance of the total annual egg production was assumed to be the weighted sum of the variance of the total daily production in each sample period (ICES, 1996, 2003).

This method is based on an estimate of the variance in egg densities for each sampling rectangle with two o more samples and non-zero values samples (ICES, 2003). It all relies on the assumption that the variance in egg counts is distributed with a constant spatio-temporal coefficient of variation (CV). Assuming a log-normal distribution of estimates, the constant CV is obtained by an Analysis of Variance of Daily egg production natural log by rectangle and period, having excluded those rectangles, which any zero value hauls, and those no-replicate rectangles (Pope and Woolner, 1984). Then CV value can be estimated as:

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

$$D = \text{Daily egg production (stage I eggs} / m^2 / \text{day)}$$

$$y = \log_{\mathrm{e}}(D)$$

Where variance of log Daily egg production (σ_y^2) can be estimated by the residual variance (Error Mean Square) from the ANOVA of log(D).

A single variance calculation was made for the survey data for the combined western and southern components of the North East Atlantic mackerel (Table 4.3.3.1). The standard error was $0.0303*10^{15}$ corresponding to a CV of 1.99. A similar calculation was made for the new western horse mackerel area (including the Cantabrian Sea). The standard error was $0.0269*10^{15}$ corresponding to a CV of 1.88. By period the estimated CV values for mackerel varied from a maximum of 2.50 in period 2 to minimum of 1.28 in period 5 (table 4.3.3.1). The estimated CV values for horse mackerel was maximum in period 4 (2.04) and minimum in period 3 (1.75) (table 4.3.3.1).

Table 4.3.3.1. Number of replicate samples and estimates of coefficient of variation (CV) for mackerel and horse mackerel by periods and for the total surveys.

Period	Number of replicate samples	Number of rectangles with replicates - mackerel	CV Mackerel	Number of rectangles with replicates – horse mackerel	CV Horse mackerel
2	63	28	2.5	18	1.9
3	59	28	1.46	20	1.75
4	28	14	1.36	10	2.04
5	8	3	1.28	1	-
6	0	-		-	-
TOTAL	158	73	**1.99**	49	**1.88**

5 Mackerel in the western and southern spawning areas: 2007 egg survey results

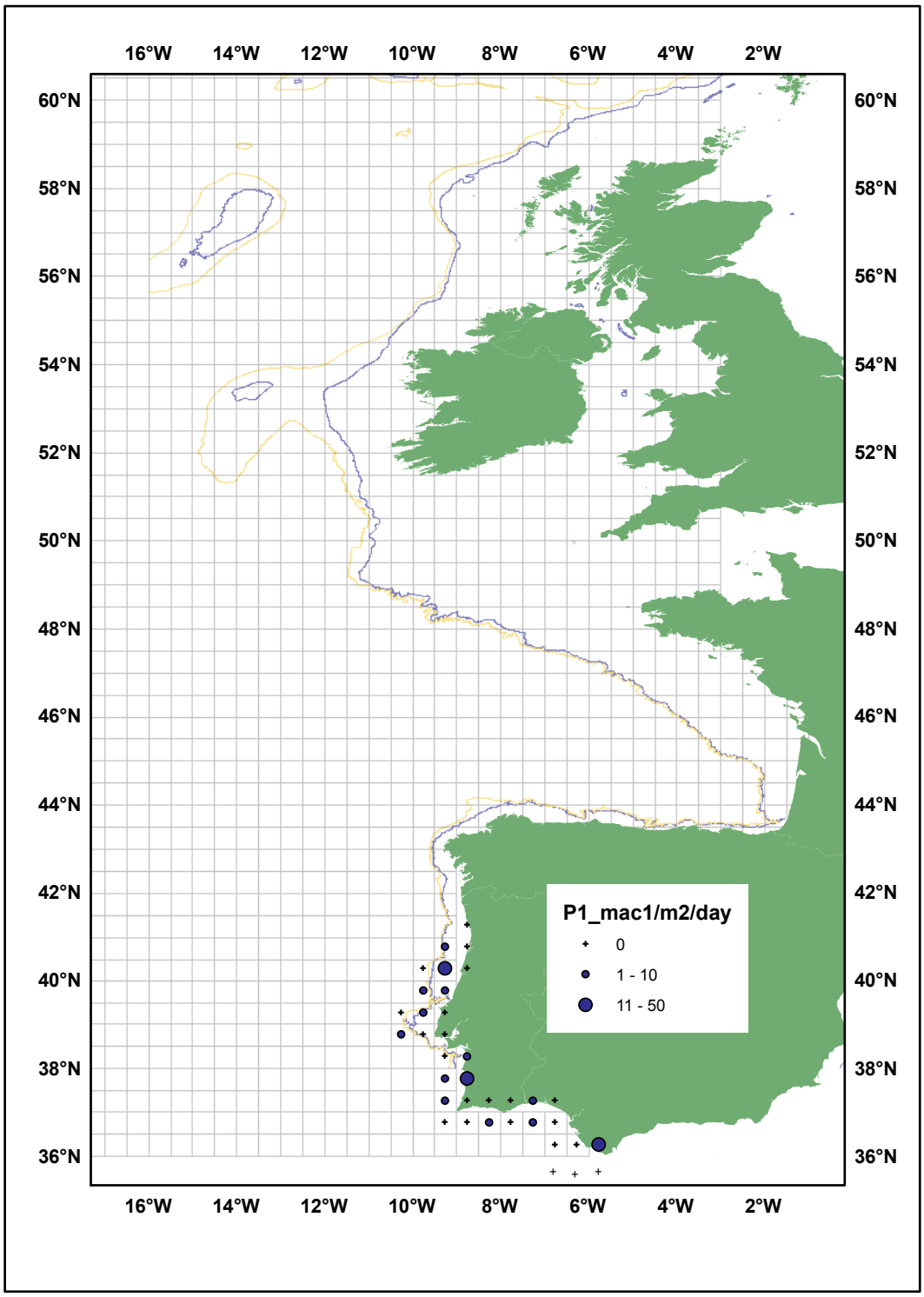
5.1 Spatial distribution of stage 1 mackerel eggs

The description of the spatial distribution of stage 1 mackerel eggs is presented for both the southern and western areas together. The subsequent calculation of the egg production curve and biomass are considered separately for the two areas.

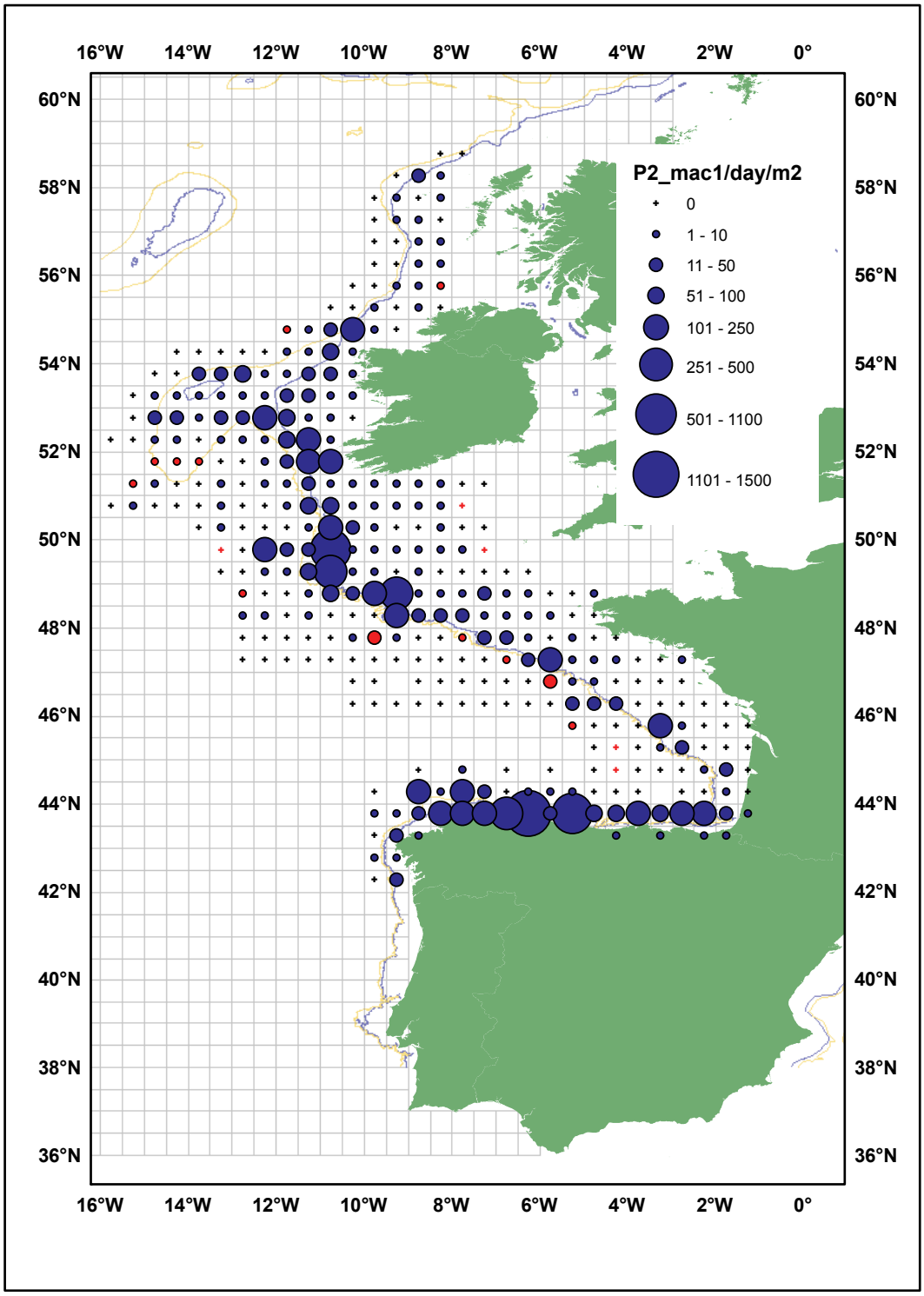
- **Period 1** - During the first Portuguese cruise surveyed the southern part of the southern area (36°00 N–42°00 N) (Figure 5.1.1a). In Portuguese waters and the Gulf of Cadiz mackerel eggs stage 1 were very sparse with very low abundance. In this period the egg production was very low. Coverage was good and there were no interpolations required.
- **Period 2** – During this period the area surveyed ran from the north coast of Portugal to the north coast of Scotland, (42°00 N–59°00 N) (Figure 5.1.1b). The area was sampled by four institutes. Significant numbers of stage 1 eggs were encountered in the Cantabrian Sea as well as along the 200m contour from 45°00 N–55°00 N in the western area. The highest concentrations of stage 1 eggs were found in the Celtic Sea. Area coverage was good and spawning boundaries were well defined. Only 14 stations were interpolated.
- **Period 3** - In Period 3 sampling again ran from the north coast of Portugal to the north coast of Scotland, (Figure 5.1.1c). Sampling was undertaken by three countries and coverage again was very good with spawning boundaries being well delineated throughout the whole area. Egg production was fairly continuous albeit at a much lower level than found in period 2 along the shelf break from Biscay to the north of Ireland (47°00 N – 56°00 N). Elsewhere, away from the shelf break but also generally in the Cantabrian Sea, south Biscay and west of Scotland above 56°00 N egg densities were low. No significant interpolation was required apart from three transects at 59° 15N, 58° 15N and 57° 15N.
- **Period 4** - Coverage in this period was quite good (Figure 5.1.1d). Three countries were originally tasked with sampling however the arrival of an additional third Scottish survey significantly improved area coverage. Widespread spawning activity was present from Biscay to NW Scotland with low numbers being encountered again in south Biscay and the Cantabrian Sea. Main concentrations of spawning were in north Biscay, to the west of Brittany, Porcupine Bank and also west of Scotland north of 56°. The main feature of this period was the large number of stage 1 eggs found as far west as Rockall by the Scottish survey from 57°00N to 60°00N. A number of transects were interpolated at 53°15N and 54°15N. Stations on the eastern edge of the Celtic Sea were also interpolated. Due to the expansion of the survey area in the northwest it proved impossible to close the northern boundary for this period.
- **Period 5** – The area was surveyed by two countries and coverage was reasonably good (Figure 5.1.1e). Sampling in the Bay of Biscay (southern 47°N) and the Cantabrian Sea was discontinued. Significant spawning activity was encountered throughout the survey area but was particularly concentrated along the 200m contour line, and on the Porcupine Shelf. Significant densities were also recorded in the Celtic Sea south of Ireland and here the boundary was not well defined. Otherwise edges of spawning

were well delineated. Interpolation was needed for three transects 47°45N, 49°15N and 50°15N.

- **Period 6** - Only one vessel was available for sampling in this period so consequently coverage was less comprehensive than in previous periods (Figure 5.1.1f). Due to the size of the sampling area only alternate transects were sampled from 47°30N to 55°00N. The intervening transects were interpolated. The southern spawning boundary was well defined but once again there was insufficient time to define completely the northern spawning boundary. The largest egg concentrations were present east of the shelf break in the Celtic Sea and to the south of Ireland and here again as in period 5 the boundary was not clearly delineated with significant numbers of eggs being recorded up to 6°W. Smaller numbers were found along the 200m contour line up along the west coast of Ireland.



5.1.1a: Mackerel egg production by half rectangle for period 1 (3rd February – 2 March). Filled blue circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.



5.1.1b: Mackerel egg production by half rectangle for period 2 (7 March – 8 April). Filled blue circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes and red crosses interpolated zeroes.

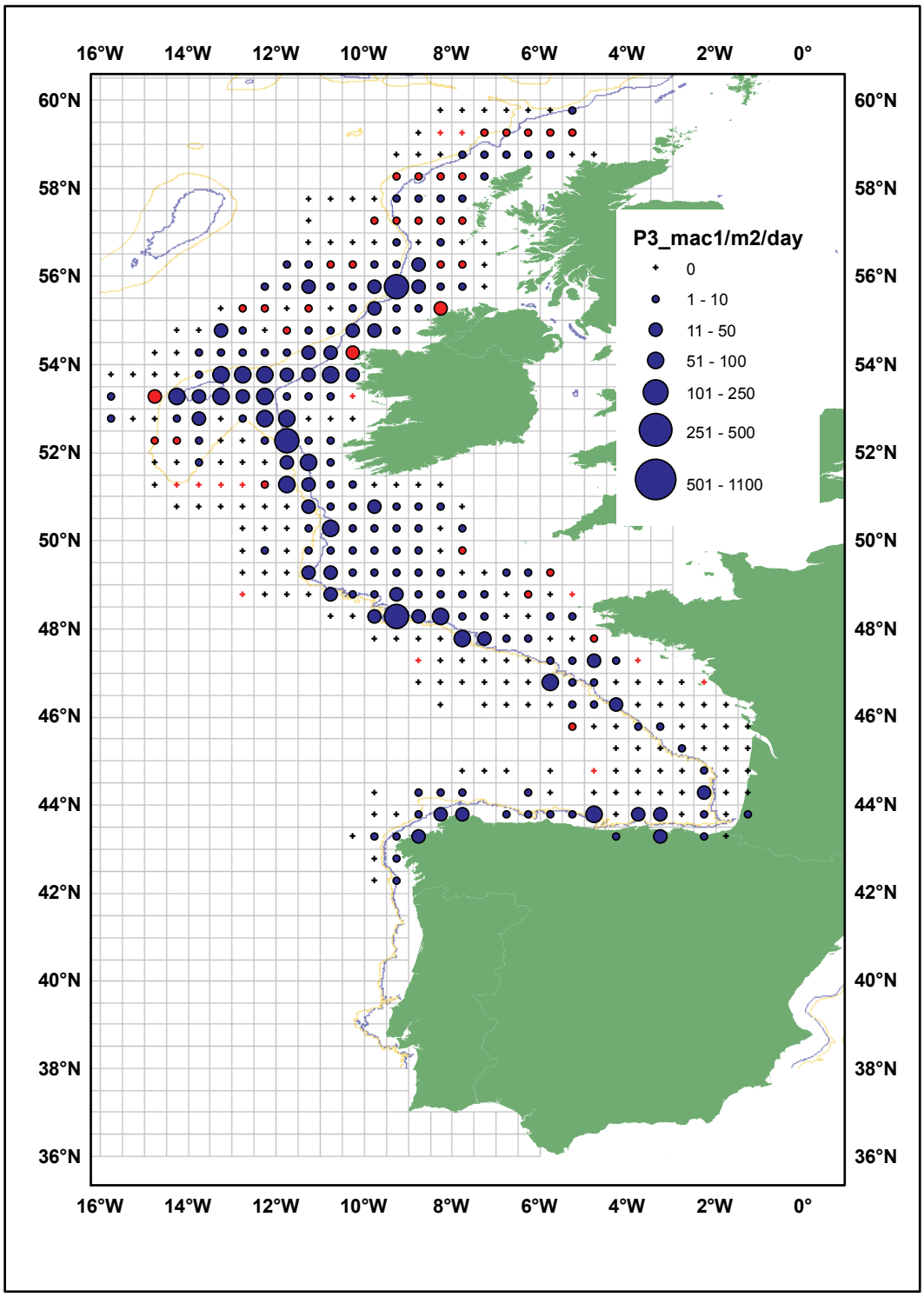


Figure 5.1.1c: Mackerel egg production by half rectangle for period 3 (9 April – 6 May). Filled blue circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

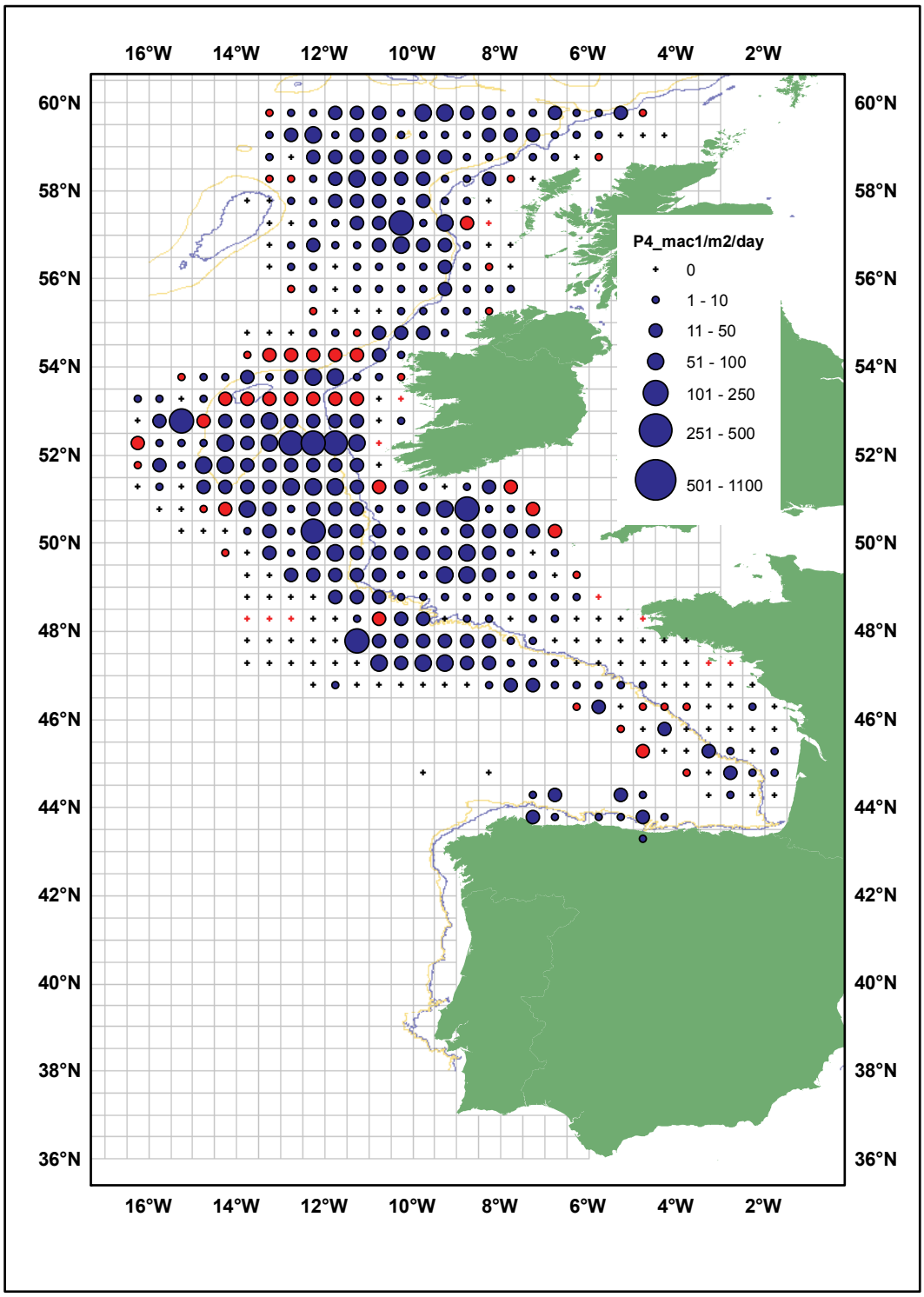


Figure 5.1.1d: Mackerel egg production by half rectangle for period 4 (7 May – 3 June). Filled blue circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

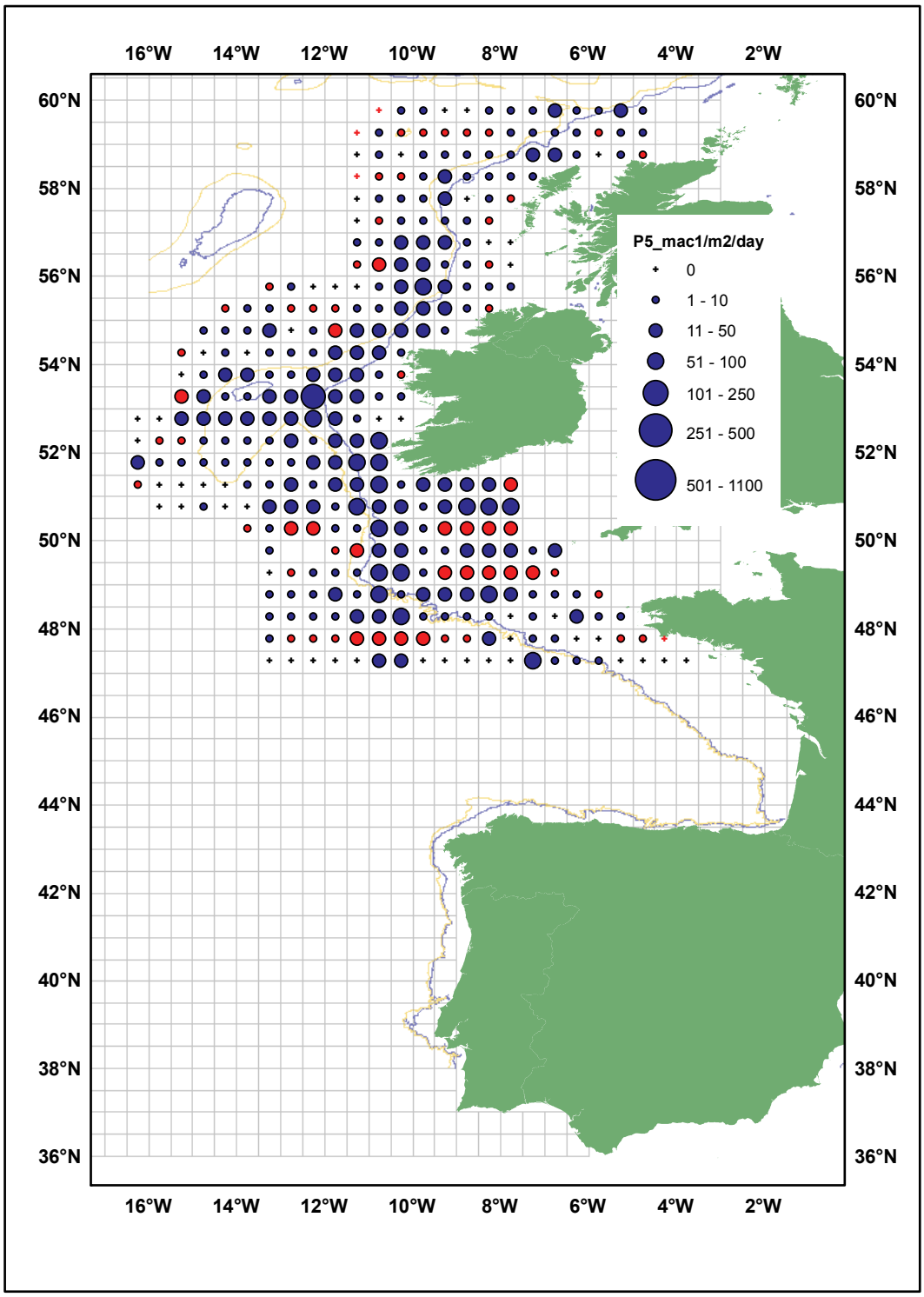


Figure 5.1.1e: Mackerel egg production by half rectangle for period 5 (4 June – 24 June). Filled blue circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

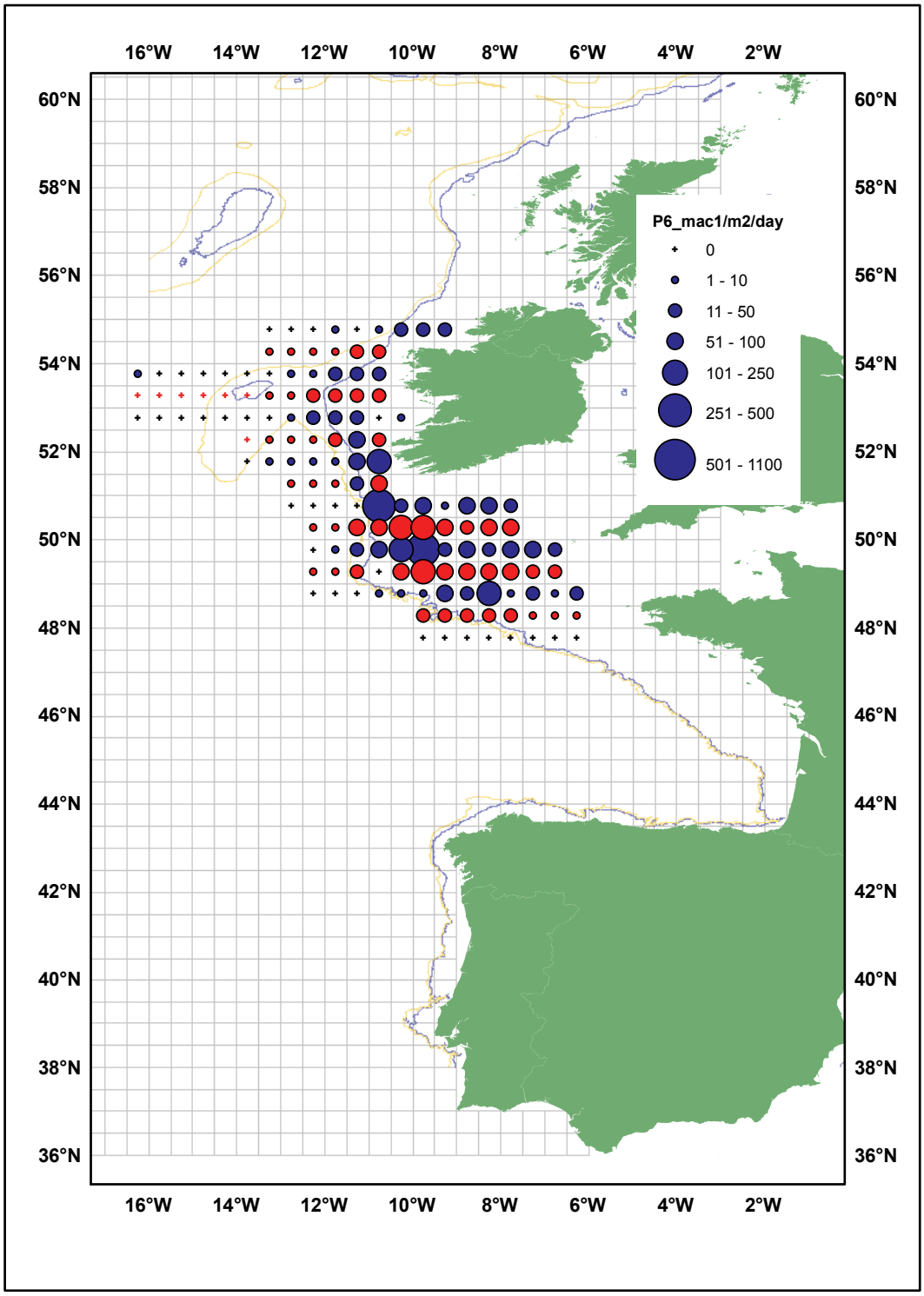


Figure 5.1.1f: Mackerel egg production by half rectangle for period 6 (25 June – 31 July). Filled blue circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

5.2 Egg production of the Northeast Atlantic Mackerel

5.2.1 Stage I egg production in Northeast Atlantic Mackerel

Figure 5.2.1.1 presents the egg production curve for the western area for the 2007 survey, along with those for the surveys in1998, 2001 and 2004 for comparison. The data values are presented in Table 5.2.1.1. The start date was assumed to be the 10 February as used since 1995. No histological or survey data were available in the western area or in the Cantabrian Sea prior to period 2 to suggest any alternative start date. The end date (31 July) is the same as that used since 1995. Egg production was low in period 6, but due to the reduced area coverage it is impossible to be sure that there was no further spawning after this survey. However, the shape of the production curve does not suggest that the chosen end date should be altered. Production estimates for the individual survey periods and the period before the surveys are presented in Table 5.2.1.1. Like 1998 and 2001, the survey periods were all completely contiguous. The standard errors are slightly lower than 2004, probably due to an increased number of duplicated samples. Total annual egg production for the western area in 2007 was calculated as 1.21×10^{15} with a standard error of 0.105×10^{15}.

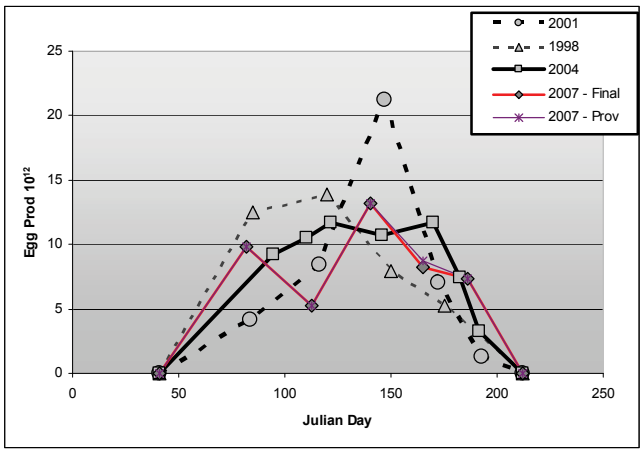


Figure 5.2.1.1: Annual egg production curve for mackerel in the western spawning component. The curve for 1998, 2001and 2004 are included for comparison. Provisional and Final curve for 2007 are presented too.

Table 5.2.1.1: Western estimate of mackerel total stage I egg production by period after integration of area under the egg production curve for 2007.

Dates	Period	Days	Annual stage I egg production x 10 15
< 7 March	Pre2	41	0.201
7 March – 8 April	2	30.5	0.229
9 April – 6 May	3	28	0.258
7 May – 3 June	4	24.5	0.262
4 June – 24 June	5	21	0.163
25 June – 31 July	6	26	0.095
Total	1.21		
Se	0.105		
CV	8.7%		

5.2.2 Stage I Egg production in southern spawning area

Table 5.2.2.1 shows the mackerel mean daily stage I egg production estimates in southern spawning area by period. The egg production curve for the southern area for the 2007 surveys is plotted in Figure 5.2.2.1. Like 2001 and 2004 there was temporal overlap between periods for 2007 surveys. Total egg production values by survey period and interpolated survey period are given in Table 5.2.2.2. The start date of spawning for mackerel was assumed on 4 February, two weeks later than in previous years. It is based on the occurrence of stage I eggs found off the Portuguese coast. The end date was assumed to be 17 July, the same as one used years before because no new data on the end of the spawning were available to suggest any alternative end data. There was 13 days gap between period 1 and 2, 9 days between period 2 and 3 and 68 days since period 4 until the end of the spawning. Although the number of interpolated days was high, its contribution to the total production in southern area was less than 1%. In 2007, the total egg production in the southern area was estimated 31.19×10^{13} (s.e. 17.09×10^{13}) more than the twice of the egg production found in 2004 but similar to the 2001 (Table 5.2.2.3).

Table 5.2.2.1. Southern Mackerel mean daily stage I egg production 10^{12}

Period	Dates	Production
1	03/02 – 02/03	0.13
2	16/03 - 5/04	9.05
3	15/04 – 06/05	0.33
4	7/05 – 10/05	0.09

Table 5.2.2.2. Southern estimate of mackerel total stage I egg production by period after integration of area under the egg production curve for 2007.

Dates	Period	Days	Annual stage i egg production x 10 13
3 February – 2 March	1	28	0.35
4 March – 15 March	*	13	6.50
16 March – 5 April	2	21	19.00
6 April – 14 April	*	9	4.28
15 April – 6 May	3	22	0.73
7 May – 10 May	4	4	0.04
11 May – 17 July	*	68	0.29
Total	31.19		
Se	17.09		
CV	7%		

Table 5.2.2.3. Annual stage I egg production for Mackerel in southern area in 1998, 2001, 2004 and 2007.

Year	Annual stage I egg production*10^{13}	
	estimate	se
1998	43.37	18.84
2001	28.31	4.67
2004	12.56	2.35
2007	31.19	17.09

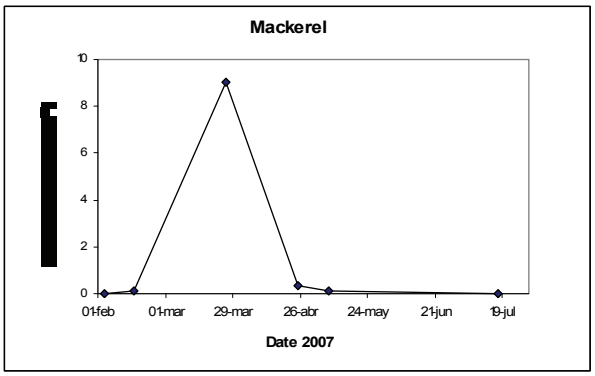


Figure 5.2.2.1: Annual egg production curve for mackerel in the southern spawning component for 2007.

5.3 Potential fecundity of Northeast Atlantic mackerel

5.3.1 Comparative Fecundity and atresia estimation 2007

5.3.1.1 Fecundity workshop report

Standardisation of fecundity estimation

Images were prepared from either an unstained whole mount sample of mackerel ovary tissue or after staining with Rose Bengal or Periodic acid Schiffs (PAS). Each analyst attending the meeting scored these images to count the number of normal, atretic and post ovulatory follicles in each preparation prior to and after discussion.

The overall CV counting both normal and atretic follicles before and after discussion of the interpretation was 3.8 and 2.3% respectively (Table 5.3.1.1.1). Comparing the scores before and after discussion for either unstained or stained images showed that the reduction in CV was greatest for the unstained sample. However after discussion the CV of the counts for each preparation was very similar with or without staining at 2.6, 1.9 and 2.3 for PAS, Rose Bengal and unstained respectively. Atretic follicles were considered to be more easily detected before staining because the stain masked the contents of the follicle and making it less easy to see the fragmented chorion. The Workshop considered that the extra time required to carry out the staining protocol was not reflected in easier identification of follicle classes. Postovulatory follicles (POF) counting also improved following discussion. It was therefore agreed that the sample should be rejected from the potential fecundity data set if 5 or more similar POF structures were found during the first stage of whole mount examination.

Following the workshop a new set of scaled Mackerel oocyte pictures were distributed to all the participating labs. This test showed rather similar results between the labs (Table 5.3.1.1.1) even though the participants now worked on different software according to what was available at the respective labs.

Standardisation of mackerel atresia estimation

The quantification of each early alpha atresia stage follicle class (yolk vesicle, yolk vesicle – yolk granule and yolk granule) stained with haematoxylin and eosin (H&E) PAS Mallory (PM) or Toluidine blue (TB) was discussed. The atretic follicle classification criteria was based on the mackerel / horse mackerel fecundity methods manual produced following the Workshop held at Lowestoft in December 2000.

Serial sections were produced from 6 mackerel ovary samples and stained with either H&E, PM or TB and scored by AZTI, CEFAS and IMARES and IMR for early alpha atresia in the 3 follicle classes.

A comparison of the alpha atresia counts, after combining the values for each stage, showed that staining method (Table 5.3.1.1.3) was not a significant factor in the results produced by the most experienced analysts (CEFAS and IMR Table 5.3.1.1.2). Although there were differences in the allocation of scores to the three follicle classes this was always between adjacent categories and the likely consequence when applied to the stereometric method under these circumstances is therefore low. Counts made (Table 5.3.1.1.4) on H&E stained sections were also not significantly different for AZTI compared with Cefas, IMARES or IMR whilst IMARES was different to both Cefas and IMR. The other scores from PM and TB stained images were very different comparing either AZTI or IMARES with Cefas or IMR and further work is required to identify the source of variation.

In summary the analysis suggests that either stain can be used for the assessment of atresia without causing serious bias to the results. Because the labs involved have different equipment and standard procedures, AZTI will use H&E whilst Cefas and IMR will use PM and TB respectively. The differences with IMARES are not likely to be important because they are not part of the effort working up atresia samples.

5.3.1.2 Comparison of mackerel fecundity data from the 2007 Triennial survey

The fecundity samples from the 2007 Triennial survey were evenly distributed between the labs. A comparison of the fecundity estimates (Table 5.3.1.2.1, Figure 5.3.1.2.1) between Scotland (FRS), Norway (IMR), Ireland (MI) and Spain (AZTI, IEO) showed significant differences from the overall mean. This happened even though all the labs followed the procedure described in the fecundity manual. However, there

were some technical aspects that need to be described in detail to avoid an arbitrary interpretation.

AZTI, IMR and FRS were not significantly different from the overall mean whilst IEO and MI were significantly higher and lower respectively. Although WGMEGS decided to accept all the data, the results show clearly that more effort should be put into ring tests (distribute parallel samples among the labs) in the preparation for the triennial survey. Also some part of the Triennial survey samples should be divided into parallels and distributed among the labs to allow for direct comparisons.

5.3.1.3 Comparison of mackerel atresia data from the 2007 Triennial survey

Both geometric mean and prevalence of early alpha atresia showed considerable differences between Institutes (Table 5.3.1.3.1). The main outliers from the mean were attributed to AZTI and IEO who reported approximately 4-5 times higher mean atretic loss than the other labs. However, the overall mean was mostly influenced by the results from Cefas, IMR and MI, since those labs analyzed all together 89 % of the samples.

The large differences in the results clearly show that we have to work more on standardizing our way of analyzing samples. Cameras, optics, image analysis software, staining procedures and way of interpretation have to be evaluated. As for the fecundity we will recommend a workshop and ring tests ahead of the triennial survey. In addition some of the samples from the survey should be taken in parallels so that the same samples can be analysed by all the labs.

Table 5.3.1.1.1 Results of the fecundity counts comparing analysts working with 3 images prepared from follicle samples stained with either Periodic acid Schiff's (PAS), Rose Bengal or unstained. Follicles were scored as normal vitellogenic (VF) atretic (atre) and post ovulatory (pof). The columns to the left of the centre line refer to results before discussion whilst those to the right were scored after discussion.

PAS

Participant	VF	pof	atre	Total
anders	227	1	0	227
merete	225	1	0	225
lorraine	222	8	1	223
Mairead	222	7	2	224
peter	226	7	0	226
cindy	232	5	0	232
Hanz	225	2	0	225
Marai S	221	0	0	221
Maria k	222	0	0	222
mean	225	3	0	225
stdev	3	4	0	3

PAS

Participant	VF	pof	atre	Total
anders	207	2	0	207
merete	215	8	0	215
lorraine	222	8	1	223
Mairead	223	6	1	
peter	224	6	0	224
cindy	218	8	0	218
Hanz	224	8	0	224
Marai S	216	2	1	217
Maria k	221	2	1	222
mean	219	6	0	219
stdev	6	6	1	6

Rose Bengal

Participant	VF	pof	atre	Total
anders	183	1	0	183
merete	190	1	2	192
lorraine	177	3	1	178
Mairead	179	2	1	180
peter	176	2	0	176
cindy	197	4	0	197
Hanz	182	2	2	184
Marai S	180	0	0	180
Maria k	184	0	0	184
mean	183	2	1	184
stdev	7			7

Rose Bengal

Participant	VF	pof	atre	Total
anders	169	1	1	170
merete	178	1	1	179
lorraine	181	1	0	181
Mairead	178	2	1	
peter	177	6	0	177
cindy	175	2	1	176
Hanz	176	4	0	176
Marai S	177	3	4	181
Maria k	177	3	3	180
mean	176	3	1	178
stdev	3	2	1	4

Unstained

Participant	VF	pof	atre	Total
anders	238	8	2	240
merete	224	5	8	232
lorraine	215	4	2	217
Mairead	207	3	1	208
peter	209	2	3	212
cindy	236	2	0	236
Hanz	233	0	0	233
Maria S	213	0	0	213
Maria k	214	0	0	214
mean	221	3	2	223
stdev	12	2	2	12

Unstained

Participant	VF	pof	atre	Total
anders	203	4	7	210
merete	211	5	4	215
lorraine	219	4	2	221
Mairead	211	5	6	
peter	207	6	6	213
cindy	205	5	6	211
Hanz	208	4	5	213
Maria S	211	9	10	221
Maria k	213	7	11	224
mean	210	5	6	216
stdev	5	6	6	5

Table 5.3.1.1.2. Counting and diameter measurements from scaled pictures distributed to all the participating labs.

	Counting		Diameter measurements of about 1/3 of the sample						
	Total number	POFs							
Institute	oocytes		Average	SD	CI_95	Max	Min	Count	LC
Picture 1									
IMR	534	1	343	100.9	14.3	729	189	199	509
IEO	511	1	337	71.2	10.9	712	211	170	454
AZTI	531	1	357	90.6	13.3	700	204	177	506
IMARES	534	1	343	100.9	14.3	729	189	199	509
MI	533	1	327	86.8	12.6	744	186	191	470
Average	529	1	341	90	13	723	196	187	490
St.dev	10	0	11	12	1	17	11	13	26
Max	534	1	357	101	14	744	211	199	509
Min	511	1	327	71	11	700	186	170	454
Picture 2									
IMR	414	2	346	109.6	16.8	791	185	171	526
IEO	414	1	340	97.4	16.6	698	187	138	501
AZTI	390	3	359	95.3	16.4	698	214	130	515
IMARES	414	2	346	109.6	16.8	791	185	171	526
MI	416	2	337	102.4	18.9	707	186	118	505

	Counting		Diameter measurements of about 1/3 of the sample						
	Total number	POFs							
Institute	oocytes		Average	SD	CI_95	Max	Min	Count	LC
Average	410	2	346	103	17	737	191	146	515
St.dev	11	1	8	7	1	49	13	24	12
Max	416	3	359	110	19	791	214	171	526
Min	390	1	337	95	16	698	185	118	501
Picture 3									
IMR	207	3	536	141.9	32.5	733	226	76	770
IEO	206	4	541	133.9	32.3	732	211	68	762
AZTI	201	>5	535	136.5	32.7	740	225	67	759
IMARES	207	3	536	141.9	32.5	733	226	76	770
MI	209	3	537	160.8	41.2	795	194	61	801
Average	206	3	537	143	34	746	216	70	772
St.dev	3	1	3	11	4	27	14	6	17
Max	209	4	541	161	41	795	226	76	801
Min	201	3	535	134	32	732	194	61	759
Picture 4									
IMR	285	0	365	106.0	21.2	611	189	100	539
IEO	273	2	373	120.8	25.3	701	188	91	572
AZTI	293	1	368	121.5	24.1	764	191	98	567
IMARES	285	0	365	106.0	21.2	611	189	100	539
MI	287	0	386	130.5	26.8	756	197	95	601
Average	285	1	372	117	24	689	191	97	564
St.dev	7	1	9	11	2	75	4	4	26
Max	293	2	386	130	27	764	197	100	601
Min	273	0	365	106	21	611	188	91	539
Picture 5									
IMR	207	0	449	133.1	28.7	777	220	86	668
IEO	201	0	437	131.9	32.2	751	183	67	654
AZTI	198	1	463	128.4	31.0	756	252	66	674
IMARES	207	0	449	133.1	28.7	777	220	86	668
MI	204	0	448	151.5	32.9	800	206	85	697
Average	203	0	449	136	31	772	216	78	672
St.dev	4	0	10	9	2	19	25	11	16
Max	207	1	463	152	33	800	252	86	697
Min	198	0	437	128	29	751	183	66	654

Table 5.3.1.1.3. Results from four Institutes scoring images for alpha atresia in three follicle classes, from six sections (between five and seven images per section) each stained with either Haematoxylin and Eosin (H&E) PAS Mallory (PM) and Toluidine blue (TB). In each case the data is presented for total of all three classes combined (all) and each class separately yolk vesicle (YV) yolk vesicle / yolk granule (YV-YG) and yolk granule (YG) respectively are presented as the mean count with the standard deviation in brackets.

	Institute				Overall
Class	AZTI	CEFAS	IMARES	IMR	mean
H&E all	3.56 (3.23)	3.92 (2.11)	2.46 (1.80)	3.68 (3.14)	3.40 (2.70)
H&E YV	1.27 (2.33)	0.31 (1.34)	0.05 (0.32)	0.24 (0.76)	0.48 (1.48)
H&E YV-YG	1.51 (1.95)	2.17 (2.46)	0.85 (1.77)	2.61 (3.52)	1.78 (2.59)
H&E YG	0.78 (1.29)	1.44 (1.87)	1.56 (1.42)	0.83 (1.30)	1.14 (1.50)
PM all	3.64 (2.09)	4.17 (2.27)	2.17 (1.40)	4.14 (2.26)	3.53 (2.18)
PM YV	1.53 (2.85)	0.56 (1.55)	0.00	1.44 (2.64)	0.88 (2.16)
PM YV-YG	0.81 (1.34)	2.19 (2.95)	0.42 (0.73)	1.33 (1.67)	1.19 (1.96)
PM YG	1.31 (1.45)	1.42 (1.72)	1.75 (1.40)	1.36 (1.89)	1.46 (1.62)
TB all	3.17 (2.74)	3.44 (2.88)	1.72 (1.59)	3.83 (4.18)	3.04 (3.06)
TB YV	0.64 (1.19)	0.36 (0.79)	0.03 (0.16)	1.86 (3.93)	0.72 (2.18)
TB YV-YG	1.58 (2.40)	2.00 (3.19)	0.64 (1.17)	1.17 (1.29)	1.35 (2.21)
TB YG	0.94 (1.32)	1.08 (1.46)	1.06 (1.52)	0.81 (1.45)	0.97 (1.43)

Table 5.3.1.1.4. P values from Paired T tests comparing counts (n=36-41) for each stain type made by each Institute.

	Toluidine			H&E			PAS		
Institute	AZTI	CEFAS	IMARES	AZTI	CEFAS	IMARES	AZTI	CEFAS	IMARES
AZTI		0.00274			0.41002			0.00000	
IMARES	0.00274	0.00071		0.13336	0.00446		0.00000	0.00000	
IMR	0.07643	0.38148	0.00368	0.67465	0.94796	0.07988	0.00045	0.89301	0.00000

Table 5.3.1.2.1 : A comparison of fecundity data prepared by AZTI, FRS, IEO, IMR, and MI showing the mean values, number of selected samples, and summary statistics including a comparison of means and 95% confidence limits.

Institute	Mean	N	SD	SE	Min	Max	P values	95 % CI
AZTI	1095	24	177	36.1	819	1496	0.8174	1021 - 1170
FRS	1127	23	396	82.6	403	1997	0.7859	956 - 1298
IEO	1355	30	260	47.5	757	2003	0.0000	1258 - 1452
IMR	1119	57	252	33.4	301	1601	0.6474	1052 - 1186
MI	870	42	169	26.1	492	1230	0.0000	817 - 922
Total	1098	176	294	22.2	301	2003		1054 - 1141

Table 5.3.1.3.1. A comparison of atresia data (geometric mean intensity and prevalence) produced by AZTI, Cefas (contracted to FRS), IEO, IMR, and MI.

	Geom. Mean intensity	95% CI		Mean prevalence	Mean atretic loss	Number of samples
AZTI	71.7	46.7	110.7	0.68	389	28
CEFAS	21.4	12.3	37.4	0.33	57	94
IEO	93.6	40.3	217.7	0.35	262	20
IMR	35.9	24.2	53.2	0.31	90	153
MI	18.4	12.4	27.4	0.42	62	123

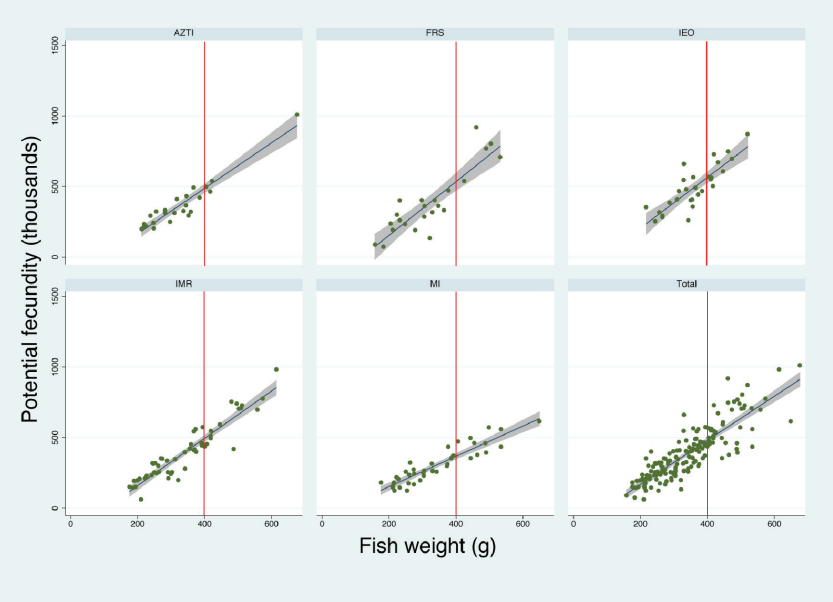


Figure 5.3.1.2.1. Potential fecundity of Mackerel by weight and laboratory. Samples were collected during the 2007 Triennial Mackerel survey period 1 and 2.

5.4 Potential fecundity in Northeast Atlantic Mackerel (western and southern combined spawning component)

Samples to determine mackerel fecundity were collected from trawl hauls made between 43 to 58 degrees North from different ships (Table 5.4.1.1). These samples were distributed between England, Norway Scotland, Ireland, and Spain and analysed according to methods described in the ICES 2007 fecundity manual. Spawning fish were excluded from the estimate of relative potential fecundity based on the presence of hydrated oocyte or postovulatory follicles in the dispersed ovary samples.

Plots of annual potential fecundity against fish length (Figure 5.4.1.1) and weight (Figure 5.4.1.2) showed a strong positive trend that was rather similar to those that was found in 2004. As also seen previous years relative fecundity against length or weight (Figure 5.4.1.3 and 5.4.1.4) only showed weak positive trends.

In 2001 and 2004 the overall estimate of relative fecundity seemed to be slightly influenced by latitude and this pattern was also repeated in 2007 also showing a small latitudinal effect. For period 1-2 slightly larger values were found in the north compared to the south (Table 5.4.1.2).

As in previous years relative fecundity was slightly higher in the early periods compared to the later periods (Table 5.3.1.3). This may be because some of the ovaries taken late in the season, and scored as pre-spawning, actually were spawning.

From the oocyte size distributions that we could estimate what is commonly called the leading cohort. In our work leading cohort was defined as the mean of the largest 10 % in the oocyte size distribution. Leading cohort may be interpreted as a proxy for stage of maturity. When plotting (Figure 5.4.1.5) relative fecundity for all periods against leading cohort we got a domed shape curve. The initial rise in relative fecundity showed that ovaries in early maturation are still recruiting new oocytes from the previtellogenic pool. This recruitment did not seem to stop before the leading cohort had reached a size of about 600 μm. The observed decrease that seemed to occur when the leading cohort was larger than 800 μm probably was caused by atresia, - or that some of these fish had started spawning. By studying oocyte frequency histograms (Figure 5.4.1.6) of pre spawning and spawning fish we could see that fish with a leading cohort of about 800 μm was likely to have started spawning.

We calculated the overall relative fecundity using two different sets of criteria, the standard (as also used in 2004) and the alternative. Using the standard criteria all samples from period 1 and 2 were used if the leading cohort was larger than 400 μm and there was no sign of spawning (presence of POF´s or hydrated oocytes). The alternative criterion was similar to the standard criteria except that the leading cohort should be in the range of 600-800 μm.

Using the alternative set of criteria (period 1 and 2) the relative fecundity estimates was increased compared to the standard criteria (Table 5.4.1.4), but the difference was small (1132 versus 1098). However, when using the alternative leading cohort criteria for periods 3-6 the differences were larger, and always the estimates using the alternative criteria was the largest (Table 5.4.1.4).

Earlier triennial estimates of fecundity (2001 and 2004) used the standard criteria. Even thought the alternative criterion possible may give a more correct and consistent result the standard criteria was used for the fecundity estimate also this year. The main reason for this was that the fecundity estimates from the triennial surveys adds to a time series. It is then extremely important to be consistent both when sampling, analysing samples, and calculating. However, using the alternative criteria for period 1 and 2 for this year would only change the final results by about 3 %.

The overall relative fecundity in 2007 (Table 5.3.1.4) was then estimated to be 1098 (SE = 22), which was very similar to what was reported for 2004 (1127 ± SE 27) and 2001 (1097 ± SE 23) and 2001.

Table 5.4.1.1. Number of fecundity and atresia samples collected by each vessel.

Ship	No. Samples
Celtic Explorer	150
Commercial samples.	60
Cornide de Saavedra	66
Costa Brava	20
Costera	20
El Bosco	40
Emma Bardan	90
Johan Hjort	70
Noruega	7
Thalassa	40
Tridens	120
Unity	190
Walther Herwig	169
Total	1042

Table 5.4.1.2. Mean realized fecundity shown by latitude for all periods combined (A) and restricted to periods 1-2 (B).

A Latitude	Mean	N	SD	SE
40-45	1029	87	280	30.0
45-50	924	58	416	54.7
50-55	948	87	377	40.4
55-60	1077	64	379	47.3
Total	995	298	362	21.0

B Latitude	Mean	N	SD	SE
40-45	1051	83	256	28.1
45-50	1140	24	351	71.6
50-55	1082	32	362	64.0
55-60	1203	35	249	42.0
Total	1098	176	294	22.1

Table 5.4.1.3. Mean realized fecundity shown for each period and for all periods in total.

Period	Mean	N	SD	SE
1	1067	71	231	27.4
2	1119	105	329	32.1
3	819	48	372	53.7
4	840	29	363	67.5
5	869	36	459	76.6
6	925	9	446	148.7
Total	995	298	362	21.0

Table 5.4.1.4. Summary of relative fecundity and atresia from the 2007 surveys. The table shows calculations done for each period both for leading cohort (LC) >= 400 µm and for LC between 600 and 800 µm. For the final fecundity estimate only data from period 1 and 2 with LC >= 400 µm was used.

	Relative potential fecundity						Relative Atresia				
	LC >= 400			LC 600 - 800							
Period	Average	St. dev	Count	Average	St. dev	Count	Geom. Mean	95% Conf	Count	Prevalence	Mean atretic loss
1	1066	230	71	1080	229	45	13.8	-	10	0.10	11.0
2	1119	323	105	1179	285	49	43.6	26.5	83	0.42	147.2
3	819	373	48	957	413	19	37.3	28.1	129	0.57	168.7
4	840	363	29	950	327	19	27.0	10.5	67	0.18	38.7
5	869	459	36	936	422	19	22.2	12.6	93	0.26	45.9
6	925	446	9	1156	393	5	4.1	1.7	34	0.32	10.6
1-2	**1098**	**294**	**176**	**1132**	**263**	**94**					
1-6	**995**	**362**	**298**	**1065**	**326**	**156**	**29.6**	**23.6**	**416**	**0.38**	**88.8**

	Realised fec.	Realised fec.
	LC >= 400	LC 600 - 800
1-2	**1009**	**1043**
1-6	**906**	**976**

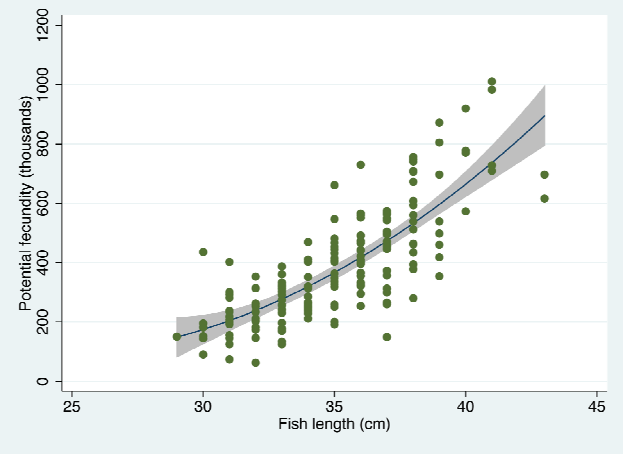


Figure 5.4.1.1. Potential fecundity (Fp) of Mackerel versus length (L) for the 2007 Triennial survey period 1 and 2. Regression line: Fp = 1249 – 99.4 • L + 2.12 L² (R^2 = 0.624).

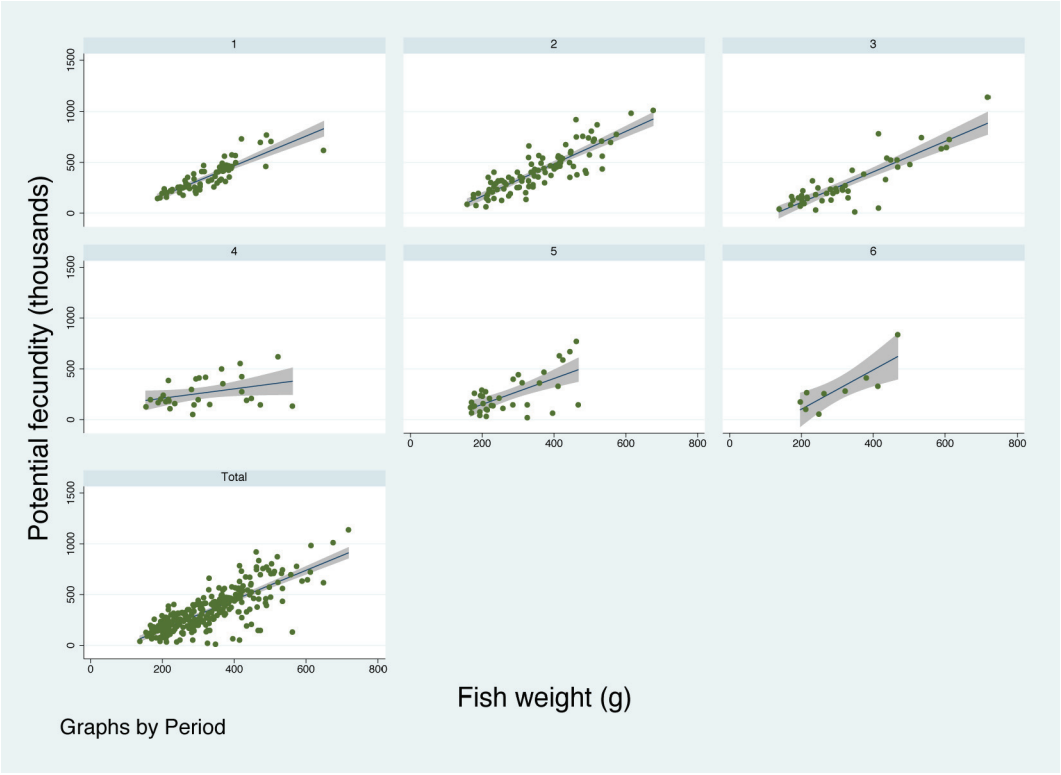


Figure 5.4.1.2. Potential fecundity (Fp) of Mackerel versus weight (Wf) and period for the 2007 Triennial survey. Regression line for total material: Fp = -134 + 1.45 • Wf (R^2 = 0.637).

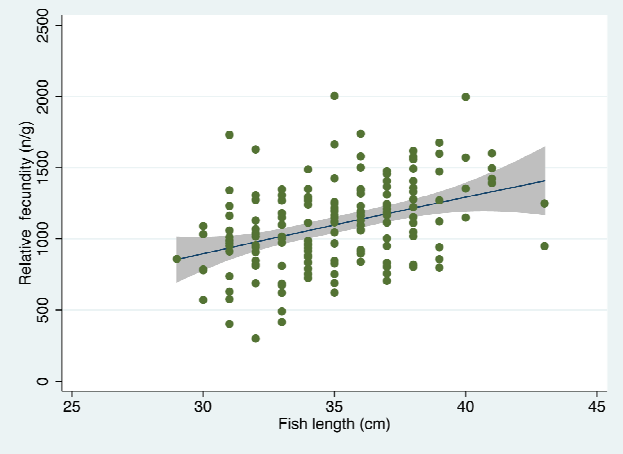


Figure 5.4.1.3. Relative potential fecundity (RFp) of Mackerel versus length (L) for the 2007 Triennial survey period 1 and 2. Regression line: RFp = -446 + 48.6 • L – 0.127 L² (R^2 = 0.151).

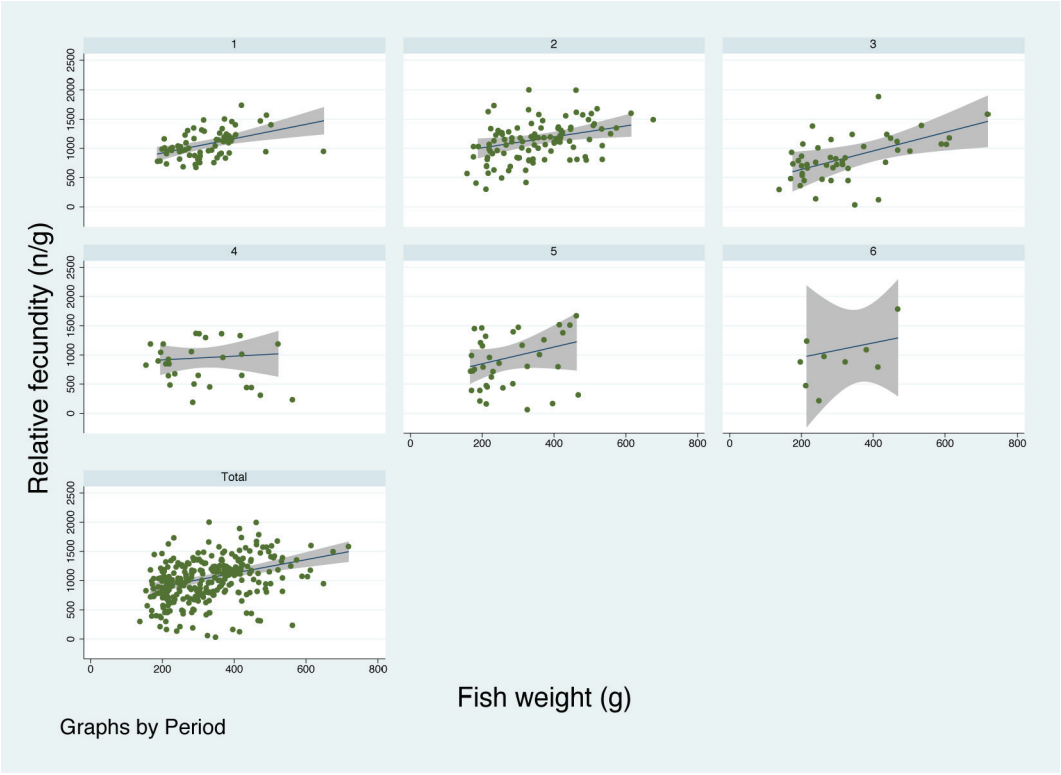


Figure 5.4.1.4. Relative potential fecundity (RFp) of Mackerel versus weight (Wf) and period for the 2007 Triennial survey. Regression line for total material: RFp = 596 + 1.23 • Wf (R^2 = 0.140).

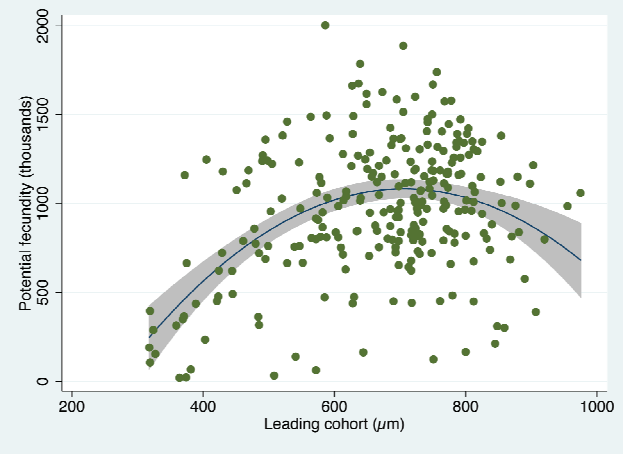


Figure 5.4.1.5. Relative potential fecundity of Mackerel versus oocyte leading cohort for the 2007 Triennial survey. Leading cohort was defined as the mean of the upper 10 % of the maturing oocyte size distribution.

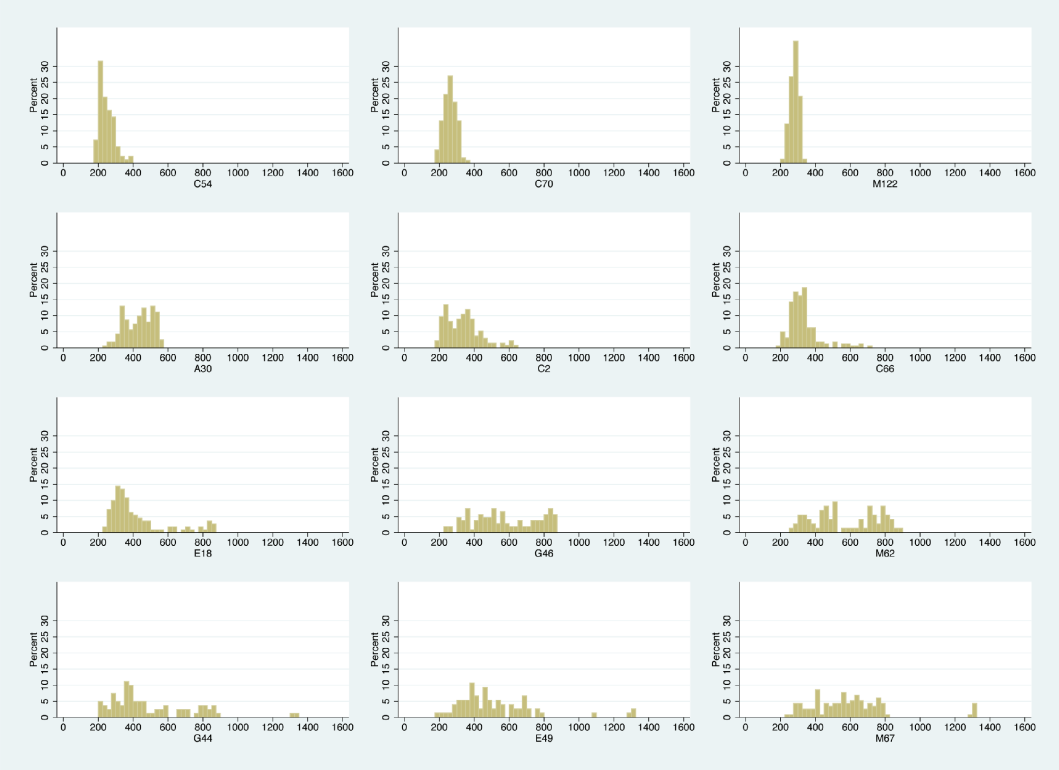


Figure 5.4.1.6. Selected oocyte size distributions of Mackerel from the 2007 Triennial survey. First row shows early maturing fish, second row shows fish in advanced vitellogenesis, third row shows fish ready to start spawning, and fourth row shows fish that has started spawning.

5.5 Atresia and realised fecundity in Northeast Atlantic Mackerel (western and southern combined spawning component)

The samples used for analysis of atresia were collected from the entire survey area and during all periods. The samples were processed into histological sections and analysed by AZTI, IEO, MI, CEFAS and IMR (Table 5.5.1). The sections were used to determine the prevalence (proportion of fish with early alpha atresia) and relative intensity of atresia (number of atretic eggs per g female). These numbers were used to determine the amount of potential fecundity that did not contribute to the annual egg

production of the stock. The loss of potential fecundity through atresia was calculated from the following equation (Horwood 1990):

Ar = Ag x P x D x S

Where Ar = loss of potential fecundity through atresia
Ag = geometric mean of relative atresia.
P = prevalence of atresia
D = duration of alpha atresia (7.5 days)
S = duration of mackerel spawning (60 days)

Samples where atresia was detected were evenly distrubuted both time and space (Figure 5.5.1). The atretic loss (Table 5.4.1.4) was small in period 1 (11 n/g), but was considerable higher in period 2 and 3 (147 and 169 n/g respectively) before it decreased towards smaller values in the later periods. However, the overall geometric mean of relative atresia, which was used to adjust the potential fecundity to realised fecundity (Table 5.3.1.4), was 30 atretic follicles g-1 female and prevalence 38 (%). This resulted in an overall relative loss by atresia (Table 5.4.1.4) of 89 (g-1 female). This reduced the potential fecundity by 9 % so that the realised fecundity (Table 5.4.1.4) was 1009 (g-1 female). Comparing the estimated realised fecundity for 2007 with historical estimates back to 1998 indicated only small differences (Table 5.4.2).

Table 5.5.1. Mackerel atresia results by lab for the 2007 Triennial survey.

	Geom. Mean intensity	95% CI		Mean prevalence	Mean atretic loss	Number of samples
AZTI	71.72	46.7	110.7	0.68	389	28
CEFAS	21.44	12.3	37.4	0.33	57	94
IEO	93.62	40.3	217.7	0.35	262	20
IMR	35.86	24.2	53.2	0.31	90	153
MI	18.41	12.4	27.4	0.42	62	123

Table 5.5.2. Results of fecundity analysis in the assessment years 1998, 2001, 2004 and 2007.

	Assessment year			
Parameter	1998	2001	2004	2007
Number of samples analyzed for fecundity	96	187	205	176
Number of samples analyzed for atresia	112	290	348	416
Potential fecundity	1206	1097	1127	1098
Prevalence of atresia	0.55	0.20	0.28	0.38
Geometric mean Relative intensity of atresia	46	40	33	30
Number of potential fecundity lost per day	3.37	1.07	1.25	1.48
Number of potential fecundity lost over an individual's spawning season	202	64	75	89
Realised fecundity	1002	1033	1052	1009
Percentage of potential fecundity lost	17	6	7	9

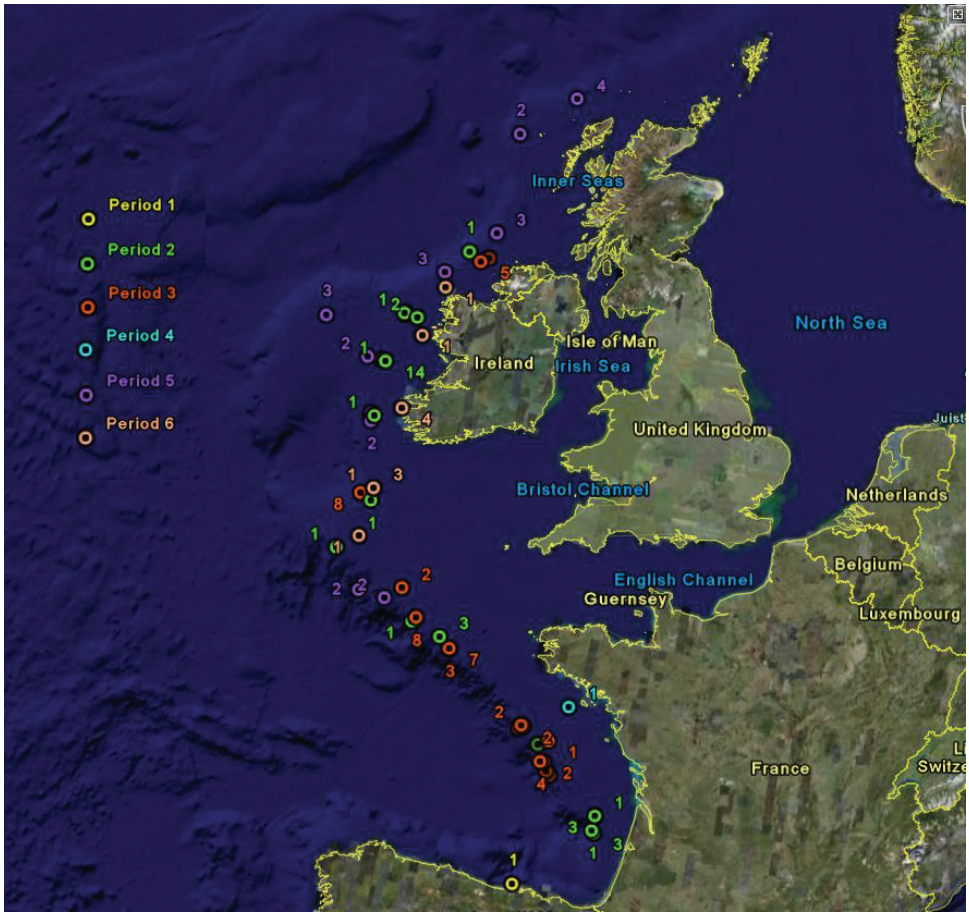


Figure 5.5.1. Number of samples with atresia by period and position.

5.6 Mackerel Biomass estimate

Total stage I egg production is given in Tables 5.2.1.1 and 5.2.2.2. Total spawning stock biomass (SSB)

was estimated using the fecundity estimate of 1,009 oocytes/g female, corrected for atresia (see Sections 5.4 and 5.5), a sex ratio of 1:1 and a raising factor of 1.08 (ICES,

1987) to convert pre-spawning to spawning fish. This gave an estimate of spawning stock biomass of:

i) 2,590 million tonnes for western component, with a variance of approximately 787, 510 tonnes. The variance in the estimate due to the egg survey was 9% and 91% to the fecundity estimate.

ii) 667, 909 tonnes for southern component, with a variance of approximately 414,852 tonnes. The variance in the estimate due to the egg survey was 78% and 22% to the fecundity estimate.

iii) 3,254 million tonnes for western and southern components combined, with a variance of approximately 1,042 million tonnes. The variance in the estimate due to the egg survey was 17% and 83% to the fecundity estimate.

Comparative data from earlier years are shown in Table 5.6.1 for western and southern areas separately and for combined areas in Table 5.6.2. The increase in biomass was a 5% and a 137 % for western and southern components respectively compared to year 2004. For the total area (western and southern component combined) the increase was a 18% compared to the previous egg survey estimate in 2004. This increase in the estimate of biomass has resulted mainly from a rise in the egg production to that found in 2004 (1.33 x 1015 and 1.52 x 10 15 in 2004 and 2007 respectively).

Table 5.6.1. Spawning stock biomass for western ands southern spawning areas of mackerel separately. Spawning stock biomass estimates are corrected for atresia. A sex ratio of 1:1 is assumed. The SSB was calculated from the total egg production based on arithmetic mean of unsampled rectangles if available.

ANNUAL EGG PRODUCTION METHOD – MACKEREL (WESTERN AREAS)					
Total egg prod ($x10^{-15}$) (mean for unsampled rectangles)		Total fecun-dity (eggs/g female)	Total fecundity corrected for atresia	Pre-spawning stock biomass ($x10^{-6}$ tonnes)	Spawning stock biomass ($x10^{-6}$ tonnes)
Geometric	Arithmetic	(atresia oo-cytes/gm female)	(eggs/g female)		(conv f 1.08)
Annual egg production method – mackerel (western areas)					
1.98		1526 [211]	1315	3.01	3.25
1.48 a		1526 [211]	1315	2.25	2.43
1.84 b		1526 [211]	1315	2.8	3.02
1.5	1.53	1526 [211]	1315	2.33	2.51
1.15	1.24	1457 [211]	1246	1.99	2.15
1.45	1.52	1608 [326]	1282	2.37	2.56
1.83	1.94	1569 [138]	1431	2.71	2.93
-	1.49	1473 [171]	1302	2.28	2.47
-	1.37	1206 [203]	1003	2.73	2.95
-	1.21	1097 [64]	1033	2.34	2.53
-	1.2	1127 [75]	1052	2.28	2.47
-	1.21	1098 [89]	1009	2.4	2.59

a. Egg survey data for period 3 included. b. Egg survey for period 3 excluded.

Table 5.6.1 continued:

ANNUAL EGG PRODUCTION METHOD – MACKEREL (SOUTHERN AREAS)						
Year	Total egg prod ($x10^{-15}$) (mean for unsampled rectangles)		Total fecun-dity (eggs/g female)	Total fecundity corrected for atresia	Pre-spawning stock biomass ($x10^{-6}$ tonnes)	Spawning stock biomass ($x10^{-6}$ tonnes)
	Geometric	Arithmetic	[atresia oo-cytes/gm Female]	(eggs/g female)		(conv f 1.08)
Annual egg production method – mackerel (southern areas)						
1995	-	0.207	1344[161]	1183	0.350	0.378
1998	-	0.461	1276[105]	1171	0.741	0.800
2001	-	0.283	1647[78]	1569	0.344	0.371
2004	-	0.126	1016[52]	964	0.259	0.280
2007	-	0.310	1098 [89]	1009	0.614	0.664

Table 5.6.2: Spawning stock biomass of mackerel for western and southern spawning areas combined . Spawning stock biomass estimates are corrected for atresia. A sex ratio of 1:1 is assumed. The SSB was calculated from the total egg production based on arithmetic mean of unsampled rectangles if available.

ANNUAL EGG PRODUCTION METHOD – MACKEREL (WESTERN AND SOUTHERN AREAS COMBINED)					
Year	Total egg prod ($x10^{-15}$) (mean for unsampled rectangles)	Total fecun-dity (eggs/g female)	Total fecundity corrected for atresia	Pre-spawning stock biomass ($x10^{-6}$tonnes)	Spawning stock biomass ($x10^{-6}$tonnes)
	Arithmetic	(atresia oocytes gm /female)	(eggs/g female)		(conv f 1.08)
Annual egg production method – mackerel (western and southern areas combined)					
1998	1.81	1206 -1276 *	1003-1171*	3.517	3.8
2001	1.49	1097- 1647 *	1033-1569*	2.684	2.9
2004	1.33	1127-1016*	1052-964*	2.545	2.75
2007	1.52	1098 [95]**	1009**	2.99	3.25
*** Data for western and southern area separated**					
**** Data for western and southern area combined**					

6 Western horse mackerel: 2007 survey results

6.1 Spatial distribution of stage I horse mackerel eggs

The western egg survey results include the Cantabrian Sea and the previous western area.

- Period 2 – This period marked the start of surveying in the western area. Coverage was very good for almost the entire area west of Scotland and the North of Spain. Only 6 rectangles were interpolated. Edges of

spawning were generally well defined with zero values throughout almost the entire area. Two major spawning areas were identified: One in the Cantabrian Sea between 3° and 6° W and another above the shelf break west of Brittany between 47° and 48° N.

- Period 3 – Again coverage of the entire survey area was very good with only 11 half rectangles having to be interpolated. The distribution of egg production was more evenly spread over the European shelf edge from the southern boundary of the survey area up to 53°30′ N than during period 2. Two major spawning areas could be discerned of which one was close to the Cantabrian coast between 2° and 5° W. The second was again west of Brittany above the shelf edge but had moved slightly further North following the 200 depth contour. The edges of spawning were generally well defined by zero observations.
- Period 4 – Sampling during this period excluded the Cantabrian Sea and had its southern boundary at 44° N. Coverage of the area was again very good, but 21 half-rectangles had to be interpolated. The overall contribution of those rectangles to total egg production of that period was negligible. The western and northern edges were well defined within the survey area, however, a southern boundary of horse mackerel spawning could not be established which raised the possibility that further egg production might have been missed in the Cantabrian Sea. Two smaller areas of high egg production were observed above the shelf break in the Celtic Sea at Great Sole Bank and southwest of the Kerry Peninsula in the vicinity of Porcupine Seabight. Another conspicuous feature was an area of increased spawning activity far up on the Celtic shelf that had not been observed on any of the previous surveys.
- Period 5 – Sampling was confined to an area north of 47° N, therefore a southern boundary of horse mackerel spawning could not be established. Given the observed pattern of horse mackerel egg distribution during this period, a significant impact of the fact is unlikely. Overall, total horse mackerel egg production peaked in this period. Highest values of up to 2200 eggs/m²/day were observed west of Ireland's Kerry Peninsula. Other areas of high spawning activity could be observed south of Sole Bank, east and west of Porcupine Bank and once again on the Celtic Shelf.
- Period 6 – Due to the reduced sampling effort in this period, only about a third of the total potential horse mackerel spawning area could be investigated. Sampling was limited to an area between 47° and 55° N with the result that in particular the northern boundary of horse mackerel spawning could not be established. The 6th period contains also the highest amount (57) of half-rectangles where egg production had to be interpolated from neighbouring production values. These circumstances could provide a potential source of error in the calculation of total horse mackerel egg production for this particular period and this perception was reinforced because some adults were still maturing egg batches and therefore contributing to fecundity. However with respect to the total horse mackerel seasonal egg production the decline shown in the last period suggests the overall under estimate of total egg production may be small. Again, major spawning activity was centred above the Celtic shelf break and west of the Kerry peninsula.

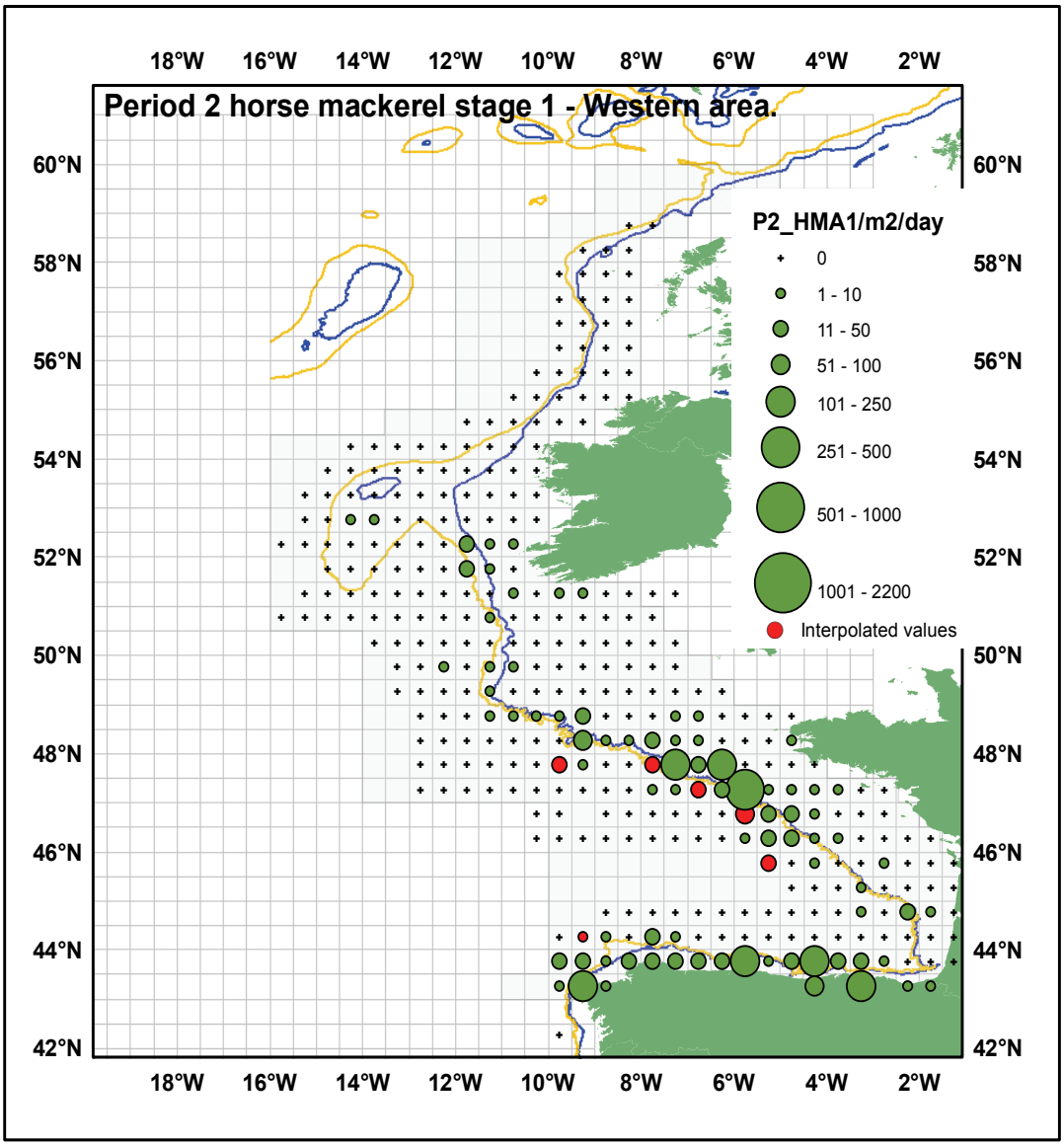


Figure 6.1.1a: Horse mackerel egg production by half rectangle for period 2 (7 March – 8 April). Filled green circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

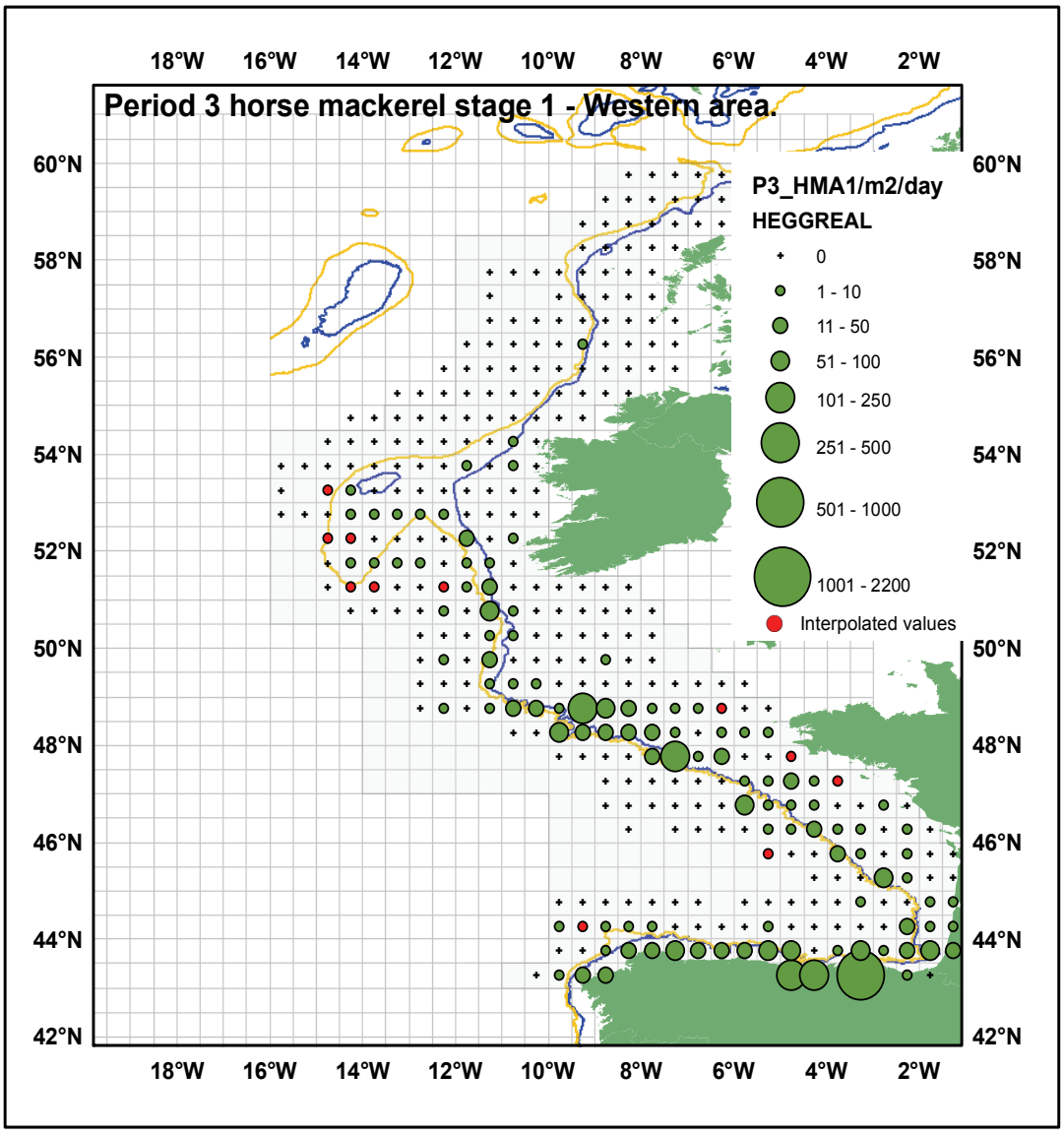


Figure 6.1.1b: Horse mackerel egg production by half rectangle for period 3 (9 April – 6 May). Filled green circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

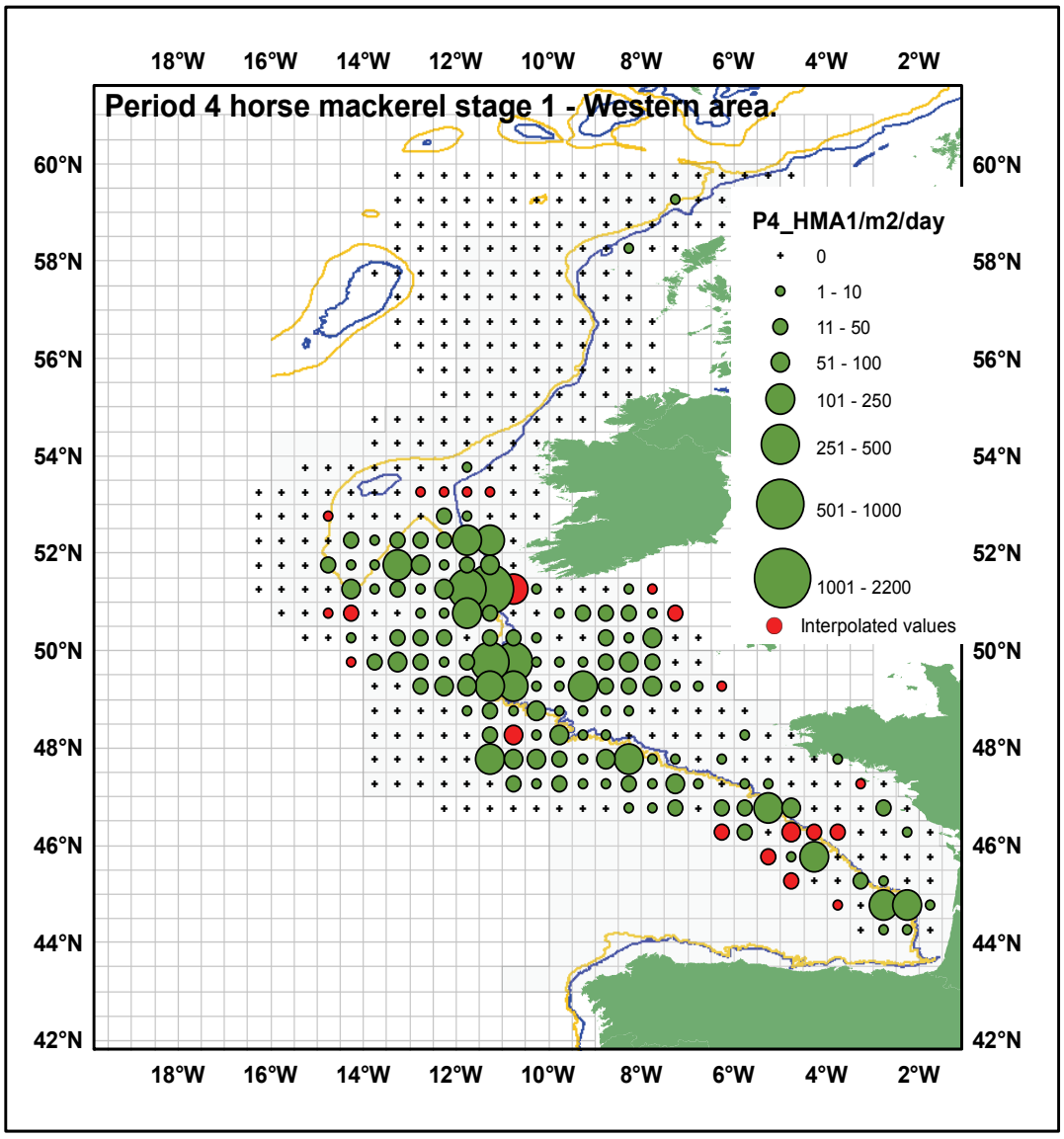


Figure 6.1.1c: Horse mackerel egg production by half rectangle for period 4 (7 May – 3 June). Filled green circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

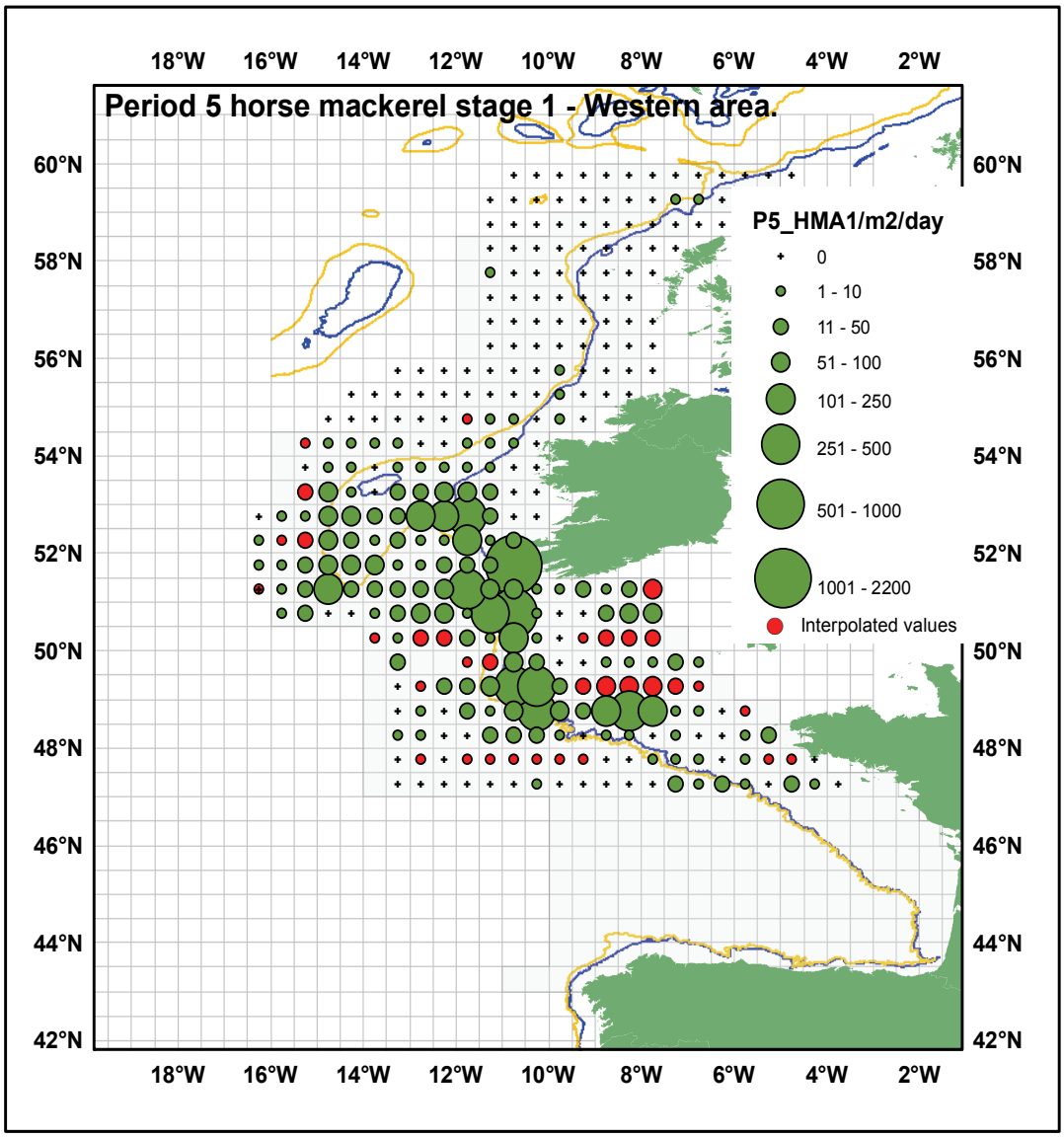


Figure 6.1.1d: Horse mackerel egg production by half rectangle for period 5 (4 June – 24 June). Filled green circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

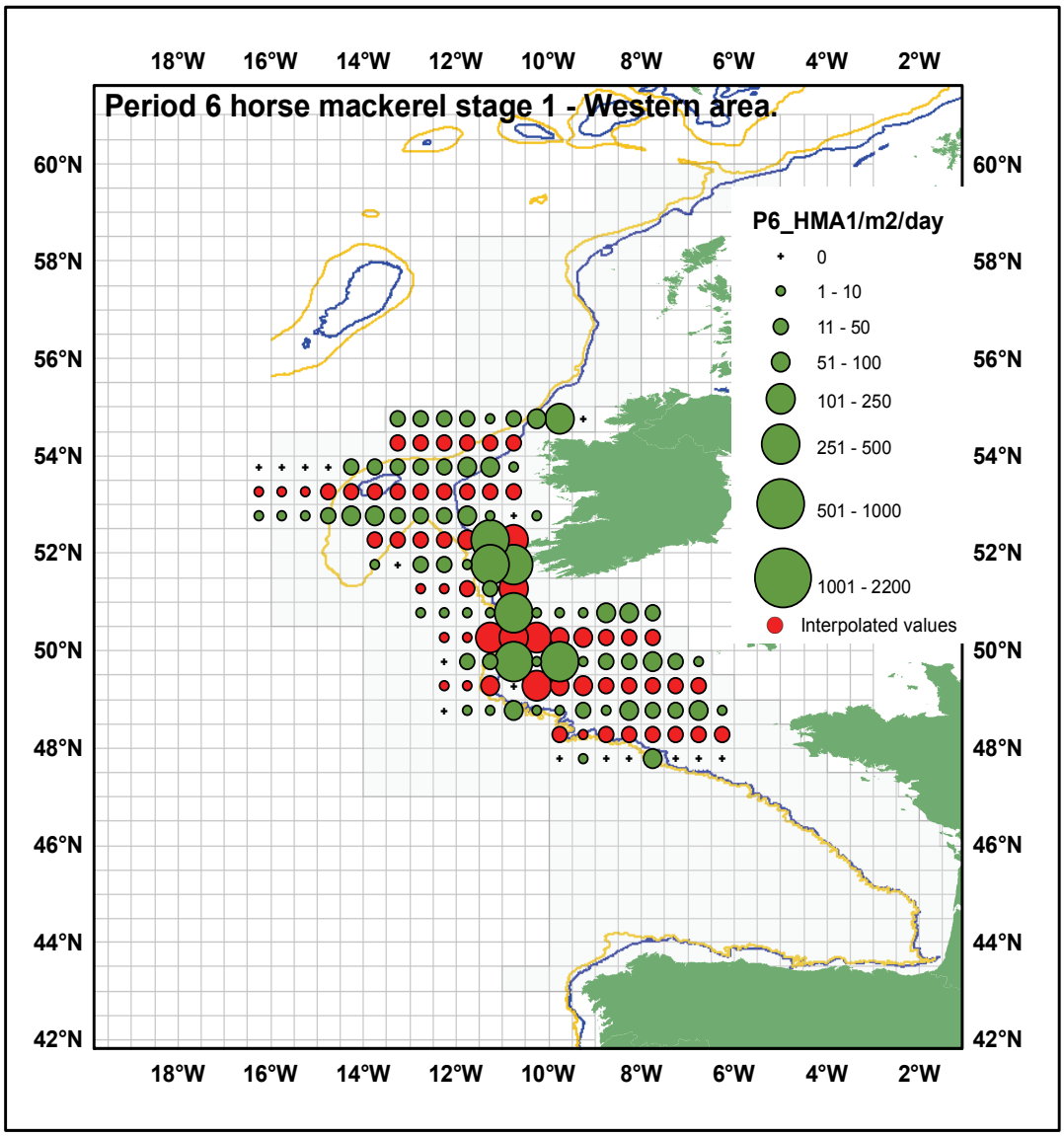


Figure 6.1.1e: Horse mackerel egg production by half rectangle for period 6 (25 June – 31 July). Filled green circles represent observed values, filled red circles represent interpolated values, black crosses represent observed zeroes, red crosses interpolated zeroes.

6.2 Stage I egg production of western horse mackerel

Figure 6.2.1 shows the mean daily stage I egg production estimates (DEP) for each survey period plotted against the mid-period days. For comparison the results of 1998, 2001 and to 2004 are included in the figure. The actual values are presented in Table 6.2.1. Since 1995 the start date is assumed to be the 10th of February and the end date to be the 31st of July.

Compared to previous years the daily egg production per mid period day is higher almost for all periods (Figure 6.2.1). The overall pattern of the curve for 2007 is quite similar to that in 2004 albeit period 2 DEP is disproportionately larger. Integration of the area under the daily egg production curve revealed an estimate of total annual egg production of 1.43 * 1015 with a standard error of 0.269* 1014 for the western area in 2007 (Table. 6.2.2). This is more than double the estimate from 2004 (0.684 * 1015, SE = 0.15* 1015).

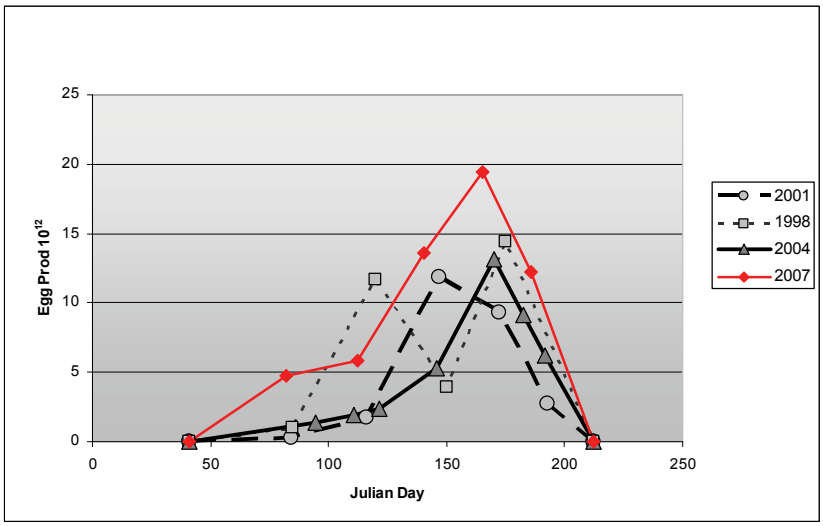


Figure 6.2.1: Daily egg production per mid period day for western horse mackerel 2007 (red diamonds), 2004 (grey triangles), 2001 (grey dots) and 1998 (grey rectangles).

Table 6.2.1: Western horse mackerel mean daily stage1 egg production

Period	Dates	Mean Daily Egg Production
Pre 2	before 7 March	0
2	7 March–8 April	$4.76 * 10^{12}$
3	9 April–6 May	$5.84 * 10^{12}$
4	7 May–3 June	$1.36 * 10^{13}$
5	4 June–24 June	$1.94 * 10^{13}$
6	25 June–31 July	$1.22 * 10^{13}$
Post 6		0

Table 6.2.2: Western estimate of horse mackerel total stage I egg production by period calculated by the integration of the area under the egg production curve for 2007. Days are indicating the duration from one mid period day to the following mid period day.

Dates	Period	Days	Annual stage I egg production x 10^{14}
10 Feb – 6 Mar	Pre 2	**41**	**0.98**
7 Mar – 8 April	2	**30.5**	**1.62**
9 April – 6 May	3	**28**	**2.72**
7 May – 3 June	4	**24.5**	**4.05**
4 June -24 June	5	**21**	**3.32**
25 June – 31 July	6	**26**	**1.59**
Total			**14.27×10^{14}**
SE			**0.269**
CV			**1.88%**

6.3 Fecundity of western horse mackerel

Problems associated with the fecundity of horse mackerel including the debate whether horse mackerel is a determinate or indeterminate spawner have been highlighted in previous planning meetings (ICES, 2003) and sample protocols have been prepared to address these problems.

A total of 726 fish samples were collected during the 2007 western egg surveys from March until July with a good spatial coverage from 43°N to 57°N. Extra 300 fish samples were collected monthly for lipid analysis prior to the egg survey, from October onwards. Sample details included fisheries parameter and are given in the ICES planning meeting (2006a). Quadruplicate ovary samples were taken from each fish and samples were analyzed by Ireland, Netherlands, Norway and Spain (IEO). Samples were analyzed for oocyte frequency and mean oocyte diameter and total standing stock of vitellogenic oocytes and (batch) fecundity derived by the gravimetric method. Threshold oocyte diameter to be included in the counts was 185◎m.

Tubes with formaldehyde fixative were weighted before and directly after the survey. Mean sample weight is 0.0185 ± 0.003 gram. Results from a CEFAS experiment with fresh pipette sample showed sample weight to be 0.0260 ± 0.0002 gram. The low sample weight found in the survey samples is probably due to formaldehyde vapour loss and loss of liquid opening the vials on board the ship. Some institutes used plastic vials with screw caps on and these preformed better. Sample weights in these vials were 0.0260 ± 0.0010 gram. Based on the CEFAS experiment and the screw cap samples pipette sample weight was assumed to be 0.026 gram.

Based on the results for the 2006 maturity workshop (ICES, 2006b) it was decided that for the whole mount analysis the samples would not be stained. Samples of 4 fish were used for calibration between the four institutes, IEO, IMARES, IMR and MI. Fecundity estimates and oocyte diameter measurements varied greatly between institutes as indicated in figure 6.3.1.3 although most of the variance can be attributed to IEO compared to the other three analysts. Part of the variation can be explained by differences in methods used for the estimation of fecundity. IEO samples are spread out wider, whereas the IMR and IMARES samples are denser. Probably smaller

oocytes are picked out more easily in the less dense samples resulting in a lower mean oocyte diameter and higher fecundity estimation (Figure 6.3.1 & 3).

The mean oocyte and leading cohort diameter are similar over the 6 periods although there are differences when comparing across the latitudinal range of the survey (Figure 6.3.4 & 5). Both mean oocyte diameter and leading cohort increased from 43 to 46 degrees north and decreased afterwards up to 57 degrees.

Results of the previous surveys showed (ICES, 2005), total and relative fecundity within the western population is increasing after the onset of spawning up to period 4 whilst in the 2007 survey there was a decrease in periods 5 and 6 (Figure 6.3.6). Over the latitudes there is considerable variation in total: fecundity (Figure 6.3.6). This does not necessarily mean that the fecundity for an individual female increases after the onset of spawning, because some fish within the population might be spawning early and some might be late. However, this may also be an indication of horse mackerel being an indeterminate spawner. In figure 6.3.7 oocyte frequency distributions are shown for horse mackerel in periods 3 to 6. In period 3 there are only a few large oocytes, but in the other periods a batch of hydrated oocytes, can be seen indicating the fish was close to spawning. Fish N37 in period 4 shows one developing batch and no smaller vitellogenic oocytes, indicating the fish were already close to being spent.

Given the variation in fecundity over time and latitude and the probable indeterminacy the WG decided not to use fecundity data in an AEPM biomass estimate for the western area.

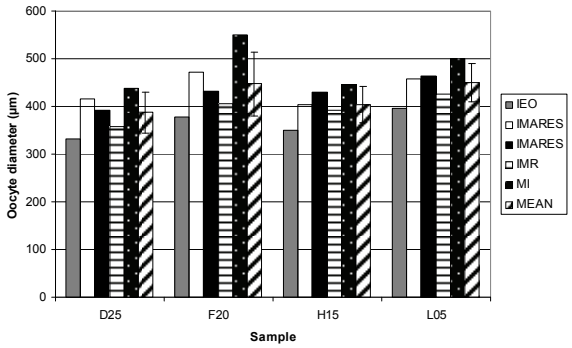


Figure 6.3.1. Variation in horse mackerel average oocyte diameter estimates in the western area during the 2007 egg survey.

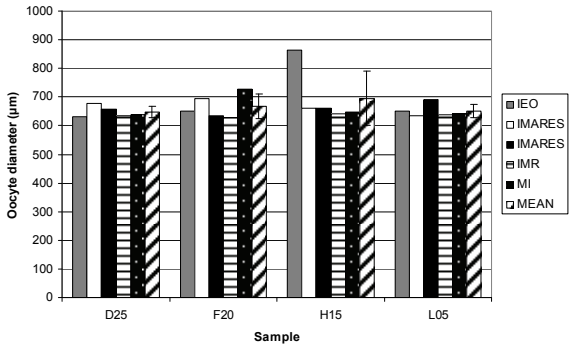


Figure 6.3.2. Variation in horse mackerel maximum oocyte diameter estimates western area during the 2007 egg survey

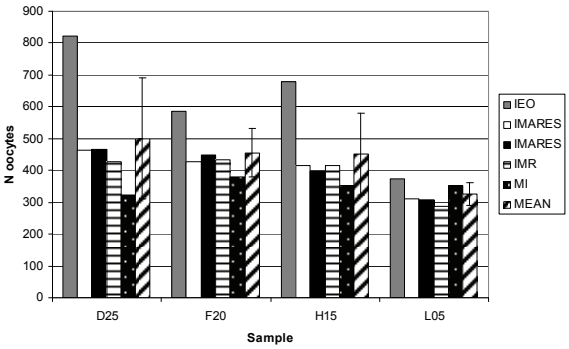


Figure 6.3.3. Variation in horse mackerel total number of vitellogenic oocytes in the western area during the 2007 egg survey

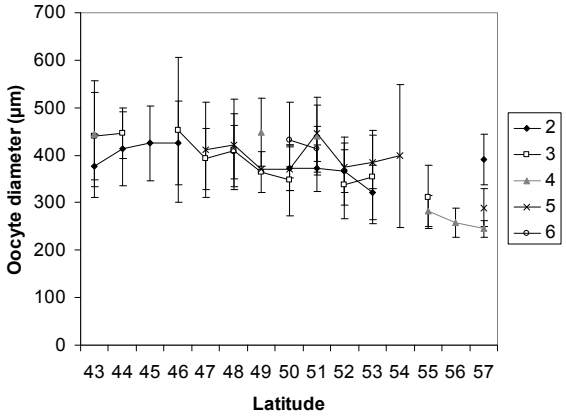


Figure 6.3.4. Horse mackerel mean oocyte diameter over the different periods and latitudes.

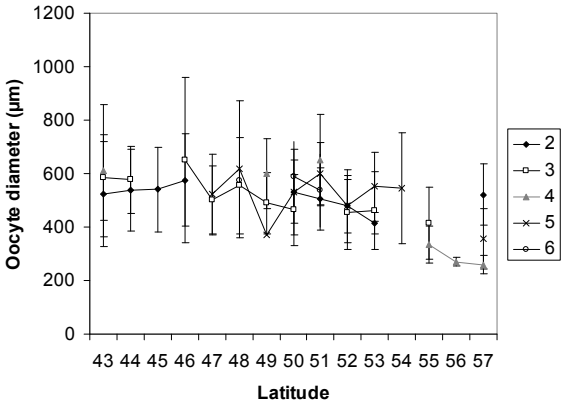


Figure 6.3.5. Horse mackerel leading cohort (10% biggest oocytes) over the different periods and latitudes.

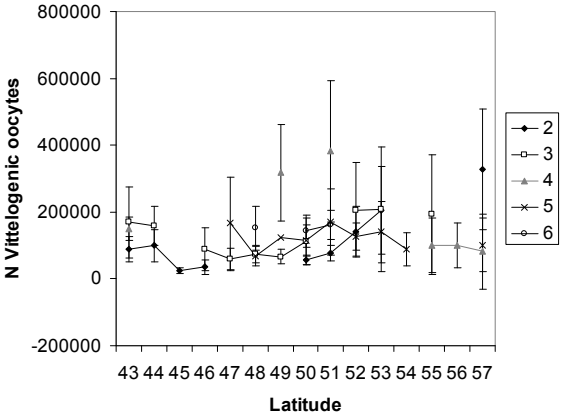


Figure 6.3.6. Horse mackerel fecundity over the different periods and latitudes

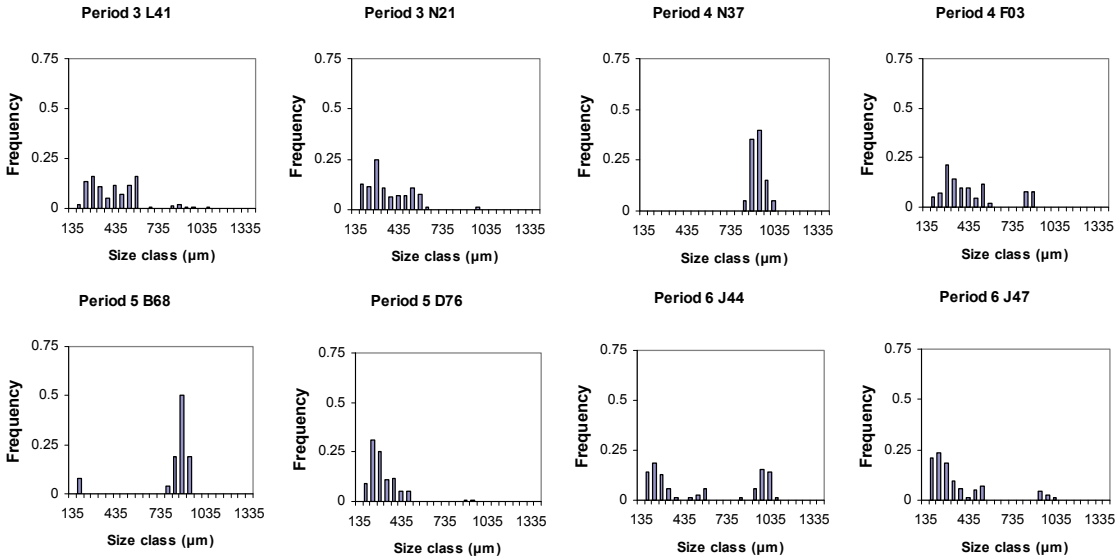


Figure 6.3.7. Horse mackerel oocyte frequency distributions for the different periods. (For each period a fish with large and small mean oocyte diameter is shown.)

6.4 Energy content and fecundity of western horse mackerel

Prior to the egg survey in the western area 300 extra horse mackerel were collected from Dutch pelagic trawlers for lipid analysis, from October to March.

Lipid content is declining from October until the end of the spawning season in July. Over the latitudes there is an increase with the highest lipid contents around 50 to 53 degrees North, followed by a decrease and then increase again at 57 degrees North (Figure 6.4.1). Fulton K shows the same trend over time and period, but the variation is smaller than in lipid content (Figure 6.4.2). Liver weight shows large variations over time and latitude (Figure 6.4.3).

There is no clear relationship between lipid and fecundity (Figure 6.4.4), but there seems to be a relation between Fulton K and liver weight with fecundity (Figure 6.4.5 & 6).

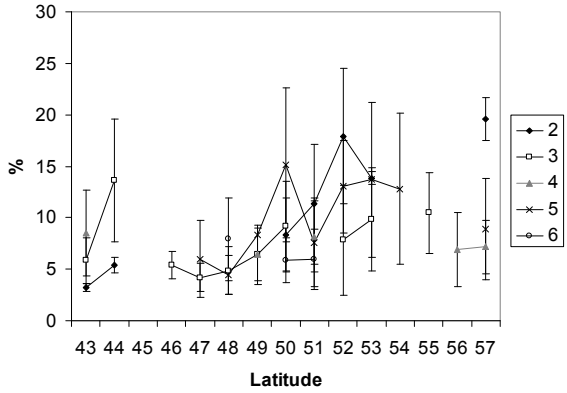


Figure 6.4.1. Horse mackerel lipid content over the different periods and latitudes.

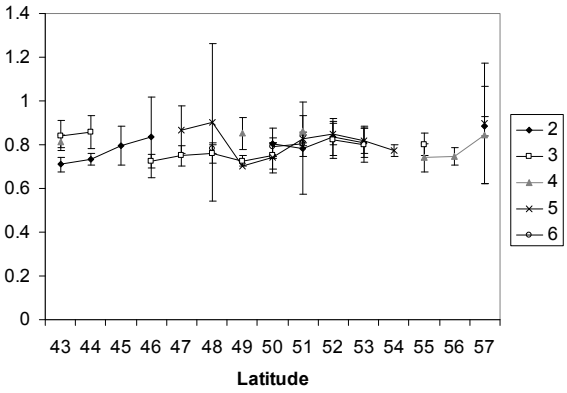


Figure 6.4.2. Horse mackerel Fulton K over the different periods and latitudes.

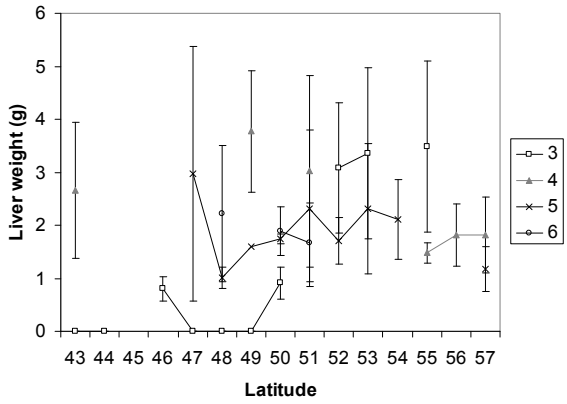


Figure 6.4.3. Horse mackerel liver weight over the different periods and latitudes.

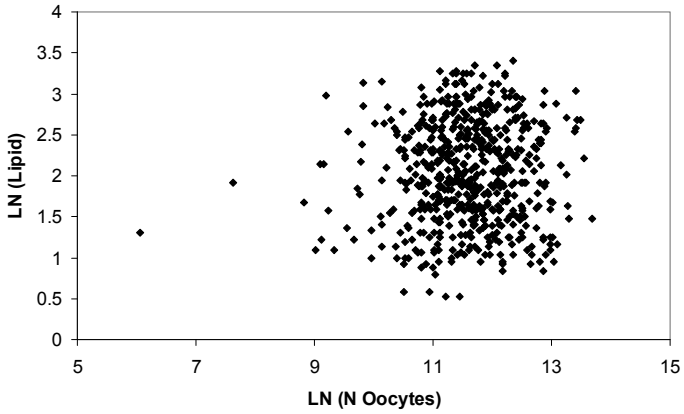


Figure 6.4.4. Horse mackerel lipid content and fecundity in the western area.

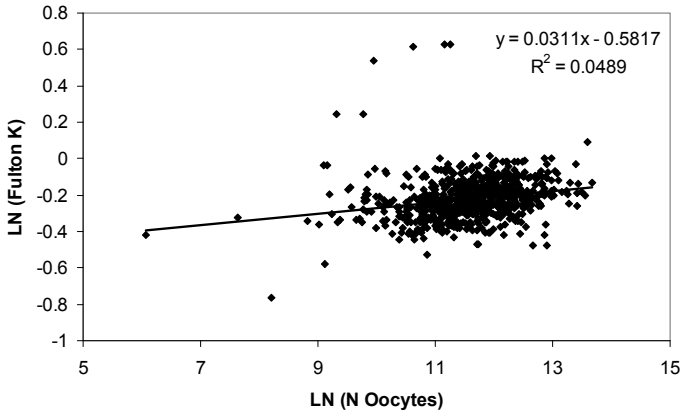


Figure 6.4.5. Horse mackerel Fulton K and fecundity in the western area.

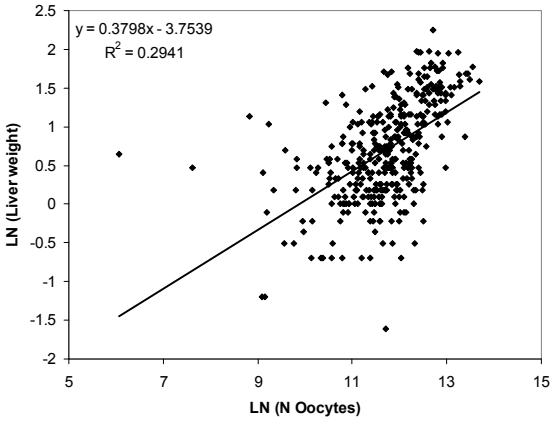


Figure 6.4.6. Horse mackerel Liver weight and fecundity in the western area.

6.5 Developing an index of Horse mackerel SSB based on spawning rates derived from image analysis data

Analysis of Western Horse mackerel fecundity samples collected in periods 1 to 6 confirmed that the duration of this and probably previous egg Triennial surveys did not cover the complete annual production (Figures 6.2.1 and 6.1.1.e). Also in period 6, some stage 4 females still contained a substantial standing stock of fecundity whilst other females only contained hydrated follicles probably equating to their last batch (Figure 6.3.7). Accepting this situation it is therefore necessary to develop an index of SSB based on DEPM taking into account conclusions from a previous comparison of AEPM and DEPM (ICES 1993) carried out following the 1992 Triennial survey. In the present report the horse mackerel DEPM index was based on estimates of spawning fraction and batch fecundity (section 7.4) that had high variance following methodology described in ICES 1993. However it is likely that the variance of daily egg production would be reduced if the spawning fraction parameter forecast egg production for several days rather than for one day using the prevalence of migratory nuclei stage follicles (section 7.4) in ovary samples taken from trawl caught fish. Similarly it is possible to identify batch fecundity formation by image analysis before the ovary appears hydrated (stage 4) by macroscopic observation (Figure 6.5.1) so that a greater number of observations will be available for the same trawling effort (section 4.3.1). Further analysis of the 2007 horse mackerel fecundity samples is required in order to determine the criteria for using image analysis follicle frequency data to determine spawning fraction and batch fecundity.

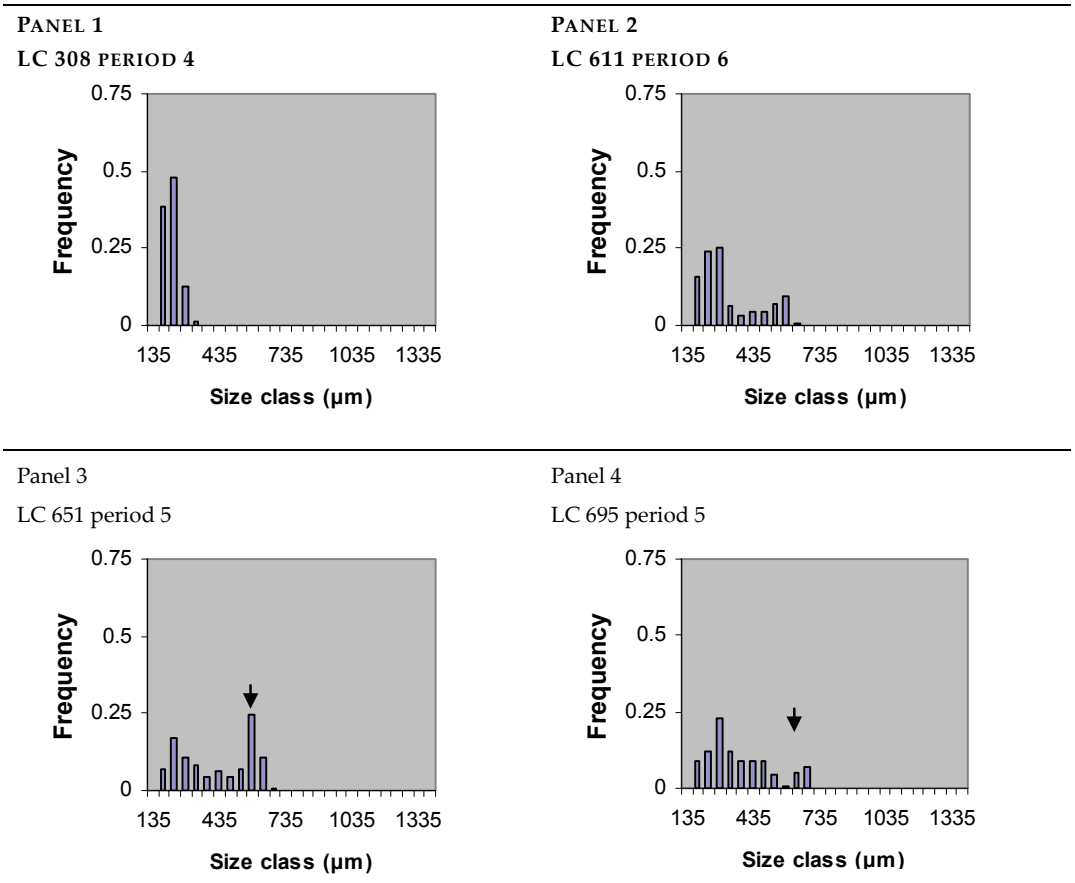


Figure 6.5.1: Frequency distributions (panels 1-4) showing the standing stock of fecundity and an increasing leading cohort (LC arrow panels 3, 4) illustrating the formation of the batch fecundity (Figure 6.3.7).

6.6 Egg production method time series for new western horse Mackerel stock

Since 2004 (ICES, CM 2005/ACFM:08) a new geographic definition of the horse mackerel southern stock has been adopted, corresponding to ICES Division IXa (from Gibraltar to Finisterre). This new definition was based on research carried out during the EU funded HOMSIR project.

The 2007 egg surveys were planned using the new definition of the southern horse mackerel stock corresponding to Divisions IXa and western stock corresponding to. However, for surveys for periods 1995-2004 eggs production data had been recalculated separately for the different areas affected by this change (ICES, CM2005/G:09). The total annual egg production for VIIc used a production curve starting on the 15 February and ending on the 17 July, based on the observations of spawning activity in the Cantabrian Sea during the early periods of the 2001 egg survey.

The time series of the total annual egg production estimates for new western horse mackerel stock is given in the figure 6.6.1. In 2007 the total annual egg production was 1.47*1015 (see section 6.2). This represents an increase of 61% compared to 2004 estimate (0.889 *1015).

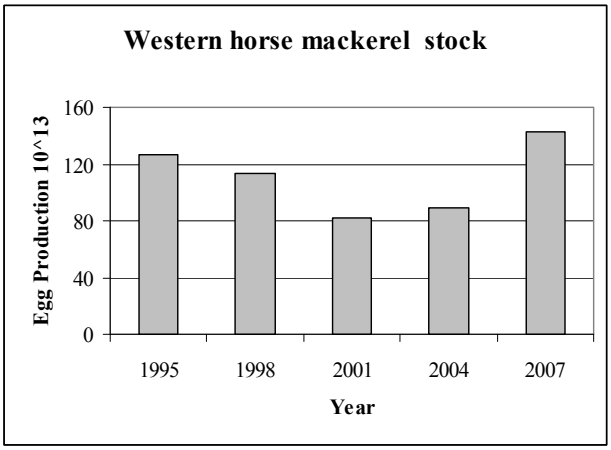


Figure 6.6.1: Time series for total annual egg production based on new definition of the western horse mackerel stock.

The temporal evolution of horse mackerel egg production in the last 12 years shows a decrease from 1995 to 2001, where the minimum value in the series (0.821 *1015) is reached and progressive increase until 2007, with the highest egg production for this time series.

7 Southern horse mackerel stock: 2007 egg survey result

7.1 Spatial distribution of horse mackerel eggs

The spatial distribution of horse mackerel eggs is shown in Figure 7.1.1. In the southern part of the sampling area the eggs were distributed preferably closer to the coast than the 200 meter isobath, while in the northern area eggs were found in deeper waters. In some transects eggs were still found at the most offshore station, which indicates that the spawning area was not totally covered. Due to the bad weather, there was a coverage gap between 41º N and 42º N. Given the high number

of eggs found to the north of the 42º latitude, it is likely that the sampling of that area could have contributed to increase the egg production estimate.

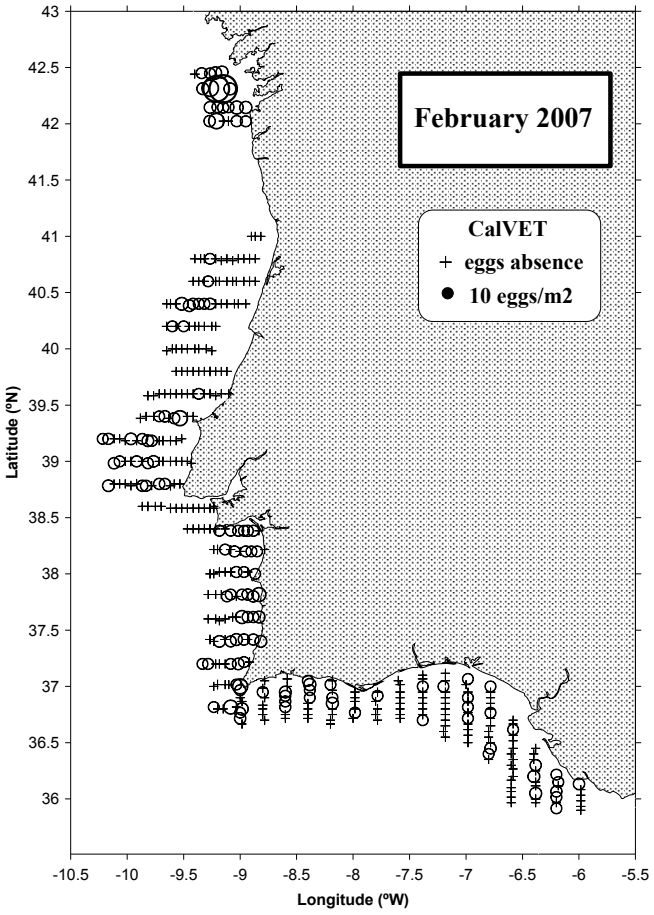


Figure 7.1.1. Spatial distribution of the sampling effort and of the egg abundance.

7.2 Horse mackerel egg ageing

Some fish species have a well defined peak of spawning at a determined time of the day, which is information that is usually taken into account, together with the time of sampling, to increase the accuracy of the age estimation procedure. Other species, such as horse mackerel, do not show this type of synchronicity (Gonçalves *et al.*, 2008), and methods that do not assume a spawning time must be applied to age their eggs. The only method, currently described, that can be applied to asynchronous spawners is the one described by Lo (1985). However this method fails to account for the variability in development at age and for the age distribution at each development stage. Moreover it uses the incubation data as if the sampling was stratified by development stage, when in fact it is age stratified.

A Working Document was presented to WGMEGS (Murta and Vendrell, 2008) describing an application of the Expectation-Maximisation (EM) algorithm (Dempster *et al*., 1977) to obtain maximum-likelihood estimates of the distribution of ages in a sample of fish eggs. The EM procedure followed here was adapted from a method previously described by Kimura and Chikuni (1987) that was originally developed to apply inverse age-length keys to fish length distributions. This method has the same data requirements as the other egg ageing methods described in the literature, namely data from incubation experiments and egg abundance at each stage.

However, it is simpler to apply, with little statistical assumptions and may be applied to species without spawning synchronicity.

When comparing the results obtained with this new method with the ones obtained with the method described by Lo (1985), the former provided more precise estimates of the number of eggs at each age, although the estimates of mean number at age were not very different between methods (Figure 7.2.1). The method based on the EM algorithm was therefore used to combine the egg abundance data from the 2007 survey with data from incubation experiments. An incubation experiment was carried out recently with horse mackerel (Cunha *et al.*, 2008), which provided estimates of the probability of eggs in each stage, given being of a certain age, for several different temperatures (Tables 7.2.1-7.2.6). Each probability matrix was then applied iteratively to the eggs caught in each haul with a water temperature similar to the one used in the corresponding incubation experiment. This procedure classified the stage-classified egg sample into stage and age (hour) classes. The resulting estimates of egg number at age per square meter, for each haul, are shown in Figure 7.2.2.

Table 7.2.1. Distribution of development stages at each age (hours) in an incubation tank at an average temperature of 16.0 degrees Celsius.

	Tank 1 (mean temperature: 16.0 deg.C)										
Age (hours)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11
0	1.00										
1	1.00										
2	1.00										
3	1.00										
4	1.00										
5		1.00									
6		1.00									
7		1.00									
8		1.00									
9		1.00									
10		1.00									
11		1.00									
12		1.00									
13	0.31	0.41	0.28								
14		0.40	0.60								
15			1.00								
16			0.92	0.08							
17			0.35	0.65							
18				1.00							
19				1.00							
20.5				1.00							
22.5				1.00							
24.5				0.22	0.78						
26.5				0.20	0.80						
28.5				0.18	0.82						
30.5					0.17	0.83					
32.5						1.00					
34.5						1.00					
36.5							1.00				
38.5							1.00				
41								1.00			
42.5								1.00			
44.5								1.00			
46.5								0.58	0.42		
48.5								0.06	0.94		
50.5								0.14	0.19	0.68	
52.5										1.00	
54.5										1.00	
56.5										1	
58.5										1.00	
60.5										0.31	0.69

Table 7.2.2. Distribution of development stages at each age (hours) in an incubation tank at an average temperature of 14.9 degrees Celsius.

	Tank 2 (mean temperature: 14.9 deg.C)										
Age (hours)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11
0	1.00										
1	1.00										
2	1.00										
3	1.00										
4	1.00										
5	0.04	0.96									
6		1.00									
7		1.00									
8		1.00									
9		1.00									
10		1.00									
11		1.00									
12		1.00									
13		1.00									
14		0.15	0.85								
15			1.00								
16		0.28		0.73							
17		0.18		0.82							
18		0.23		0.77							
19		0.17		0.83							
20.5				1.00							
22.5				1.00							
24.5				0.35	0.65						
26.5					1.00						
28.5					1.00						
30.5						1.00					
32.5						1.00					
34.5						1.00					
36.5						1.00					
38.5						1.00					
41							1.00				
42.5							1.00				
44.5							1.00				
46.5								1.00			
48.5								1.00			
50.5								1.00			
52.5									1.00		
54.5									1.00		
56.5										1	
58.5										1.00	
60.5										1.00	
62.5										1.00	
64.5										1.00	
66.5										1.00	
68.5							0.03			0.89	0.08
70.5										0.40	0.60
72.5										0.14	0.86

Table 7.2.3. Distribution of development stages at each age (hours) in an incubation tank at an average temperature of 12.5 degrees Celsius.

Tank 3 (mean temperature: 12.5 deg.C)

Age (hours)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11
0	1.00										
1	1.00										
2	1.00										
3	1.00										
4	1.00										
5	1.00										
6	1.00										
7	1.00										
8	1.00										
9	1.00										
10	1.00										
11		1.00									
12		1.00									
13		1.00									
14		1.00									
15		1.00									
16		1.00									
17		1.00									
18		1.00									
19		1.00									
20.5		1.00									
22.5		0.14	0.86								
24.5			0.35	0.65							
26.5			0.05	0.95							
28.5				1.00							
30.5				1.00							
32.5				1.00							
34.5				1.00							
36.5				1.00							
38.5				1.00							
41				1.00							
42.5					1.00						
44.5					1.00						
46.5					1.00						
48.5					0.32	0.68					
50.5					0.03	0.94		0.03			
52.5						1.00					
54.5						1.00					
56.5						1					
58.5						1.00					
60.5						1.00					
62.5						1.00					
64.5						0.10	0.90				
66.5							1.00				
68.5							1.00				
70.5							1.00				
72.5							1.00				
74.5								1.00			
76.5								1.00			
78.5								1.00			
80.5								1.00			
82.5								1.00			
84.5								1.00			
86.5									1.00		
88.5								0.06	0.94		
90.5								0.07		0.93	
92.5									0.07	0.93	
94.5								0.05	0.05	0.91	
96.5										1.00	
98.5										1.00	
100.5										1.00	
102.5										1.00	
104.5										1.00	
106.5										1.00	
108.5										1.00	
110.5										1.00	
112.5										1.00	
114.5										1.00	
116.5										1.00	
118.5										1.00	
120.5										0.71	0.29
122.5										0.63	0.38
124.5										0.60	0.40
126.5										0.46	0.54
128.5											1.00
130.5											1.00

Table 7.2.4. Distribution of development stages at each age (hours) in an incubation tank at an average temperature of 10.6 degrees Celsius.

	Tank 4 (mean temperature: 10.6 deg.C)										
Age (hours)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11
0	1.00										
1	1.00										
2	1.00										
3	1.00										
4	1.00										
5	1.00										
6	1.00										
7	1.00										
8	1.00										
9	1.00										
10	1.00										
11	1.00										
12		1.00									
13		1.00									
14		1.00									
15		1.00									
16		1.00									
17		1.00									
18		1.00									
19		1.00									
20.5		1.00									
22.5		1.00									
24.5		1.00									
26.5		1.00									
28.5		1.00									
30.5		1.00									
32.5		1.00									
34.5		1.00									
36.5			1.00								
38.5			1.00								
41			0.20	0.80							
42.5			0.06	0.94							
44.5				1.00							
46.5				1.00							
48.5				1.00							
50.5				1.00							
52.5				1.00							
54.5				1.00							
56.5				1							
58.5				1.00							
60.5				1.00							
62.5				1.00							
64.5				1.00							

Table 7.2.5. Distribution of development stages at each age (hours) in an incubation tank at an average temperature of 17.4 degrees Celsius.

	Tank 5 (mean temperature: 17.4 deg.C)										
Age (hours)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11
0	1.00										
1	1.00										
2	1.00										
3	1.00										
4	1.00										
5		1.00									
6		1.00									
7		1.00									
8		1.00									
9		1.00									
10			1.00								
11				1.00							
12				1.00							
13				1.00							
14				1.00							
15				1.00							
16				1.00							
17				1.00							
18				1.00							
19					1.00						
20.5					0.64	0.36					
22.5				0.11	0.89						
24.5					1.00						
26.5						1.00					
28.5						1.00					
30.5							1.00				
32.5							1.00				
34.5							1.00				
36.5								1.00			
38.5								1.00			
41								0.50	0.50		
42.5								0.18	0.82		
44.5								0.03	0.97		
46.5									0.08	0.92	
48.5								0.05		0.95	
50.5									0.06	0.94	
52.5										0.95	0.05
54.5										1.00	
56.5										0.47	0.53
58.5										0.50	0.50

Table 7.2.6. Distribution of development stages at each age (hours) in an incubation tank at an average temperature of 19.0 degrees Celsius.

Tank 6 (mean temperature: 19.0 deg.C)

Age (hours)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11
0	1.00										
1	1.00										
2		1.00									
3		1.00									
4		1.00									
5		1.00									
6		1.00									
7		1.00									
8		1.00									
9		0.16	0.84								
10			0.14	0.86							
11			0.55	0.45							
12			0.63	0.38							
13				1.00							
14				1.00							
15				1.00							
16					1.00						
17					1.00						
18					0.06	0.94					
19						1.00					
20.5						1.00					
22.5						1.00					
24.5							1.00				
26.5							1.00				
28.5								1.00			
30.5								1.00			
32.5								1.00			
34.5								0.36	0.50	0.14	
36.5								0.05	0.05	0.90	
38.5								0.04	0.29	0.67	
41									0.07	0.93	
42.5								0.10		0.90	
44.5									1.00		

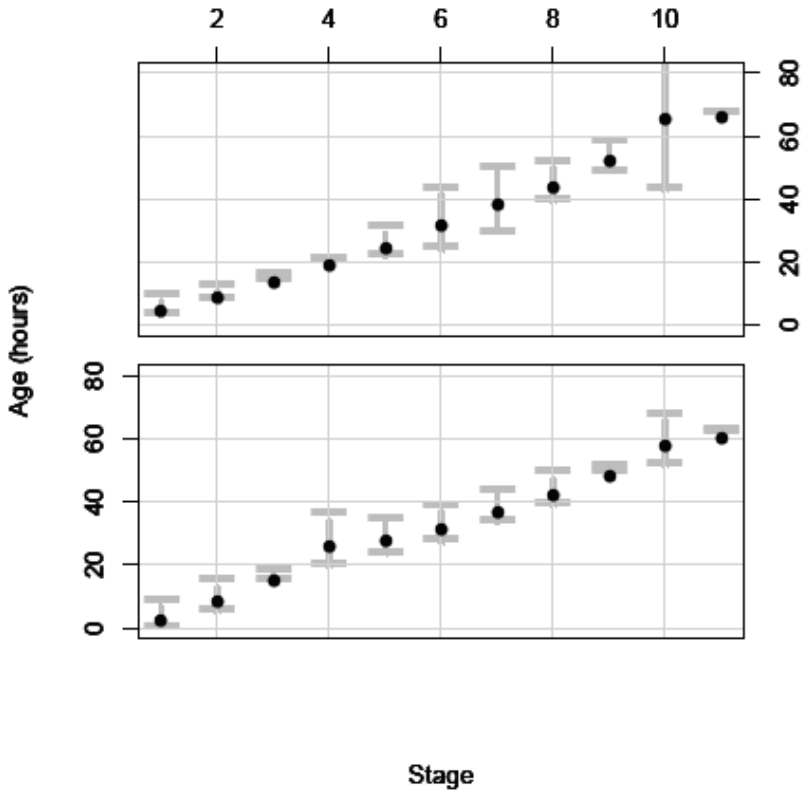


Figure 7.2.1. Mean age at each development stage obtained with the method by Lo (1985, top panel) and with the method based on the EM algorithm (bottom).

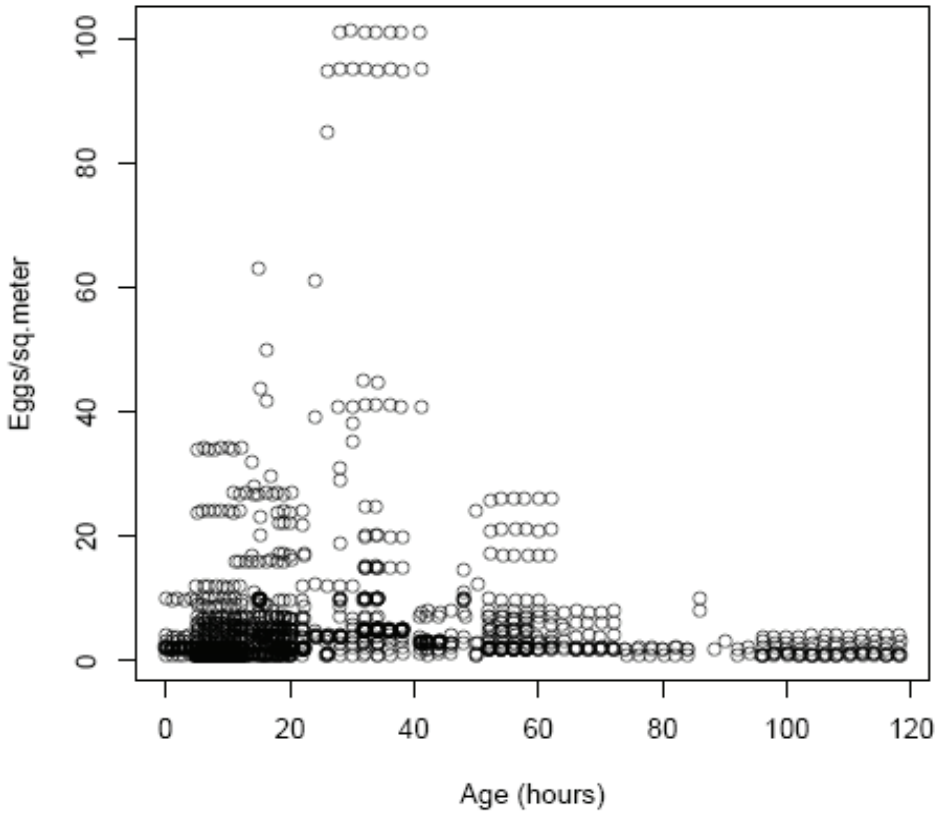


Figure 7.2.2. Number of eggs of horse mackerel per square meter by age in the 2007 egg survey.

7.3 Batch fecundity and spawning fraction estimates for southern horse mackerel in 2007

The mean batch fecundity value obtained with the hydrated oocytes methodology was of 146.8 oocytes/g (N=38), with a standard error of 10.91 oocytes/g. The spawning fraction, and respective standard deviation, estimated with the 4 different criteria (migratory nucleus, hydrated oocytes, POF criterion assuming POFs last for 2 days, and POF criterion assuming POFs last for 3 days) are shown in Table 7.3.1.

Table 7.3.1. Calculation of the spawning fraction with four different criteria.

Sample	n	MN	HO	POF 2days	POF 3 days
1	55	0.2000	0.1820	0.0550	0.0367
6	90	0.4330	0.0000	0.3055	0.2037
7	64	0.0625	0.0156	0.2190	0.1460
8	9	0.1580	0.0350	0.1140	0.0760
9	85	0.2940	0.2830	0.2590	0.1727
10	36	0.3060	0.6390	0.1390	0.0927
11	36	0.1390	0.0830	0.0415	0.0277
12	47	0.1700	0.0000	0.0850	0.0567
13	60	0.1330	0.0670	0.1750	0.1167
14	62	0.1130	0.0000	0.0725	0.0483
	N	10	10	10	10
	Mean	0.2009	0.1305	0.1466	0.0977
	Mean var.	0.0149	0.0077	0.0118	0.0085

7.4 Egg production estimate for southern horse mackerel in 2007

To obtain the egg production (P) for the total stock area, the exponential decay model:

$$Nt = P0 \cdot \exp(-Z.t)$$

where Nt is the mean number at age t of eggs per square meter and P0 is the production of eggs per square meter, was fitted by non-linear regression to the mean number of eggs/sq.meter per age class (Figure 7.4.1). Mean number at age was used instead of the raw data in order to smooth the highly variable data, and to avoid that outliers could have a high leverage on the fitting of the model. Also, the eggs younger than 12 hours of age were not included in the model fitting. Due to their high aggregation during about the first 12 hours after spawning, the eggs of these ages are rarely caught (Figure 7.4.1). Therefore, their apparently low abundance "pulls" down the initial part of the mortality curve, which results in artificially low mortality (Z) and production (P0) estimates.

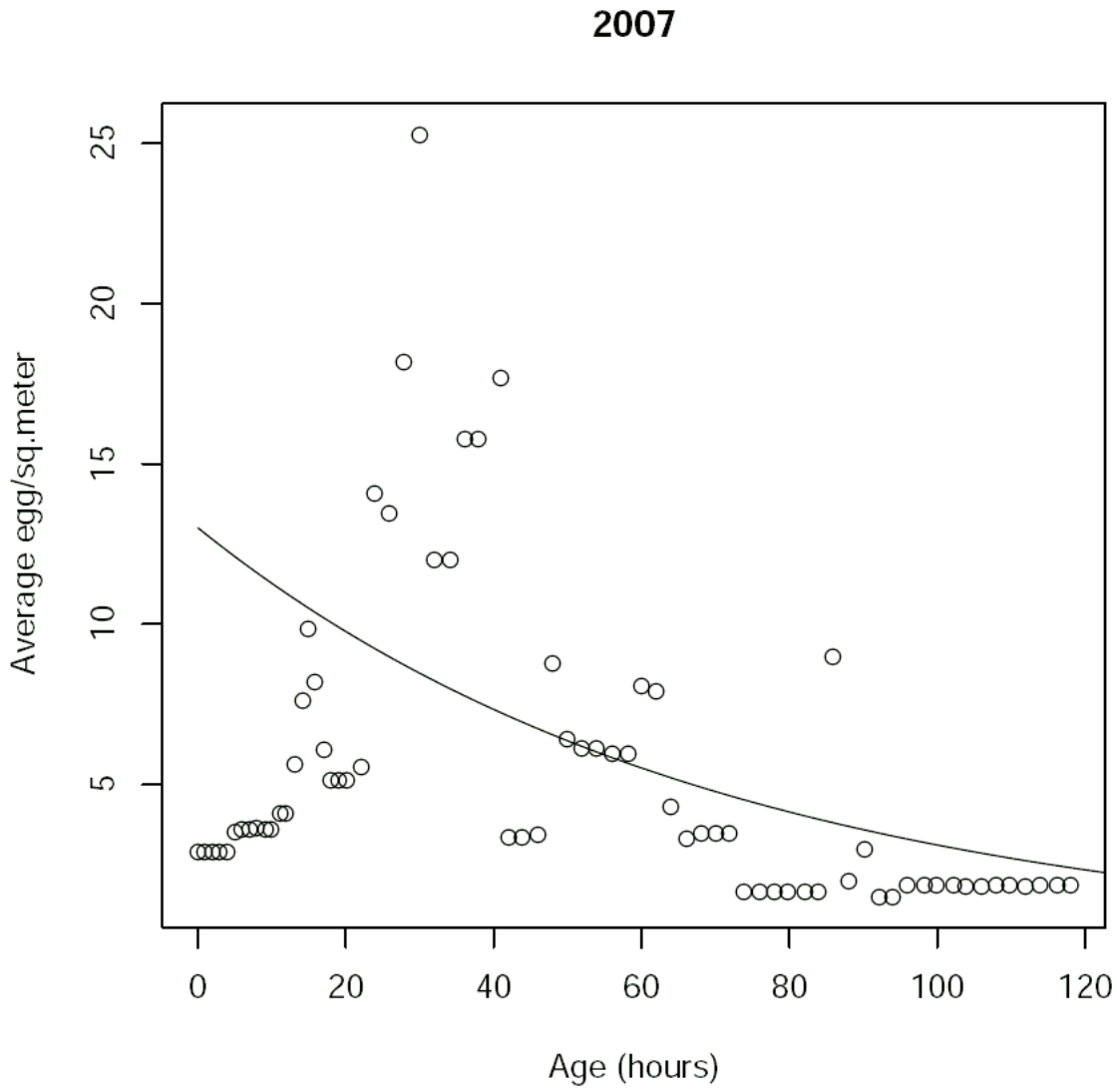


Figure 7.4.1: Mortality curve fitted to the mean number of eggs/sq.meter at age. Eggs aged less than 12 hours where not used for fitting the model.

The model fitting indicated a clear minimum of the sum-of-squares surface corresponding to a production of 13 eggs/sq.meter (SD = 2.0) and a mortality rate of 0.014/hour (SD = 0.075) (Figure 7.4.2). By analysing the plot in Figure 7.4.1, it is expected that the model fitting has produced high positive residuals, corresponding to observations in the range of ages from 20 to 40 hours. This is confirmed by the q-q plot of the residuals in Figure 7.4.3.

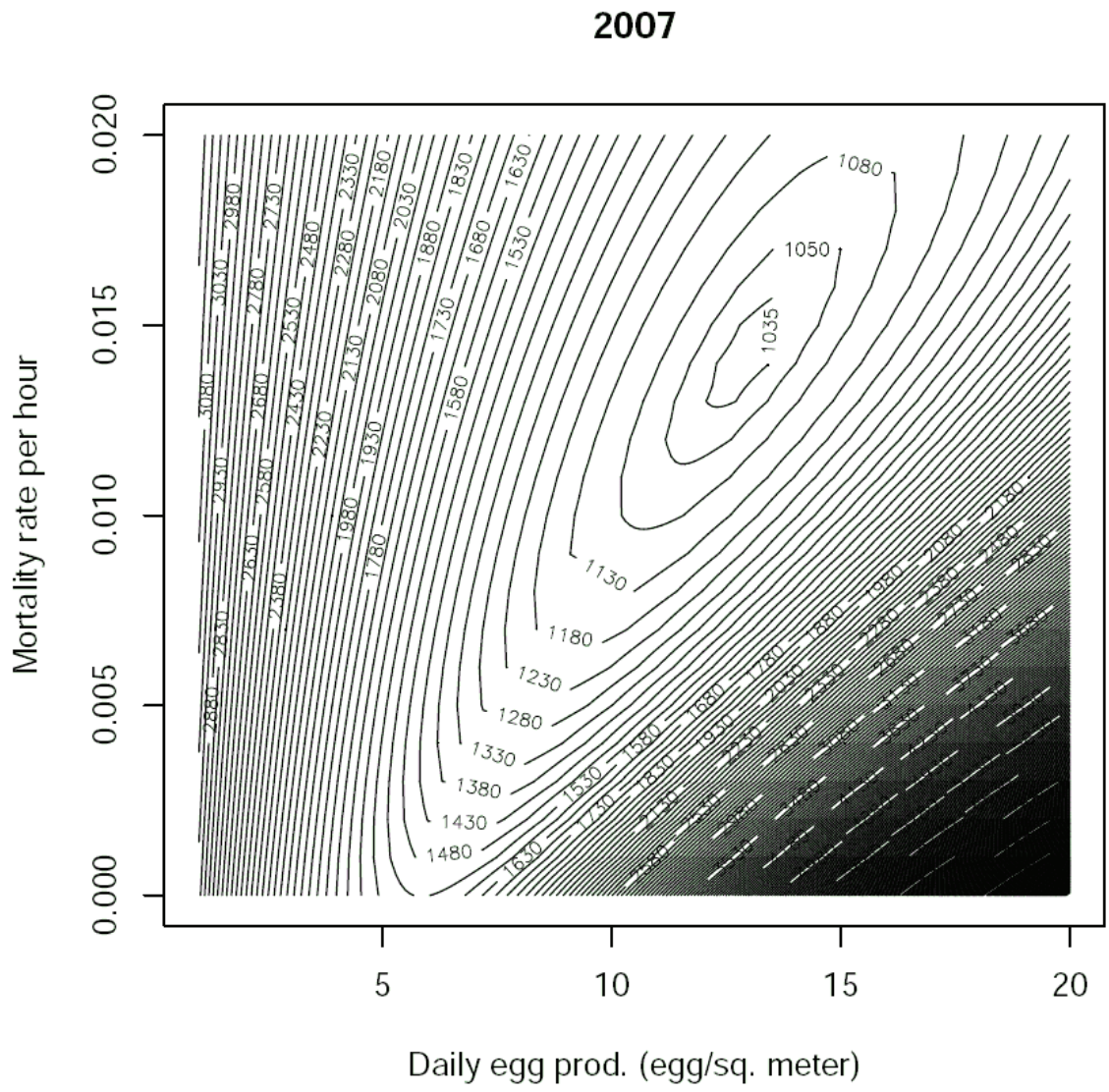


Figure 7.4.2: Sum of squares surface of the non-linear model fitted to the egg abundance data.

The data used to fit the exponential decay model only included non-zero observations, therefore the estimated P_0 corresponds in fact to the production of eggs/sq.meter given the presence of eggs. Assuming that the proportion of sampling stations with at least 1 egg is a good estimator for the proportion of the stock area in which spawning occurred, the production of eggs in the whole stock area can then be calculated as:

$$P = A \cdot Q \cdot P_0$$

where A is the area (in sq.meters) where the stock is distributed and Q is the proportion of sampling stations with 1 or more eggs. For A=1.72e11 sq.meter and Q=0.32, the estimate of P for the 2007 DEPM survey was 7.15e11 eggs. The approximate variance of P, assuming that Q and P_0 are independent, is given by:

$$var(P) = (A \cdot P_0)^2 \cdot var(Q) + (A \cdot Q)^2 \cdot var(P_0) = 1.1e24.$$

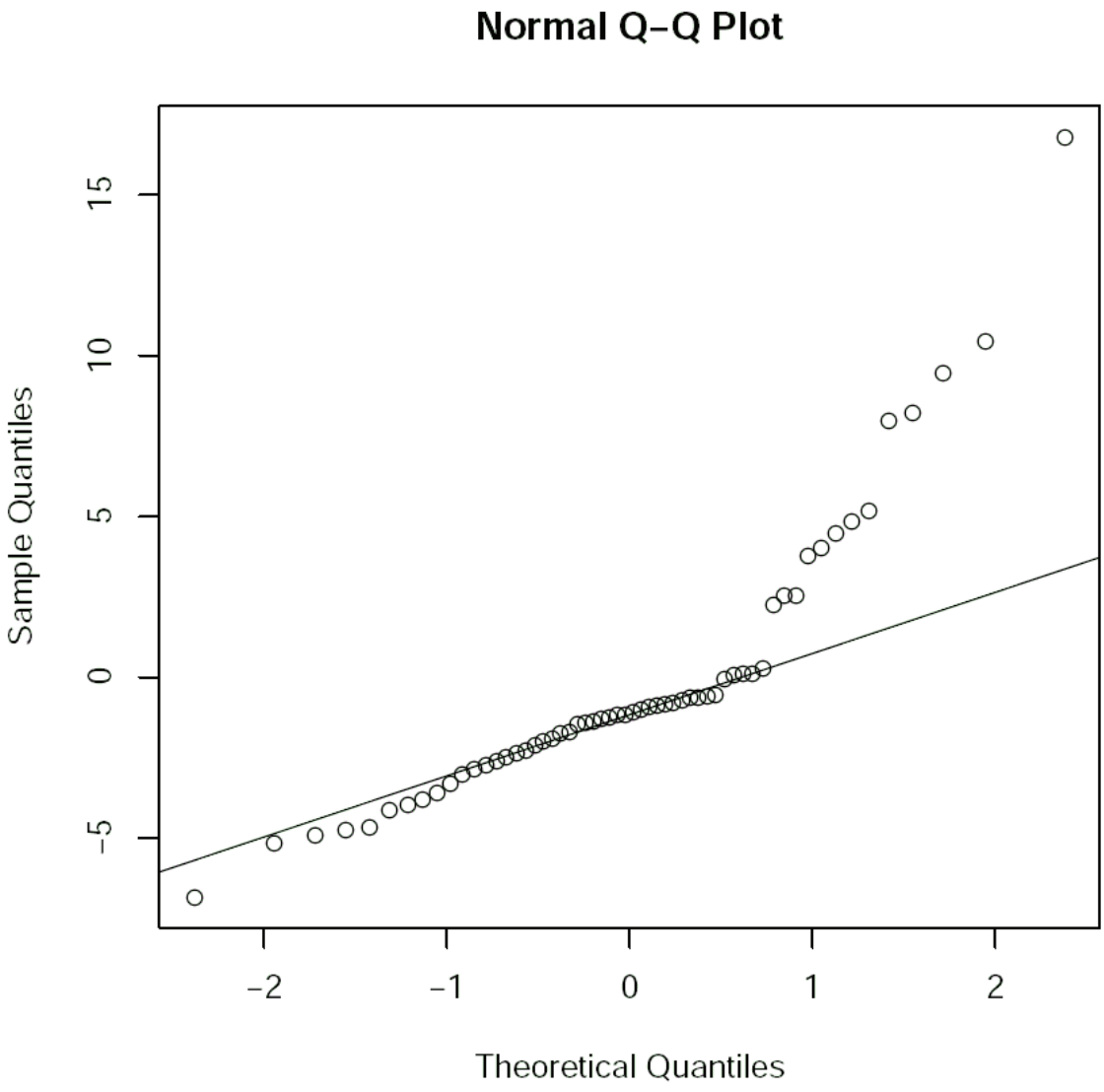


Figure 7.4.3: Q-Q plot of the residuals of the exponential decay model.

7.5 Biomass estimate for southern horse mackerel in 2007

The DEPM SSB estimate was calculated as:

SSB = P / (F . S . R)

where P is the daily egg production for the total area, F is the female batch fecundity per tonne, S is the spawning fraction of females and R is the sex-ratio, taken here as a constant of 0.5. The variance of the SSB estimate was approximately calculated with the expression:

var(SSB)= (F.S.R)^-2 . var(P) + P^2 . (F^2.S.R)^-2 . var(F) +
P^2 . (F.S^2.R)^-2 . var(S)

assuming that the covariances between P, F and S are zero. The estimates obtained were dependent on the criterion chosen for estimating the spawning fraction (Table 7.5.1).

Table 7.5.1. Estimates of SSB, and respective variance and CV, from the 2007 DEPM survey for southern horse mackerel, according to each criterion followed for estimating the spawning fraction.

Spawning fraction criterion	Migratory nucleus	Hydrated oocytes	POFs (2 days)	POFs (3 days)
SSB (ton.)	11 335	17 439	15 113	22 670
Variance	5.2e7	1.5e8	1.3e8	4.5e8
CV	63%	70%	74%	94%

8 Deficiencies and Recommendations

8.1 Deficiencies

The results of the triennial egg surveys are used by the ICES Mackerel, Horse Mackerel, Sardine and Anchovy Assessment Working Group as tuning data series in the assessment of mackerel and horse mackerel stocks. The assessments provide estimates of stock size and catch options from which the ACFM provides advice on the management of these stocks. The advice is subsequently used by the management authorities to set annual TAC's and national quotas. The quality of the data used for the assessments is therefore extremely important as a basis for the provision of accurate and thus reliable advice.

There is a need to maintain consistency and accuracy of egg identification to species and then staging of those eggs. To help harmonise procedures WGMEGS have put in place a series of workshops, held immediately before the surveys to address these issues. The first of these was held in 2000 with the most recent workshop being hosted at Cefas, Lowestoft in 2006 (ICES, 2006c). The outcomes have produced an improvement in agreement between egg readers and a consistency and standardisation of approach. These workshops need to be held before every survey to ensure the quality of the sample processing.

A review of the sampling gear and deployment methods following the 2004 survey (ICES, 2005) showed some differences between the participating institutes, e.g. the use of Gulf III, Gulf VII and other national variants of the Gulf "high speed" plankton sampler in the western area. For the 2007 survey Scotland changed from using a Gulf III to a Gulf VII type sampler providing greater consistency between participants. In **section 4.3.2**, **Table 4.3.2.1** describes the various designs of these samples used by each participating country.

In 2007, both Spanish participants (IEO and AZTI) used a standardised design of Bongo sampler was used to fulfil a recommendation of WGMEGS, 2006 (ICES, 2006). The aperture of the Bongo sampler was 40 cm in diameter with a mesh net of 250 μm. In previous experiments there were found to be no significant differences in performance between Gulf III or Bongo sampler (Coombs *et al*., 1996). Therefore no preference could be expressed for either a Gulf III or Bongo sampler for use on the ICES co-ordinated surveys.

The Portuguese changed from a bongo to a 'double CalVET' sampling device for the 2007 survey. The 'double CalVET' is comprised of two adjoining, 20cm diameter ring nets hauled vertically. This gear allowed quicker deployments in Portuguese waters, over a much closer sampling grid, to better describe the egg distributions in an area where the continental shelf is narrow and the shelf slope is steep.

8.1.1 2007 Western Area Survey Programme – Period overlaps

There were no interpolated survey periods during the 2007 western area survey programme with all the periods being contiguous. A consequence of this was that on 4 occasions surveys overlapped with an adjacent sampling period. In all but period 2 the overlap was no more than a few days however for each case the impact of excluding these out of period data were analyzed and the results and subsequent impact on the total annual egg production estimate (TAEP) for both species were calculated and the results documented below. In each of these cases excluding these data or indeed moving the period dates to bring the data back within period would have compromised survey coverage elsewhere for that period and indeed reduced the accuracy of the survey programme.

Period 2: The first AZTI survey straddled period 2 and 3. Indeed by the time the survey dates were finalised the survey occupied more effort in period 3 than period 2. Given the proximity to the start of the surveys the decision was made to retain the survey within period two rather than splitting it between the periods which risked disrupting an otherwise settled survey plan. The extremely large egg production estimate for period 2 compared to period 3 required us to look at the contribution made by these out of period AZTI stations that were sampled within period 3. The out of period stations were located in northern Biscay.

Mackerel - The contribution made by these out of period stations to the daily egg production (DEP) estimate for period 2 was approximately 5% ($4.9*10^{11}$) with most of the mackerel spawning activity in this period taking place further north in the Celtic Sea and Porcupine Bank. We can therefore safely assume that the impact of including these out of period stations on the overall total annual egg production estimate (TAEP) is negligible.

Horse mackerel – For horse mackerel the contribution made by the out of period stations was higher at $1.48*10^{12}$ eggs/m^2/day, which is approximately 28% of the total daily egg production (DEP) estimate for period 2. It is worth noting that 3 stations contributed around 70 % of the out of period abundance during this period and that these stations were undertaken on the 13th April, which was only 5 days into period 3. Since this was early in the horse mackerel spawning season removal of these stations would have resulted in a decrease to the TAEP of only 4 %. Therefore, it can be assumed that the impact of including the out of period stations was minimal.

Period 3: The Scottish survey dates were brought forward unexpectedly by 5 days. Consequently this resulted in the survey overlapping into period 2 by 5 days. The out of period stations were located in the area west of Scotland.

Mackerel – The contribution may by these out of period stations amounted to approximately 10% of the DEP for period 3. The subsequent impact of excluding the data on the TAEP was less than a 1% decrease. The decision was taken to therefore include the data in the analysis for period 2.

Horse mackerel - There was virtually no evidence of horse mackerel spawning in the area concerned at this time therefore the impact of including the data in the TAEP are zero.

The IEO survey in the Cantabrian Sea overlapped into period 4 by 3 days.

Mackerel - The out of period mackerel data were excluded from period 3 and incorporated into the period 4 egg production estimate. The southern spawning component which includes the Cantabrian Sea was calculated separately in 2007 and

including observed period 4 data subsequently reduced the period of interpolation by creating an additional sampling period. Sampling in this region was due to cease after period 3.

Horse mackerel - The out of period stations for this survey contributed approximately 15% ($8.62*10^{11}$ eggs/m^2/day) to the DEP for period 3. Removal of these stations would have resulted in a corresponding decrease to the TAEP of less than 2%. Therefore the impact overall of including the out of period stations again was minimal.

Period 4: During this period the Norwegian survey overlapped into period 5 by 3 days. The out of period stations being located west of Ireland and on the Porcupine Bank.

Mackerel – Although this was the period of peak mackerel spawning, significant spawning activity was dispersed over a wide area (see figure 5.1.1.d.) which helped to downplay the importance of the out of period stations to the estimate. The out of period stations contributed 15% ($2.06*10^{12}$ eggs/m^2/day) to the DEP for this period. This translated into a decline in the TAEP of 4%. Once again including the data was seen as being the least problematic solution. Moving the period back to accommodate the out of period data would have resulted in loss of survey coverage along the northern survey boundary in period 5. The effect of including the data was judged as minimal and the data was included.

Horse mackerel - The out of period stations contributed 8% ($1.06*10^{12}$ eggs/m^2/day) to the DEP for this period. The impact of excluding these data on the TAEP saw a decrease of 2%. Moreover over 98% of the out of period abundance was recorded less than 1 full day out of period. Yet again on balance it was decided to include the data.

8.2 Recommendations

WGMEGS recommends:

- to arrange routine workshops (WKMHMES) immediately before the surveys to harmonise egg identification, (species and development stage) and determination of realised fecundity in mackerel, and spawning rates in horse mackerel. The next WKMHMES are scheduled for late 2009.
- to redraft and update the survey and fecundity manuals at the 2009 WGMEGS planning meeting and the subsequent meeting of WKMHMES.
- that participants should try to provide adult samples for fecundity studies of North Sea mackerel in May 2008. These samples will be processed by IMR, Bergen to determine spawning rates (batch fecundity, spawning fraction and residual fecundity) by image analysis.
- image analysis data (batch fecundity and residual fecundity) from western horse mackerel fecundity samples should be examined (by IMARES, Netherlands) to produce an index of SSB. Attention should be directed to reducing the variance attributed to spawning fraction and batch fecundity, compared to the traditional DEPM method, which is based on the presence of migratory nuclei (ICES 1990 and ICES 1993) or hydrated follicles (this report). These parameters should be redefined according to data produced from automated image analysis of follicles larger than 400 μm.
- screw cap plastic tubes with an 'O' ring seal should be evaluated on the 2008 North Sea survey for the storage of mackerel fecundity samples. Comparisons on evaporation rates (which may affect sample weights) can then be made between tubes fitted with screw cap and flip-top lids.

Refrigerated storage (between 1–5°C) of sample tubes (of both types) is recommended, to reduce evaporation as far as possible.

- that an MS Excel template is produced (by IMR, Norway and IMARES, Netherlands) to store all available adult parameter data for both mackerel and horse mackerel. This is to include a summarised version of follicle size distributional data. This would enable the compilation of an historical dataset which would allow for a greater range of statistical analyses. These data should be held in a common share point available to all participants.
- that further work is conducted (by IEO and AZTI, Spain) on the end-point of mackerel and horse mackerel egg production south of 47ºN during 2008. A working document is to be produced for discussion at the 2009 WGMEGS planning group meeting. Sampling is also required west of Ireland to confirm the assumed end of spawning of horse mackerel in that area. This should include two plankton transects during early August.
- that an exchange of samples (ring trial) is conducted for fecundity and atresia in mackerel (IMR, Norway), and fecundity in horse mackerel (IMARES, Netherlands) before the 2009 WKMHMES workshop. The results are to be compiled and a presentation prepared to enable discussion at this workshop.
- that research is undertaken to update the calculation of 'spawning duration' (currently estimated at 60 days) and 'atresia duration' (7.5 days) in the mackerel atresia calculation. The Matre facility in Norway would provide a suitable facility for this work.

8.3 Proposed Terms of Reference for 2009

The Working Group on Mackerel and Horse Mackerel Egg Surveys [WGMEGS] (Chair: Jens Ulleweit, Germany) will meet in Hamburg, Germany, 20–24 April, 2009 to:

a) Coordinate the timing and planning of the 2010 Mackerel/Horse Mackerel Egg Survey in the ICES Sub-areas VI to IX.

b) Coordinate the planning of the sampling programme for mackerel/horse mackerel fecundity and atresia.

c) Review and report on procedures for egg sample sorting, species identification and staging.

d) Review and report on procedures for fecundity and atresia estimation.

e) Analyse and evaluate the results of the 2008 mackerel egg survey in the North Sea.

f) Update the survey manual and make recommendations for the standardization of all sampling tools, survey gears and procedures.

g) Evaluate and report on the use of the Triennial egg survey to provide an abundance index for horse mackerel, and make recommendations for the 2010 survey for data collection and analysis.

9 Working documents presented to the Working Group

1) Sampling variability in mackerel egg abundance

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Abstract

Variability of mackerel egg abundance was studied during the German, Scottish and Irish participation in MEGS 2007. In total, 5 transects were sampled at least twice either within sampling period 2 or 3. Time lag between each sampling replicate was between 1 and 11 days. Variability per each station in egg abundance and in developmental stage composition was high, particularly when time lag between samplings was large. But also at stations that were re-sampled at short time intervals a large variability was observed when mean abundance was high as well. These differences in abundance by station were balanced when calculating the daily egg production by transect, but only in those replicates at short time lags, i. e. 1 – 3 days. Daily egg production estimates for these transect were always within the same order of magnitude in differed only by a maximum factor of 2.4. In contrast, in those replicates with a time lag of 11 days the daily egg production estimate was 1 order of magnitude higher or lower and differed by factor of 8.6 and 5.9, respectively. We conclude that this observed high variability is expectable in these rather short lived egg stages. A survey that aims at the determination of mean daily egg production rates per each sampling period should, therefore, reflect this variability over the time span of each period. Given the known problems in ship time allocation and co-ordination, the current alternate transect sampling scheme appears to be a reasonably appropriate strategy.

2) Distribution of Snake Pipefish during the 2007 MEGS

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Abstract

Snake pipefish (*Entelurus aequoreus*) have gained increasing interest in marine science because of their sudden increase in abundance in the Northeast Atlantic as well as in the North Sea and the subsequent effects on breeding success in some sea bird populations. Therefore, during the 2006 Workshop on Mackerel and Horse Mackerel Egg Staging all participants in the Mackerel and Horse Mackerel Egg Survey (MEGS) were asked to look for specimens of snake pipefish in their plankton samples and report the results to the authors of this document. We present first results of snake pipefish sampling during the 2007 MEGS. All results so far available were grouped by sampling period according to the MEGS sampling scheme. Snake pipefish were found in an area stretching from the Bay of Biscay northwards up to west of Scotland. No snake pipefish were caught in the Cantabrian Sea and west of the Iberian Peninsula. The overall pattern of snake pipefish distribution was generally much the

same for all sampling periods with snake pipefish being more abundant in the southern areas. North of the Bay of Biscay snake pipefish were concentrated above the shelf edge and beyond it, over deep waters. In contrast, in the Bay of Biscay the centre of distribution apparently moved westward and away from the shelf edge to areas entirely above deep waters. A table is presented that contains all data supplied so far and lists data that are still needed in order to complete the analysis.

3) Mackerel and Horse mackerel egg production in 2007 in western component and western stock respectively

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Egg production for NEA mackerel and western horse mackerel stock was estimated in 2007. Stage I egg abundance data were provided by the different institutes which completed surveys between March and July adhering to a survey plan devised at the 2006 planning meeting in Vigo 2006. The data were split into 6 periods. Overall survey coverage was good for all periods. Deviations from the initial plan were analysed and their impact recorded. Egg production curve for mackerel showed a bimodality, with a decrease in the production in the second western survey period (Period 3). The total eggs production estimated for western area was $1.21*10^{15}$, quite similar to that obtained for 2004 ($1.20*10^{15}$) Period 4 saw peak spawning although spawning activity was recorded throughout all periods recorded. The egg production curve for horse mackerel showed similar pattern to the curve for 2004 although much larger. Estimate of total annual egg production for western area was 1. 43*1015, that is more than double the estimate for 2004. The peak of spawning occurred in period 4, but high values were observed in period 3 and 4 as well.

4) Investigation of bias introduced by change of plankton torpedo

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Abstract

In 2004 the Dutch changed from the use of a Gulf III plankton torpedo for the herring larvae surveys to the Gulf VII torpedo. However nothing is know about difference in catchability of herring larvae between these two torpedoes. To investigate the possible bias introduced by the change of gear it was decide to perform a real-time comparing fishing trial during the September 2006 herring larvae survey. In May 2006 a frame with both torpedoes was performed to investigate the stability of the structure. This frame was used, later in September surveys. Sea water volume filtered by the Gulf VII was significantly lower then the Gulf III. There is an influence of the Gulf VII on the Gulf III. When comparing the flows from previous herring larvae surveys, where the torpedoes were towed separately, the volume filtered by the torpedoes is significantly different. Comparing the flows from the 2006 survey to the previous years, there is no significant differences for both torpedoes.

5) Taking horse mackerel, *Trachurus trachurus*, as an indeterminate spawner: estimates of batch fecundity, spawning fraction and time of spawning for the southern stock (ICES div. IXa)

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The Annual Egg Production Method (AEPM) has been applied triennially, since 1995, to the southern stock of horse mackerel in the northeast Atlantic. This method has the assumption that fecundity is determinate, however there has been mounting evidence that horse mackerel is an indeterminate spawner. In this study we present further evidence supporting that hypothesis: the number of oocytes by unit area along the 1998 spawning season (January to March) shows an increasing trend, while there was a decrease on the mean diameter of the oocytes during the same time. The Daily Egg Production Method (DEPM) does not rely on the assumption of a determinate fecundity, which makes it the appropriate method in this case. Therefore, we re-analysed samples collected in previous years in order to obtain a time series of estimates for DEPM parameters (batch fecundity and spawning fraction). The estimates of batch fecundity were very variable between years (or periods within the same year). Regarding spawning fraction, the analysis of post-ovullatory follicles, assuming a degeneration time of 3 days, gives similar results to the hydrated oocytes criterion, but with a much lower variance. Our results also suggest that horse mackerels do not have a well-defined spawning time during the day, therefore being considered as asynchronous spawners.

6) Using the EM algorithm for ageing eggs from fihsh without daily spawning synchronicity

By Alberto G. Murta and Catarina Vendrell

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Abstract

We describe an application of the Expectation-Maximisation (EM) algorithm to obtain maximum-likelihood estimates of the distribution of ages in a sample of _sh eggs. This EM procedure was adapted from a method developed to apply inverse age-length keys to _sh length distributions. The data necessary to apply it are the number of sampled eggs in each development stage and a set of matrices (one for each water temperature) giving the probability of an egg to be in a given development stage given it is in a certain age class. In order to compare this method with another widely used method that is suitable for asynchronous spawners, we applied both methods to the same data sets. The mean age estimates are slightly different between methods, however it is the variability of the ages in each stage that shows the highest differences. The exponential decay egg mortality model was better fitted to the age data obtained with the EM algorithm method. Also, the back- calculated time of spawning for sardine, which is considered a synchronous spawner, much more well-defined for the EM method than for the traditional method. The application of the method described here is not restricted to egg ageing, but can also be used for other purposes, such as giving ages to post-ovulatory follicles, using data on follicle degradation at different water temperatures.

7) Mackerel egg production in ICES Division VIIIc and IXa in 2007

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Abstract

From 3 February to 10 May 2007, mackerel in ICES Divisions VIIIc and IXa were investigated by ichthyoplankton surveys, in order to apply the Annual Egg Production Method (AEPM) to estimate the southern component of the North Atlantic mackerel stock. The surveys were carried out by Portugal (IPIMAR) and Spain (IEO and AZTI).

The annual mackerel stage I egg production in the southern component was estimated at 31.19×10^{13} (s.e. 21.5×10^{13}).

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Annex 2: WGMEGS Survey manual

[All changes to the last version of the survey manual (ICES, 2003) and recommendations are highlighted in bold text].

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

This annex now incorporates the current protocols (together with recent changes) for the collection and analysis of adult fish parameters required for the AEPM method. It is recommended that this annex is updated on a regular basis and is distributed for use by all participants on the 2007 and future triennial **surveys.**

1. Sampling areas and sampling effort

The spatial and temporal distribution of sampling is designed to ensure an adequate coverage of both mackerel (*Scomber scombrus* L.) and horse mackerel (*Trachurus trachurus* L.) spawning. Sampling effort is targeted at producing estimates of stage 1 egg production for both species.

The 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 is regarded as being from 36°N to 45°N. It includes southern Biscay, the Cantabrian Sea and from the Portuguese coast to 11°W. Sampling usually begins in January in this area and continues until June in the Cantabrian Sea.

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

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

Since the surveys began in 1977 considerable changes have been made to the 'standard' sampling area and some of these were described in Section 8.4 (ICES, 1994). Based on the expansion of the "standard area" since 1977, it was agreed (ICES, 2002a) 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 given below (Section 2.). The core areas for the western and southern surveys together, are presented in Figure 1. The sampling area in the south has been modified from the design used in 2001 and previously (Figure 2). Figures 1 and 2 are provided as a

planning guide only. The limits of the survey in both areas should be established on the basis of two consecutive zero samples, and not by the boundaries on these maps.

2. Sampling strategy

The sampling strategy in the western and southern areas will be targeted at the AEPM only. However, Portugal will collect both plankton and adult fish samples to produce a DEPM estimate for horse mackerel in their waters, in 2007.

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

The amount of ship time available and the size of the area to be covered will determine the spacing and omission of sampling transects. During periods when several ships are available it should be possible to sample all transects, 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 concentrate in areas where high densities were observed in the first pass. The cruise leader should be aware of edge definition problems where the contours run east-west. In this way, weather problems, equipment failure and vessel breakdown need not seriously prejudice results. Such a strategy, furthermore, enables better evaluation of distributional change with time, which is likely to be important in modelling the results. An example of an appropriate sampling strategy where one in two transects is fully sampled is given in Figure 6.16 in ICES (1994).

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

3. Standardisation of survey gears

The standard plankton samplers for use on these surveys are national variants of 'Gulf type' or Bongo 'high-speed' samplers (Nash *et al.*, 1998). These samplers

generally incorporate conductivity, temperature and depth probes (CTD's) and are fitted with either mechanical or electronic flowmeters to enable the volume of water filtered on each deployment to be calculated. These sensors either relay 'real-time' environmental data back to a shipboard computer or log the information, ready for downloading once the station has been completed.

It would be preferable to use a standard survey sampler for the triennial surveys. **As a first step, it is therefore recommended that each participating nation should review the design of their sampling equipment (including flowmeters) against published sampler designs. This information will be collated by C. van Damme (for Gulf type samplers) and G. Costas (Bongo samplers). It will be presented at the next meeting of WGMEGS in 2008, and included in an updated version of this annex.** Nash *et al.*, 1998, provides a comprehensive description for a Gulf type sampler, which they call a Gulf VII. A useful review of Bongo designs and a suggested standard is given by Coombs *et al.* (1996) in an annex to the final report of EU AIR project AIR3 CT94 1911. **Each participant is requested to compare their samplers against these suggested designs, report the differences at the next WGMEGS meeting and attempt to modify their sampler designs to make them more similar to the published standard.**

The estimation of volume of water filtered by each sampler is critical in the calculation of egg abundance. Again, the suggestions provided by Nash *et al.* (1998), and Coombs *et al.* (1996) provide an acceptable standard. **It is recommended that participants follow these standards as closely as possible.** It is also critical that participants understand the importance of calibrating flowmeters and changes in flowmeter performance when they are mounted in the apertures of plankton samplers (EU AIR3 CT94 1911). **It is recommended that all participants review the performance of their flowmeters and regularly their check their calibration in-situ (i.e. within the sampling device)**. The current flowmeters used in the survey are largely considered as state-of-the-art; however, new developments are being made in non-intrusive 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 aperture is adequate for sampling mackerel and horse mackerel eggs, a nylon mesh with an aperture between 250 and 280 microns is the recommended size for these surveys. This allows the plankton samples to be more widely used for investigations on other species and taxa. In the North Sea surveys, where clogging is a problem, a 500 micron aperture mesh is used by both the Netherlands and Norway. Norway is the only participant to use 500 micron aperture mesh in the western (or southern) area.

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

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

4. Plankton sampler deployment

It is recommended that the Gulf type samplers are deployed on a double oblique tow, at **4 knots, (note change from 5 knots)**, from the surface to maximum sampling depth (see below) and return. The Bongo samplers are deployed at 2–3 knots on similar, double oblique tows. The aim is for an even (not stepped) 'V' shaped dive profile, filtering the same volume of water from each depth band. The aim is to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band (ICES, 2001). At shallow stations, multiple double-oblique dives may be necessary to enable a sufficient volume of water to be filtered. **A minimum sampler deployment time of 15 minutes is recommended.**

Norway uses the Gulf type samplers in the western area but deployed a Bongo in the North Sea until the 2005 survey when a Gulf VII sampler was used. **Both Norway and the Netherlands now use Gulf VII samplers on the North Sea surveys and this is now the recommended sampling device for this survey. Norway has also changed from a stepped tow profile (used with the Bongo) to the recommended double oblique tow used by all other nations. This is a welcome standardisation both in terms of gear design and in deployment method, and is to be encouraged.**

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 in 10m depth, sampling can be confined to a maximum depth of 20m below the base of the thermocline.

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

5. Plankton sample collection and fixation

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

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

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

c) Always wash down from the nosecone end last.

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

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

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

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

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

i) Put labels containing station details in pencil into all jars.

The standard fixative for use on these surveys will be a 4% solution of buffered (pH 7 - 8) formaldehyde in either distilled or fresh water. (420g of sodium acetate trihydrate is dissolved in 10 litres of 4% formaldehyde, ICES, 2001). This solution is approximately iso-osmotic with seawater and will 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%.

There was some discussion at WGMEGS 2006 about the suitability of the sodium acetate buffer for the preservation of fish eggs. It is recommended that all participants review their preservation methods and present any results for discussion at WKMHMES 2006. Any conclusions will be included in the WKMHMES report and will be available for the next meeting of WGMEGS in 2009.

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

6. Plankton sample sorting

Following practical demonstrations and trials with a 'spray technique' for the removal of fish eggs from plankton samples at WKMHMES (ICES, 2004b), it was recommended that this technique was used on samples collected during the 2004 triennial survey. Since then, enhancements have been made to the equipment and methods (WD, Eltink), which will again be evaluated at WKMHMES in 2006. **It is recommended, that where suitable, the spray technique be used at sea to quickly remove the majority of fish eggs from plankton samples. This will allow a rapid decision to be made on whether to continue sampling along a transect or to move to the next transect line.**

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

All sorted eggs should be kept in tubes, in, 4% buffered formaldehyde, inside the sample container for future reference and use. Usually only the eggs of mackerel and horse mackerel need be identified to species and staged.

7. Egg identification and staging

This is a key area for standardization and has been the subject of considerable attention by the working group. Egg staging was the subject of a detailed workshop held at Cefas, Lowestoft in 2000 (WKMHMES, ICES, 2001). This workshop produced

a detailed manual on plankton sample handling and analysis, which was used by all survey participants during the 2001 surveys. A subsequent exchange programme on plankton sorting, species identification and staging revealed some deficiencies, mainly in the species identification (see section 9.3). It should be noted that this was a small-scale exercise, and was mainly intended to highlight areas for further work rather than as an analysis exercise in itself. Based on these findings a further WKMHMES (ICES, 2004b) was held in 2003, which included, sample sorting, species identification and egg staging. The results of this workshop were very re-assuring and a further WKMHMES is planned in 2006, to train and evaluate the performance of the plankton analysts involved with the 2007 survey. The results of this workshop will be presented to ICES by the end of 2006.

The eggs and larvae of most of the species found in the area are well described by Russell, 1976. This book is well known and used by all the participants of the ICES triennial surveys. It is generally regarded as the definitive work on the subject in this area.

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

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

Within WGMEGS the eggs of mackerel are classified into one of five morphological stages (I, II, III, IV and V) (Lockwood *et al.*, 1981) (Figure 3), following the development criteria described for plaice (Simpson, 1959). For horse mackerel the description of stages is the same with the exception of stage V, which does not exist. Horse mackerel larvae hatch at the end of egg stage IV (Pipe and Walker, 1987).

For the estimation of daily egg production for both mackerel and horse mackerel, only the counts of stage I eggs are used. This is recognised as a conservative estimate of the total eggs spawned because of mortality which occurs during development. However until there is consistency in the identification of the other stages, between all countries, the other stages cannot be used for the estimation of mortality rates and backtracking to total eggs spawned.

8. Calculation of daily egg production

To convert abundance of eggs into daily egg production, data on the rate of development is required. For mackerel the relationship between egg development rate and temperature was described by Lockwood *et al*. (1977, 1981). This has been used as the basis for calculating daily egg production of stage I eggs on all the surveys from 1977. For horse mackerel similar egg development data are given by Pipe and Walker (1987) and have also been used for the calculation of stage I egg production since 1977. The formula for calculating the duration of stage I mackerel eggs from the sea temperature (T°C) is:

$$\text{Log}_e \text{ time (hours)} = -1.61 \log_e (T°C) + 7.76$$

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

$$\text{Log}_e \text{ time (hours)} = -1.608 \log_e (T°C) + 7.713$$

Work aimed at reviewing the existing calculation to estimate the rate of development is taking place (see section 11). 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.

9. Standardisation of plankton data analysis

Detailed procedures for the post analysis of egg abundance data to produce daily and, finally, annual egg production estimates are given below. This analysis has previously been carried out by two data coordinators (one for the western and one for the southern area), using data submitted in a standard format. **However, F. Burns, FRS, Aberdeen will manage the results for the entire 2007 survey.** This analysis is subject to examination and approval by the full working group and will ensure a standard approach and methodology. **It is recommended that participants will supply their plankton data either in a standard MS Excel spreadsheet or Paradox database input form, to be distributed by the data co-ordinator.**

To convert the number of eggs in each sample (or sub-sample) to the number of eggs per m^2, the following calculations are made. Firstly the volume of sea water filtered by the sampler during the haul is calculated.

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

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

$$\text{Eggs/m2} = \frac{\text{Eggs counted x Factor}}{\text{Volume Filtered (m3)}} \times \text{Depth Sampled}$$

Where:

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

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

For stage I **mackerel** eggs:

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

For stage I **horse mackerel** eggs:

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

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

Area (m^2) = (cos(latitude) x 30 x 1853.2) x (30 x 1853.2)

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

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

The method was modified for use in the analysis of the 1995 survey data. It is fully described in section 5.3.3 of the report of those surveys (ICES, 1996b). The same methods will be used for the analysis of the 2007 survey data. **It is recommended that the flowmeters and sampling devices deployed in the survey should be calibrated in terms of the volume of water filtered**. There are two aspects to calibration: The first requirement is to know and understand the relationship between flowmeter revolutions and distance travelled through the water. The second is to relate flowmeter revolutions, (whilst mounted *in-situ* in the aperture of a plankton sampler), to volume filtered by the sampler. The only way in which the second aspect can be accurately determined is to calibrate the flowmeter and sampler under controlled conditions in a circulating water channel or in a large towing tank. These facilities provide independent measures of water or towing speed and also 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.**

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

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

10. Standardization of adult sampling – data collection and analysis

The working group prepared an updated protocol for the collection and analysis of adult parameters; fecundity, atresia, and parameters for condition and feeding in the case of horse mackerel. These are detailed in Sections 3.4 to 3.6 (ICES, 2003). The analysis of these samples, particularly with reference to fecundity estimation, the use of the Auto-diametric approach and oocyte diameter determination, were standardised at WKMHMES (ICES, 2004). This fecundity and atresia manual will again be updated at the next meeting of WKMHMES to be held at Cefas, Lowestoft, in October 2006.

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

Following WGMEGS decision to use only formaldehyde fixative (ICES 2003) it will be possible to provide a unified sampling scheme for fecundity and atresia for use on the 2007 survey. **Following the experience of the 2004 survey the Auto-diametric method, (although useful where the fecundity sub-sample weight is not known) produces more variable fecundity data compared to the Gravimetric method (Hunter *et al.* 1989). The Working Group recommends that the latter technique is used for the 2007 survey.** All changes in the sampling protocol and methods between the 2004 and 2007 surveys are given in table 10.1.1.

Table 10.1.1. Changes for 2007 compared to 2004.

2004	2007
Auto-diametric method (Thorsen and Kjesbu 2001)to estimate fecundity was more variable than Gravimetric results	Gravimetric fecundity (F) method (Hunter *et al.* 1989). F = O * C*S where O= ovary weight ± 0.1g, C=count of vitellogenic follicles in the sub-sample weight S (± 0.0001g)
Fecundity sub-sample weight assumed equivalent to pipette displacement (0.026mg)	Tubes + fixative weighed prior to survey and after filling with sample. 4 replicates should be taken.
No instruction to add sample into the tube	Ensure sub sample is covered by fixative
Non standardized staining of slides for mackerel atresia	Staining of slides stained by agreed protocol following October 2006 workshop.
No exchange of atresia samples for mackerel in the Southern area	Fecundity and atresia samples from Southern and Western spawning components shared between all Institutes participating in the analysis

Samples for estimation of mackerel potential fecundity and atresia will be mostly taken on vessels participating in the egg survey or from commercial fishing vessels. Recognising the constraints of the egg survey, cruise leaders should try to distribute trawl stations across the whole survey area. Details on the numbers, timing and spatial coverage of the samples required will be provided by participants of each relevant WGMEGS, planning working group (e.g. tables 3.1.2 a-b, this report).

If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes in Table 10.1.2 below. In order not to concentrate the sampling on spawning fish it is preferable that trawling is not concentrated on the 200 metre depth contour but is adapted to fit in conveniently with the egg survey along the transects over the continental shelf. In 2007 Cefas will not be contributing towards the collection and analysis of mackerel fecundity and atresia so the samples will be redistributed to Norway, Scotland, and Spain. Ireland has been requested to take over allocation of samples that were

previously processed by Cefas. Details of preparation for fecundity sampling at sea are shown in Table 10.1.3.

Table 10.1.2. Weight classes for sampling females of maturity stages 2-6 (Walsh *et al.* 1990)for Potential fecundity and atresia.

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

Table 10.1.3. Protocol for processing and distribution of mackerel ovary sub-samples for either fecundity or atresia analysis, 2007.

Prior to cruise departure: **Norway (Merete Fonn)** will coordinate the analysis of mackerel fecundity samples and assign tube reference numbers to cruise leaders for labeling the eppendorf tubes used on their cruises.
Co-ordinators to assign unique codes to each participating cruise.
Procure Eppendorf type tubes and place in suitable racks.
Attach a spot label to the eppendorf lid and add 1.2 ml of 3.6% formaldehyde buffered with 0.1m sodium phosphate (referred to below as 'fixative') to each tube using a dispensor. The label should contain 3 alpha or numeric characters for a primary key in the fecundity database. Prepare 4 replicates for each tube label and colour the replicate white, red, blue and green respectively. Measure and record the weight of each tube including fixative (±0.0001 g) using the tube label code and colour for reference.
Procure sample bottles for the remaining ovary tissue should have parallel walls and without a restricted neck opening (otherwise we cannot extract the ovary without cutting of the jar top). The largest ovaries will require 250 ml sample bottles but in many cases a 100 ml or smaller capacity jar will be adequate. Label the bottle with the eppendorf code and cruise.
Procure 25-50 µl capillary pipettes and test performance of the pipette by taking 25 µl water samples and weighing the dispensed fluid.

Procedures to follow at sea to collect samples and for sample analysis in the laboratory are shown in Tables 10.1.4 and 10.1.5 respectively. In order to compare estimates of fecundity made by each country 100 samples should be analysed by all participants but, for the remainder, at least 2 of the quadruplicate samples should be analysed. Overall targets for estimating realized fecundity are shown in Table 10.1.6. Provisional reporting of estimates for potential fecundity and atresia are required for the 2007 Mackerel Horse Mackerel Working group in September and final results for WGMEGS in the spring of 2008. If the participants or fecundity coordinator are not certain of the data quality the concern should be passed on to the Working Group Coordinator (Finlay Burns).

Table 10.1.4. Adult mackerel sampling programme Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2007

MACKEREL SAMPLING

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

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

Area	Sampling by	Period/samples 1	2	3	4	5	total no. of samples	
Southern	POR/IPIMAR	40					40	
	ESP/IEO	80	20				100	
	ESP/AZTI			40			40	**180**
Western	ESP/AZTI	60		40			100	
	GER/BFA Fi	80	40				120	
	IRL/MI	120				100	220	
	ESP/IEO	20	60				80	
	SCO/FRS		100		60		160	
	NED/IMARES			60	60		120	
	NOR/IMR			40	20		60	**860**

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

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

c) remove ovaries ***undamaged!***, weigh ovaries take 4 parallel pipette samples of one ovary **(25 µl)** in pre-filled Eppendorf tubes and put the other ovary in formalin jars, weigh liver, guts (including contents) and carcass → ovary, liver, guts, carcass weight

d) send on one of every parallel sample to Vigo, Aberdeen, Bergen,Galway

histological analysis at IEO, FRS, IMR, MI → potential fecundity, atresia, realised fecundity

For the desired temporal and spatial distribution of the fecundity samples please refer to Table 3.1.2; for further instructions please refer to paragraphs 3.1.

Collection of samples for genetic population analysis (refering to 3.4)

a) Collect a larger sample of mackerel

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

c) Freeze 50 fish individually in plastic bags

d) Send the Eppendorf tubes and the frozen fish to IMR (Bergen)

Sample analysis targets for Ireland, Norway, Scotland and Spain participating in estimation of mackerel fecundity and atresia 2007. Each country carrying out the various is responsible for distributing their sample collection alternately to the countries carrying out the fecundity analysis. Norway will coordinate mackerel fecundity sample analysis in 2007.

Table 10.1.5. Processing ovary and pipette samples on return from sea.

After a minimum of 1 week fixation, cut cross-sections 4 mm thick from the ovary not previously sampled and place them in labelled histological cassette. The cassettes should be engraved with an indelible label corresponding to each replicate set of Eppendorf tubes. Cefas can provide engraved cassettes under contract but procurement locally would be more convenient.
Cover the cassettes with fixative or 70% ethanol and pack them in a leak proof bottle. Pack the consignments for each country with a maximum volume of 1000 ml solution in each package. On the outer cover of the package indicate the volume of fixative and that it is within the limits for unclassified transport. Retain the remaining ovary until analysis of data is completed at the 2008 WGMEGS.
Record weight of the Eppendorf tubes, fixative and added tissue 1 week and 4 weeks after return to estimate quantity of tissue taken by the pipette.

Table 10.1.6. Protocol for laboratory analysis of mackerel fecundity samples.

Tasks	Countries	Timing for work completion
Training coordinated by Cefas	England, Ireland, Norway, Scotland and Spain	October Workshop
Examine Eppendorf samples to identify and select pre-spawning fish based on the absence of spawning markers such as hydrated follicles or <5 POF type structures in the sample. Apply image analysis protocol based on the fecundity manual to determine fecundity (number of follicles >0.185mm) using the gravimetric method ((Hunter *et al.* 1989). The outputs from the image analysis macro should configured to fill all the fields in the Gravimetric sampling table of the fecundity database. The fecundity manual will be revised during the 2006 Workshop based on procedures developed during the 2004 survey. Ensure that at least 100 tube samples are analysed by all institutes for quality control and that each fish has at least 2 replicate fecundity estimates. Ovaries that have either commenced the annual spawning or are recently spent should be processed to estimate atresia below.	Ireland, Norway Scotland and Spain	Provisional results completed for 2007 Assessment Working in September. Completed results for WGMEGS 2008
Prepare resin sections from all mature fish identified as either in spawning or spent to determine the intensity and prevalence of atresia. Each Institute will process ¼ of the atresia samples.		
Determine atresia in mature fish identified as either spawning or spent above by Stereometric analysis using the protocol in the fecundity manual.Configure the macro used to process the atresia analysis results to complete all the columns in the histology table of the fecundity database.	All participating countries	

10.2 Sampling for horse mackerel fecundity in the Western area

Following the experience of the 2004 survey and discussion at the Vigo planning meeting, 2006 the following changes have been recommended for the 2007 survey. **In this context the Auto-diametric method, although useful where the fecundity sub-sample weight is not known, produces more variable fecundity data especially in the case of horse mackerel compared to the Gravimetric method (Hunter *et al.* 1989). The Working Group recommends that the latter technique is used for the 2007 survey.** All changes in the sampling protocol and methods between the 2004 and 2007 surveys are given in table 10.2.1

Table 10.2.1. Changes for 2007 compared to 2004.

2004	2007
Auto-diametric method (Thorsen and Kjesbu 2001)to estimate fecundity was unreliable for horse mackerel	Gravimetric fecundity (F) method (Hunter *et al.* 1989). F = O * C*S where O= ovary weight ± 0.1g, C=count of vitellogenic follicles in the sub-sample weight S (± 0.0001g)
Fecundity sub-sample weight assumed equivalent to pipette displacement (0.026mg)	Tubes + fixative weighed prior to survey and after filling with sample. 4 replicates should be taken
No instruction to add sample into the tube	Ensure sub sample is covered by fixative
Lipid content determined on whole body homogenate after solvent extraction and gravimetric determination of extracted fat carried out by all countries collecting horse mackerel	Fat content determined using a fat meter at IMARES. Fish sampled for fecundity (table 3.2.2) to be frozen and sent to IMARES (after consultation) for lipid analysis.
Lipid levels determined in the Southern and Western spawning components	Lipid levels determined in early maturing fish collected from commercial sources in October and November 2006 and from mature fish caught in the Western area surveys from March to July.
Standing stock of fecundity determined in fish selected as pre-spawning from collections made in the Southern and Western spawning areas	Standing stock of fecundity determined in mature fish collections made in the Southern and Western spawning areas Table 3.2.2 a-b by Ireland, Netherlands Norway and IEO Spain. This data will provide information on trends in ovary weight, batch fecundity, spawning fraction and residual standing stock of fecundity.

In 2007 horse mackerel will be collected from the Southern and Western spawning components selecting fish in maturity stages 3-6 fish > 25 cm collected on trawl hauls spread both temporally and spatially throughout the survey.

Protocols for the 2007 horse mackerel sampling preparations, sampling at sea and analysis in the laboratory and analysis are shown in tables 10.2.2-10.2.4 respectively. **Cindy Van Damme from the Netherlands** will coordinate the analysis of horse mackerel fecundity samples. 50 samples will be analysed by all 4 countries for quality assurance but at least 2 sub-samples should be analysed for all the remaining fish.

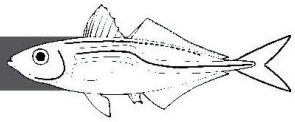
Table 10.2.2. Protocol for processing and distribution of mackerel ovary sub-samples for either fecundity or atresia analysis.

Prior to cruise departure: **Cindy Van Damme (Netherlands)** will coordinate the analysis of horse mackerel fecundity sample and assign tube reference numbers to cruise leaders for labeling the Eppendorf tubes used on their cruises.
Procure Eppendorf type tubes and place in suitable racks.
Attach a spot label to the Eppendorf lid and add 1.2 ml of 3.6% formaldehyde buffered with 0.1M sodium phosphate (referred to below as 'fixative') to each tube using a dispensor. The label should contain 3 alpha or numeric characters for a primary key in the fecundity database. Prepare 4 replicates for each tube label and colour the replicate red, blue and green respectively. Measure and record the weight of each tube including fixative (±0.0001 g) using the tube label code and colour for reference.
Procure 25-50 µl capillary pipettes and test performance of the pipette by taking 25 µl water samples and weighing the dispensed fluid.

Table 10.2.3. Flowchart for selecting and processing horse mackerel samples.

Mackerel and Horse Mackerel Egg Survey 2007

HORSE MACKEREL SAMPLING



1 Estimation of lipid content in pre-spawning fish

Market sampling

Area	Sampling by	Month/samples 10	11	12/06	01/07	02	03	total no. of samples
Western	NED	50	50	50	50	50	50	**300**

a) obtain length distribution of larger sample ----------► length distribution

b) (thaw if frozen), weigh (total), gut, select mature, pre-spawning females ----------► lenght, weight (total), sex, maturity

c) homogenize carcass and organs together, analyze fat content per dry weight ----------► fat content, dry weight

or (depending on availability)*

use a fat-meter to analyze the fat content ----------► fat content

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

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

Stock Comp.	Sampling by	Period/samples 1	2	3	4	5	total no. of samples	
Southern	POR/IPIMAR	40					**40**	
Western	ESP/AZTI	30		40			70	
	GER/BFA Fi	40	20				60	
	IRL/MI	60				50	110	
	ESP/IEO	50	90				140	
	SCO/FRS		50		30		80	
	NED/IMARES			30	30		60	
	NOR/IMR			20	10		30	**500**

a) obtain length distribution of larger sample (>100 fish) ----------► length distribution

b) measure, weigh (total), gut, select randomly mature females >= 25cm, weigh (gonad, carcass), determine stomach fullness ----------► lenght, weight (total, gonad, carcass, [gut]), sex, maturity (1-6, Walsh scale), stomach fullness (1-4: empty, filled, full, almost bursting)

c) take 4 parallel pipette samples of ovary **(25µl)** in pre-filled Eppendorf tubes

e) send on one of every parallel sample to Vigo, IJmuiden, Bergen, Galway

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

* *fish can be frozen (carcass and organs together!) between these two steps for further processing but keep in mind that pipette samples and frozen fish needs the same indication for later identification*

d) (thaw), homogenize carcass and organs together, analyze fat content per dry weight **at sampling lab** to avoid transfers (IPIMAR, IEO, MI, FRS, IMARES, IMR, BFAFi) ----► fat content, dry weight

or (depending on availability)*

send the frozen sample to IMARES for analyzing the samples with a fat-meter ----------► fat content

* will be clarified Oct 2006 (Fecundity Analysis Workshop)

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

Table 10.2.4. Protocol for Laboratory analysis of horse mackerel.

Tasks	Countries	Timing for work completion
Training coordinated by Cefas	Ireland, Netherlands Norway and IEO Spain	October Workshop
Examine Eppendorf samples to identify and note presence or absence of spawning markers such as hydrated follicles or <5 POF type structures in the sample. Apply image analysis protocol based on the fecundity manual to determine follicle size frequency distribution. The threshold to identify the standing stock of fecundity will be determined for the 2006 Fecundity Workshop. Use the using the gravimetric method ((Hunter *et al.* 1989). The fecundity manual will be revised during the 2006 Workshop based on procedures developed during the 2004 survey. Ensure that at least 100 tube samples are analysed by all Institutes for quality control and each fish has at least 2 replicate fecundity estimates.	Ireland, Netherlands Norway and IEO Spain All participating countries	Completed results for WGMEGS 2008

10.3 Methodology for taking samples from mackerel and horse mackerel ovaries

10.3.1 Use of a capillary pipette to take fecundity samples from horse mackerel or mackerel ovaries and associated equipment

Table 10.3.1. Details of equipment and suppliers.

Equipment	Catalogue reference	Supplier
Transferpettor capillary	307/5502/05	VWR International Dublin Critical Environment Business City west Business Campus Naas Road Dublin 22 Ireland Tel: ++3531 4660111 Fax: ++3531 4660380
Transferpettor capillary	307/5502/15	VMX as above
Eppendorf type tubes	LA-MCT-200-C	Biohit Ltd, Unit 1 Barton Hill Torquay, Devon, TQ2 8JG England Tel. O800 685 4631 email sales@biohit.demon.co.uk
Racks for tubes	LL-9200–0	Biohit above
Laser tough spots, 0.375"	SPOT-1000	Web Scientific Ltd, Business and Technology, Centre Radway Green Venture Park, Radway Green, Crewe, Cheshire CW2 5PR Tel +44 (0) 1270 875172Fax +44 (0) 1270 878186 Website www.webscientific.co.uk

Method

The capillary pipette will remove an ovary sample of standard weight CV 3% from a stage 3 to 5 ovary but not stage 6. In the case of Stage 4 running ovaries squeeze out all the loose eggs before taking the sample. In the case of stage 6 ovaries take a small piece with forceps from the centre of the ovary similar to that removed by the pipette. Repeat for each of the tube replicates.

Operation

- In the case of mackerel take the replicate samples out of the rear half of one of the ovaries leaving the remaining ovary intact for taking histology samples after fixing for 1 week.
- Make a small hole in the ovary tunica
- Depress the piston to the bottom of the capillary
- Push the tool through the hole in the ovary into the centre of ovary
- With the pipette end held within the ovary pull the plunger wire out of the tube until the base of the piston reaches the first blue line on the capillary (see below).
- Push the sample out of the capillary into a 2.5 ml Eppendorf tube containing 1.2 ml 3.6 % formaldehyde buffered with 0.1 M sodium phosphate.
- Take 3 more replicate samples as above
- After each station wash the capillary and piston.
- Place the other unsampled ovary in a bottle for atresia estimation (mackerel only).

The Piston can be used 300 + times but eventually piston ware causes a drop in suction power and it must cut off and replaced by pushing the plunger wire into a new piston held in the assembly plate. The amount of sample can be controlled by the distance the piston is pulled up the capillary tube. A second blue line indicates the distance to pull out the piston for twice the standard sample volume.

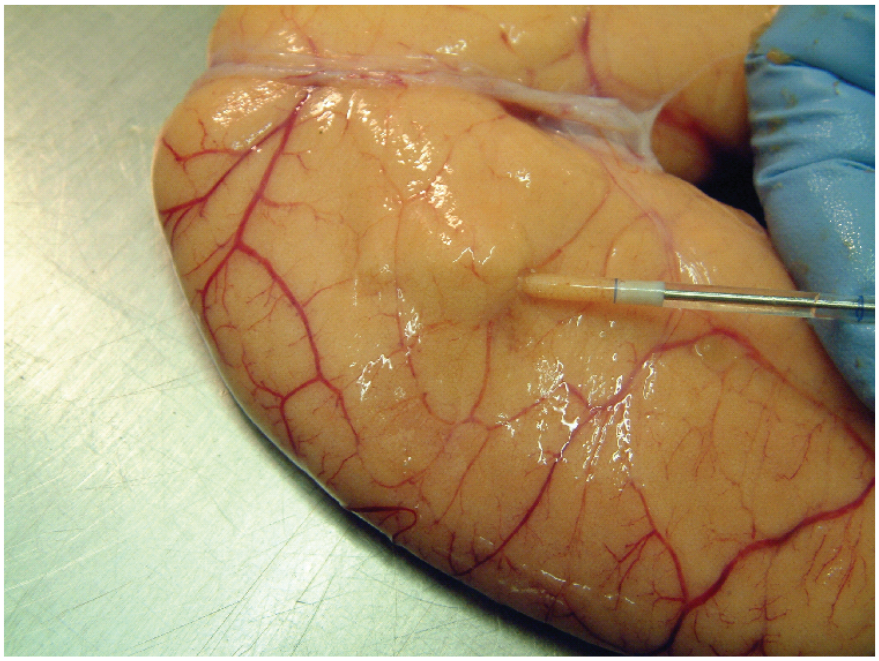


Figure 10.3.1. Method to use a capillary pipette to remove an ovary sample.

Push the plunger to the bottom of the glass tube and then push the tube into the hole previously made in the tunica. Pull up the plunger until the **sample reaches the lowest line** on the glass pipette (see figure 10.3.1). This will provide a sample of 26 mg of tissue. Ensure there are no air pockets in the sample sucked from the ovary and

that it is expelled into the 3.6% formaldehyde solution held in the tube. Ovaries that are nearly spent will not readily provide samples and in these cases use forceps to remove a similar sized sample from the centre of the ovary. Before the cruise ensure operators are familiar with the pipette operation by dispensing water into a container weighed to ±0.0001g

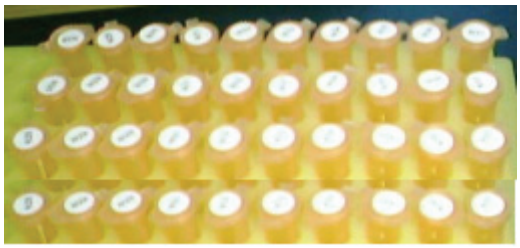


Figure 10.3.2. Picture of a rack holding Eppendorf like tubes for 10 fish with 3 replicates identified by spot labels on the lids. During storage a lid fits on top of the rack to keep the tubes in order during transport.

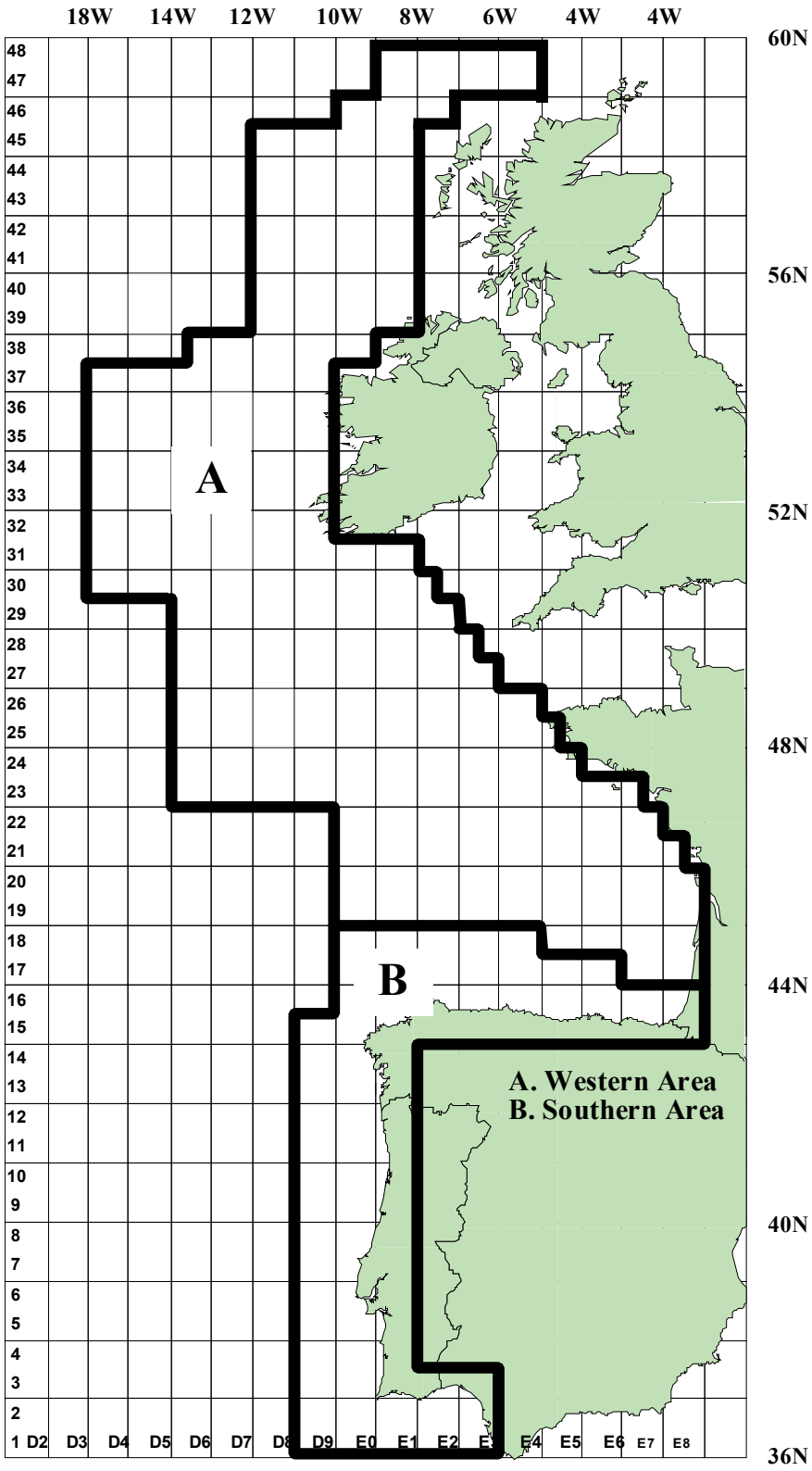


Figure 1. Core sampling areas for mackerel and horse mackerel eggs in the western and southern areas for 2004. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines.

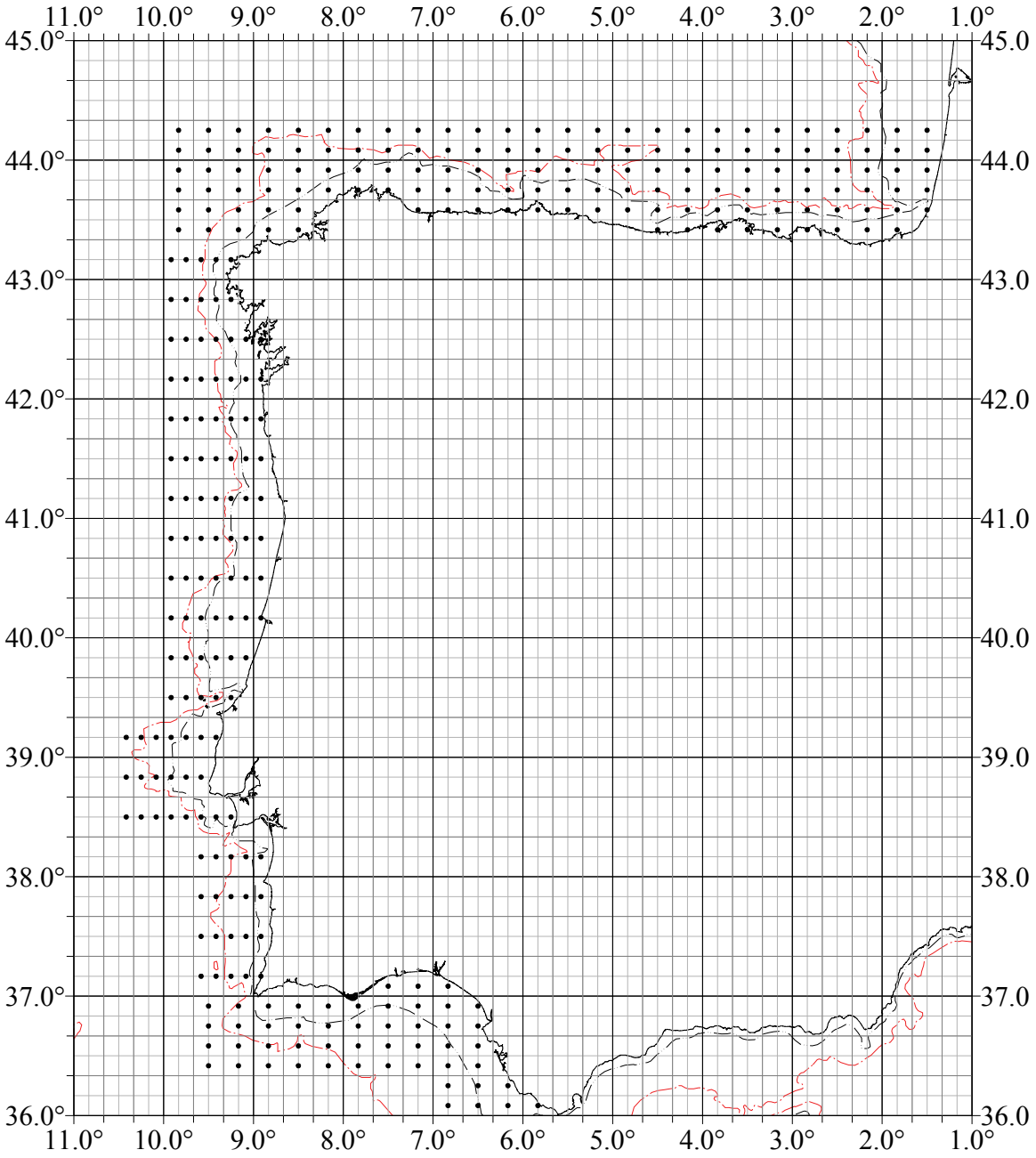
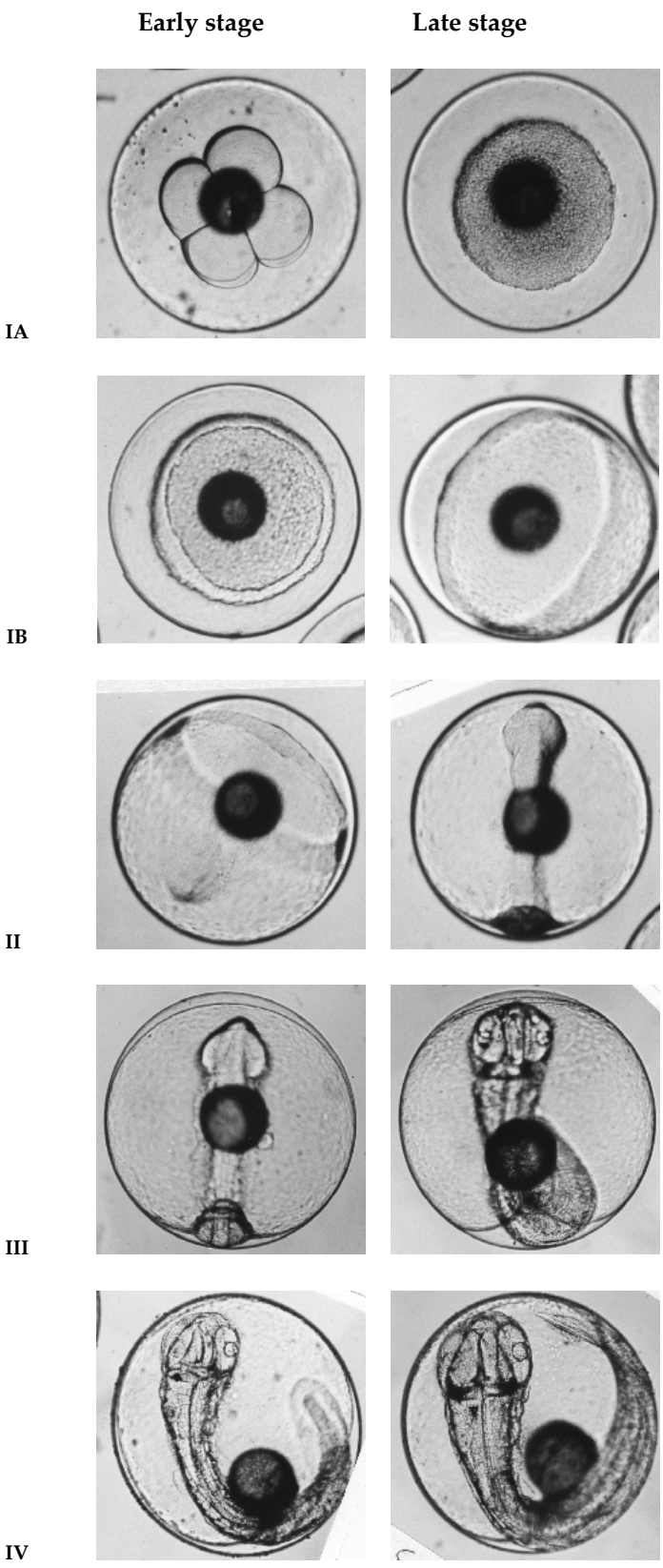


Figure 2. Provisional station location for mackerel and horse mackerel egg surveys in the southern area in 2004. Offshore boundaries will be based on two consecutive zero rectangles.


Early stage
Late stage
IA
IB
II
III
IV

V

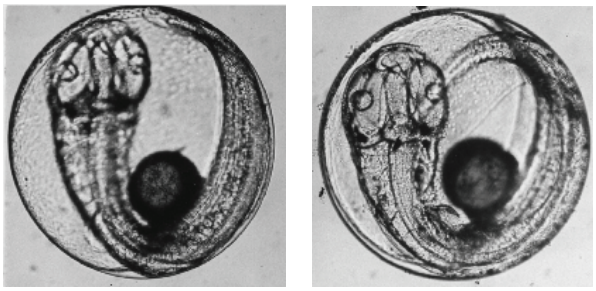


Figure 3. Mackerel eggs at the beginning and end of the six development stages.