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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. The meetings are held in the years before and after the surveys themselves, the WG works by correspondence in the survey years themselves. The main activity for this meeting was the reporting and analysis of the 2004 survey. The terms of reference and the outcomes were as follows:

a) analyse and evaluate the results of the 2004 mackerel and horse mackerel egg surveys of the western and southern areas;

The 2004 surveys were carried out according to the plan laid out in the 2004 report of WGMEGS, and were modified and adapted by the survey coordinators during the surveys themselves. Within the periods chosen for the surveyed, the spatial and temporal coverage was generally good, although there were some periods where additional sampling would have been helpful – particularly the Cantabrian Sea and the western area south of 52°N in period 2, and across the western area in period 7. In general, sampling appeared to cover the bulk of the spatial range of both mackerel and horse mackerel spawning, and reached zero samples along most of the edges of the distribution.

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

- Total annual egg production for mackerel in the western area in 2004 was calculated as 1.2018×10^{15} with a standard error of 0.10947×10^{15}. This can be compared to the 1.209×10^{15} in 2001.
- Total annual egg production for mackerel in the southern area in 2004 was calculated as 0.126×10^{15} with a standard error of 0.0235×10^{15}. This can be compared to the 0.283×10^{15} in 2001.
- Total annual egg production for horse mackerel in the western area in 2004 was calculated as 0.678×10^{15} with a standard error of 0.150×10^{15}. This can be compared to the 0.684×10^{15} in 2001.
- Total annual egg production for horse mackerel in the southern area in 2004 was calculated as 0.248×10^{15} with a standard error of 0.121×10^{15}. This can be compared to the 0.171×10^{15} in 2001.

Recent work has indicated that the geographical split between southern and western horse mackerel should change, placing Division VIIIc in the western area. New time series of egg production were calculated based on this change up to and including 2004, and included in the report.

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

WGMEGS set up a detailed adult sampling scheme for fecundity in both species and for atresia in mackerel. Western mackerel fecundity samples were collected between 48° and 53°N, the main area of spawning, during periods 3 and 4 – the start of spawning in this area. Southern samples were collected on the Cantabrian coast during period 1. Unlike previous years the samples were collected in triplicate from each fish and then divided between analysis groups, allowing a detailed examination of variation, within and between institutes and areas and times. The calculated potential fecundity for the western component was 1127 (se 27) eggs per gram female compared to 1097 (se 23) eggs per gram female reported in 2001.

The overall prevalence of atresia in the western component as a percentage of the population was 28% and the relative intensity was 33.5 eggs per gram. This reduced the potential fecundity by 7% giving a realised fecundity was 1052 eggs per g female.

The overall prevalence of atresia in the southern component as a percentage of the population was 6% and the relative intensity was 105 eggs per gram. This reduced the potential fecundity by 5% giving a realised fecundity was 964 eggs per g female.

Horse mackerel fecundity remained difficult to determine in the early part of spawning it was calculated at 215 eggs per gram female rising to a maximum of 1152 eggs per g female by the time of peak spawning. It is not possible currently to use this estimate to provide a realistic estimate of the spawning biomass

d) evaluate the results of studies on horse mackerel fecundity determination and proxies on the basis of data collected during the 2004 surveys and in other relevant work;

WGMEGS identified two candidate proxies for fecundity in horse mackerel that may have had value in providing a biomass estimate. These were feeding state and lipid content. In order to assess energy intake the stomach content of the horse mackerel was monitored throughout the spawning season. However, results showed no evidence of feeding during spawning and there was no sign of regurgitation, indicating that this could not be used as a proxy. Large numbers of fish were collected and frozen for analysis of total lipid content. The results of this analysis showed a considerable variation in both fecundity and lipid content during the spawning season. These results suggest that it is not currently possible to derive an index to convert egg production into SSB in this species.

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

Based on the total egg production, fecundity and atresia data given above, the analysis gave an estimate of western component spawning stock biomass for 2004 of 2.468 million tonnes, with a variance of approximately 723,500 tonnes. The equivalent value for the southern spawning component was 280,300 tonnes with a variance of 70,900 tonnes.

f) evaluate the quality and reliability of the 2004 survey in the light of the previous surveys.

In general the quality and reliability of the surveys was good. There was a reduction in survey effort in 2004 compared to 2001, when additional EU funding was made available. This led to a small increase in the variance in the estimate of the egg production. The fecundity sampling was considerably improved. The deployment of the new Gilsons free methodology made it possible to collect large numbers of good quality samples for both fecundity and atresia. The triplication and analysis in a range of laboratories improved the reliability of the estimate, which was broadly similar to that in 1998 and 2001.

As in 2000 the WG held an egg identification and staging workshop prior to the surveys. This meant that these aspects of the analysis were as consistent as possible across the participating institutes. The workshop was also expanded to include fecundity estimation and procedure. Both activities led to an improvement in the quality of the estimate.

Some aspects of the area coverage were weaker than in previous years, notably in the Cantabrian Sea, and in the western area in the final period. This will have resulted in the estimate being very slightly negatively biased.

It was discovered that there some small differences in the operation of the egg sampling procedure on the surveys themselves. These differences were small and were not believed to have

had any significant impact on the estimate. Notwithstanding this the Survey Manual will be reviewed in 2005 and every effort will be made to harmonise sampling protocols.

1 Introduction

1.1 Terms of Reference

At the ICES Annual Science Conference in Vigo, Spain, in September 2004 it was decided that (C.Res. 2004/2G07) the Working Group on Mackerel and Horse Mackerel Egg Surveys [WGMEGS] (Chair: D. Reid, UK) will meet in Bergen, Norway, 4–8 April 2005 to:

a) analyse and evaluate the results of the 2004 mackerel and horse mackerel egg surveys of the western and southern areas;

b) calculate the total seasonal Stage 1 egg production estimates for mackerel and horse mackerel separately for the western and southern areas;

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

d) evaluate the results of studies on horse mackerel fecundity determination and proxies on the basis of data collected during the 2004 surveys and in other relevant work;

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

f) evaluate the quality and reliability of the 2004 survey in the light of the previous surveys.

WGMEGS will report by 1 June 2005 for the attention of the Living Resources and the Resource Management Committees.

1.2 Participants

A list of participants can be found in Annex 1 of this report.

2 General aspects

2.1 Summary of WGMEGS activities in 2003 and 2004

WGMEGS met in Lisbon 1–4 April 2003 to plan the ICES Triennial Mackerel and Horse Mackerel Egg Survey in 2004. The report was published as ICES CM 2003/G:07 and presented to the joint session of LRC and RMC at the ASC in Tallinn, Estonia in September 2003.

A Workshop on Mackerel and Horse Mackerel Egg Staging and Identification was held from 20–25 October 2003 at CEFAS, Lowestoft, England. Details of the workshop are presented below in section. The report was published as ICES CM 2004/G:01 and presented to the joint session of LRC and RMC at the ASC in Vigo, Spain in September 2004.

The surveys were carried out from January to July 2004 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 (D. Reid, Scotland) and southern areas (C. Franco, Spain). WGMEGS prepared a report by correspondence summarising this process (ICES CM 2004/G:10). Survey data (egg abundances and ancillary data plus preliminary fecundity and atresia estimates) were collated in August 2004 and spawning stock biomass information presented for use in the annual assessment to the September meeting of WGMHSA in Copenhagen, (ICES CM 2005/ACFM:08). This was the first time that the survey estimate was available 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. However, a small-scale plankton sample exchange programme, carried out after the 2001 survey, showed that there may also have been some problems in the identification of eggs to species (WD Milligan and Shaw, ICES, 2003). It was therefore proposed to extend the scope of the 2003 workshop (prior to the 2004 survey) to address all aspects plankton analysis, including removal of eggs from the samples, identification and 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.

2.2.2 Results and recommendations from WKMHMES 2003

Egg sorting

Following the problems encountered with the plankton sample exchange (WD Milligan and Shaw, ICES, 2003) and in an attempt to standardise the egg sorting procedure, a 'new' mechanical method for effectively removing fish eggs from plankton samples was devised by Dr A Eltink of RIVO-DLO, Netherlands. The development of the 'Spray technique' would also make this task less time-consuming and less prone to human error. This technique was fully evaluated at WKMHMES. 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 2004 triennial surveys.

Egg identification

The identification of mackerel and horse mackerel eggs was also considered to be a potential problem following the plankton sample exchange in 2001/02. Consequently, a literature review was conducted during WKMHMES in 2003 and a table was produced summarising published descriptions of mackerel, horse mackerel and other species of eggs with similar morphological features. In addition, photographs of mackerel and horse mackerel eggs (from artificial fertilisations) were produced, with a view to aiding egg identification. WKMHMES also recommended that further Quality Assurance exercises were conducted during the 2004 sampling season. In order to address this issue and check on the consistency of egg identification between participants, an egg measuring exercise was conducted during 2004 (Annex 2). The results show that there is very little overlap in the egg diameters of mackerel and horse mackerel and it is therefore unlikely that mis-identification of eggs is a significant source of error for these surveys.

Egg staging

WKMHMES also explored the potential problem of mis-allocation of eggs to the various development stages. The results were very reassuring, showing that there was 94% agreement between participants in the allocation of mackerel eggs to Stage 1 (1a + 1b combined) and 97% agreement in the allocation of horse mackerel eggs to Stage 1. Further clarification of stage descriptions was also produced to help analysts allocate eggs to stage from samples collected on the 2004 survey.

Recommendations and Terms of Reference

WGMEGS recommends that the next meeting of WKMHMES (Chair: S. Milligan), should take place at CEFAS, Lowestoft, during October 2006, with the following terms of reference:

a) To review the results of the egg measuring exercise conducted in 2004 and to better define the morphological differences between mackerel and horse mackerel eggs.

b) To review available documentation on identifying fish eggs to species and define standard protocols.

c) To review any information available on other egg identification procedures – particularly DNA probes.

d) To review the effectiveness of the 'Spray technique' for removing eggs from plankton samples and to define standard procedures.

e) To carry out a comparative egg staging trial following the pattern used in the 2003 egg-staging workshop.

2.2.3 Report of WKMHMF (Workshop on mackerel and horse mackerel Fecundity Lowestoft October 2003)

In order to implement the new fecundity protocols described for mackerel in ICES 2003 a workshop was held in Lowestoft immediately following WKMHMES (see report in Annex 3) The aims of this workshop were to demonstrate all of the equipment and protocols required to carry out both sampling at sea and fecundity determination using the Gravimetric (Hunter *et al*., 1989) and Auto-diametric (Thorsen and Kjesbu 2001) methods in both mackerel and horse mackerel. An additional aim of the Work shop was to inter-calibrate the Stereometric fecundity method used prior to 2001 for horse mackerel with the Gravimetric method. A provisional conclusion was that including developing eggs larger than 0.185 mm would give equivalent fecundity estimates irrespective of the method used. However in the course of the inter-calibration exercise differences in horse mackerel fecundity were found depending on which Country completed the stereological analysis. Independent evidence based on the morphology of follicles indicated that previtellogenic follicles were smaller than 0.185 mm (the same determined for mackerel reported previously (ICES, 2002) and follicles comprising the fecundity were larger than 0.185mm. It was recommended that further inter-calibration work was carried out and that each Institute should provide an Auto-diametric model to determine fecundity.

2.3 Summary of the egg measuring exercise

The full report of the egg measuring exercise is given at Annex 3 and an abstract is given in Section 11 of this report. This exercise was conducted to fulfil a recommendation of WKMHMES, 2004 and was designed to help analysts distinguish between mackerel and horse mackerel eggs. A total of 9,400 mackerel and 5,600 horse mackerel eggs and oil globule diameters were measured by all the participants who took part in the 2004 survey during periods 3–7. The results show very similar egg and oil globule sizes for each participant and only slight decreases in egg diameters during the spawning season. The results also show that there

is very little overlap in the egg diameters of mackerel and horse mackerel and it is therefore unlikely that mis-identification of eggs is a significant source of error for these surveys.

It is recommended that future measuring exercises utilise eggs from artificial fertilisations (or natural spawning by captive fish) to ensure that the eggs are definitely from either mackerel or horse mackerel. It is also recommended that participants use image analysis systems to measure egg and oil globule diameters to ensure consistency in egg measurement and for producing the greatest possible resolution to the data.

2.4 Absolute versus relative: comments from WGMEGS

In its 2004 October meeting ACFM modified the NEA mackerel assessment from that of the assessment WG (WGMHSA), by using the mackerel egg survey based SSB estimates as relative instead of absolute. This resulted in a very different perception of the stock dynamics where the SSB in 2003 was estimated to be 40% lower than estimated in the previous year and in a substantially higher fishing mortality.

The rationale put forward by ACFM for this was: *The estimate of SSB from the 2004 egg survey is the lowest in the series (since 1992). With the new SSB estimate for 2004, there is a downward trend in the SSB over the time period covered by the series. The estimates of SSB derived from the catch data throughout the 1990s were considerably lower than the survey estimates. In previous assessments, the survey estimates have been treated as absolute measures of SSB. In order to reproduce the trend in the survey estimates in the assessment, the survey data have to be interpreted as a relative index. This implies that the survey data are considered to be overestimates and the SSB estimates derived from the catches are taken as absolute. SSB estimates are normally used as relative estimates in assessments. This revision has led to a substantial change in the perception of the recent history of the stock. The previous rationale for using SSB estimates from egg surveys as absolute was based on the experience with the western mackerel stock component which suggested catchability close to unity. This allowed the stock to be assessed with the short time series available for North East Atlantic mackerel (four estimates or less).*

The ACFM report does not discuss or give any indications of why the egg surveys might significantly overestimate the SSB. WGMEGS have always considered that the egg production estimates, from which the SSB is derived, were likely to be *underestimated*. This is firstly because the total spawning area and season is probably not completely covered during the different surveys. Secondly, and probably more importantly, the egg production estimate is not adjusted for egg mortality in the 1A and 1B stages used to derive biomass. An analysis carried out by Portilla for this group (WD 2005) indicates that this mortality is in the order of 30%, and would lead to a corresponding underestimate of the biomass. Furthermore, an additional study by Mendiola and Alvarez (WD 2005) indicated a faster egg development time than that used in the calculation of egg production by the WGMEGS. This was calculated to lead to an underestimate of the egg production by between 7 and 12%. The study was carried out on mackerel from the southern spawning component, and a replication of this study in the western component would be desirable. However these two studies indicate that the egg production might be underestimated by as much as 40%.

A possible source of overestimation might be the estimates of fecundity and atresia used to convert egg production to mackerel SSB. However, this aspect of the work has been given considerable attention in the more recent surveys. This has led to an increase in the sampling, and the spatio-temporal spread of those samples, as well as improved and more accurate methodologies and QA procedures. It is the opinion of the WGMEGS that these parameters have been accurately estimated, although with some variance. Between 1995 and 1998 there was a substantial change in the realised fecundity for this species, from 1302 to 1002 eggs.g.female^{-1}, with resulting changes in calculated biomass. Lower fecundity leads to a

higher biomass for a given egg production. The egg production calculated in 1998 (1.37×10^{15}) was lower than 1995 (1.49×10^{15}) but the lower fecundity led to a higher biomass estimate (2.95 m tonnes in 1998 compared to 2.47 in 1995). As this was also the highest biomass estimate in recent years, there was a popular perception that the biomass estimate was too high in this, and possibly other years. Recent work (Slotte WD WGMHSA 2003) has indicated that the fecundity change may be explained by lower condition factor in the previous autumn. Lower condition at this time might be expected to lead to a reduced scale of development of eggs and a concomitant reduction in potential fecundity in the following year. It should be noted that the realised fecundity has stayed low since 1998, providing further confirmation that the change was real.

Therefore WGMEGS still considers the present egg survey based SSB estimates as likely underestimates.

2.5 Potential uses of additional egg survey in interim year

The triennial Spawning Stock Biomass (SSB) survey of the North East Atlantic (NEA) mackerel stock provides an essential source of information to estimate the current level of stock biomass and fishing mortality with the Integrated Catch at Age method (ICA). The Mackerel horse mackerel sardine and anchovy working group (WGMHMSA) acknowledges that a three years time interval between the surveys has implications on the precision with which the status of the stock is estimated: the accuracy of the estimation of the fundamental variables used for management is best during the year of the SSB survey and decreases the further away from this measurement the stock assessment is performed.

An additional survey to be performed in years other than the triennial sequence currently employed should assist this situation and improve the assessment of this stock, in particular in years when no survey data are available. To help designing this additional survey, we conducted a simulation study to determine the most beneficial timing for an additional survey as well as to investigate the range of precision on the estimation of the SSB index of mackerel that would provide improved assessment of the stock.

The results show that an additional survey (AS) with better or slightly poorer precision will improve our knowledge of the status of the stock in all years and particularly in the year of the AS survey. The benefit for stock assessment of gathering additional information on the NEA mackerel SSB using an AS with poorer precision depends on timing of the assessment. Availability of such information during the year the further away from the current survey (CS), when no other SSB estimation is available (1 gap year between surveys), provides a more precise characterisation of the stock: a noisy source of information, up to 4.5 time as uncertain as the CS, provides better estimation of fishing mortality, SSB and TSB than no information at all. The other cases presented in this article, namely the assessment of the stock performed the year after the CS or the year of the CS, showed that large variability of the AS produces more uncertain estimation of the stock status unless the precision of the additional survey is known and incorporated into the model. WD.

2.6 INDICES recommendation and survey data inventory

INDICES Recommendation

The WG noted the successful outcome of the EU funded project INDICES to use the samples collected in 1998 to study egg and larval abundance distribution for a wider range of species. Therefore, the working group recommends that the plankton samples collected during the 2004 egg survey should be further analysed in the same way in order to obtain maximum value from international egg surveys. Continuing from the work carried out under the INDICES project, target species for further analysis should be mackerel and horse mackerel larvae

as well as eggs and larvae of sardine, anchovy, hake, megrim and blue whiting. Ireland and Spain (and possibly other potential partners) have included the work under their national programs of the EC data directive and subject to funding, plan to analyse plankton samples from the whole survey area and for all periods. The working group recognises the need for financial support for this work under EU provisions.

Data inventory for WGMEGS

The working group acknowledged the fact that the historical data collected during the past mackerel and horse mackerel egg surveys is invaluable for long-term ecosystem studies. Data collected in the past have not only provided egg abundances of mackerel and horse mackerel, but also provided samples for egg and larval studies on hake, sardines, anchovies and other species in a number of projects including SEFOS, INDICES and SEAMAR. In order to continue the application of this data, the working group decided to carry out an inventory of the historical data collected during past surveys before the next planning meeting. The inventory will be divided into two sections:

- A sample inventory, which describes the location and status of historical plankton samples with details on location and timing of collection, status of egg sorting, status of larval sorting and status of any additional specimen removed e.g., zooplankton.

- A data inventory that describes the details of historical data collection with time and location of data collection, details of environmental data collected in the horizontal and/or vertical dimension, biological data collected in terms of species, type of life stage (egg or larvae), size measurement and developmental categories.

3 North Sea egg survey 2005

3.1 Countries and Ships participating

Until 1990 egg surveys in the North Sea were carried out usually every second year. Since then surveys were carried out in 1996 (ICES, 1997), in 1999 (ICES, 2000a) and 2002 (ICES, 2003). Based on these surveys the SSB was estimated at 78000 tonnes in 1990 (Iversen *et. al*., 1991), 110,000, 68,000, and 210,000 tonnes in 1996, 1999 and 2002 respectively.

As in 1999 and 2002 the Netherlands and Norway will carry out a mackerel egg survey in the North Sea in 2005. The total survey period, 6 June–3 July, will not cover the total spawning period. However, historically the main spawning period has been observed about mid June, and will therefore probably also be covered during the survey period in 2005. In 1996, 1999 and 2002 two vessels carried out the egg survey by covering the area three times in a three week period. In order to ensure that the peak of spawning will be detected, this sampling strategy is changed. In 2005 the spawning area will be covered four times with the same amount of available ship time (see Table 3.1.1.). One vessel will cover the whole North Sea spawning area in the first week; two vessels will cover this area in the second and again in the third week. In the fourth week again one vessel covers the whole area. The planned deployment of research vessel effort is given below:

The first and fourth coverage will be restricted due to survey time, but will include the main part. R/V “Tridens” will break for the first weekend in Aberdeen and the next one in IJmuiden. R/V “Johan Hjort” will have a break in Stavanger 19–20 June.

3.2 Sampling area and survey design

Based on the results from the later surveys the suggested area to be covered in 2005 is given in the above text table.

During the second and third coverage's R/V "Tridens" will start in the south working northwards and R/V "Johan Hjort" will start in the north working southwards. The survey grid during the second, third and fourth coverage's 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.

Plankton samples will be collected in the middle of half ICES rectangles. The Netherlands and Norway will use a Gulf 7 towed in double oblique hauls with a towing speed of 5 knots. Both vessels will apply a net with a mesh size of 500 microns, as nets with smaller mesh size will easily become clogged.

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. The volume of seawater filtered on each of the plankton stations should also be recorded. Thereby the number of mackerel eggs produced per m^2 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 WGMHSA meeting in September 2005. The final results will be reported to the next WGMEGS meeting in 2006.

3.4 Biological sampling of mackerel

Norway and Netherlands will collect mackerel samples from pelagic trawl hauls for the estimation of the age composition of the North Sea mackerel as well as the estimation of the mean weights at age, which are needed for assessment purposes (mean stock weights at age of North Sea mackerel are needed for the estimation of the stock weights of NEA mackerel).

3.5 Fecundity and atresia

A small scale fecundity and atresia study is planned to be carried out by Norway during this season. The intention is to investigate 50 ovaries for potential fecundity and 50 ovaries for atresia. The samples will be taken, handled and analysed as described in ICES (2003 G:07). However, since there are hardly any mackerel fisheries going on in the North Sea during May it might be difficult to collect ovaries in a pre-spawning state for the fecundity estimation. If there are surveys in east part of the North Sea in May-early June this year the WG recommends that they should try to provide samples for potential fecundity studies of North Sea mackerel.

The ovaries for atresia studies should be collected during the whole survey period by collecting 12–13 ovaries per coverage.

Table 3.1.1: Timings and areas for North Sea mackerel egg survey in 2006.

VESSEL/COVERAGE	PERIOD			
	1	2	3	4
R/V "Tridens"	6–11 June	13–16 June	20–24 June	-
R/V "Johan Hjort"	-	13–19 June	20–26 June	27 June–3 July
Suggested area to be covered	54.30–57.30°N 1°W–2°E	54–57.30°N 1°W–3°E	54–58°N 1°W–4°E	54–58°N 1°W–4°E

4 Western and southern egg surveys in 2004

4.1 Countries and ships participating

As for previous surveys, the 2004 mackerel and horse mackerel egg survey was designed to cover the whole spawning area of the two species within 7 sampling periods of differing geographical coverage (Table 3.1, ICES, 2003). The deployment of research vessel effort in 2004 in the western mackerel and horse mackerel sampling area is given in Table 4.1.1. Table 4.1.2 shows research vessel effort for the southern area in 2004. A total of 208 ship days were invested in the western area survey in 2004, which was a decrease of 36 ship days (15 %) compared to the 2001 survey. A total of 83 ship days were invested in the southern area survey in 2004, which was a decrease of 55 days (40%) compared to the 2001 survey. However, a total of 291 ship days were invested in the complete 2004 mackerel and horse mackerel egg survey, which is a slight increase on the number of ship days employed during the 1998 survey (275 days). The increased number of ship days available in 2001 was exceptional and was only possible due to additional financial support from the EU (ICES, 2002).

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 is presented in Figures 4.2.1.a-f. It should be noted that the rectangles in the western area and in Div IXa are 30' north-south, and 30' east-west. In area VIIIc and in the Gulf of Cadiz, IXa are 15' north-south, and 1° east-west. The figures also include those rectangles where egg production was calculated by interpolation from neighbouring, sampled, rectangles. As for previous surveys, the 2004 Mackerel and Horse Mackerel Egg Surveys were designed to cover the area within seven sampling periods of differing geographical coverage, allowing full coverage of the expected spawning area and season. In periods 1 and 2 only the western and southern seaboard of the Iberian Peninsula were surveyed. In period 3 it was planned to cover the entire southern area, plus the western area as far north as 58°N. In period 4, the Galician and Cantabrian Sea areas were surveyed as well as the western area to 60°N. In period 5 the surveys covered the Cantabrian Sea and the western area to 61°N. In period 6 the surveys were restricted to the western area between 47 and 61°N. In period 7 the surveys were restricted to the western area between 48° 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 the Cantabrian Sea and the western area south of 52°N in period 2, and across the western area in period 7. In general, sampling appeared to cover the bulk of the spatial range of both mackerel and horse mackerel spawning, and reached zero samples along most of the edges of the distribution. Slight exceptions to this were seen in;

- Period 1 – Sampling for this period was planned to cover the area from Gibralter to 42°N on the Portuguese coast. Coverage was good, with a small number of interpolated rectangles. All rectangles were sampled at least twice.
- Period 2 – Sampling for this period was planned to cover the area from the Gulf of Cadiz to 43°N on the Galician coast. There were some interpolated samples in the middle of the Portuguese coast around 41°N. Most rectangles were sampled at least twice.
- Period 3 – This was the first period where sampling was planned beyond the west Iberian coast, to include the western shelf to 62°N. The area between 38 and 42° was not sampled due to bad weather. There were very few interpolated samples. Most rectangles on the north Spanish coast were sampled more than once and good numbers of rectangles across the rest of the area.

- Period 4 – Sampling in this period was planned from 42 to 60°N and did not include the Portuguese coast. A small number of interpolated mackerel samples were required, mostly in the SE corner of the Bay of Biscay. One transect at 62° 15'N was unsampled and was filled in by interpolation. Again most rectangles in the Cantabrian Sea and the southern part of Biscay were sampled more than once. In the western area, there were only small numbers of rectangles sampled twice.
- Period 5 – Sampling in this period was planned from 43 to 61°N and again did not include the Portuguese coast. There were a small number of interpolations scattered across the area, particularly in Biscay and west of Scotland. Only small numbers of rectangles were sampled more than once, mainly in Biscay and west of Scotland.
- Period 6 – Sampling in this period was planned from 47 to 62°N and did not include the southern area or the southern part of Biscay. There was slightly more interpolation in this period but mostly scattered and at the periphery. Only four rectangles in the Celtic Sea were sampled more than once.
- Period 7 – Due to lack of ship time, sampling in this period was restricted to the area from 48° 30' to 55°N, believed to be the main spawning area at this time. Three transects were interpolated from adjacent transects. Sampling was at one station per rectangle throughout.

4.3 Sampling and data analysis

As in the previous survey, the 2004 survey was carried out in accordance with the modified sampling strategy described in detail for the 1995 survey (ICES, 1996; 1997). An appraisal of how this method has been developed and applied was presented in the 2003 report of this WG (ICES, 2003).

4.3.1 Sampling strategy (Southern area)

The sampling rectangle design in the south has been modified from that used in previous surveys. Effectively, the stations have been placed closer in the onshore/offshore direction and further apart in the alongshore direction. This means 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 1° east-west.

Otherwise, sampling protocols remained as standard.

4.4 Replicate sampling

The estimation of mackerel and horse-mackerel biomass from the egg surveys based on the assumption that the number of eggs spawned per year by females (Total Annual egg Production) is directly proportional to their biomass. Currently no allowance is made for any egg mortality between spawning and when the eggs are actually sampled. Recent work on mortality rates using egg survey data collected on standard survey designs using birth-death models has suggested, however, that mortality rates are considerable (WD-2 Portilla *et al.*). In order to enhance our confidence in the mortality rates we were estimating, an intensive period of sampling was undertaken during the 2004 egg survey. Sampling took place over 24 hours in one location at a high rate following the same sampling strategy as that used to estimate Total Annual Egg production on the standard survey. Using these data it was possible to quantify mortality rates more accurately with average values for mackerel of 1.12 d^{-1}. Mortality rates showed a diel variation within the 24h period possibly related to sunlight variation and average age of the samples. Nevertheless, the values were higher, but of the same order of magnitude, than that of previous estimates (0.0.55 d^{-1}. Estimates of egg mortality carried out by the traditional method also provided lower estimates of mortality (0.4d^{-1}) than those obtained with the birth-death models. For horse mackerel, egg mortality using birth death models was computed by bootstrapping the available samples. Estimate of mortality was higher (1.63 d^{-1}) than

that obtained by the traditional method (1.17 d^{-1}). The spatial and temporal patterns of egg mortality rates and the implications for absolute abundance estimation will continue to be investigated.

4.5 Sampling gears and procedure

In the western area plankton sampling was carried out using national versions of a Gulf III or Gulf VII type samplers with the exception of Spain which used a Bongo sampler. Each Gulf III or 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 5 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 Portugal and Spain. The samplers were again 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.

4.6 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; 1997). The detailed steps of the data analysis were also updated for the 2003 WGMEGS report (ICES, 2003). For all sampling in the western area, individual countries supplied data in an electronic database 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 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). In the western area standard errors were calculated for both mackerel (s.e. 0.1095×10^{15} corresponding to a CV of 9.1%) and horse mackerel (s.e. 0.1503×10^{15} corresponding to a CV of 22.2%). Replicate rectangle samples were taken mainly in periods 3 and 4, with a small number in period 5. For both species, the coefficient of variation σ were estimated by the residual standard deviation from an analysis of variance of log (stage I eggs/m2/day) by rectangle (ICES, 1996). The estimated σ values were then used to estimate variance, standard deviation and CV.

In 2004, replicate rectangle samples were taken mainly in periods 3 and 4 in the southern area. In these periods, for both species the square of the coefficient of variation (CV^2) were estimated by the mean residual squared error from an analysis of variance of log (stage I eggs/m^2/day) by rectangle, as they are analogous as can be proved by the Delta method. To avoid the influence of zero egg counts, any rectangles with any zero counts were excluded. The estimated CV values for period 3 (1.43 for mackerel; 1.60 for horse mackerel) were used

to estimate variance of both species in the southern area in periods 1, 2 and 3, and estimated values for period 4 (1.63 for mackerel; 1.80 for horse mackerel) were used in periods 4 and 5.

Table 4.1.1: Deployment of research vessel effort in the 2004 western mackerel and horse mackerel egg survey.

PERIOD	COUNTRY	VESSEL	CRUISE DATES	AREA COVERAGE	SHIP DAYS
3	Spain (IEO)	Cornide	19/03 – 06/04	44º15' – 45º15'N	3
18/03–18/04	Spain (AZTI)	Investigador	24/03 – 11/04	44º00' – 48º00'N	15
	Germany	Walther Herwig	20/03 – 18/04	46º15' – 58º15'N	30
4	Spain (IEO)	Cornide	12/04 – 29/04	44º15' – 45º45'N	3
20/04–10/05	Ireland	Celtic Explorer	21/04 – 8/05	48º45' – 51º45'N	18
	Scotland	Scotia	21/04 – 8/05	52º45' – 59º45'N	18
	England & Wales	CEFAS Endeavour	26/04 – 10/05	46º15' – 51º45'N	15
	Spain (AZTI)	Vizconde de Eza	7/05 – 10/05	44º00' – 45º15'N	4
5	England & Wales	CEFAS Endeavour	11/05 – 18/05	49º45' – 51º45'N	8
11/05–8/06	Netherlands	Tridens	11/05 – 25/05	47º15' – 49º15'N	15
	Norway	Johan Hjort	20/05 – 8/06	52º15' – 59º45'N	20
	Spain (AZTI)	Vizconde de Eza	11/05 – 20/05	44º00' – 46º15'N	10
6	Norway	Johan Hjort	8/06 – 10/06	59º45' – 60º45'N	2
9/06–27/06	Netherlands	Tridens	9/06 – 22/06	47º15' – 49º15'N	14
	Scotland	Scotia	9/06 – 27/06	50º45' – 59º45'N	19
7	Ireland	Celtic Voyager	3 – 16/07	48º45' – 54º45'N	14
3/07–16/07					
Sum of realised ship days					**208**

Table 4.1.2: Deployment of research vessel effort in the 2004 southern mackerel and horse mackerel egg survey.

PERIOD	COUNTRY	VESSEL	CRUISE DATA	AREA COVERAGE	SHIP DAYS
1 15/01 – 26/01	Portugal	Capricornio	15/01 – 26/01	36º00'– 41º25' N	12
2 19/02 – 02/03	Portugal	Capricornio	19/02 – 02/03	36º25'– 42º45'N	13
3 7/03 – 10/04	Portugal Spain (IEO) Spain (AZTI)	Capricornio Cornide *Investigador*	07/03 – 20/03 19/03 – 06/04 07/04 – 10/04	36º00' – 38º45'N 42º15' – 45º00'N 43º25' – 44º15'N	14 16 4
4 12/04 – 6/05	Spain (IEO) Spain (AZTI)	*Cornide* Investigador	12/04 – 29/04 02/05 – 06/05	42º15' – 45º00'N 43º15' – 44º00'N	15 4
5 21/05 – 27/05	Spain (AZTI) Netherlands	*Investigador* Tridens	21/05 – 22/05 25/05 – 27/05	43º30' – 44º00'N 43º30' – 44º00'N	2 3
			Sum of realised ship days		83

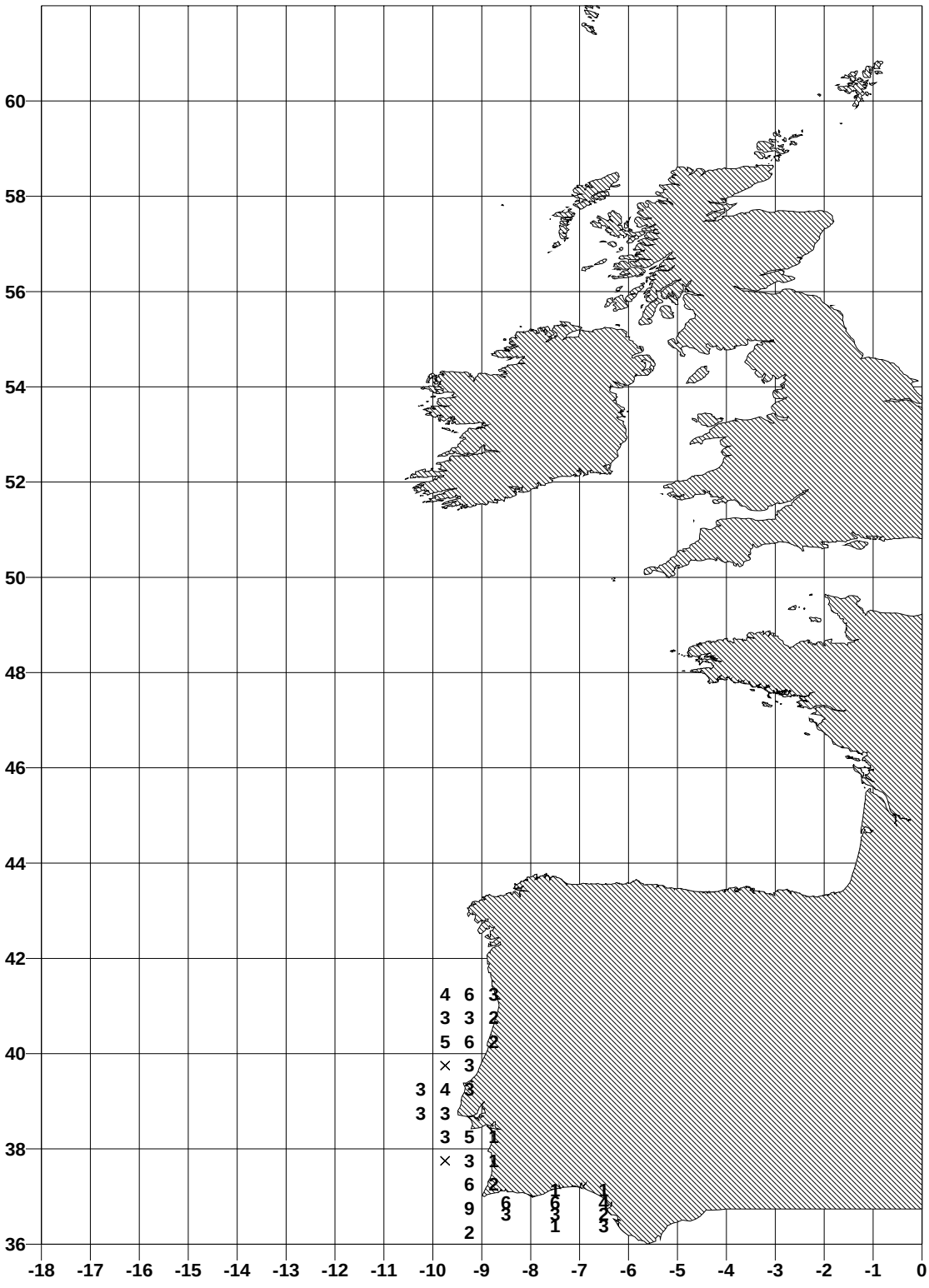

Figure 4.2.1a: Number of observations per rectangle in period 1 (15 January – 26 January) – X represents interpolated rectangles.

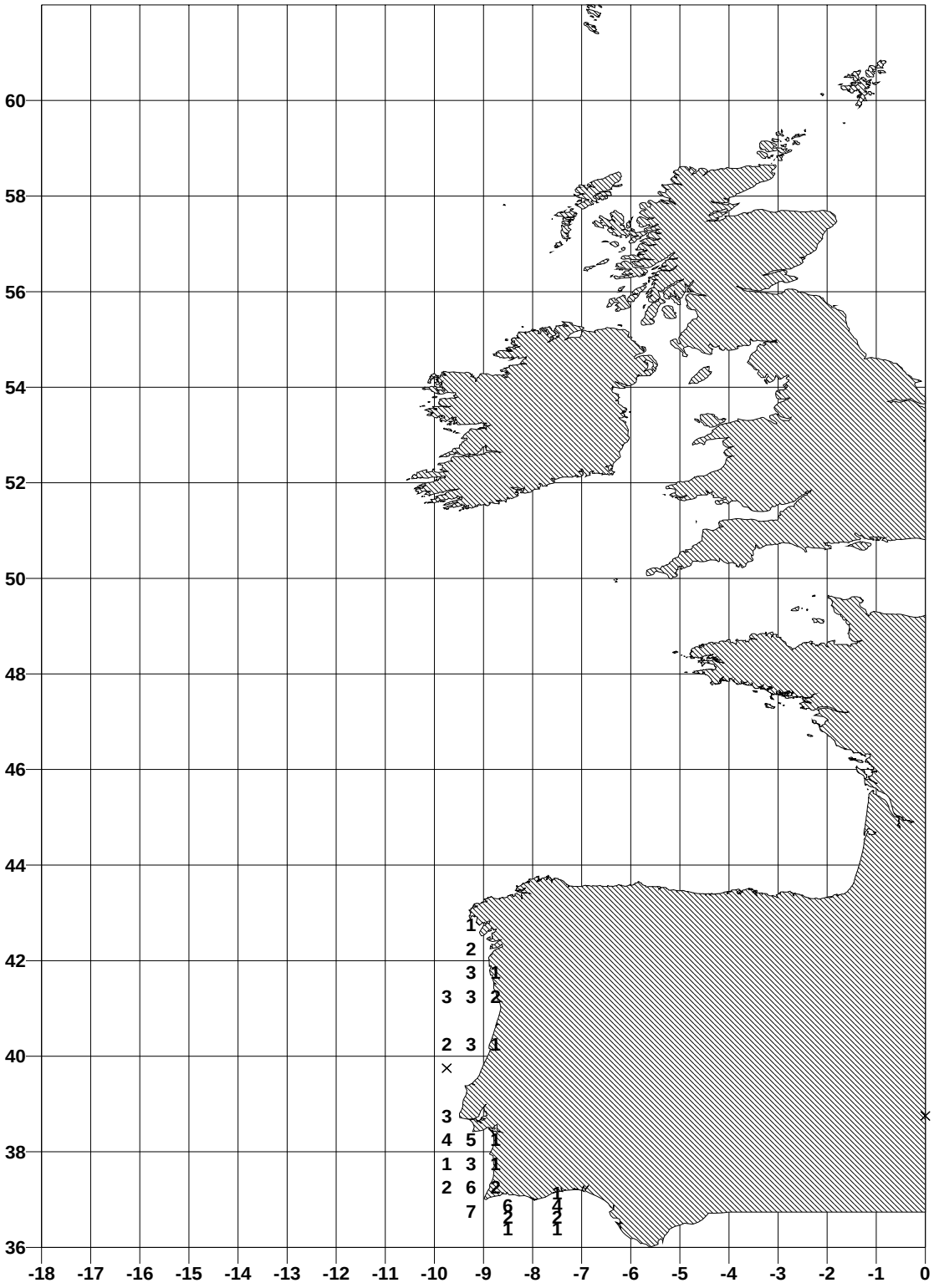

Figure 4.2.1b: Number of observations per rectangle in period 2 (19 February – 2 March) – X represents interpolated rectangles.

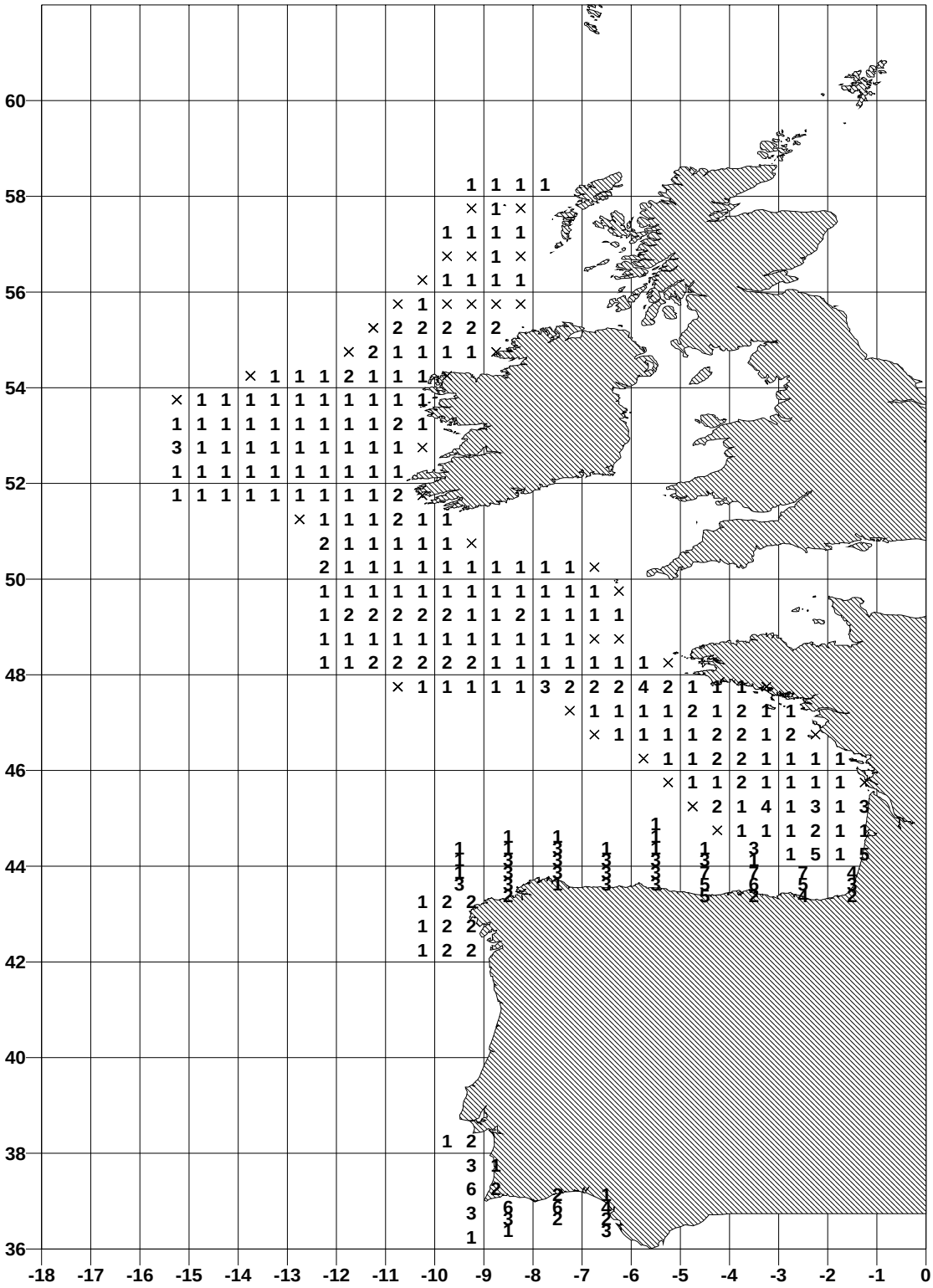

Figure 4.2.1c: Number of observations per rectangle in period 3 (7 March – 10 April in southern area; 18 March – 18 April in western area) – X represents interpolated rectangles.

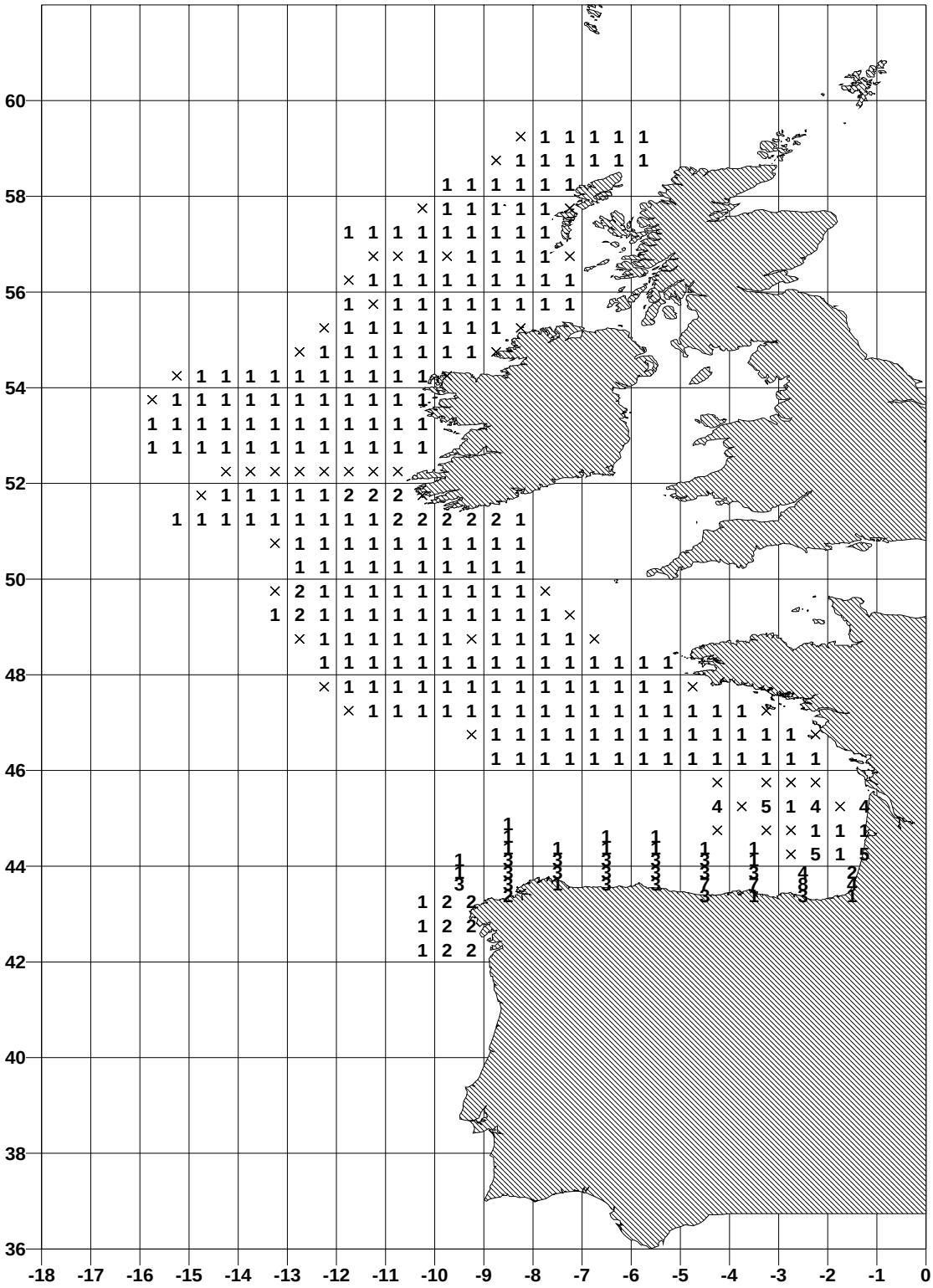

Figure 4.2.1d: Number of observations per rectangle in period 4 (12 April – 6 May in southern area; 20 April – 10 May in western area) – X represents interpolated rectangles.

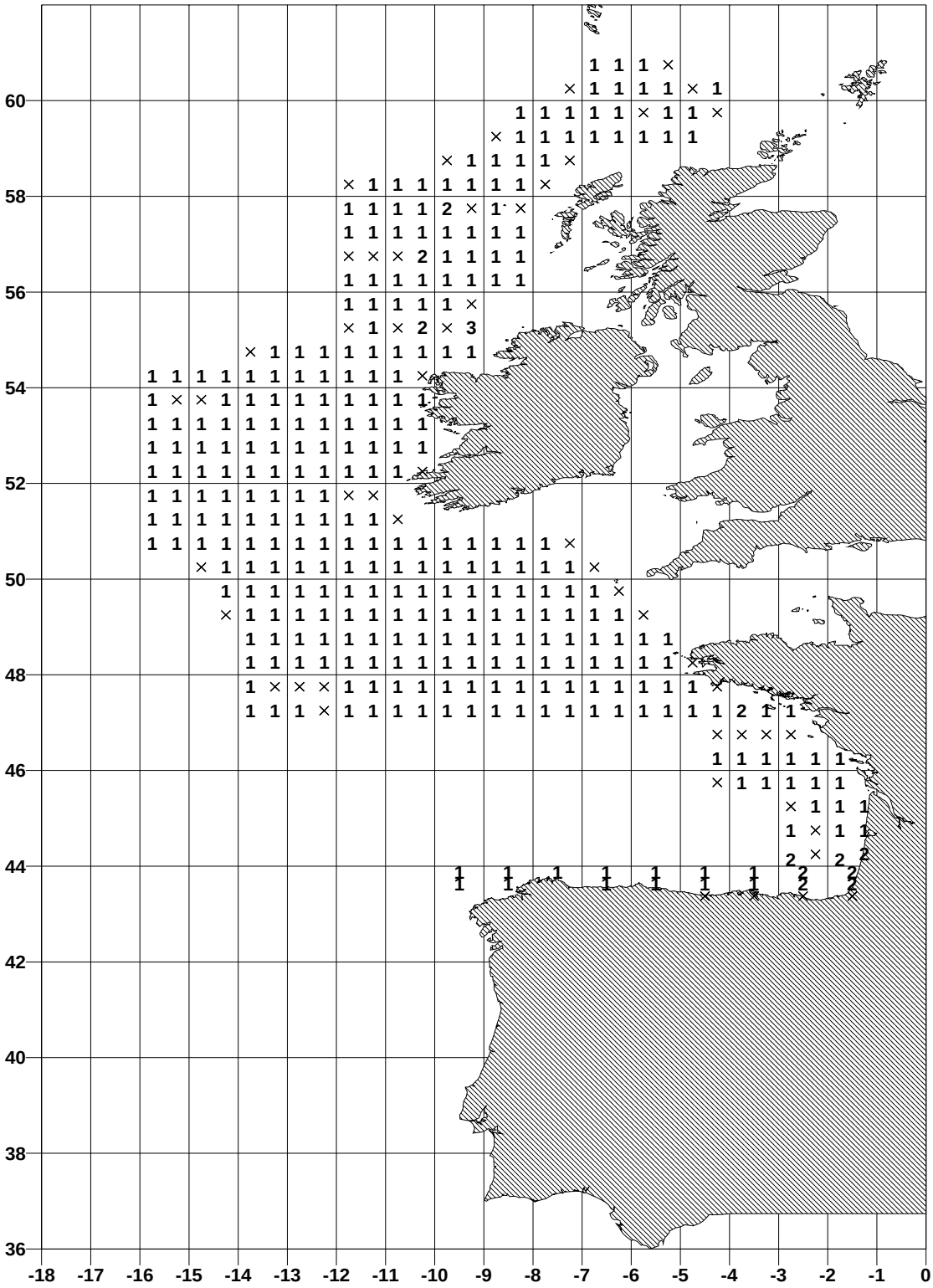

Figure 4.2.1e: Number of observations per rectangle in period 5 (21 – 27 May - in southern area; 11 May – 8 June in western area) – X represents interpolated rectangles.

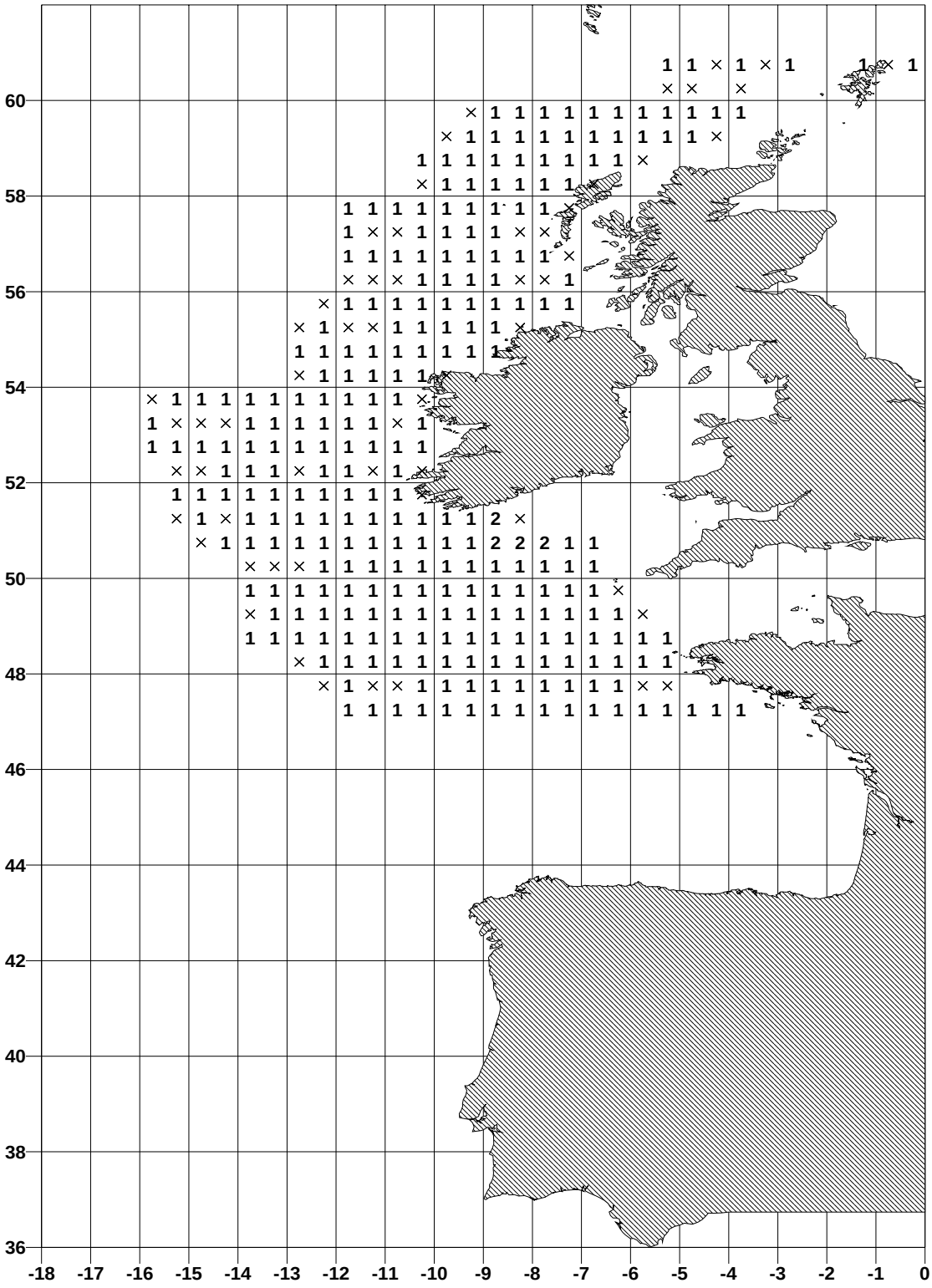

Figure 4.2.1f: Number of observations per rectangle in period 6 (9 – 27 June) – X represents interpolated rectangles.

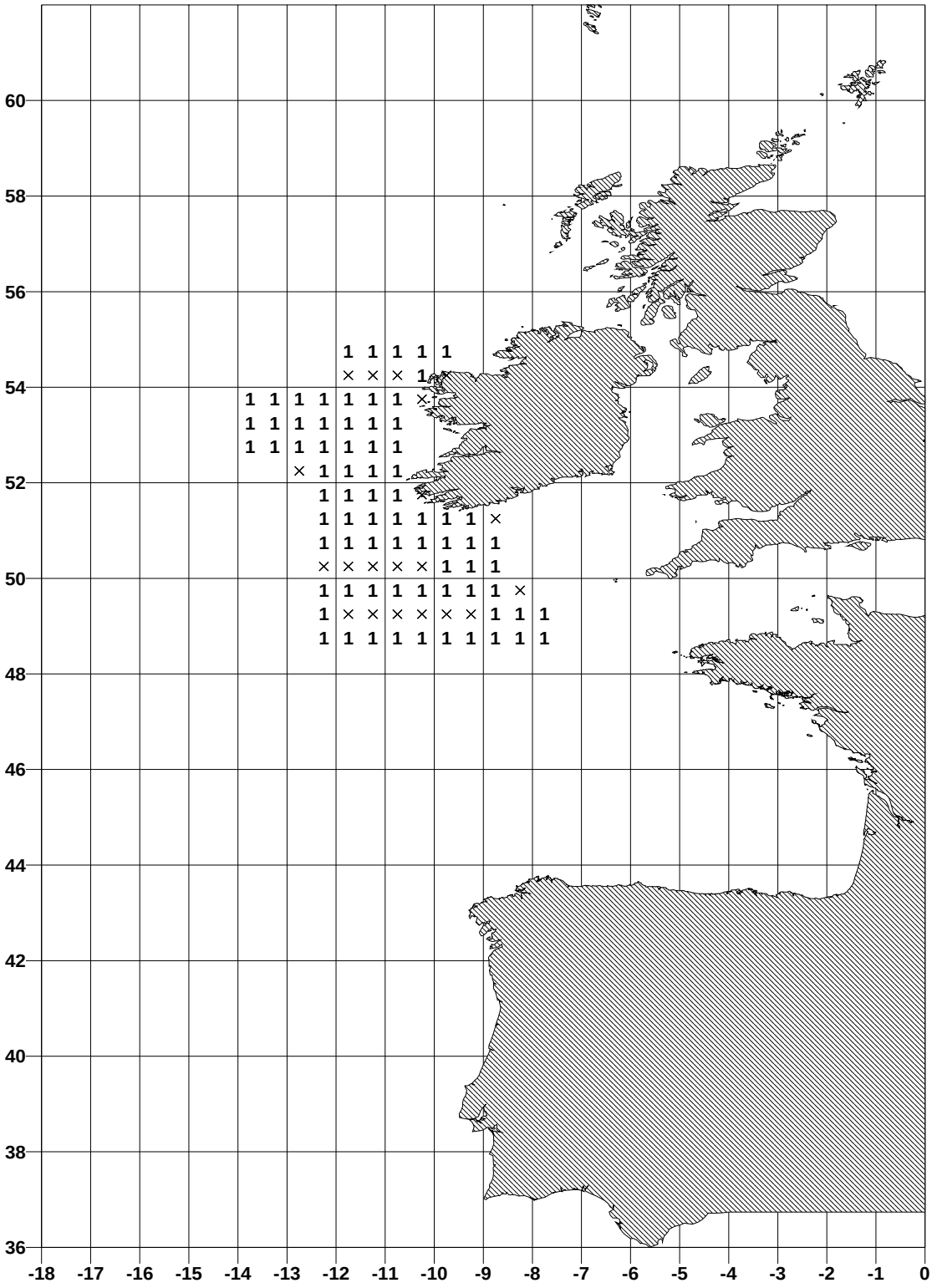

Figure 4.2.1g: Number of observations per rectangle in period 7 (3 – 16 July) – X represents interpolated rectangles.

5 Mackerel in the western and southern spawning areas: 2004 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 (36º00 N - 41º30 N) (Figure 5.1.1a). In Portuguese waters, mackerel eggs stage I were very sparse with very low abundance. In the area of Gulf of Cadiz no eggs were found. In this period the egg production was very low. Coverage was good and there were no significant interpolations required.
- **Period 2** – During this period the whole Portuguese area was surveyed (36º15 N - 43º00 N) (Figure 5.1.1b). A very low abundance of Stage 1 mackerel eggs were found across the sampled area, and were located closer to the coast. Again, mackerel eggs were rare in the Gulf of Cadiz. The egg production was slightly higher than in period 1. Some interpolation was required on the transect at 40º 45'N.
- **Period 3** – The first survey in the western area was in Period 3 (Figure 5.1.1c). Coverage was very good over most of area, from the Gulf of Cadiz to the North of Scotland. The main gap was on the coast of Portugal where weather curtailed sampling. The outside edges of sampling were well established throughout the area. Egg production was fairly continuous along the shelf break from NW Spain to Brittany. There was another concentration around Grand Sole Bank, south west of Ireland, and lower production on the Porcupine Bank. Production continued north of there up to 55º 15'N. No significant interpolation was required.
- **Period 4** – Coverage in this period was also very good (Figure 5.1.1d). There was still significant egg production in the Cantabrian Sea in this period, but lower production in Biscay. The main concentration of spawning was in the area of Grand Sole Bank, west of Brittany and Porcupine Bank. Further spawning was apparent along the 200m contour north of 48ºN, although this was patchy west of Scotland. The edges were mostly well defined by zero observation except at the northern end of the area. One transect was missed at 52º 15'N, and this was interpolated.
- **Period 5** – Coverage was slightly less comprehensive than in the previous periods (Figure 5.1.1e). The Cantabrian Sea was well covered and showed reduced production from period 4. Coverage in Biscay was patchy in the south, and the outside boundary was not established in the area immediately south of 47ºN. The remainder of the area was well covered although part of the transect at 51º 45'N required interpolation. Production was concentrated along the 200m contour and at Porcupine Bank. Again, there was patchy productivity west of Scotland.
- **Period 6** – Coverage was also reasonably good (Figure 5.1.1f). Egg production was again mostly concentrated along the 200m contour from 47º 45' to 59º N, although again patchy west of Scotland. The major problem was the strong eastward extension of spawning in the Celtic Sea south of Ireland. This extension had not been seen in previous surveys. It seems likely that there was further egg production to the north and east of the surveyed area. Apart from this area, interpolation was mostly minor, the few large interpolations were well established.
- **Period 7** – Only one vessel was available for sampling in this period so the coverage was necessarily less complete than in previous periods (Figure 5.1.1g). The aim was to survey in the likely main area of spawning. However it is likely that there would have been significant egg production to the north of the survey area, and possibly to the south. The possibility of spawning further east in the Celtic Sea as seen in period 6 cannot be discounted. Significant numbers of rectangles were interpolated, and in several cases these were at the edges of the survey, and may be open to doubt. For example, at 52º 15'N, 12º 45'W, and in the Celtic Sea

5.2 Egg production of the Northeast Atlantic Mackerel

5.2.1 Stage I egg production in western spawning area

Figure 5.2.1.1 presents the egg production curve for the western area for the 2004 survey, along with those for the surveys in 1998 and 2001 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 3 to suggest any alternative start date. The end date (31 July) is the same as that used since 1995. The egg production was low in period 7, 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 5.2.1.2. Unlike 1998 and 2001, the survey periods were not all completely contiguous. There was a two day gap between periods 3 and 4 and a six day gap between periods 6 and 7. Egg production for these periods was calculated by linear interpolation, following the protocols published in previous WG reports (ICES, 1995).

Total annual egg production for the western area in 2004 was calculated as 1.2018×10^{15} with a standard error of 0.10947×10^{15}.

5.2.2 Stage I Egg production in southern spawning area

The mackerel mean daily stage I egg production estimates for each survey period (Table 5.2.2.1) are plotted against the mid-cruise dates to provide the egg production curve (Figure 5.2.2.1). Total egg production values for survey periods and interpolated periods are given in Table 5.2.2.2.

The start of spawning for mackerel was assumed to be on the 15 January, two days earlier than in previous years. It is based on the occurrence of stage I eggs found off the Portuguese coast during period 1. The end of the spawning was assumed to be the 17 July, as used in previous years.

Total annual egg production for the southern area in 2004 was calculated as 0.126×10^{15} with a standard error of 0.0235×10^{15}.

This value was considerably lower than previous estimations and has decreased over the last 3 surveys (Table 5.2.2.3). This may be explained by distribution changes within the overall NEA stock

5.3 Potential fecundity of Northeast Atlantic mackerel

5.3.1 Potential fecundity in the western spawning component

Samples to determine mackerel potential fecundity were collected on CEFAS "Endeavour" and "Walther Herwig" in periods 3 and 4 (Table 5.3.1.1) from trawl hauls made between 48 to 53 degrees North. These samples were distributed between England, Norway Scotland and Spain and analysed according to methods described in ICES, 2003. Spawning fish were excluded from the estimate of relative potential fecundity based on the presence of hydrated oocytes or post ovulatory follicles in the dispersed ovary samples. Plots of annual potential fecundity and relative potential fecundity against fish length are shown in Figures 5.3.1.1 and 5.4.2.2d respectively. The overall estimate of relative fecundity was weakly influenced by the effect of latitude both in 2001 and in the current survey (Section 5.4.2). However as most of the egg production was also recorded north of 48 degrees the potential fecundity should be considered as representative of the spawning population. Based on this assumption the overall

relative fecundity in 2004 was 1127 se 27 female compared to 1097 se 23 eggs per g female reported in 2001 (ICES, 2002).

5.3.2 Potential fecundity in the southern spawning component

The sampling of adult fish to estimate potential fecundity of mackerel took place in 2004 in the VIIIc area, following the procedures agreed upon by the WMEGS planning group (ICES, 2003). IEO contracted a commercial vessel "Bosco" to collect the adult samples which were then analysed by IEO, FRS & IMR. A total of 100 ovaries were collected from fish that were classified as being in maturity stage 3 with no hydrated oocytes. 97 were processed and finally 55 were selected to estimate the potential fecundity (Table 5.3.2.1). The total weight of fish ranged from 197 to 768g, and the mean total weight for fish and ovary was 465 g and 42 g respectively. The relationship between weight and annual potential fecundity is shown in Figure 5.3.2.1. The mean estimated relative fecundity was 1016 oocytes/g cv 0.17.

5.4 Atresia and realised fecundity in the Northeast Atlantic mackerel

5.4.1 Atresia and realised fecundity of the western spawning component

Details of the number of fish collected over the latitudinal range of the Western mackerel spawning component during periods 3 to 7 are shown in Table 5.4.1.1. These samples were processed into histological section and analysed by CEFAS and IMR to select spawning and recently spent females. This subset of the sample was used to determine the prevalence (proportion of fish with early alpha atresia) and relative intensity (number of atretic eggs per g female) in order to determine the amount of potential fecundity that did not contribute to the annual egg production of the stock. Variances were determined by bootstrap sampling (n=5000) from the data and the loss of potential fecundity through atresia from the following equation (Horwood 1990):

$A_r = A_g \times P \times D \times S$

Where A_r = loss of potential fecundity through atresia

A_g = geometric mean of relative atresia.

P = prevalence of atresia

D = duration of alpha atresia (7.5 days)

S = Duration of mackerel spawning (60 days)

The overall prevalence of atresia as a percentage of the population was 28 se 2.4% and the relative intensity was 33.5 se 3.4 eggs per g whole body weight. This reduced the potential fecundity by 7% so that the realised fecundity was 1052 eggs per g female. A comparison of historical estimates of fecundity is shown in Table 5.4.1.2 and Figure 5.4.1.1.

5.4.2 Variance estimation and sources for fecundity and atresia estimates

Western Mackerel – Potential fecundity

Four different institutes were involved in the analysis of samples for the estimation of fecundity in the western mackerel component. The results for potential fecundity are presented in Table 5.4.2.1.

There are observable differences between institutes in these summary data, but could this be explained as simply due to institute? The pictures presented in Figure 5.4.2.1 also suggest that there may have been, i.e., eggs per gram female are highest for Fisheries Research Services (FRS) at first sight; however, there are also possible differences for latitude.

To test whether this apparent institute difference was statistically significant we fitted a series of 'nested' generalized linear models with log link of the form:

1) y=1
2) y=latitude
3) y=institute
4) y=latitude+institute

Where y=eggs per gram female, latitude enters the models as a continuous variable whereas institute is obviously categorical. The ANOVA table, which assesses the deviance/variance explained by each additional term, is given in Table 5.4.2.2.

The conclusion from this series of models is that both latitude and institute are significant by themselves, i.e., they each explain significant quantities of variance when tested against the NULL model. However, when institute is tested against a model with latitude already included (4.) it is not significant. Interactions between latitude and institute were also examined. At first it appeared that they were important, i.e., the latitudinal gradients of mean eggs per gram female varied between institutes. Closer investigation, however, revealed that the 'interaction' was probably an artefact and due to the non-linearity in the latitudinal dimension, see (c) above. In conclusion, there was no significant institute effect.

Western Mackerel – Atresia

A similar procedure was used to gauge whether different institutes (in this case IMR and CEFAS) might be measuring atresia differently. These data were more challenging since we measure both the intensity of atresia and its prevalence. Generally, atresia will only occur in a relatively small proportion (*ca* 20%) of the female population. The relationships between atresia and period, latitude, institute and fish weight are shown in figure 5.4.2.2.

The significance of the institution affect on the atresia data was treated using two different model formulations. The total relative atresia (intensity) was tested using GLMs from the Gaussian 'family' with log link. The 'prevalence' of fish with atretic ovaries was examined using GLMs from the binomial 'family' because the data consist simply of zeros and ones, i.e., presence=1 and absence=0. The models (1:4) below were fitted separately to both the total relative atresia (intensity) and the prevalence data.

1) y=1
2) y=period
3) y=period+weight class
4) y=period+weight class+institute

This first group of models (Table 5.4.2.3) suggested that there was no institutional effect on intensity, i.e., given that atresia was recorded for a particular fish, it did not matter which institution analysed the sample.

There was, however, an institutional effect on the prevalence of atresia according to these statistics (Table 5.4.2.4). Period explains significant quantities (7.5) of deviance, weight class less so (5.56) but the two institutes (CEFAS and IMR) were very different. The coefficients from model 4 (intercept=-0.09872; period=-0.22223; weight class 2=0.11,weight class 3=0.28, weight class 4=0.72, inst=-0.78185), for example, show that IMR recorded a much lower prevalence of atresia in the samples it analysed. According, then, to model (4), in period 5 and for weight class 4; for example, IMR noted a prevalence of 22% and CEFAS 50%.

Western and southern component combined

Large differences were noted between the southern (IEO) and western (CEFAS and IMR) data collected for atresia. Overall atretic loss in the population has been estimated in the past by calculating the geometric means for the intensity part of the data and multiplying that figure by the prevalence/proportion of atretic ovaries noted in the population. This has been done by bootstrap see (Table 5.4.2.5 and Figure 5.4.2.3).

5.4.3 Atresia and realised fecundity in the southern spawning component

The IEO surveys (PELACUS and CAREVA) were carried out on board R/V "Cornide Saavedra" and "Thalassa" during periods 3 and 4 in IXa and VIIIc Divisions (Figure 5.4.3.1). In these surveys a total of 157 ovaries were collected and processed of which 145 ovaries were used to estimate atresia. The numbers of fish sampled in periods 3 and 4 were 58 and 85 respectively. The mean total female weight was 465g and the mean ovary weight was 42 g. (Table 5.4.3.1).

Division IXa North was sampled during period 3. In this area a total of 21 ovaries were collected. Eleven ovaries were in spawning condition and 17 were rejected because they were in prespawning condition and contained no spawning markers (post ovulatory or hydrated follicles). Neither early alpha atresia ovaries nor ovaries with a massive atresia were observed.

Division VIIIc was sampled during periods 3 and 4. 124 ovaries were collected of which 93 were in spawning condition and 7 contained massive atresia.

In total 104 samples were in spawning condition and used for the estimation of atresia. The prevalence estimated in these samples was 6% and the total intensity of atresia was 105 ocytes per gram. The relative atresia and the relative realised fecundity were 52.9 oocytes and 964 oocites/g respectively. In 2001 the relative intensity of atresia and prevalence lower at 68 oocytes/g and 8% respectively.

5.5 Mackerel biomass estimate

5.5.1 Estimate of the western spawning component

Total stage I egg production is given in Table 5.2.1.2. Total spawning stock biomass (SSB) was estimated using the fecundity estimate of 1,052 oocytes/g female, corrected for atresia (see Sections 5.3 and 5.4), a sex ratio of 1:1 and a raising factor of 1.08 (ICES, 1987b) to convert pre-spawning to spawning fish. This gave an estimate of spawning stock biomass for 2004 of 2.468 million tonnes, with a variance of approximately 723,500 tonnes. The variance in the estimate due to the egg survey was 27% and 73% to the fecundity estimate. Comparative data from earlier years are shown in Table 5.5.1.1. These indicate a 2% decrease in biomass compared to the previous egg survey estimate in 2001. This decrease in the estimate of biomass has resulted mainly from a slight rise in realised fecundity to that found in 2001 (1033 and 1052 oocytes/g female in 2001 and 2004 respectively).

5.5.2 Estimate of the southern spawning component

In 2004, the total egg production in the southern area was estimated at 12.56×10^{13} (CV = 18.68%), 56% lower than in 2001. Total spawning stock biomass (SSB) was estimated using the realised fecundity estimate of 964 oocytes/g female with a coefficient of variation of 17.1%, a sex ratio of 1:1 and a raising factor of 1.08. The realised fecundity was estimated using samples of the divisions VIIIc and IXa processed by IEO. This realised fecundity was 41% lower than in 2001, and more in line with that of the western area.

In 2004 the spawning stock biomass estimate of the southern mackerel component was 280,307 t (CV = 25.3%). This estimation is 25% lower than the SSB estimated in 2001 (Table

5.5.2.1). The decrease in realised fecundity means that the 56% drop in egg production translates to a 25% drop in SSB. The decrease in SSB in the southern area may be the result of migration from the southern to the western area.

Table 5.2.1.1: Western mackerel mean daily Stage 1 egg production 10^{-12}.

PERIOD	DATES	ESTIMATE
3	18/3 – 18/4	8.22
4	20/4 – 10/5	11.41
5	11/5 – 8/6	9.92
6	9/6 – 27/6	10.83
7	3/7 –16/7	3.34

Table 5.2.1.2: Western mackerel total Stage 1 egg production estimates by time period for 2004.

Dates	Period	Number of days	Annual Stage 1 egg production.10^{-15}
10/2 – 17/3	*	39	0.11583
18/3 – 18/4	3	30	0.24674
19/4 – 20/4	*	2	0.020229
20/4 – 10/5	4	20	0.22827
11/5 – 8/6	5	29	0.28778
9/6 – 27/6	6	19	0.20580
28/6 – 3/7	*	6	0.038908
3/7 –16/7	7	12	0.040255
17/7 – 31/7	*	15	0.017971
Total			1.2018
Standard deviation			0.1095
CV			11.0%

Table 5.2.2.1: Southern mackerel mean daily stage I egg production in 2004.

PERIOD	DATES	PRODUCTION (X 10^{-12})	SE
1	15 – 26/01	0.02	0.004
2	19/02 – 02/03	0.08	0.04
3	07/03 – 10/04	1.90	0.41
4	12/04 – 06/05	1.45	0.40
5	21 – 27/05	0.24	0.18

Table 5.2.2.2: Mackerel total stage I egg production estimates by time period for 2004 in southern area.

DATES	PERIOD	Nº OF DAYS	ANNUAL STAGE I EGG PRODUCTION (x 10^{-13})
15 January – 26 January	1	12	0.02
27 January – 18 February	*	23	0.11
19 February – 2 March	2	13	0.10
3 March – 6 March	*	4	0.25
7 March – 10 April	3	35	6.64
11 April	*	1	0.16
12 April – 6 May	4	25	3.62
7 May – 20 May	*	14	0.92
21 May –27 May	5	7	0.17
28 May – 17 July	*	51	0.56
	Total	185	12.56
	Se		2.35
	CV		0.19

Table 5.2.2.3: Total mackerel egg production in the southern spawning area from 1998 to 2004.

YEAR	ANNUAL STAGE I EGG PRODUCTION (x10^{-13})	
	estimate	Se
1998	43.37	18.84
2001	28.31	4.67
2004	12.56	2.35

Table 5.3.1.1: Number of fecundity samples by collecting vessel and analysing institute.

VESSEL	DATE	TOTAL
CEFAS Endeavour	Count of CEFAS Sept 04	199
	Count of FRS Sept 04	45
	Count of IMR Sept 04	13
	Count of IMR Feb 05	
	Count of IEO Feb 05	
Walther Herwig	Count of CEFAS Sept 04	14
	Count of FRS Sept 04	71
	Count of IMR Sept 04	57
	Count of IMR Feb 05	46
	Count of IEO Feb 05	112
	Total Count of CEFAS Sept 04	213
	Total Count of FRS Sept 04	116
	Total Count of IMR Sept 04	70
	Total Count of IMR Feb 05	46
	Total Count of IEO Feb 05	112

5.3.2.1:. 2004 Mackerel fecundity from the southern area used to estimate potential fecundity in Div. VIIIc. (Results from CEFAS, FRS, IMR, IEO).

		Position		Weights					
Ship	Fish Reference number	Lat	Long	Fish Total length (mm)	Total (g)	Ovary	Liver	Guts including contents	Fecundity gravimetric oocyt/g
Bosco	S1-1	43.5667	3.3000	441	714	54.60	11.98	21.29	996
Bosco	S1-2	43.5667	3.3000	405	620	64.67	12.22	19.42	1224
Bosco	S1-5	43.5667	3.3000	417	595	60.50	10.52	15.42	1073
Bosco	S1-7	43.5667	3.3000	429	647	52.14	13.78	18.29	949
Bosco	S1-9	43.5667	3.3000	380	459	51.83	7.46	12.26	1357
Bosco	S1-16	43.5667	3.3000	374	377	39.86	5.99	15.15	833
Bosco	S1-19	43.5667	3.3000	435	768	97.56	12.99	17.52	858
Bosco	S2-2	43.5667	3.3000	410	563	59.77	10.92	12.44	914
Bosco	S2-9	43.5667	3.3000	437	688	43.92	13.54	20.48	963
Bosco	S2-12	43.5667	3.3000	368	374	32.20	5.15	10.57	1049
Bosco	S2-16	43.5667	3.3000	304	197	12.04	2.80	7.09	603
Bosco	S2-38	43.5667	3.3000	362	405	24.69	6.02	12.68	881
Bosco	S2-39	43.5667	3.3000	328	235	14.47	3.16	8.89	714
Bosco	S2-45	43.5667	3.3000	432	747	76.98	11.55	18.82	1047
Bosco	S2-49	43.5667	3.3000	434	703	36.02	10.61	15.81	1061
Bosco	S2-50	43.5667	3.3000	389	449	62.34	8.38	12.12	1338
Bosco	S2-59	43.5667	3.3000	322	232	20.31	3.45	7.42	1007
Bosco	S2-60	43.5667	3.3000	347	311	20.03	5.54	8.82	947
Bosco	S2-61	43.5667	3.3000	322	259	22.75	4.17	6.96	933
Bosco	S2-63	43.5667	3.3000	361	381	32.68	7.28	10.15	1121
Bosco	S2-69	43.5667	3.3000	374	397	38.36	7.80	14.13	990
Bosco	S2-71	43.5667	3.3000	334	264	17.56	5.11	8.95	1012
Bosco	S2-74	43.5667	3.3000	415	670	102.39	12.17	14.10	1255
Bosco	S3-2	43.6000	3.2400	348	312	31.80	6.36	10.50	1147
Bosco	S3-6	43.6000	3.2400	449	656	37.12	11.09	23.90	1145
Bosco	S3-8	43.6000	3.2400	352	307	14.90	5.12	10.08	708
Bosco	S3-9	43.6000	3.2400	345	278	23.97	5.10	10.42	1215
Bosco	S3-13	43.6000	3.2400	341	288	16.69	4.90	12.18	946
Bosco	S3-14	43.6000	3.2400	334	255	15.40	3.58	10.74	953
Bosco	S3-15	43.6000	3.2400	410	607	52.50	11.69	15.28	1059
Bosco	S3-16	43.6000	3.2400	421	611	52.34	12.01	18.02	1222
Bosco	S3-17	43.6000	3.2400	363	418	32.76	9.17	14.36	878
Bosco	S3-18	43.6000	3.2400	450	545	46.93	10.00	17.28	1182
Bosco	S3-20	43.6000	3.2400	435	734	87.09	12.02	17.85	538
Bosco	S4-1	43.6167	3.3000	412	564	42.98	9.51	17.13	1356
Bosco	S4-2	43.6167	3.3000	338	311	19.85	5.64	12.53	911
Bosco	S4-3	43.6167	3.3000	384	502	33.70	8.52	14.92	967
Bosco	S4-4	43.6167	3.3000	343	292	16.47	4.72	11.25	812
Bosco	S4-6	43.6167	3.3000	414	553	35.17	10.20	18.00	1042
Bosco	S4-8	43.6167	3.3000	387	435	43.62	5.26	15.26	1145
Bosco	S4-11	43.6167	3.3000	394	472	43.44	8.68	12.66	922
Bosco	S4-13	43.6167	3.3000	422	599	50.48	12.26	16.70	921
Bosco	S4-14	43.6167	3.3000	357	340	21.67	5.69	11.96	705
Bosco	S4-16	43.6167	3.3000	415	512	67.57	7.93	16.85	1213
Bosco	S4-18	43.6167	3.3000	336	272	16.40	3.60	13.21	1062
Bosco	S4-19	43.6167	3.3000	391	387	23.48	6.41	15.40	1118
Bosco	S4-20	43.6167	3.3000	417	560	43.81	8.81	15.68	1169
Bosco	S4-23	43.6167	3.3000	345	297	23.98	5.22	15.38	1088
Bosco	S5-1	43.5333	3.1320	383	436	32.40	7.87	13.70	920
Bosco	S5-2	43.5333	3.1320	394	489	40.58	9.49	13.75	1112
Bosco	S5-3	43.5333	3.1320	408	465	31.04	7.75	13.69	1055
Bosco	S5-4	43.5333	3.1320	409	571	72.93	10.10	13.38	1048
Bosco	S5-10	43.5333	3.1320	404	548	72.19	7.41	15.92	1199
Bosco	S5-11	43.5333	3.1320	433	652	81.50	9.33	18.44	990
Bosco	S5-20	43.5333	3.1320	329	266	30.58	4.58	8.00	987

Table 5.4.1.1: Details of the numbers of mackerel fecundity samples collected in period 3 and 4 showing where and when they were collected in the Western spawning area.

	Weeks from January 1 2004					
Latitude degrees	10	12	13	14	15	Grand Total
48	3			2		5
49	7		44	64		115
50		9	16			25
51	5	4	36			45
52					12	12
53					3	3
54						
Grand Total	15	13	96	66	15	205

Table 5.4.1.2: Results of fecundity analysis in the assessment years 1998, 2001 and 2004.

	Assessment year		
Parameter	1998	2001	2004
Number of samples analysed: potential fecundity	96	187	205
atresia	112	290	348
Potential fecundity	1206	1097	1127
Prevalence of atresia	0.55	0.20	0.28
Geometric mean Relative intensity of atresia	46	40	33
Number of potential fecundity lost per day	3.37	1.07	1.25
Number or potential fecundity lost over an individual's spawning season	202	64	75
Realised fecundity	1002	1033	1052
Percentage of potential fecundity lost	17	6	7

Table 5.4.2.1: Mean eggs per gram female recorded by each participating institute. N= number of samples; se=standard error.

	CEFAS	FRS	IEO	IMR
Mean eggs g^{-1}	1186	1273	978	1093
N	90	88	92	72
Se	89	114	116	92

Table 5.4.2.2: The effect of the institute on the mean number of eggs per gram female.

MODEL	RESID.DF	RESID.DEV.	DF	DEVIANCE	P(>|CHI|)
1	341	38523990	NA	NA	NA
2	340	38267348	1	256642	0
3	338	34219266	2	4048082 1	0
4	337	34218530	1	737	1

Table 5.4.2.3: The effect of period, weight class and institute on the atresia intensity data for the western component of the mackerel stock (Ns =not sig.). These models were fitted to the intensity data using a GLM from the Guassian family with a log link.

MODEL	RESID. DF.	RESID. DEV.	DF.	DEV. EXPLAINED	P(>|CHI|)
1	69	109814			
2	78	109579	1	235	Ns
3	75	98965	3	10613	0.046
4	74	98645	1	320	Ns

Table 5.4.2.4: The effect of period, weight class and institute on the atresia prevalence data for the western component of the mackerel stock (Ns =not sig.). These models were fitted to the prevalence data (zeros and ones) using a GLM from the binomial family with a log link.

MODEL	RESID. DF.	RESID. DEV.	DF.	DEV. EXPLAINED	P(>|CHI|)
1	347	375			
2	346	368	1	7.5	0.01
3	343	362	3	5.56	0.14
4	342	351	1	11.26	0.0008

Table 5.4.2.5: Average intensity, prevelance and atretic loss estimated for the 3 institutes.

	CEFAS	IMR	IEO
Geometric mean intensity (se)	269 (6)	226 (14)	884.9 (35)
Mean prevalence (se)	0.28 (0.002)	0.12 (0.003)	0.05 (0.002)
Mean atretic loss (se)	75.15 (0.49)	26.87 (0.747)	45.85 (0.788)

Table 5.4.3.1: Southern mackerel atresia period 3 and 4 in VIIIc IXa Division.

												Histology analysis				
Ship		Date	Sample reference	Position		Maturity stage	Length (mm)	Total Fish (g)	Ovary (g)	Liver (0.1g)	Guts inc. conten	Presence of spawning markers			Early alfa atresia	
	day	nonth		Lat	Long							Mig nuc	Hyd	POF	Prev	Number
Thalassa	4	apr	T13-20	42.92	9.24	5	293	161	2.6	1.8	10.0	0	0	0	0	0
Thalassa	4	apr	T13-21	42.92	9.24	5	292	150	4.8	3.0	12.4	0	0	0	0	0
Thalassa	4	apr	T13-35	42.92	9.24	3	289	148	8.4	2.2	9.4	0	0	0	0	0
Thalassa	4	apr	T13-40	42.92	9.24	3	279	138	12.2	4.0	8.6	0	0	0	0	0
Thalassa	4	apr	T13-42	42.92	9.24	4	330	219	13.8	5.0	14.6	0	0	0	0	0
Thalassa	4	apr	T13-5	42.92	9.24	5	287	135	7.0	3.4	10.6	0	0	0	0	0
Thalassa	4	apr	T14-1	43.25	9.18	3	290	157	10.0	4.4	15.2	0	0	0	0	0
Thalassa	4	apr	T14-3	43.25	9.18	3	290	155	8.4	3.4	18.4	0	0	0	0	0
Thalassa	4	apr	T14-7	43.25	9.18	3	286	154	10.8	4.2	17.2	0	0	0	0	0
Thalassa	3	apr	T9-5	42.77	9.27	5	282	151	3.8	3.2	16.4	0	0	0	0	0
Thalassa	4	apr	T13-16	42.92	9.24	5	285	148	4.2	2.8	10.2	1	0	1	0	0
Thalassa	4	apr	T14-14	43.25	9.18	3	277	136	10.2	3.0	11.2	1	0	0	0	0
Thalassa	4	apr	T14-16	43.25	9.18	4	330	215	21.2	4.8	18.2	1	0	0	0	0
Thalassa	4	apr	T14-17	43.25	9.18	3	294	150	11.6	3.0	14.8	1	0	0	0	0
Thalassa	5	apr	T16-2	43.53	8.39	3	285	135	11.0	3.8	10.8	1	0	1	0	0
Thalassa	7	apr	T19-15	43.75	7.35	3	288	141	21.4	4.6	8.8	1	0	1	0	0
Thalassa	7	apr	T21-10	43.80	8.17	4	288	139	11.4	3.0	8.2	1	1	0	0	0
Thalassa	7	apr	T21-9	43.80	8.17	4	283	139	15.6	2.6	9.6	1	1	1	0	0
Thalassa	7	apr	T22-30	43.90	8.09	3	276	123	7.2	3.2	10.4	1	0	1	0	0
Thalassa	31	mar	T5-15	41.97	9.19	5	289	150	7.4	4.8	15.4	1	0	1	0	0
Thalassa	31	mar	T5-16	41.97	9.19	5	282	149	6.8	4.8	17.2	1	0	1	0	0
Thalassa	31	mar	T5-22	41.97	9.19	3	304	184	10.0	4.4	18.4	1	0	1	0	0
Thalassa	31	mar	T5-23	41.97	9.19	3	280	137	10.4	4.4	12.2	1	0	1	0	0
Thalassa	31	mar	T5-28	41.97	9.19	3	340	238	13.4	5.2	20.0	1	0	1	0	0
Thalassa	3	apr	T9-12	42.77	9.27	4	285	149	13.4	3.6	16.6	1	0	0	0	0
Thalassa	3	apr	T9-17	42.77	9.27	5	299	170	11.0	3.8	20.6	1	0	1	0	0
Thalassa	3	apr	T9-3	42.77	9.27	4	294	165	29.0	5.6	14.6	0	1	0	0	0
Thalassa	3	apr	T9-7	42.77	9.27	5	310	185	9.6	5.4	26.6	1	0	0	0	0
Thalassa	3	apr	T9-8	42.77	9.27	5	300	173	12.6	4.2	21.3	1	0	0	0	0
Thalassa	7	apr	T19-4	43.75	7.35	5	368	282	7.8	4.0	16.4	0	0	0	0	0
Thalassa	4	apr	T13-41	42.92	9.24	4	347	252	9.2	4.8	15.4	1	0	1	0	0
Thalassa	4	apr	T14-19	43.25	9.18	4	399	374	24.8	8.6	28.0	0	1	0	0	0
Thalassa	4	apr	T14-9	43.25	9.18	3	353	273	22.8	5.8	25.0	1	0	1	0	0
Thalassa	7	apr	T19-1	43.75	7.35	3	369	270	21.4	7.0	17.6	1	0	1	0	0
Thalassa	7	apr	T19-11	43.75	7.35	3	364	264	27.4	6.6	15.0	1	1	0	0	0
Thalassa	7	apr	T21-7	43.80	8.17	4	393	382	53.0	6.6	12.0	0	1	1	0	0
Thalassa	7	apr	T22-21	43.90	8.09	3	386	359	42.4	10.4	16.2	1	1	1	0	0
Thalassa	8	apr	T24-21	43.92	7.29	3	383	350	51.0	10.8	21.4	1	0	1	0	0
Thalassa	8	apr	T24-24	43.92	7.29	4	398	393	60.4	11.4	18.2	0	1	1	0	0
Thalassa	8	apr	T24-7	43.92	7.29	4	392	358	41.8	7.4	16.4	0	1	1	0	0
Thalassa	8	apr	T24-9	43.92	7.29	4	388	374	67.6	10.6	20.6	0	1	1	0	0
Thalassa	31	mar	T5-7	41.97	9.19	5	385	348	16.6	8.8	38.4	1	0	1	0	0
Thalassa	3	apr	T9-41	42.77	9.27	5	348	266	17.8	7.6	39.8	1	0	1	0	0
Thalassa	7	apr	T19-22	43.75	7.35	5	371	307	13.0	5.4	19.4	0	1	1	1	62
Thalassa	7	apr	T19-29	43.75	7.35	5	354	256	15.8	5.0	13.4	0	1	1	1	240
Thalassa	8	apr	T24-11	43.92	7.29	4	431	476	57.6	10.8	18.4	0	0	0	0	0
Cornide	4	apr	C7-2	43.98	7.31	4-5	435	513	35.7	8.8	19.9	1	1	1	0	0
Thalassa	7	apr	T9-21	43.75	7.35	5	401	411	33.8	12.4	20.0	1	1	1	0	0
Thalassa	7	apr	T19-3	43.75	7.35	5	420	448	49.6	11.0	20.0	1	1	1	0	0
Thalassa	7	apr	T19-8	43.75	7.35	5	407	413	66.8	11.4	17.8	1	1	1	0	0
Thalassa	7	apr	T21-13	43.80	8.17	4	417	412	68.6	10.8	20.8	1	1	1	0	0
Thalassa	7	apr	T21-2	43.80	8.17	4	429	475	65.6	10.0	21.2	0	1	0	0	0
Thalassa	7	apr	T22-23	43.90	8.09	5	405	416	26.6	11.6	21.8	1	1	1	0	0
Thalassa	7	apr	T22-28	43.90	8.09	4	402	417	39.4	10.4	21.4	1	1	1	0	0
Thalassa	8	apr	T24-16	43.92	7.29	5	424	466	61.2	11.6	21.0	0	1	0	0	0
Thalassa	8	apr	T24-23	43.92	7.29	5	414	420	34.2	8.4	20.6	1	1	1	0	0
Thalassa	8	apr	T24-30	43.92	7.29	4	427	476	90.8	12.6	23.2	0	1	0	0	0
Thalassa	8	apr	T/4-5	43.92	7.29	3	416	432	60.6	12.0	19.0	1	1	1	0	0
Thalassa	13	apr	T42-3	43.52	5.14	5	290	166	4.2	2.6	13.2	0	0	0	0	0
Thalassa	13	apr	T42-6	43.52	5.14	5	267	117	2.4	2.4	12.2	0	0	0	0	0
Thalassa	14	apr	T44-1	43.45	4.31	6	281	130	1.2	1.8	12.8	0	0	0	0	0

Table 5.4.3.1 Continued: Southern mackerel atresia period 3 and 4 in VIIIc IXa Division.

Thalassa	15	apr	T51-13	43.48	3.16	3	284	143	8.4	3.2	14.6	0	0	0	0	0
Thalassa	16	apr	T53-2	43.52	3.17	5	326	222	15.0	5.8	19.6	0	0	0	0	0
Thalassa	16	apr	T53-8	43.52	3.17	6	301	179	4.4	4.4	13.4	0	0	0	0	0
Thalassa	16	apr	T53-9	43.52	3.17	4	327	126	16.4	6.9	19.5	0	0	0	0	0
Thalassa	16	apr	T54-14	43.55	3.11	5	320	212	6.8	2.8	11.4	0	0	0	0	0
Thalassa	16	apr	T58-12	43.82	1.36	5-6	297	140	4.2	2.0	7.8	0	0	0	0	0
Thalassa	17	apr	T60-18	43.47	2.26	3	299	159	9.2	2.0	11.2	0	0	0	0	0
Thalassa	17	apr	T60-32	43.47	2.26	5	340	238	7.6	3.4	15.2	0	0	0	0	0
Thalassa	9	apr	T26-22	43.63	7.09	4	341	236	19.2	5.4	16.2	0	1	1	0	0
Thalassa	9	apr	T26-30	43.63	7.09	4	340	246	34.8	7.0	15.6	0	1	1	0	0
Thalassa	10	apr	T31-17	43.62	6.12	5	334	222	11.6	4.0	15.4	0	1	1	0	0
Thalassa	10	apr	T31-26	43.62	6.12	5	274	147	3.6	3.8	12.0	0	0	1	0	0
Thalassa	13	apr	T39-35	43.73	5.20	4	305	169	34.2	4.6	10.8	0	1	1	0	0
Thalassa	13	apr	T41-9	43.78	5.14	5	326	202	19.6	5.0	13.2	1	0	1	0	0
Thalassa	13	apr	T42-9	43.52	5.14	4	313	187	11.8	4.4	13.2	0	1	1	0	0
Thalassa	14	apr	T44-6	43.45	4.31	4	343	229	26.2	5.1	17.6	1	1	1	0	0
Thalassa	15	apr	T51-2	43.48	3.16	5	344	241	11.4	4.9	22.5	1	1	0	0	0
Thalassa	15	apr	T51-5	43.48	3.16	5	316	184	8.4	2.8	19.4	1	0	1	0	0
Thalassa	15	apr	T51-7	43.48	3.16	5	315	175	19.4	4.2	21.2	0	1	0	0	0
Thalassa	16	apr	T53-1	43.52	3.17	5	297	168	11.2	4.4	14.6	0	1	1	0	0
Thalassa	16	apr	T54-1	43.55	3.11	5	312	179	11.6	5.4	17.0	0	0	1	0	0
Thalassa	16	apr	T54-19	43.55	3.11	5	310	179	16.6	4.0	15.4	1	0	0	0	0
Thalassa	16	apr	T54-24	43.55	3.11	3	311	182	16.4	3.8	16.4	1	0	1	0	0
Thalassa	16	apr	T54-25	43.55	3.11	5	276	120	7.4	7.6	13.8	0	0	1	0	0
Thalassa	16	apr	T54-39	43.55	3.11	5	344	226	10.8	4.6	15.2	1	0	1	0	0
Thalassa	16	apr	T54-5	43.55	3.11	5	332	212	12.4	5.2	18.6	1	0	1	0	0
Thalassa	16	apr	T54-8	43.55	3.11	4	283	141	4.4	2.2	13.2	1	0	1	0	0
Thalassa	16	apr	T54-9	43.55	3.11	5	324	203	9.8	3.2	12.8	1	0	1	0	0
Thalassa	16	apr	T55-18	43.55	2.34	5	305	158	9.6	3.0	12.6	1	0	1	0	0
Thalassa	16	apr	T58-14	43.82	1.36	4	293	150	12.4	3.2	7.4	1	1	1	0	0
Thalassa	16	apr	T58-25	43.82	1.36	4	351	170	23.8	6.0	13.8	1	1	1	0	0
Thalassa	17	apr	T60-6	43.47	2.26	3	332	214	16.6	5.4	13.8	1	0	1	0	0
Thalassa	14	apr	T44-3	43.45	4.31	5	270	132	3.2	4.4	11.6	1	1	0	1	84
Thalassa	16	apr	T53-14	43.52	3.17	5	281	138	7.6	3.0	13.4	1	0	0	1	97
Thalassa	16	apr	T55-1	43.55	2.34	5	349	247	10.8	4.2	14.8	0	0	0	0	0
Thalassa	16	apr	T54-41	43.55	3.11	5	355	265	5.4	3.6	15.8	0	0	0	0	0
Thalassa	16	apr	T54-73	43.55	3.11	5	406	364	10.8	4.6	14.2	0	0	0	0	0
Thalassa	16	apr	T54-74	43.55	3.11	6	392	377	4.8	4.6	18.6	0	0	0	0	0
Thalassa	16	apr	T54-76	43.55	3.11	5	394	382	16.0	7.2	22.4	0	0	0	0	0
Thalassa	18	apr	T61-32	43.50	2.11	6	369	346	5.4	3.0	15.4	0	0	0	0	0
Thalassa	18	apr	T61-33	43.50	2.11	5	379	374	13.4	9.0	25.6	0	0	0	0	0
Thalassa	14	apr	T44-19	43.45	4.31	5	354	273	5.5	7.6	23.2	0	0	0	0	0
Thalassa	18	apr	T61-34	43.50	2.11	5	397	377	10.0	6.0	17.0	0	1	0	0	0
Thalassa	12	apr	T36-40	43.77	5.33	6	399	384	6.0	5.0	23.0	0	0	0	0	0
Thalassa	14	apr	T44-4	43.45	4.31	5	345	257	5.5	3.0	19.8	0	0	0	0	0
Thalassa	9	apr	T26-18	43.63	7.09	4	368	277	35.2	5.2	14.4	0	1	1	0	0
Thalassa	12	apr	T36-17	43.77	5.33	5	389	356	42.6	11.4	25.0	1	0	1	0	0
Thalassa	12	apr	T36-4	43.77	5.33	3-4	393	379	33.2	4.8	23.8	1	0	1	0	0
Thalassa	13	apr	T39-14	43.73	5.20	4	388	390	62.4	9.2	20.8	1	1	1	0	0
Thalassa	13	apr	T39-20	43.73	5.20	4	393	382	90.6	10.6	19.0	1	1	1	0	0
Thalassa	13	apr	T39-24	43.73	5.20	4	394	382	72.8	10.4	22.8	0	1	1	0	0
Thalassa	13	apr	T39-25	43.73	5.20	4	385	346	50.6	9.4	19.0	1	1	1	0	0
Thalassa	13	apr	T39-3	43.73	5.20	4	404	400	74.0	11.4	26.2	1	1	1	0	0
Thalassa	13	apr	T39-4	43.73	5.20	5	389	348	27.2	10.2	20.4	1	0	1	0	0
Thalassa	13	apr	T39-54	43.73	5.20	4	379	379	48.0	11.0	18.2	1	1	1	0	0
Thalassa	13	apr	T39-69	43.73	5.20	4	355	267	30.4	7.9	22.0	1	1	0	0	0
Thalassa	13	apr	T41-17	43.78	5.14	5	375	315	24.6	7.8	15.8	1	1	1	0	0
Thalassa	13	apr	T41-3	43.78	5.14	5	380	338	26.2	7.8	26.0	1	1	1	0	0
Thalassa	16	apr	T54-75	43.55	3.11	4	356	273	38.2	6.4	11.6	1	1	0	0	0
Thalassa	16	apr	T55-20	43.55	2.34	5	399	382	23.8	7.6	18.0	1	0	1	0	0
Thalassa	19	apr	T63-9	43.55	1.29	5	371	322	10.4	4.2	23.2	1	1	1	0	0
Thalassa	12	apr	T36-26	43.77	5.33	5	391	337	13.2	7.0	18.6	1	0	1	1	95
Thalassa	13	apr	T39-41	43.73	5.20	5	384	363	11.2	5.2	20.5	1	1	0	0	0
Thalassa	19	apr	T63-1	43.55	1.29	2	379	322	8.6	3.6	23.6	1	0	0	1	145
Thalassa	10	apr	T31-11	43.62	6.12	5	394	407	33.8	10.4	20.4	1	0	0	0	0
Thalassa	16	apr	T54-20	43.55	3.11	6	432	517	8.8	7.2	18.8	0	0	0	0	0
Thalassa	18	apr	T61-31	43.50	2.11	6	409	457	8.2	8.6	32.0	0	0	0	0	0
Thalassa	13	apr	T39-1	43.73	5.20	5	429	472	12.2	8.2	28.4	0	0	0	0	0
Thalassa	16	apr	T54-6	43.55	3.11	6	404	428	8.2	5.4	20.2	0	0	0	0	0
Thalassa	13	apr	T39-55	43.73	5.20	5	440	504	14.6	7.4	25.8	0	0	1	0	0

Table 5.4.3.1 Continued: Southern mackerel atresia period 3 and 4 in VIIIc IXa Division.

Thalassa	9	apr	T27-4	43.88	7.09	4	424	503	47.6	8.8	26.8	1	1	1	0	0
Thalassa	12	apr	T36-12	43.77	5.33	4	406	408	38.8	9.0	2.6	1	1	1	0	0
Thalassa	13	apr	T39-10	43.73	5.20	4	430	467	59.0	1.0	24.2	1	1	1	0	0
Thalassa	13	apr	T39-27	43.73	5.20	4-5	406	426	35.0	9.4	23.0	1	1	1	0	0
Thalassa	13	apr	T39-57	43.73	5.20	5	442	502	42.4	11.6	30.6	1	0	1	0	0
Thalassa	13	apr	T39-64	43.73	5.20	5	429	495	30.4	10.2	22.2	1	0	1	0	0
Thalassa	13	apr	T39-65	43.73	5.20	4	433	467	91.8	13.4	23.2	1	1	1	0	0
Thalassa	13	apr	T39-9	43.73	5.20	4	430	489	75.3	12.4	22.4	1	1	1	0	0
Thalassa	13	apr	T41-6	43.78	5.14	4	406	441	99.2	10.4	17.6	1	1	1	0	0
Thalassa	16	apr	T54-32	43.55	3.11	4	426	493	42.0	8.0	21.4	1	1	1	0	0
Thalassa	16	apr	T55-22	43.55	2.34	5	421	498	32.0	10.0	18.8	1	0	1	0	0
Thalassa	9	apr	T27-10	43.88	7.09	4	447	565	87.6	15.8	26.6	1	1	1	0	0

Table 5.5.1.1: Spawning stock biomass for the western spawning component of mackerel and western horse mackerel. 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 – WESTERN MACKEREL						
Year	Total egg prod ($x10^{-15}$) (mean for unsampled rectangles)		Total fecundity (eggs/g female)	Total fecundity corrected for atresia	Pre-spawning stock biomass ($x10^{-6}$ tonnes)	Spawning stock biomass ($x10^{-6}$ tonnes)
	Geometric	Arithmetic	(atresia oocytes/gm female)	(eggs/g female)		(conv f 1.08)
Annual egg production method – western mackerel						
1977	1.98		1526 [211]	1315	3.01	3.25
1980	1.48 a		1526 [211]	1315	2.25	2.43
1980	1.84 b		1526 [211]	1315	2.80	3.02
1983	1.50	1.53	1526 [211]	1315	2.33	2.51
1986	1.15	1.24	1457 [211]	1246	1.99	2.15
1989	1.45	1.52	1608 [326]	1282	2.37	2.56
1992	1.83	1.94	1569 [138]	1431	2.71	2.93
1995	-	1.49	1473 [171]	1302	2.28	2.47
1998	-	1.37	1206 [203]	1003	2.73	2.95
2001	-	1.21	1097 [64]	1033	2.34	2.53
2004	-	1.20	1127 [75]	1052	2.28	2.47

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

Table 5.5.2.1: Southern mackerel spawning component; Total annual egg production, realised fecundity and calculated SSB for 1995–2004.

	TOTAL ANNUAL EGG PRODUCTION ($x10^{-13}$)	FECUNDITY PER GRAMME OF FISH WEIGHT (OOCYTES/G)	SPAWNING STOCK BIOMASS (TONNES)
1998	43.37 (CV = 43.4%)	1171 (CV = 28.8%)	800 000 (CV = 68.0%)
2001	28.31 (CV = 16.5%)	1647 (CV = 12.6%)	371 279 (CV = 20.7%)
2004	12.56 (CV= 18.7%)	964 (CV = 17.1%)	280 307 (CV = 25.3%)

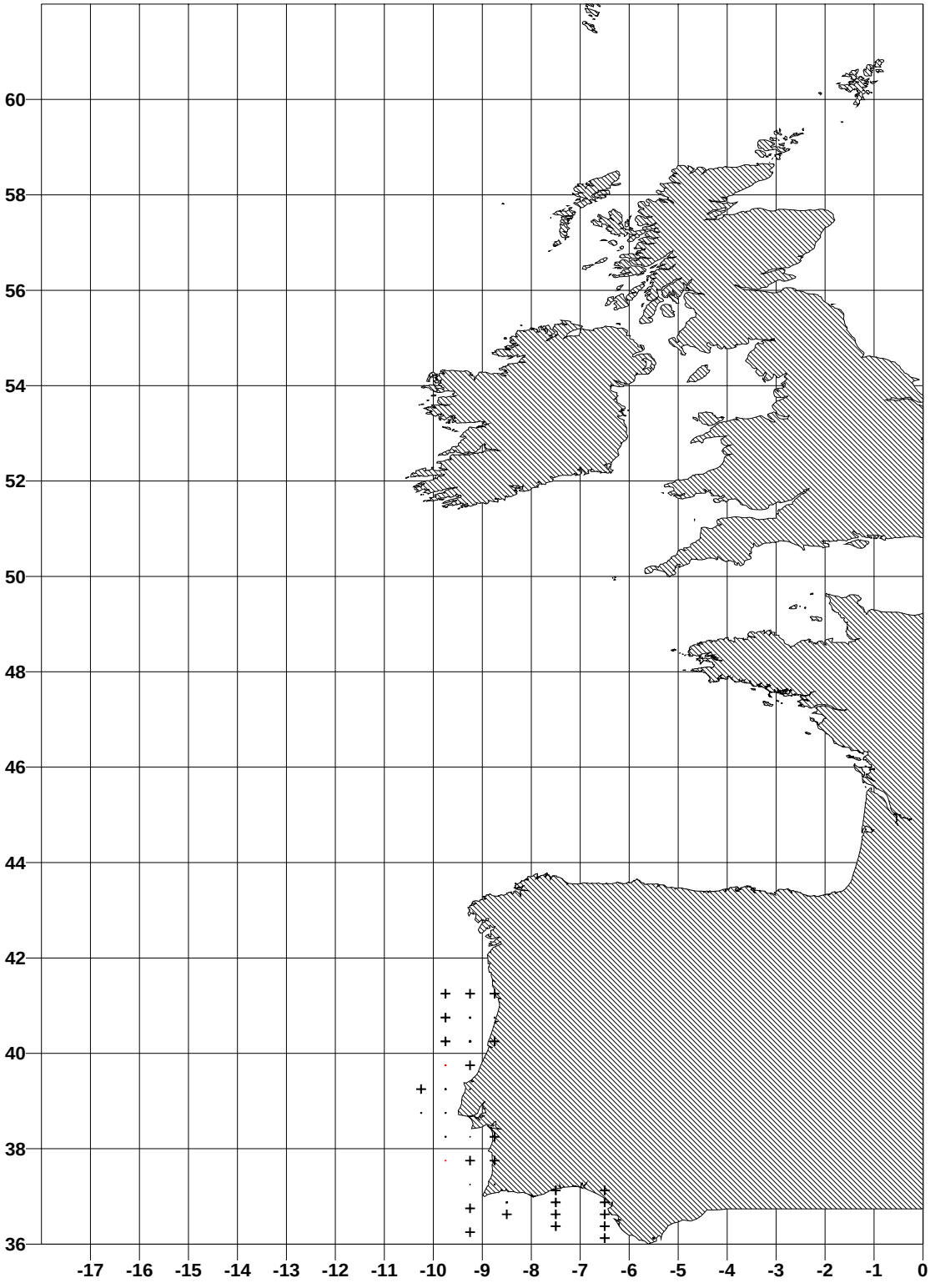

Figure 5.1.1a: Mackerel egg production by rectangle for period 1 (15 January – 26 January). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

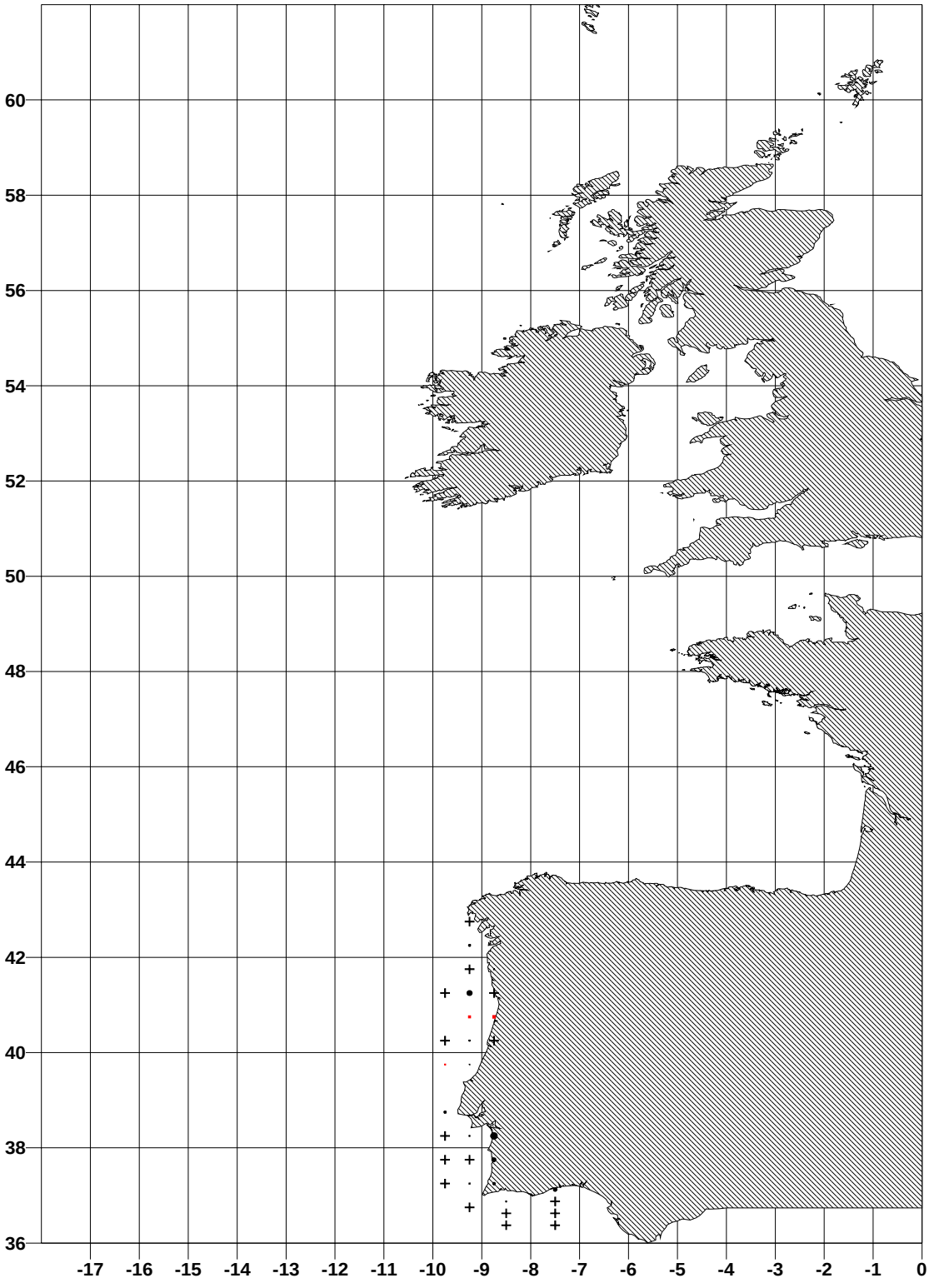

Figure 5.1.1b: Mackerel egg production by rectangle for period 2 (19 February – 2 March). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

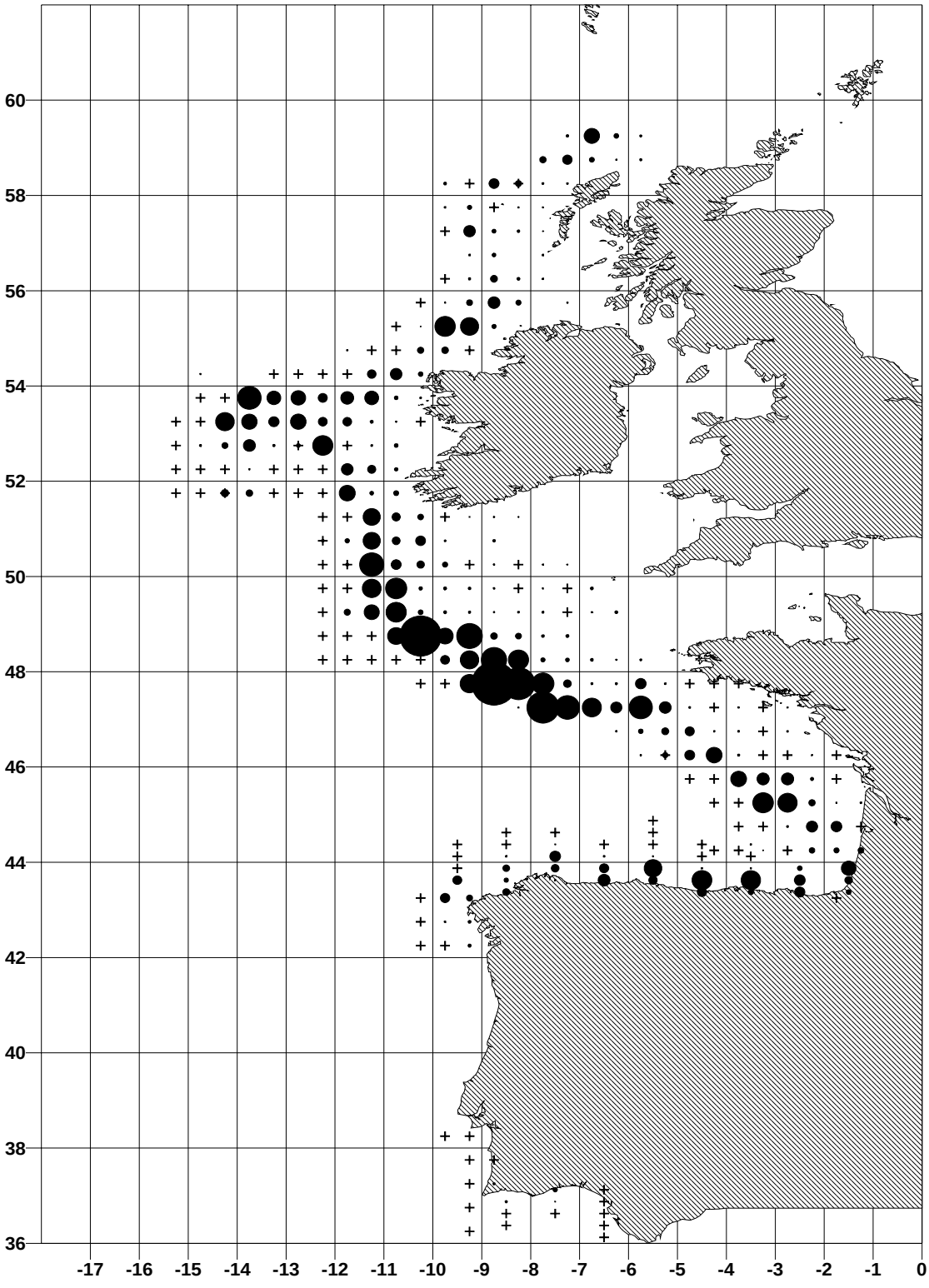

Figure 5.1.1c: Mackerel egg production by rectangle for period 3 (7 March – 10 April in southern area; 18 March – 18 April in western area). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

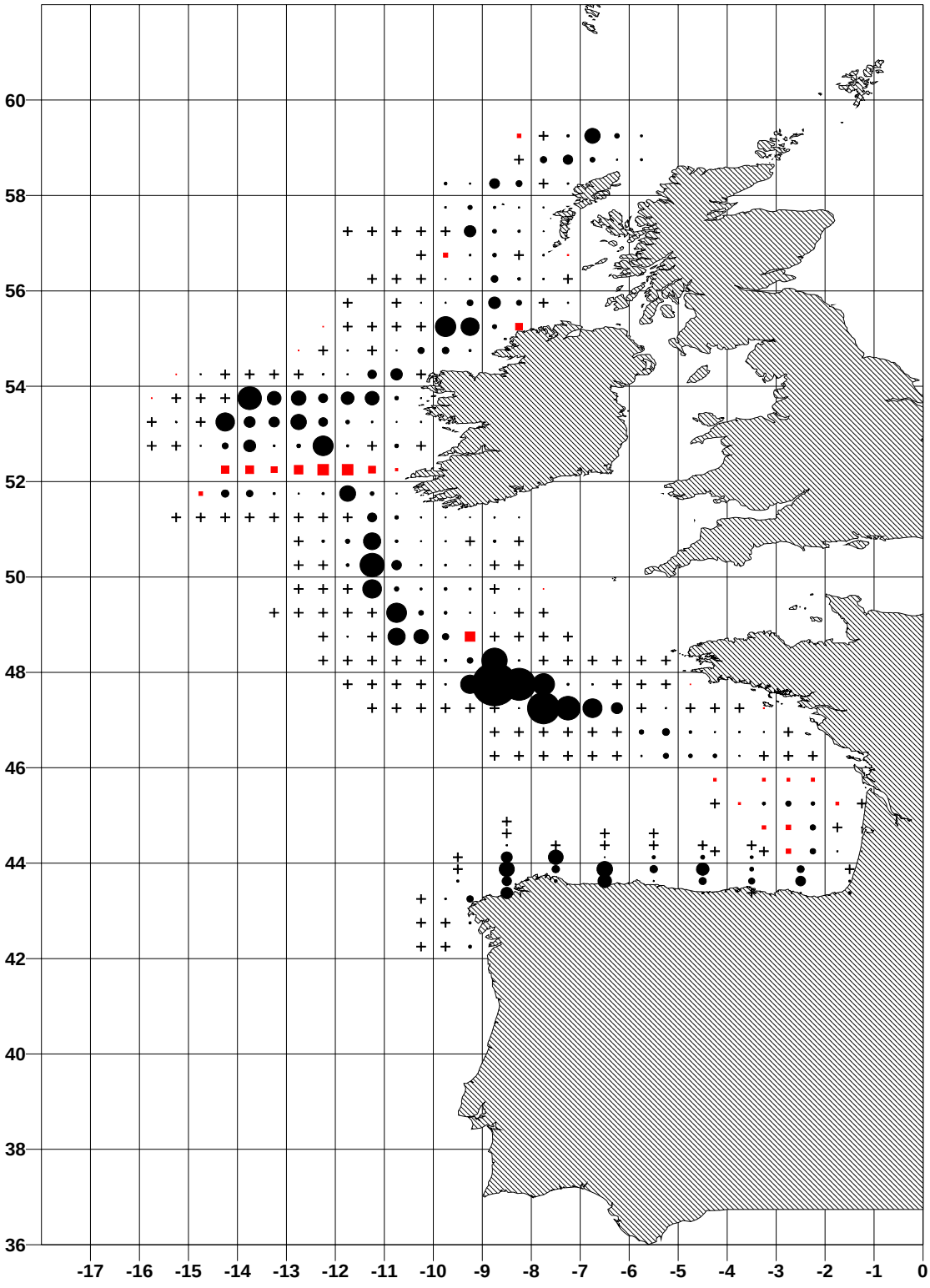

Figure 5.1.1d: Mackerel egg production by rectangle for period 4 (12 April – 6 May in southern area; 20 April – 10 May in western area). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

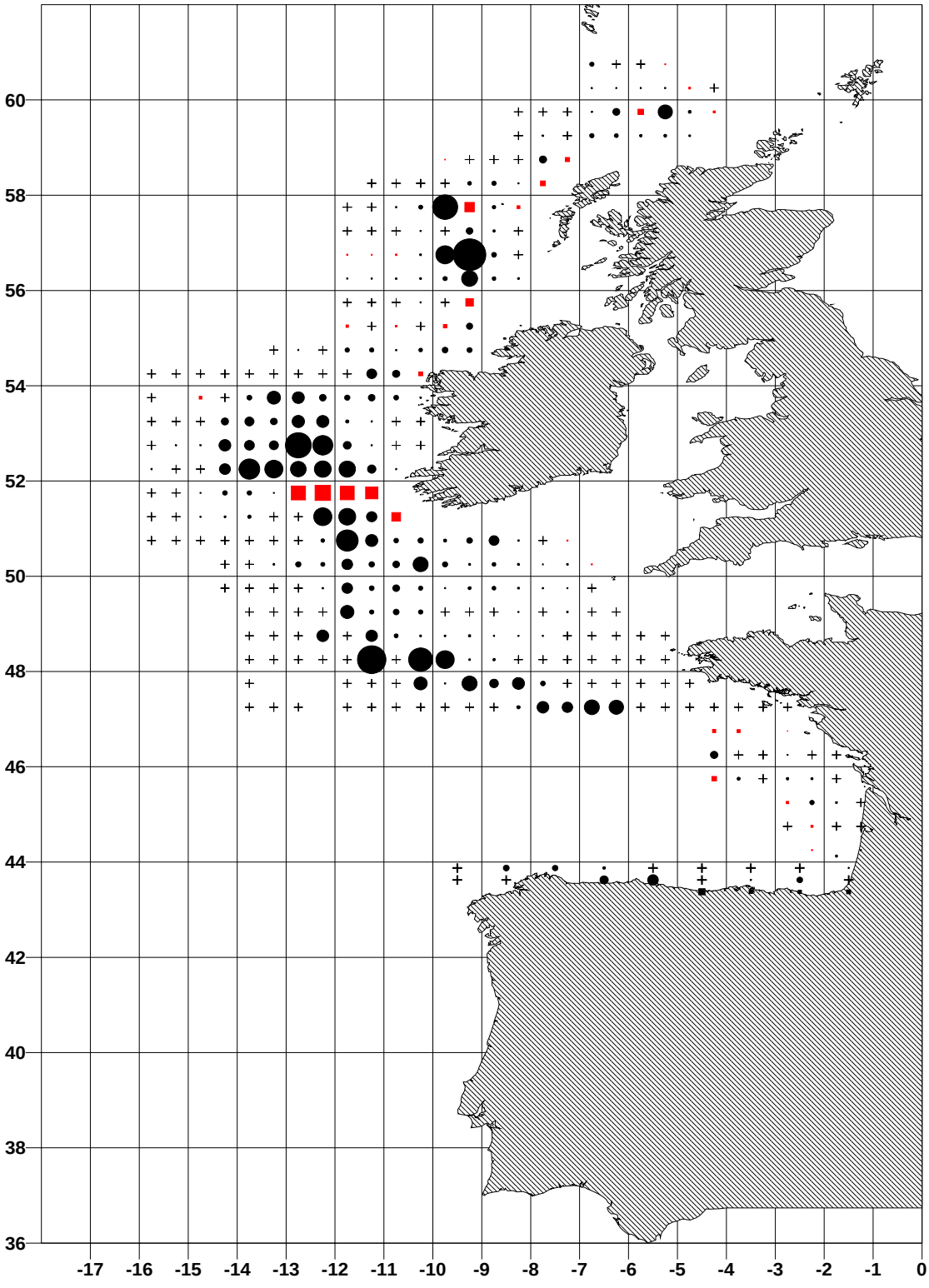

Figure 5.1.1e: Mackerel egg production by rectangle for period 5 (21 – 27 May - in southern area; 11 May – 8 June in western area). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs m^{-2}.day^{-1}.

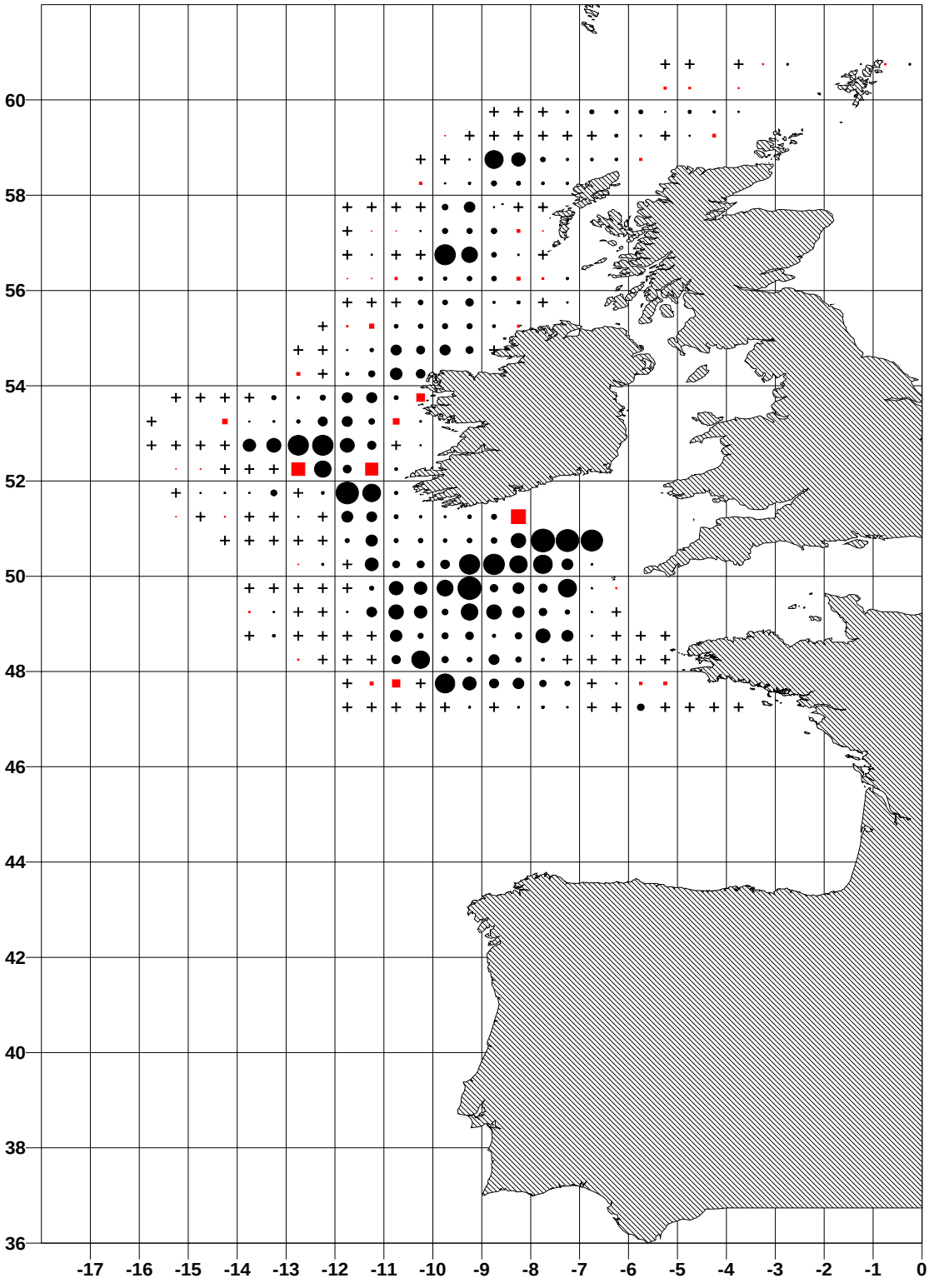

Figure 5.1.1f: Mackerel egg production by rectangle for period 6 (9 – 27 June). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

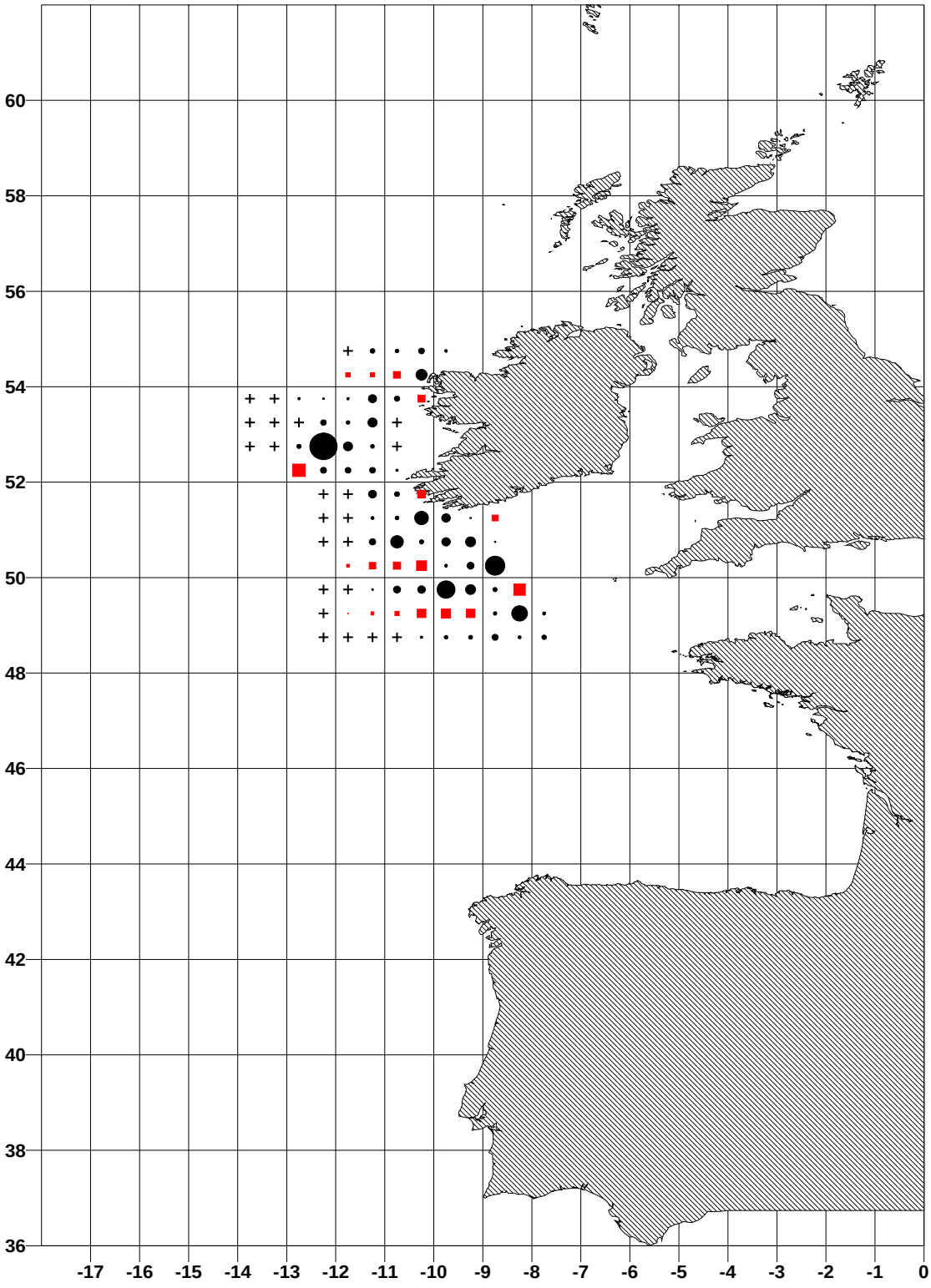

Figure 5.1.1g: Mackerel egg production by rectangle for period 7 (3 – 16 July). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

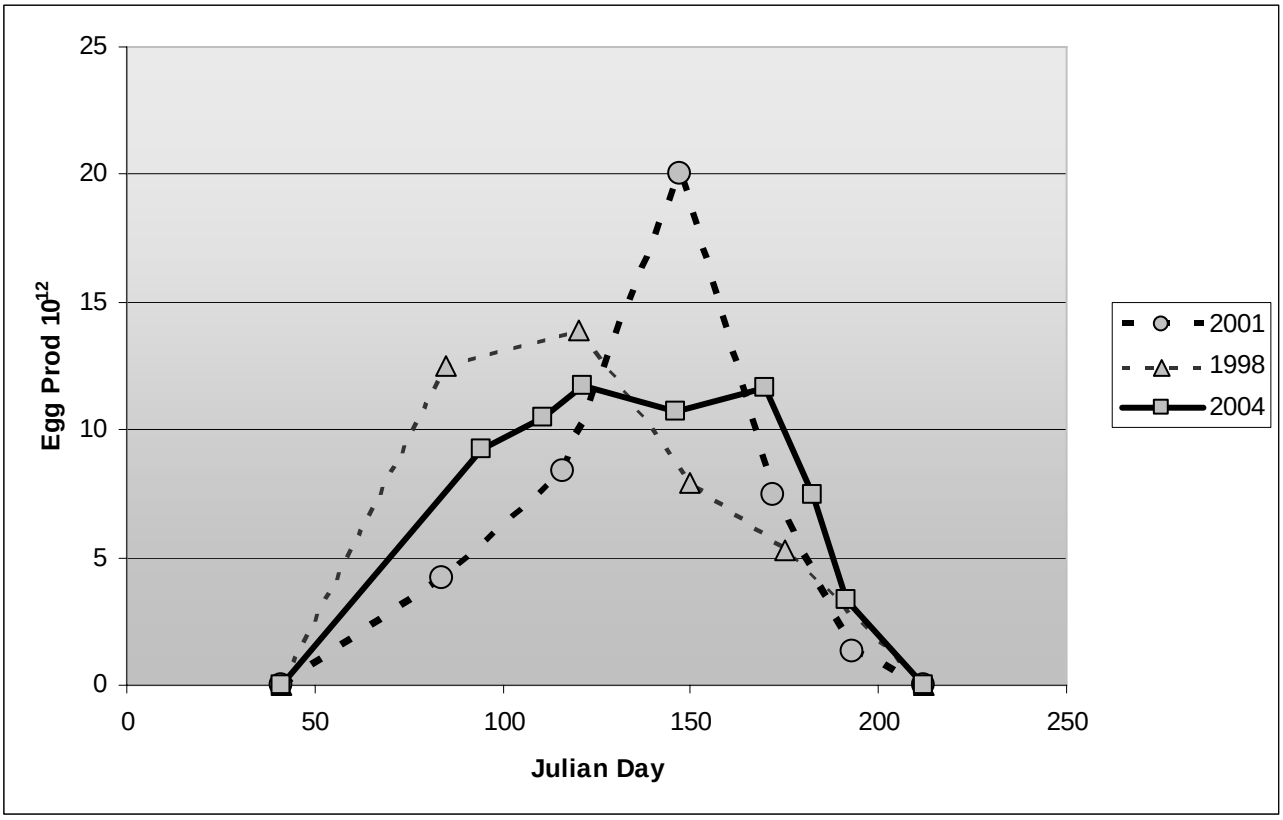

Figure 5.2.1.1: Annual egg production curve for mackerel in the western spawning component. The curves for 1998 and 2001 are included for comparison.

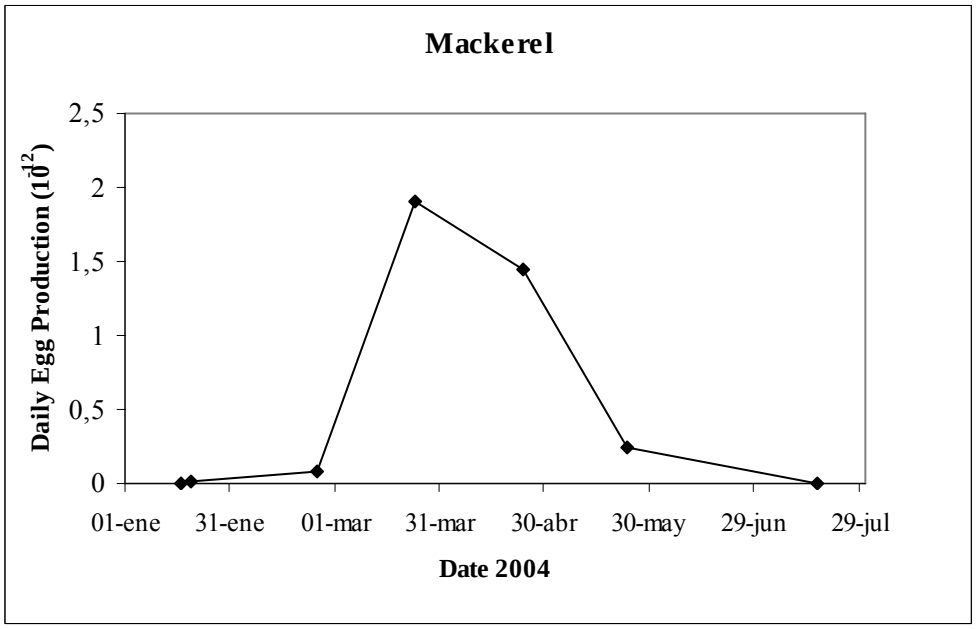

Figure 5.2.2.1: Annual egg production curve for mackerel in the southern spawning component.

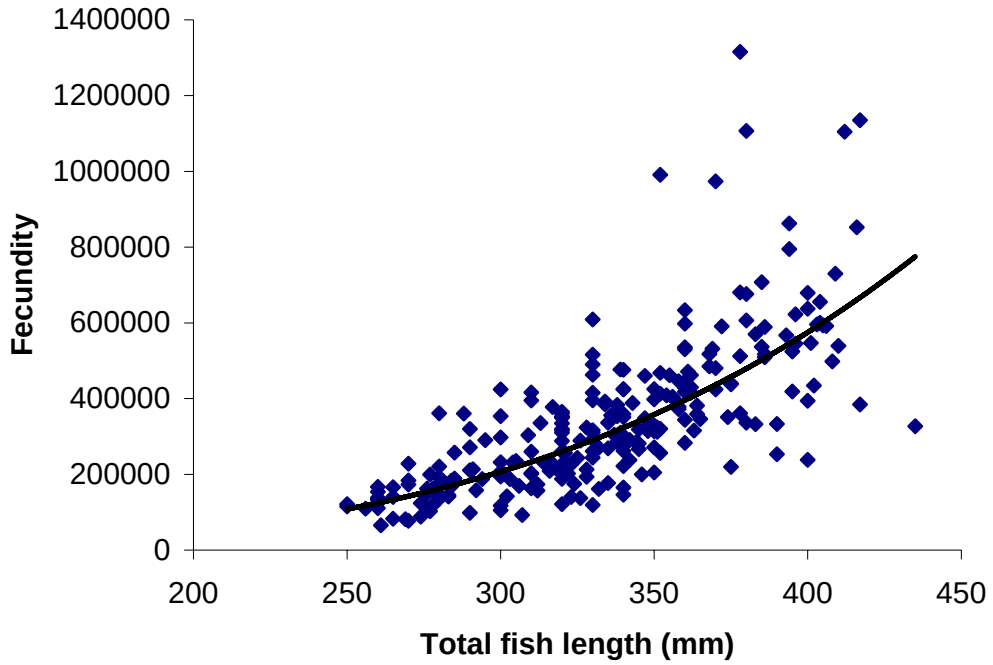

Figure 5.3.1.1: Potential fecundity of Western mackerel plotted against fish length. The equation for the fitted line is a= b $0.00033^{3.55290}$ where a = potential fecundity and b = total fish length r^2 = 0.62 n= 249.

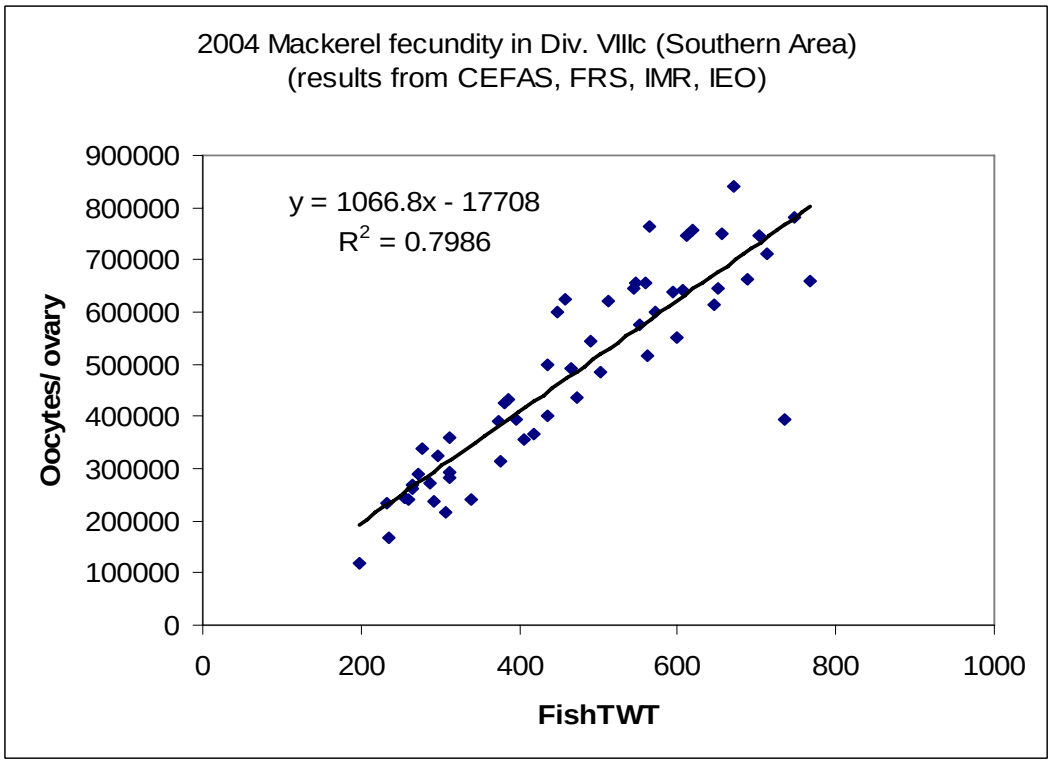

Figure 5.3.2.1: Mackerel fecundity against weight for the southern spawning component in 2004.

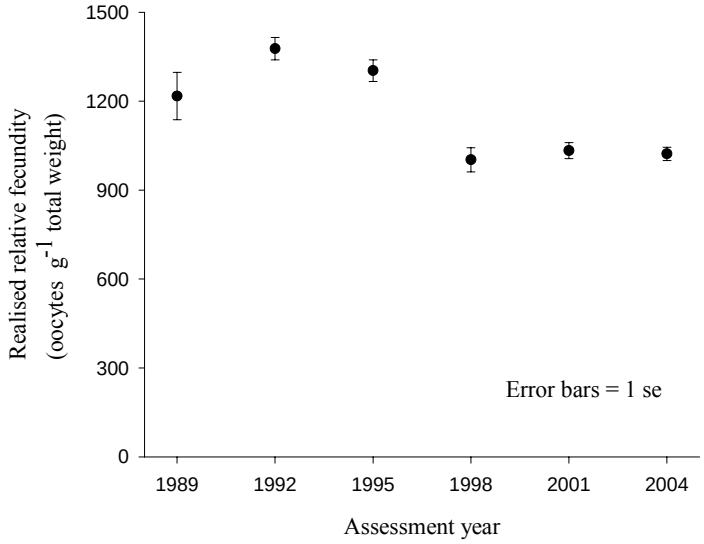

Figure 5.4.1.1: Historical time series of Western Mackerel realised fecundity since the first assessment in 1989 when atresia was deducted from the potential fecundity.

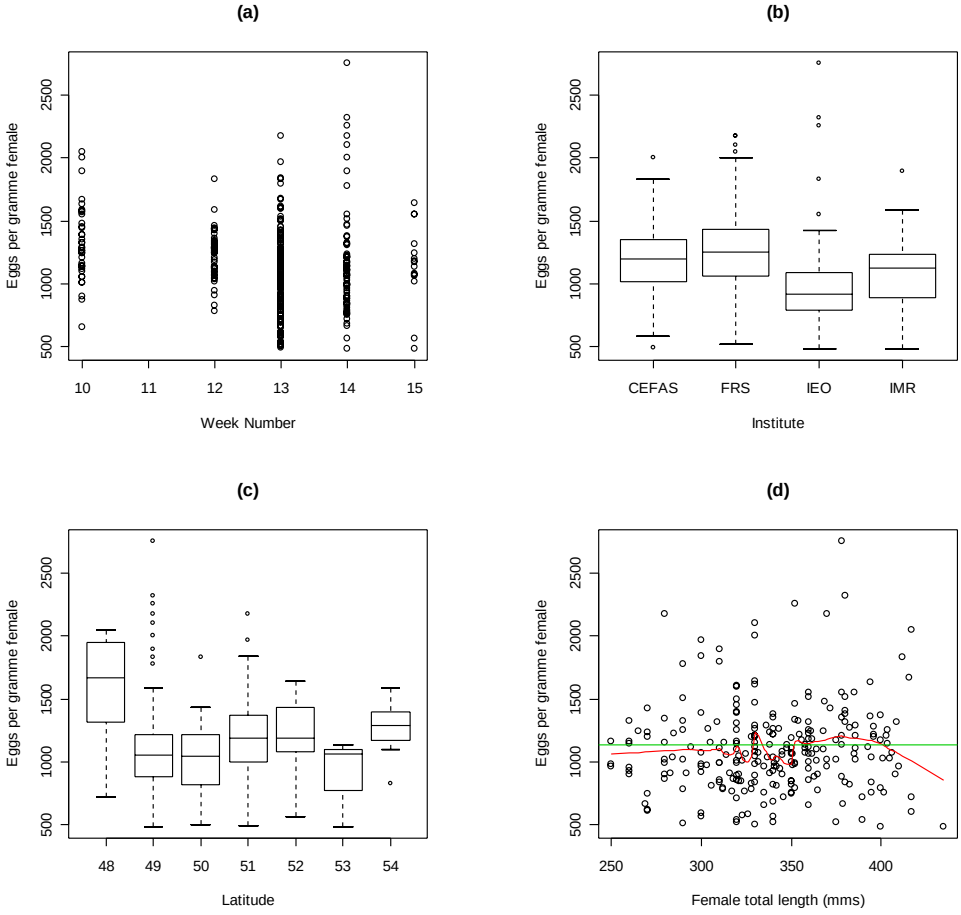

Figure 5.4.2.1: Potential fecundity in the western component of the NEA mackerel, plotted against a. week sampled. b. institute, c. latitude and d. female length.

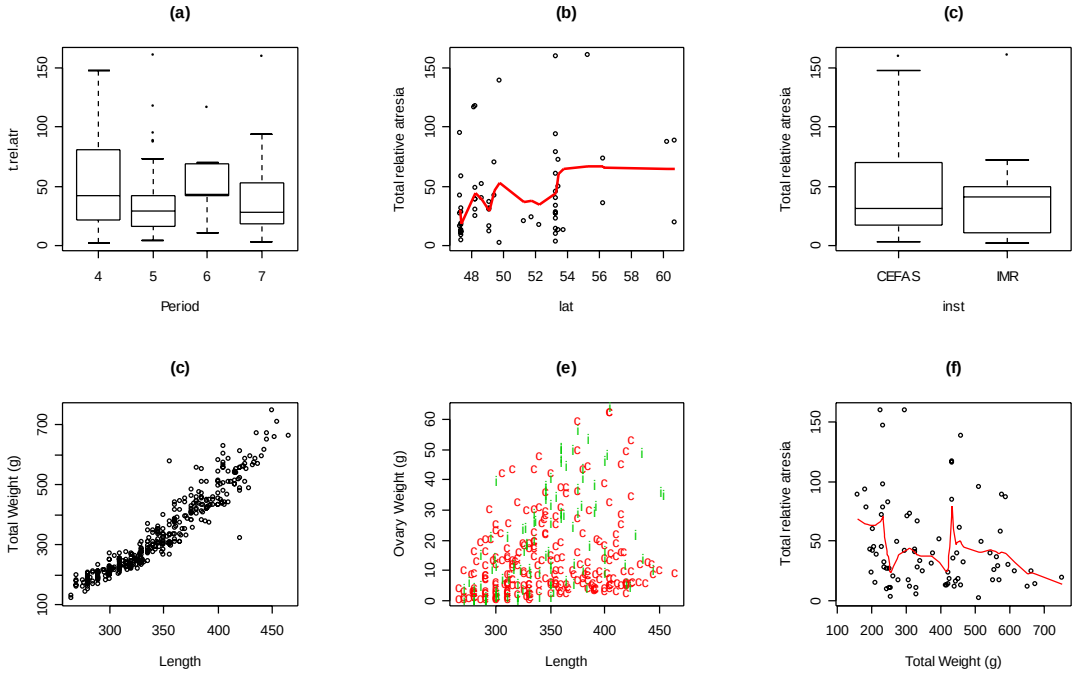

Figure 5.4.2.2: Total relative atresia in the western component of the NEA mackerel stock.

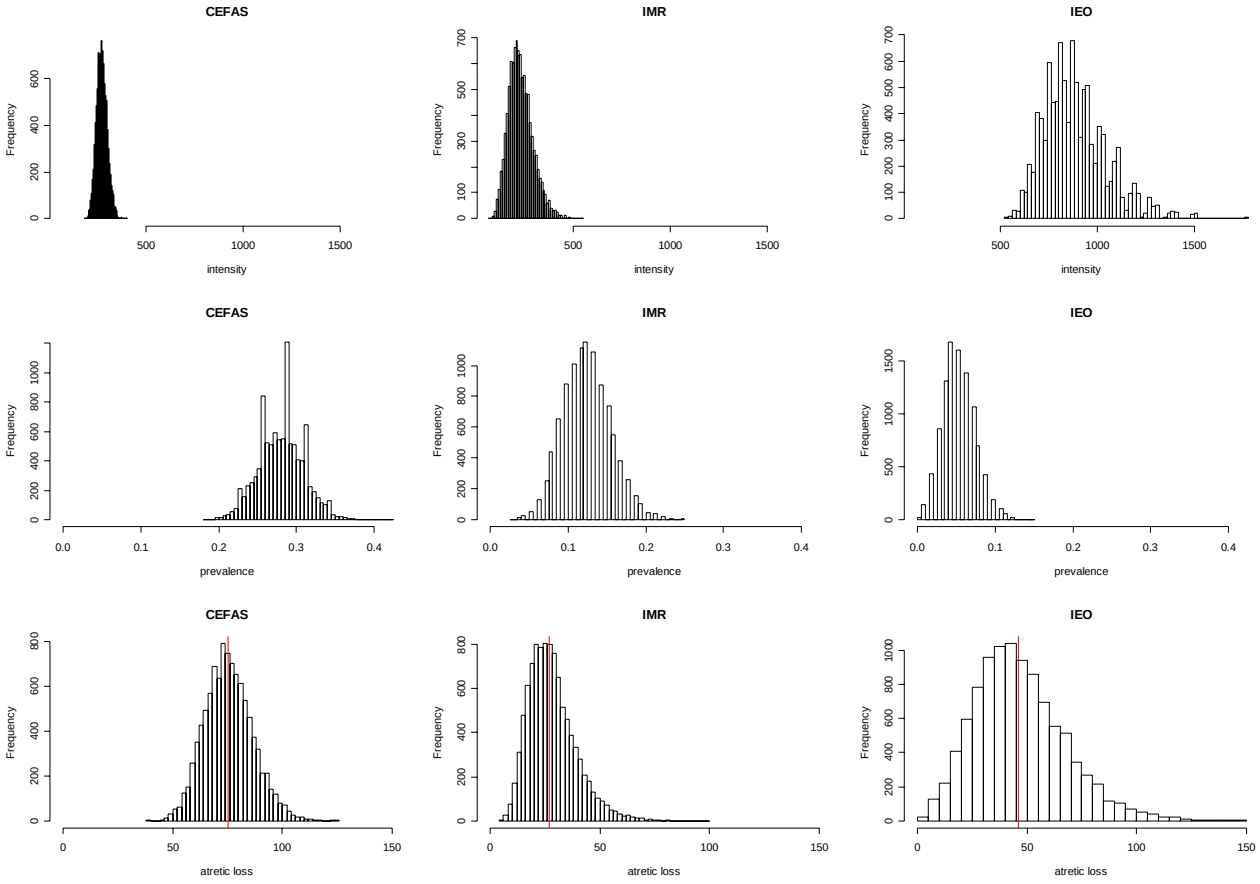

Figure 5.4.2.3: Comparison of intensity (top panel), prevalence (middle panel) and overall atretic loss (bottom panel) using 1000 bootstrap resamples. The red vertical line is the mean atretic loss. Note: the atretic loss (bottom panel) is the product of intensity and prevalence.

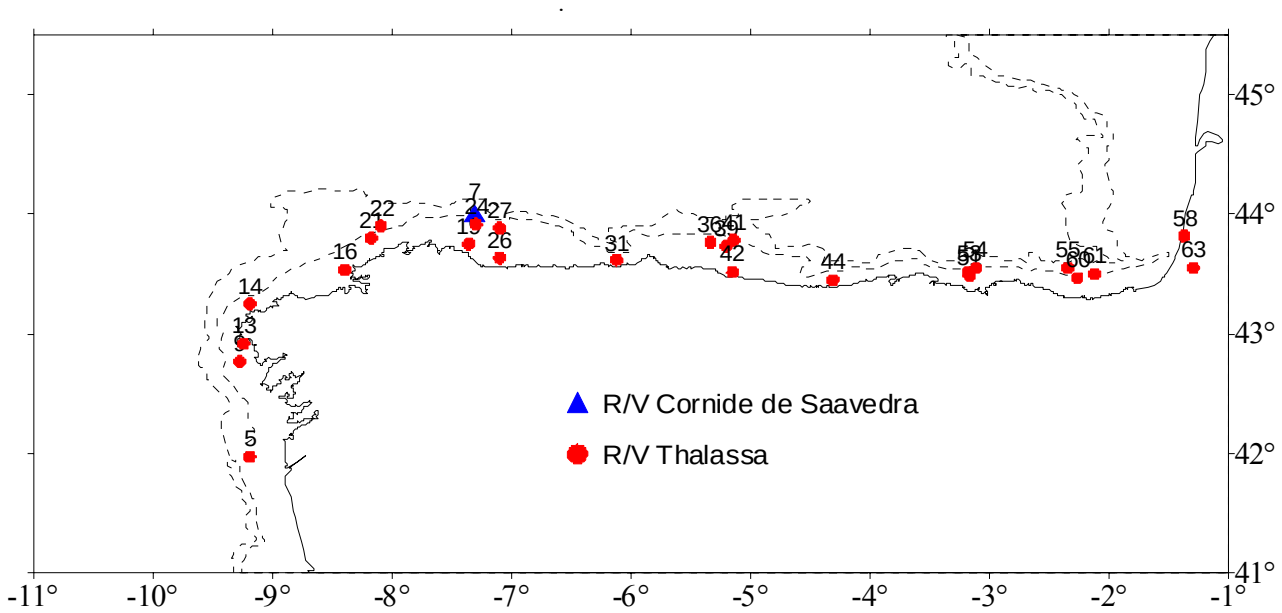

Figure 5.4.3.1: Sampling locations for atresia samples for the southern spawning component taken in periods 3 and 4 in Divisions IXa north and VIIIc

6 Western horse mackerel: 2004 egg survey results

6.1 Spatial distribution of stage I horse mackerel eggs

This description of the egg survey results is for both the previous western area and for the Cantabrian Sea which is now both included in the western stock.

- Period 3 – The first survey in the western area was in Period 3 (Figure 6.1.1c). Coverage was very good over most of area, from the Gulf of Cadiz to the North of Scotland. The main gap was on the coast of Portugal where weather curtailed sampling. The outside edges of sampling were well established for most of the area, although there was one significant interpolated rectangle on the western edge at 47° 15'N. Two main areas of high egg production were seen, in the Cantabrian Sea and particularly off Galicia, and also in the Celtic Sea along the shelf break between 48 and 50°N. The concentration off Galicia was not bounded by zero observations, raising the possibility of further production further offshore. Given the general spatial pattern of horse mackerel spawning this is probably unlikely.
- Period 4 – Coverage in this period was also very good (Figure 6.1.1d). There was again significant egg production in the Cantabrian Sea in this period, particularly, again, off Galicia. There was further scattered egg production along the shelf edge from 44 to 54°N. The edges were mostly well defined by zero observations including the hot spot off Galicia. The SE corner of Biscay had some gaps in sampling which required interpolation, except at the northern end of the area. One transect was missed at 52° 15'N, this was interpolated, but contained only zero values. .
- Period 5 – coverage was slightly less comprehensive than in the previous periods (Figure 6.1.1e). The Cantabrian Sea showed reduced production from period 4, but the edges were not defined by zero observations. Coverage in Biscay was patchy in the south, and the outside boundary was not established in the area immediately south of 47°N. The remainder of the area was well covered although part of the transect at 51° 45'N required interpolation. Production was concentrated along the 200m contour through Biscay and the Celtic Sea, with a hot spot at 48° 45'N.
- Coverage in Period 6 was also reasonably good, although probably not extended far enough south to encompass the full spawning distribution (Figure 6.1.1f). Egg production was concentrated mostly in the Celtic Sea area and west of Ireland, from 47° 45' to 53° N. As with mackerel, there was an eastward extension of spawning into the Celtic Sea south of Ireland. This extension was seen in previous surveys. As with the mackerel, it seems likely that there was further egg production to the north and east of the surveyed area in the Celtic Sea. There were some important interpolations in this period but these were well established.
- Period 7 – Only one vessel was available for sampling in this period so the coverage was necessarily less complete than in previous periods (Figure 6.1.1g). The aim was to survey in the likely main area of spawning for mackerel, not horse mackerel. It is likely that there would have been egg production to the north of the survey area, as was seen in 2001, and also to the south, although this cannot be confirmed. Significant numbers of rectangles required interpolation, and in one case produced a substantial value at 52° 15'N, 12° 45'W.

6.2 Stage I egg production of western horse mackerel

The mean daily stage I egg production estimates for each survey period are plotted against the mid-period days in Figure 6.2.1 to provide an egg production curve as presented for previous surveys. The results for 2001 are included for comparison. The data values are presented in Table 6.2.1. 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 3 to suggest any alternative start date. The end date (31 July) is the same as that used since 1995, although there was no sampling available to substantiate this in this year, the egg production curve for 2004 does not suggest any need to change this date. Production estimates for the individual survey periods are presented in Table 6.2.2. There was no temporal overlap between periods for the 1998 survey. Unlike 1998 and 2001, the survey periods were not all completely contiguous. There was a two day gap between periods 3 and 4 and a six day gap between periods 6 and 7. Egg production for these periods was calculated by linear interpolation, following the protocols published in previous WG reports (ICES, 1995).

Total annual egg production for the western area in 2004 was calculated as 0.678×10^{15} with a standard error of 0.150×10^{15}.

No data from the southern area were included in this analysis.

6.3 Western horse mackerel fecundity estimates

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

A total of 310 fish samples were collected during the 2004 western egg surveys from March until June with a good spatial coverage from 43°N to 53°N latitude. Sample details included fisheries parameters and are given in the ICES planning meeting (2003). Triplicate ovary samples were taken of each fish and samples above 48°N were analyzed by Netherlands, Norway and Ireland while samples below 48°N were analyzed by IEO. Samples were analyzed for oocyte frequency and mean oocyte diameter and fecundity derived by the gravimetric method described in Section 2.2. Threshold oocyte diameter to be included in the counts was 185µm. Sample weights were assumed to be 26mg for the 25µl pipettes and 105mg for the 100µl pipettes.

Fecundity within the western population is increasing after the onset of spawning (Figures 6.3.1 and 6.3.2). The increase is seen both in the southern and northern part of the western area but fecundity in the south is lower compared to the north. Mean oocyte diameter shows an increase from the onset of spawning for the northern part (Figure 6.3.3). However, the results from the southern part remain at the same level through the spawning season. The increase in fecundity throughout the spawning season was also apparent in the results from the 1998 and 2001 survey (ICES, 2002) and supports the assumption that horse mackerel is an indeterminate spawner; however a seasonal change in fecundity can also be due to individual females moving in and out of the spawning area.

Variation in horse mackerel fecundity estimates

Fecundity samples were collected from the Western spawning component throughout periods 3 to 7 and analysed by Ireland (MI), Netherlands (RIVO) and Norway (IMR). A comparison of replicate sub-samples showed large difference in relative fecundity between either MI or RIVO and IMR (Table 6.3.1) equating to 1.75 and 1.86 times less for the former two institutes compared to IMR. At this stage the cause of the difference has not been investigated but method was likely to be a contributory factor. Ireland and the Netherlands were using PAS-

staining for colouration of the oocytes, while Norway analysed samples without coloration and with a different image analysis system. However the large differences in fecundity results were not seen in the comparison between IMR and CEFAS or the Marine lab Aberdeen for mackerel (Figure 6.3.4) where the same procedures were used to complete the analysis. Because of the above differences the relative fecundity data has been combined for Ireland and RIVO whilst IMR is shown as a separated plot (Figure 6.3.5 – panels a and b). In both data sets relative fecundity varies a great deal within one day's collection and to a lesser extent over the season. From the start of sampling until day 90 fecundity was rather constant and then fell towards day 112 (average of 215 egg per g female n=13 fish) where upon there was a gradual increase to a maximum of 1152 eggs per g female (n=11 fish) towards the end of sampling on day 170. This value from was the highest mean relative fecundity for any sample taken through out the survey. Further analysis of this data considering also the fat content with respect to the egg production curve is required to try to understand the dynamics of egg production by this species.

6.4 Determinate versus indeterminate fecundity in horse mackerel

The question on whether horse mackerel has a determinate or indeterminate fecundity was discussed at the planning meeting for the 2004 egg survey (see Section 3.4.2.2 of ICES, 2003). At this WGMEGS meeting a working document was presented on this subject (Gordo, *et al*., WD 2005). During the HOMSIR project only one horse mackerel fecundity sample per year was collected in 7 areas (North Sea, west and south off Ireland, west and south of Portugal, off Mauritania and west Mediterranean) in 2001 and 2002. Each sample from each area was taken randomly either at the beginning, peak or end of spawning.

The mean standing stock of vitellogenic oocytes (residual fecundity) was estimated for:

1) pre-spawning ovaries: without any signs of spawning e.g., migrating nucleus stage oocytes, hydrated oocytes and post-ovulatory follicles;
2) imminent spawning ovaries: with migrating nucleus stage oocytes or with hydrated oocytes but without post-ovulatory follicles;
3) recently spent ovaries: with post-ovulatory follicles and low incidence of alpha stage oocytes.

In this study the estimated standing stock of vitellogenic oocytes (= residual fecundity) represents a certain time point in the spawning season. The residual fecundity has not been estimated as a mean over all collected ovaries, but has been estimated separately for pre-spawning, imminent spawning and recently spent ovaries. The increase in residual fecundity from pre-spawning to imminent spawning ovaries and the decrease again from imminent to recently spent ovaries indicates the occurrence of *de novo* vitellogenesis and therefore indeterminate fecundity (Figure 6.4.1. for all areas combined and Figure 6.4.2 by area). It is important to note that the difference in residual fecundity from pre-spawning to imminent spawning and back to recently spent roughly agrees to the batch size of 209 eggs per gram female, which was estimated during the 1992 international mackerel/horse mackerel egg surveys, when the annual and daily egg production methods where applied at the same time (ICES, 1993). This observed phenomenon does not seem to be a coincidence, since it is obvious in all areas. These figures indicate that a batch of pre-vitellogenic oocytes is developed to vitellogenic oocytes during the short period of migrating nucleus and hydrated oocyte stage. This indicates the occurrence of *de novo* vitellogenesis and therefore indeterminate fecundity of horse mackerel.

6.5 Lipid content of western horse mackerel

Considering the problems with the fecundity estimates in horse mackerel the WG opted for an alternative approach which was based on the fact that realized fecundity is determined by (ICES, 2003):

a) The energy indicated by lipid content and dry weight fraction prior to the onset of spawning.

b) The energy taken in as food during spawning.

Lucio and Martin (1989) showed that the condition factor does not appear to change to any great extent during the spawning season due to the replacement of fat by water. Therefore, the actual amount of lipids is regarded to reflect much better the energy content of a female fish and therefore also the expected realized fecundity. In addition, the food availability during the spawning season can be monitored by classifying the stomach fullness. This will provide information on the additional energy gained by feeding during the spawning season.

A total of 480 fish samples were collected prior to and during the 2004 egg survey. Of these 175 fish were analyzed for both lipid content and fecundity. Lipid content seems to be lower in the southern part of the western area compared to the northern part (Figure 6.5.1 and 6.5.2). Before the onset of spawning lipid content rapidly declines, while during the spawning season lipid content remains constant (Figure 6.5.3). The data of the 2004 survey shows a constant decline in lipid content suggesting that the peak occurred prior to sampling. If lipid content is to be used as an indication of fecundity sampling should be carried out during the peak period.

In order to assess energy intake the stomach content of the horse mackerel was monitored throughout the spawning season. Results (Table 6.5.1) show no evidence of feeding during spawning and there was no sign of regurgitation.

Given the fact that horse mackerel is likely to be an indeterminate spawner and that there is great variation within the fecundity and lipid content results the WG was not able to derive an index to convert egg production into SSB.

Table 6.2.1: Western horse mackerel mean daily Stage 1 egg production 10^{-12}

PERIOD	DATES	ESTIMATE
3	18/3 – 18/4	1.41
4	20/4 – 10/5	2.33
5	11/5 – 8/6	5.25
6	9/6 – 27/6	13.17
7	3/7 –16/7	6.19

Table 6.2.2: Western horse mackerel total Stage 1 egg production estimates by time period for 2004.

Dates	Period	Number of days	Annual Stage 1 egg production.10^{-15}
10/2 – 17/3	*	39	0.0199
18/3 – 18/4	3	30	0.04239
19/4 – 20/4	*	2	0.00392
20/4 – 10/5	4	20	0.0467
11/5 – 8/6	5	29	0.1524
9/6 – 27/6	6	19	0.2503
28/6 – 3/7	*	6	0.0547
3/7 –16/7	7	12	0.07436
17/7 – 31/7	*	15	0.0332
Total		0.678	
Standard deviation		0.15	
CV		22.0%	

Table 6.3.1: Comparison of horse mackerel fecundity estimates between institutes based on analysis of replicate sub-samples from 19 (Netherlands and Norway) and 16 fish (Norway).

	Netherlands RIVO	Ireland MI	Norway IMR
Standing stock of fecundity(mean)	142152	151691	267017
Standing stock of fecundity (se)	19092	18101	38286
Number of sample replicates	19	19	16
Ttest Netherlands Rivo compared	----	0.367	0.002
Ttest Ireland (MI) compared	0.367	----	0.004

Table 6.5.1: Stomach fullness of spawning horse mackerel.

	Stomachs	%
empty	235	87.0
partially full	24	8.9
full	10	3.7
stuffed	1	0.4
Total	270	100

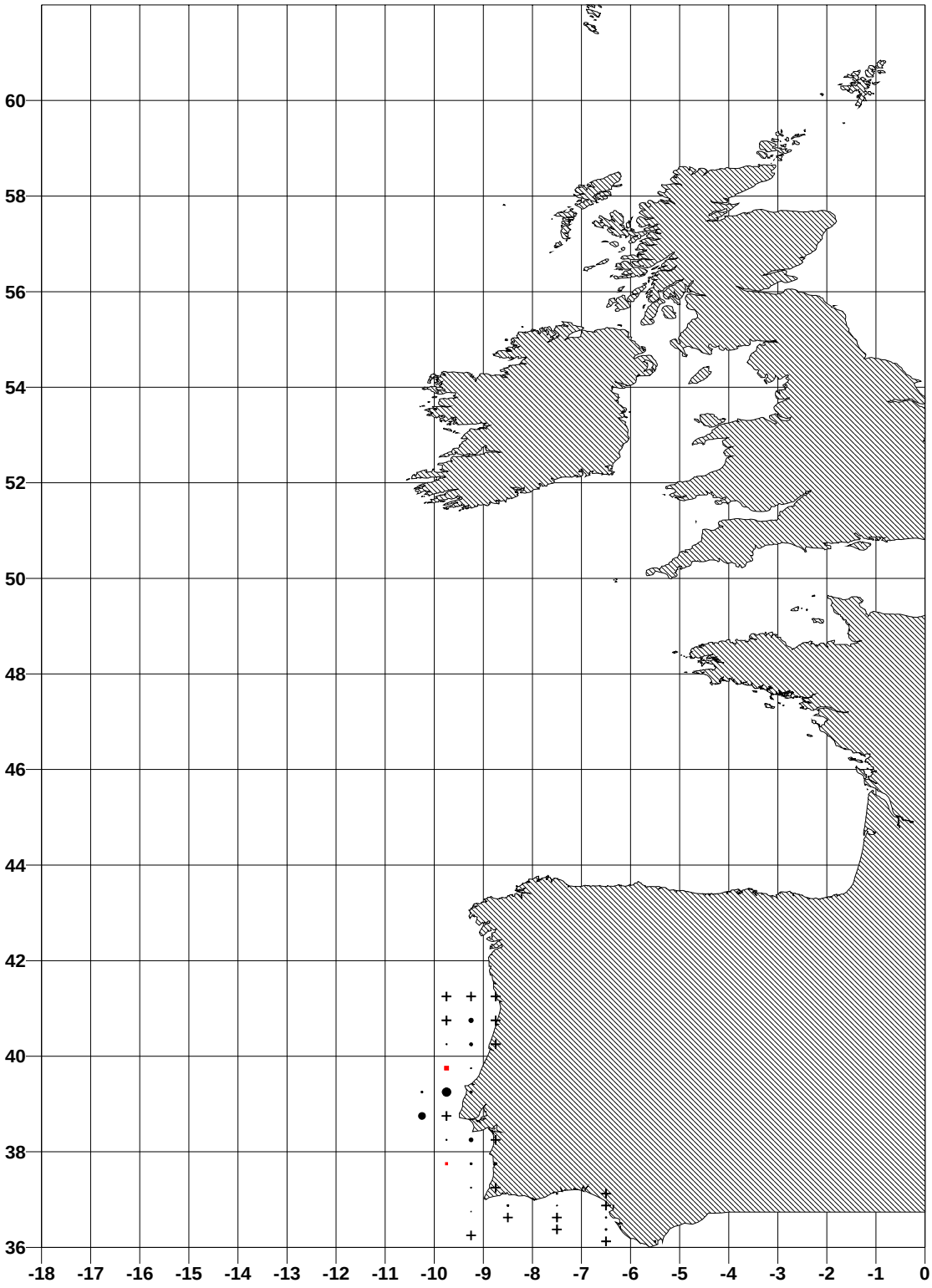

Figure 6.1.1a: Horse mackerel egg production by rectangle for period 1 (15 January – 26 January). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

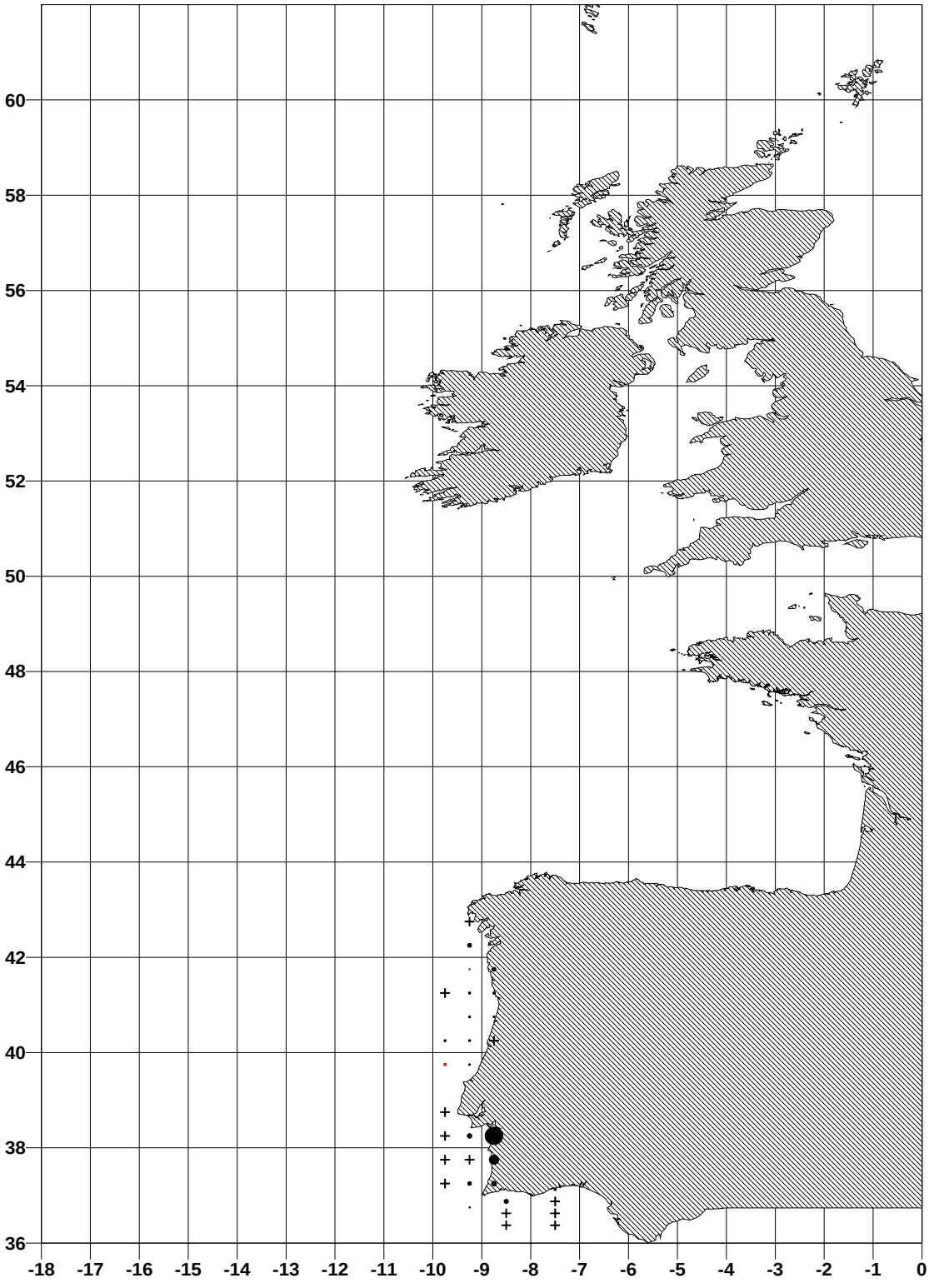

Figure 6.1.1b: Horse mackerel egg production by rectangle for period 2 (19 February – 2 March). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

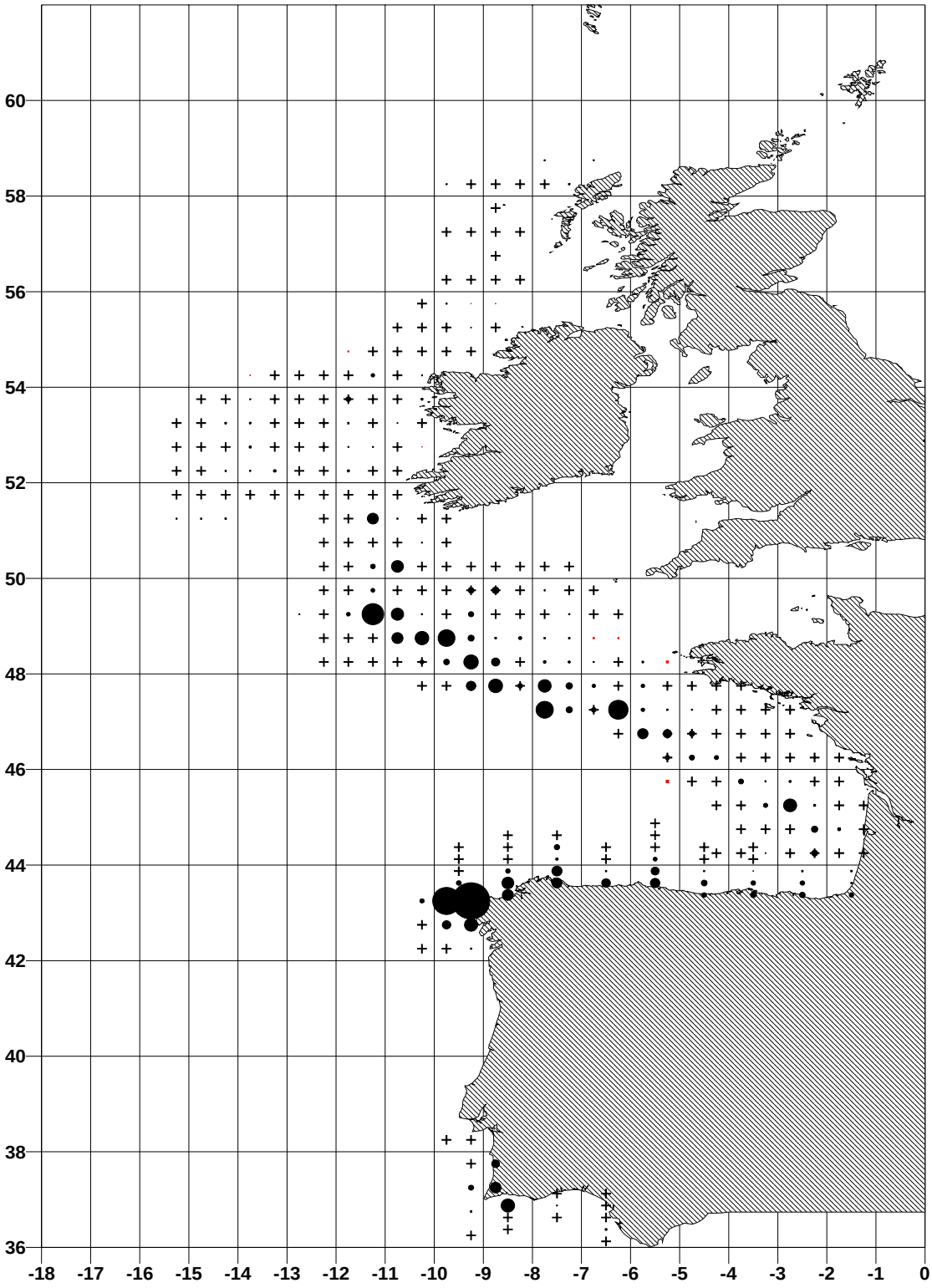

Figure 6.1.1c: Horse mackerel egg production by rectangle for period 3 (7 March – 10 April in southern area; 18 March – 18 April in western area). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^2.day^{-1}$.

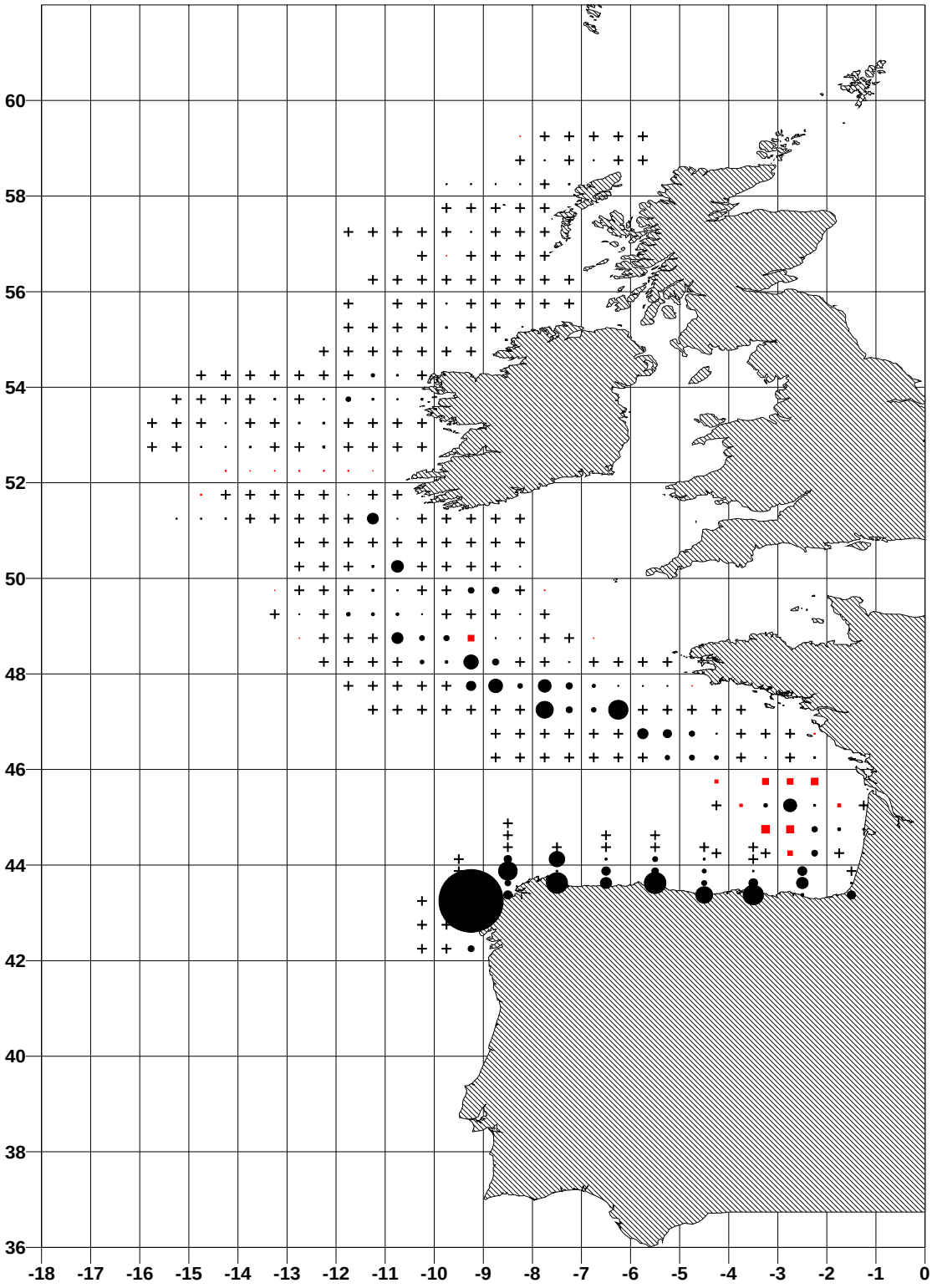

Figure 6.1.1d: Horse mackerel egg production by rectangle for period 4 (12 April – 6 May in southern area; 20 April – 10 May in western area). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

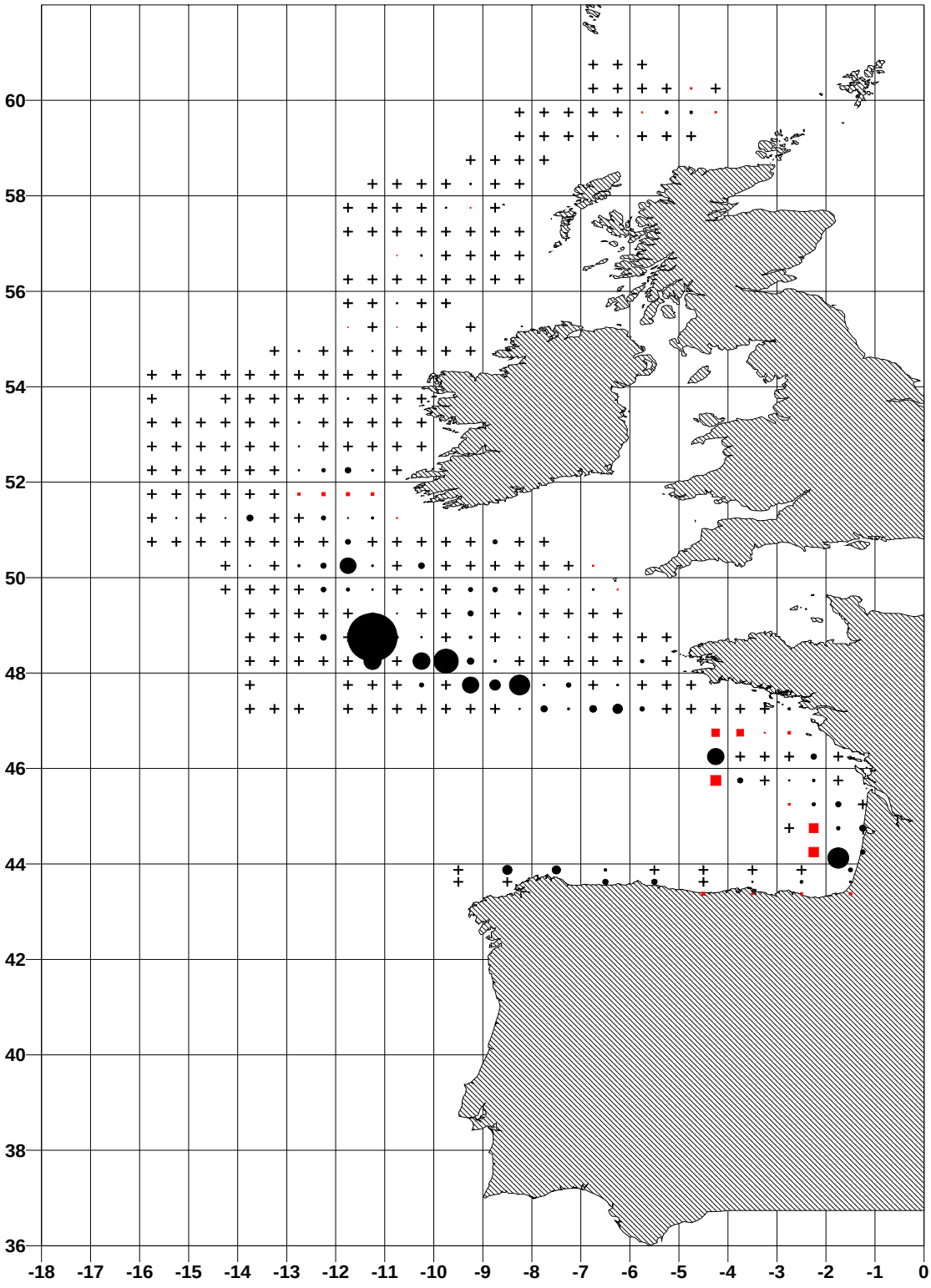

Figure 6.1.1e: Horse mackerel egg production by rectangle for period 5 (21 – 27 May - in southern area; 11 May – 8 June in western area). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

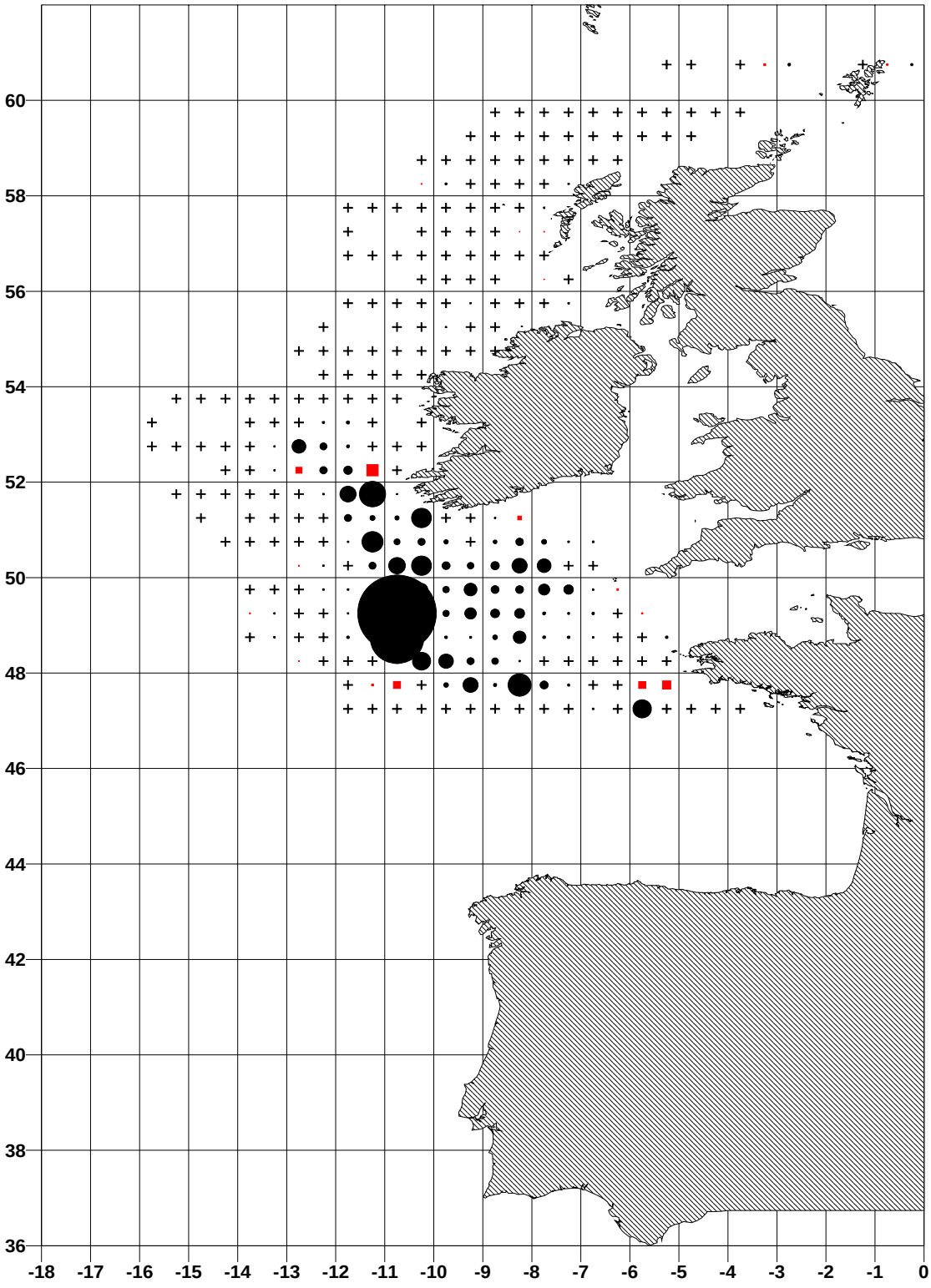

Figure 6.1.1f: Horse mackerel egg production by rectangle for period 6 (9 – 27 June). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

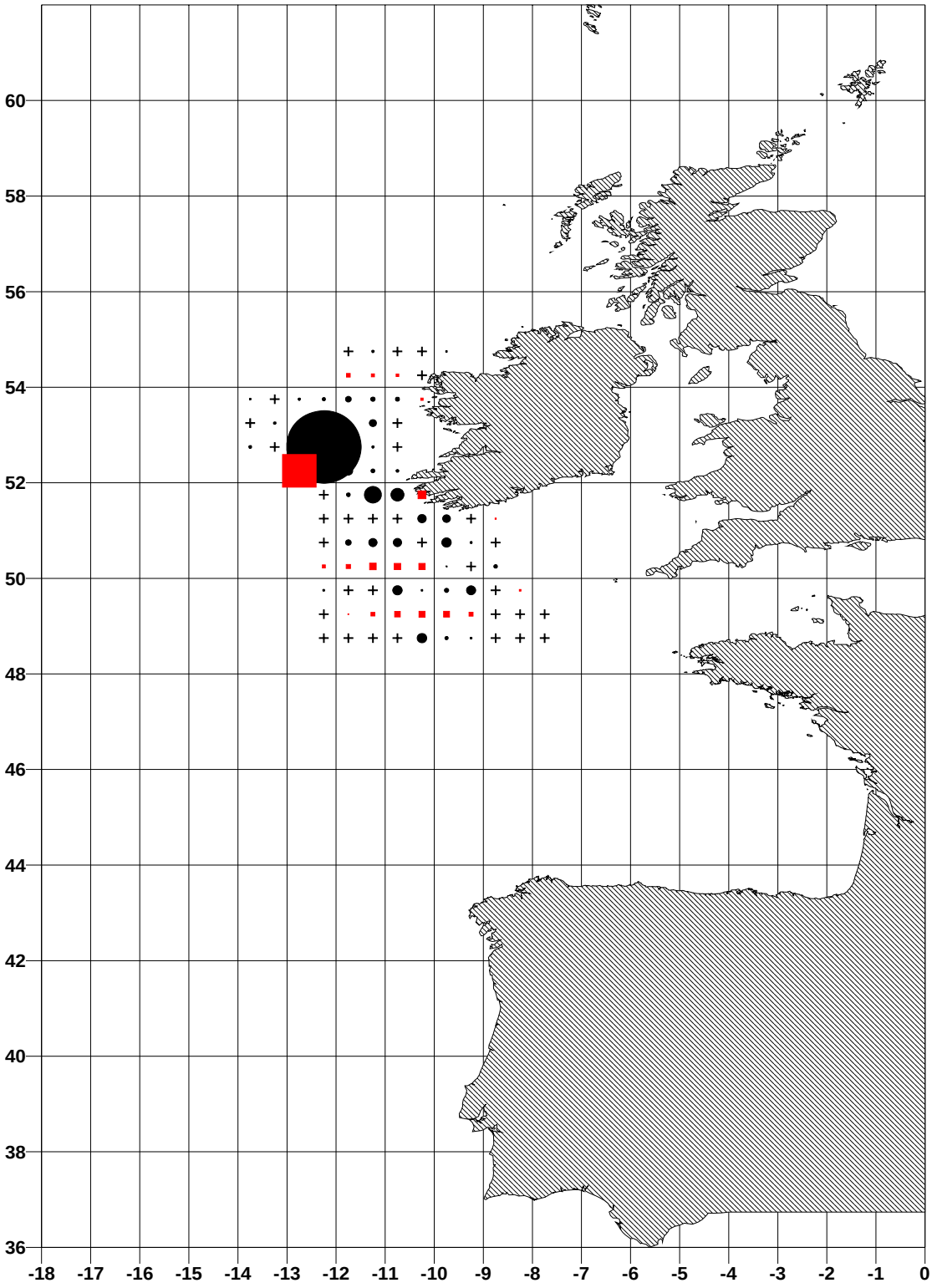

Figure 6.1.1g: Horse mackerel egg production by rectangle for period 7 (3 – 16 July). Filled circles represent observed values, filled squares represent interpolated values, and crosses represent observed zeroes. Interpolated zeroes are not included. Circles and squares are square root scaled to a maximum of 750 eggs $m^{-2}.day^{-1}$.

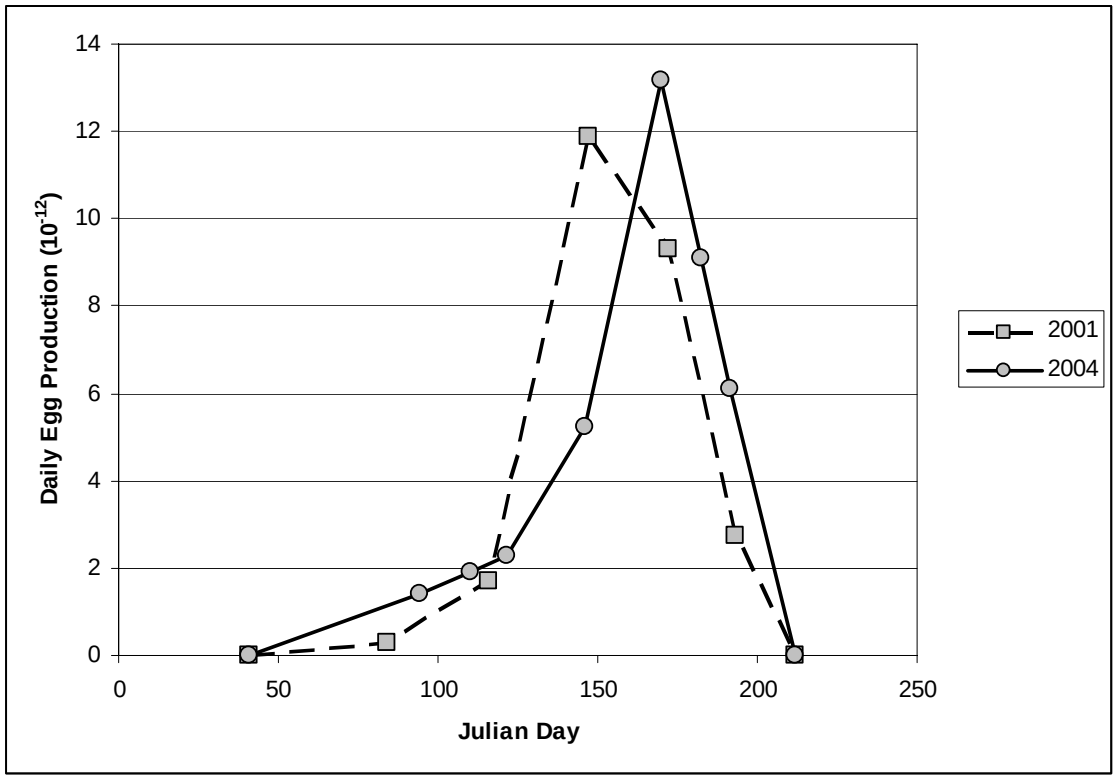

Figure 6.2.1: Annual egg production curve for western horse mackerel. The curve for 2001 is included for comparison.

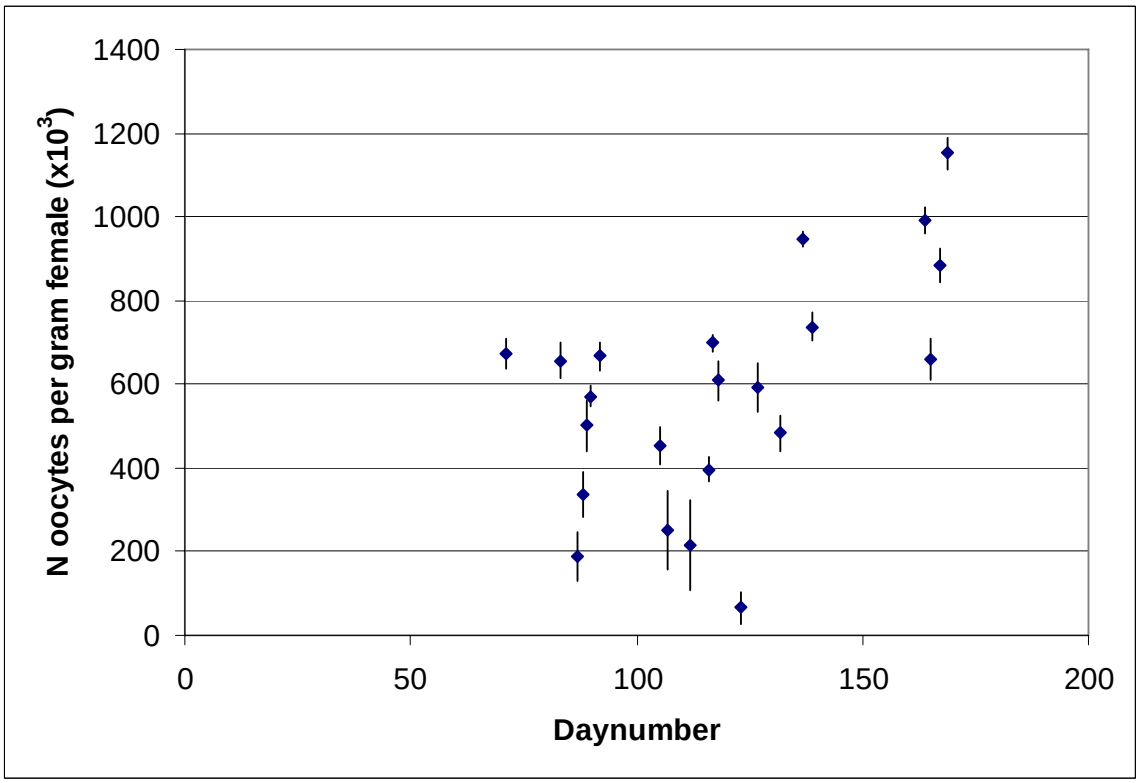

Figure 6.3.1: Variation in horse mackerel fecundity estimates in the northern part of the western area during the 2004 egg survey

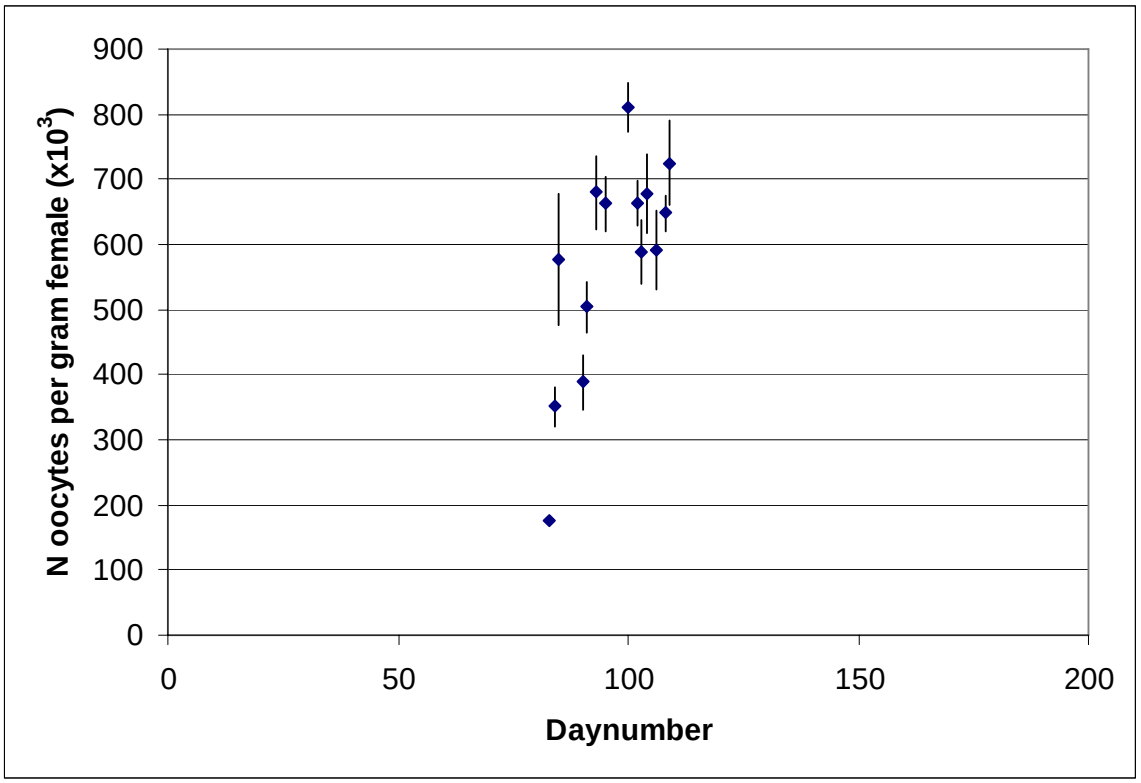

Figure 6.3.2: Variation in horse mackerel fecundity estimates in the southern part of the western area during the 2004 egg survey.

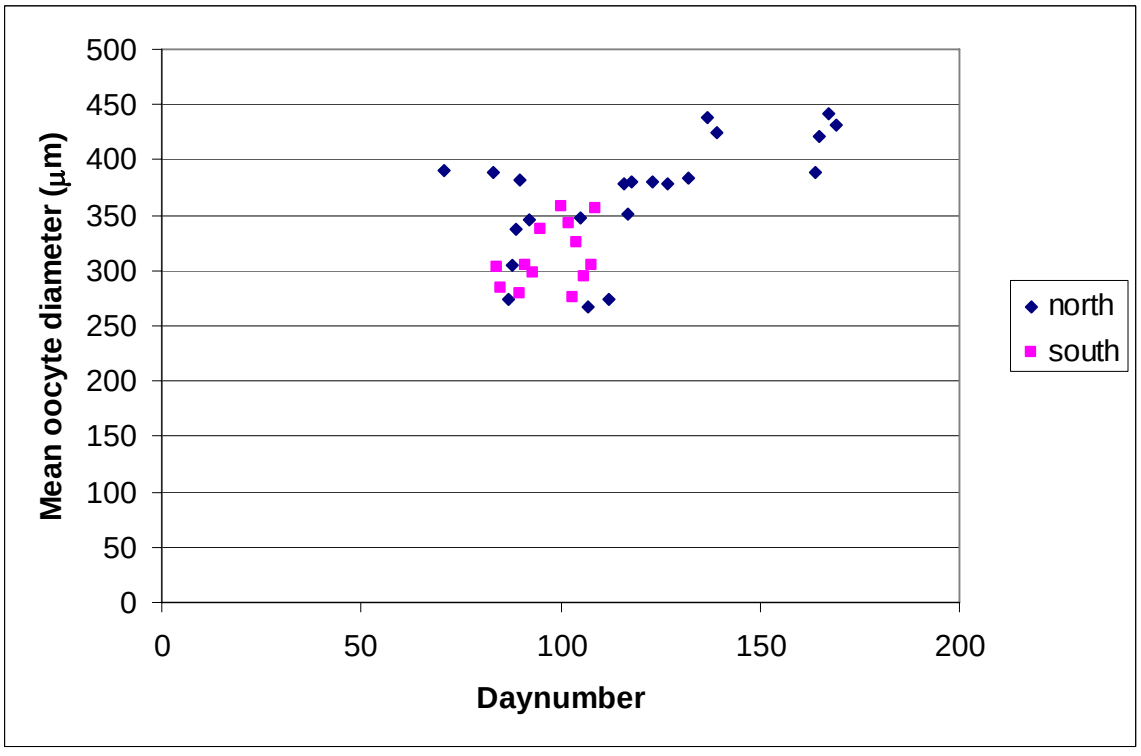

Figure 6.3.3: Variation in horse mackerel oocyte diameter in the western area during the 2004 egg survey

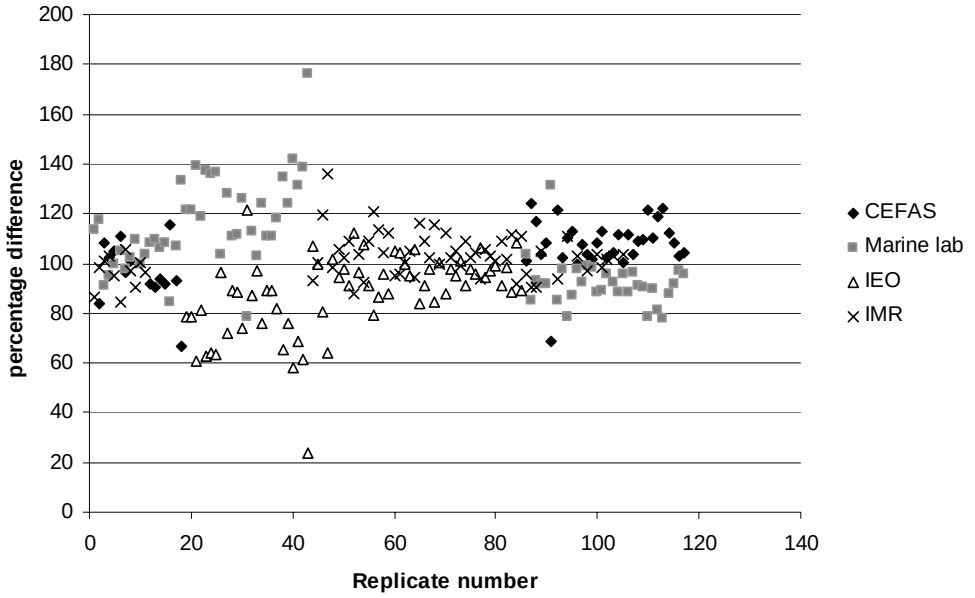

Figure 6.3.4: Comparison of replicate fecundity estimates by country expressed as a percentage difference from the sample mean.

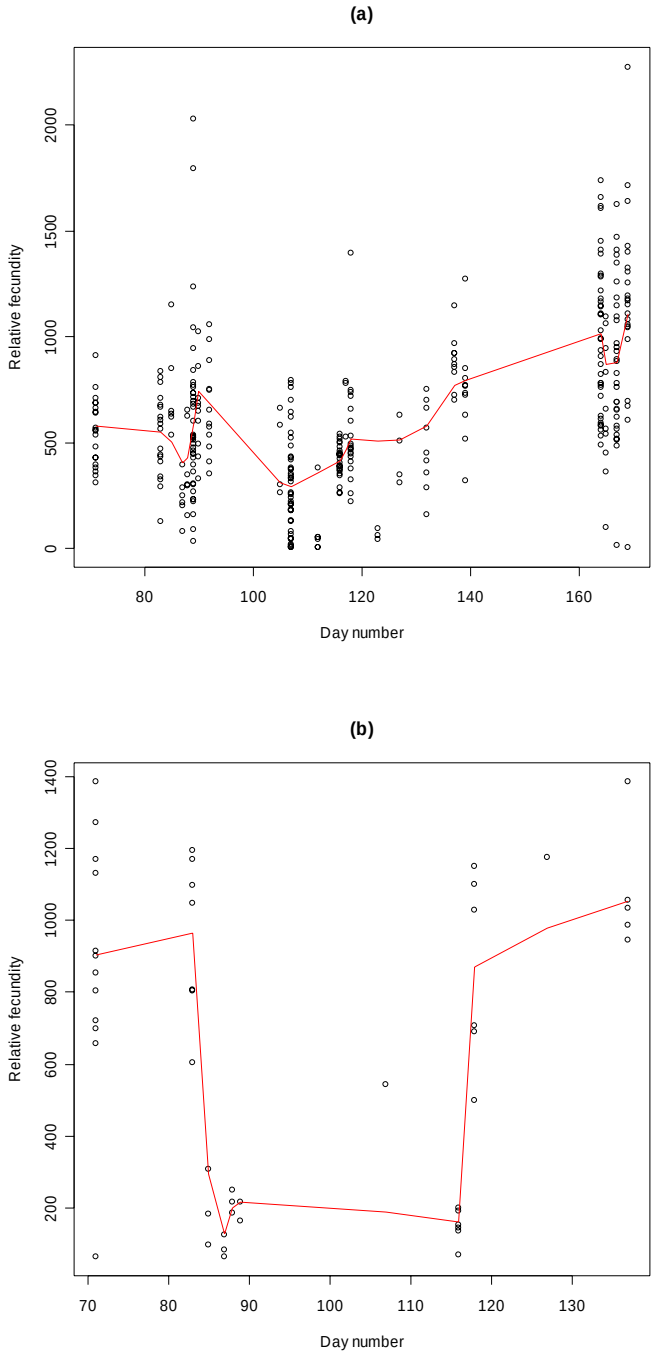

Figure 6.3.5: Variation in horse mackerel fecundity estimates in the northern part of the western area during the 2004 egg survey shown in the combined RIVO –Ireland data (panel a) and Norway (Panel b). The line was fitted using a smoothing function (Freidland 1984 1 and 2).

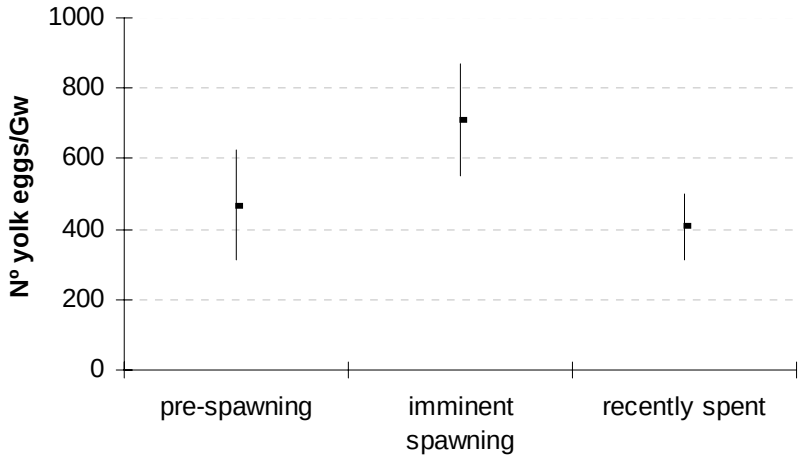

Figure 6.4.1: The number of vitellogenic oocytes per gram of female present in pre-spawning, imminent to spawning and recently spent ovaries for all areas combined.

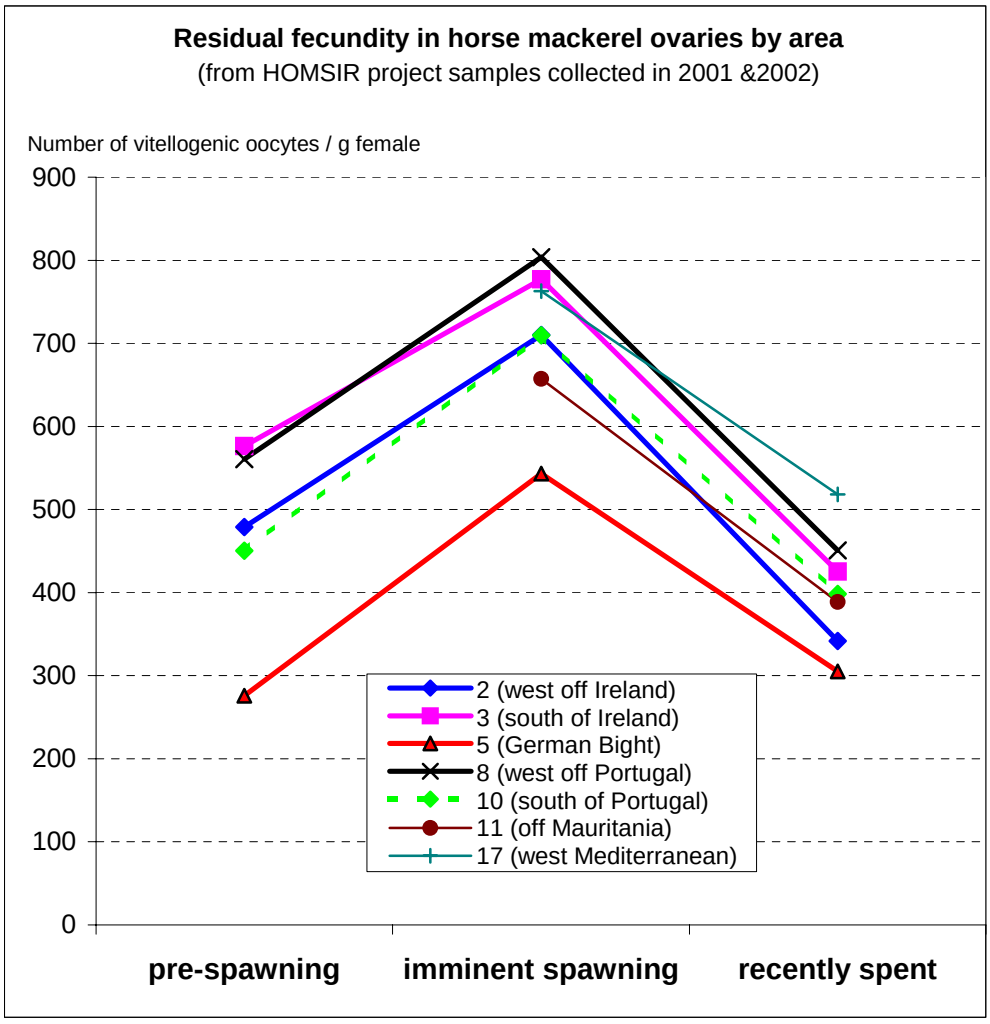

Figure 6.4.2: The number of vitellogenic oocytes per gram of female present in pre-spawning, imminent spawning and recently spent ovaries in each of the sampled areas.

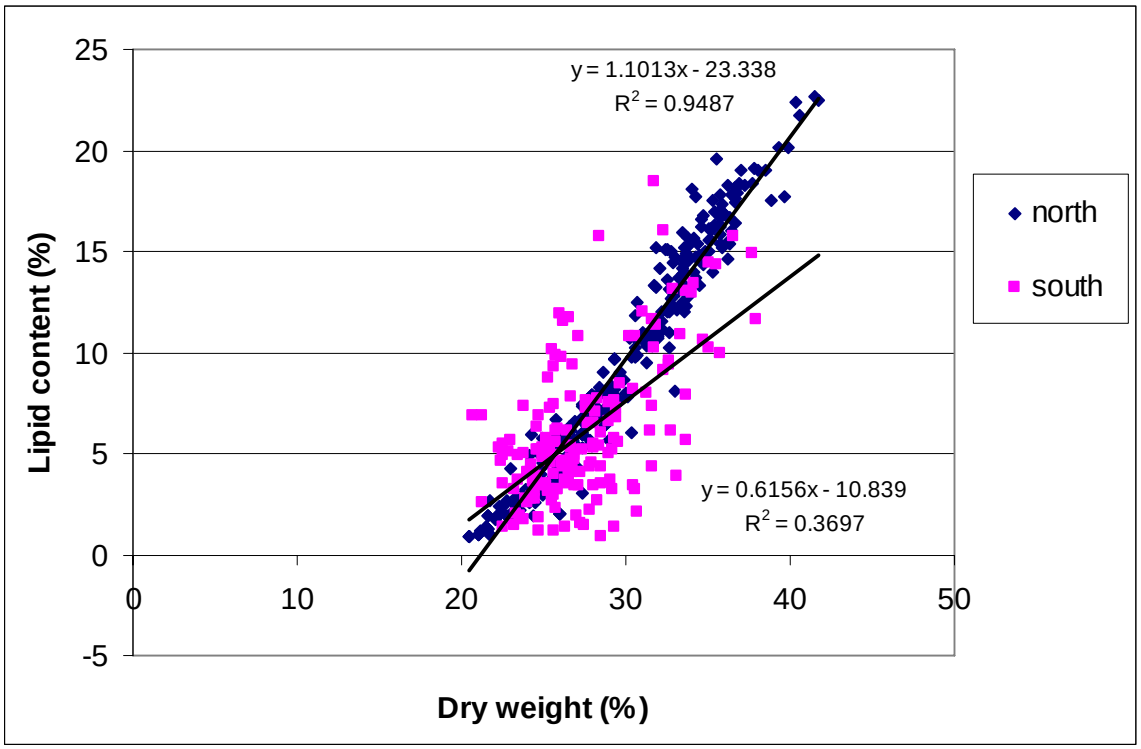

Figure 6.5.1: Horse mackerel lipid content in the western area.

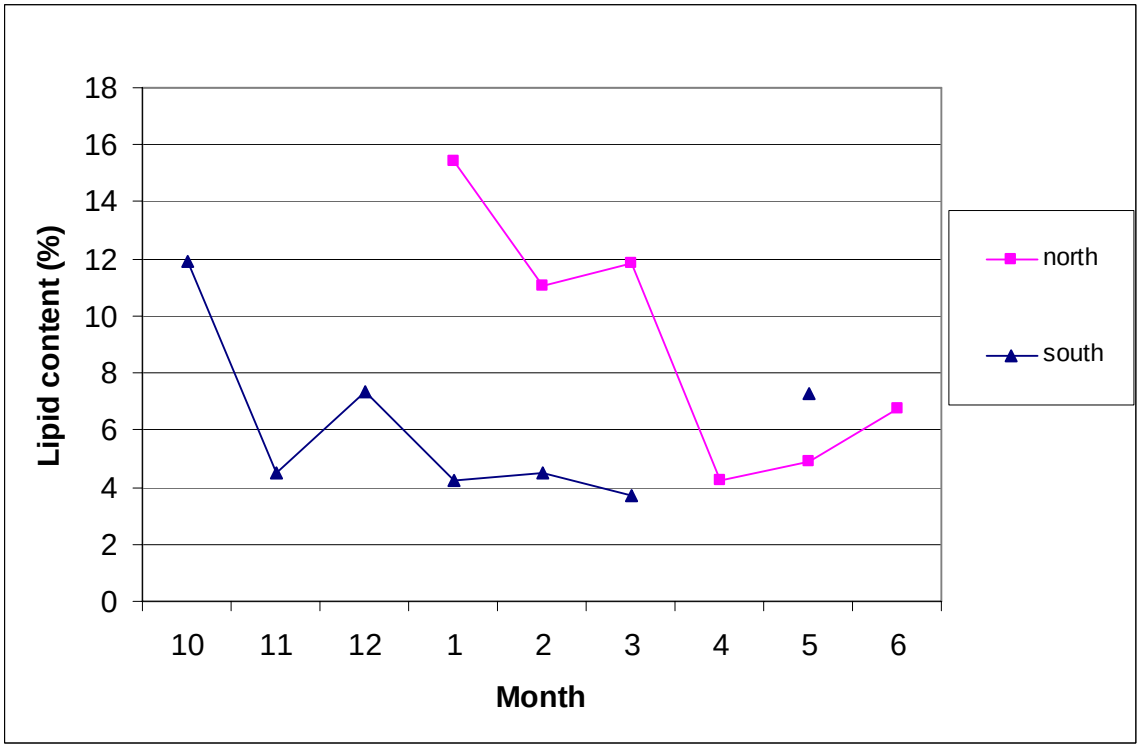

Figure 6.5.2: Horse mackerel lipid content in the western area over time.

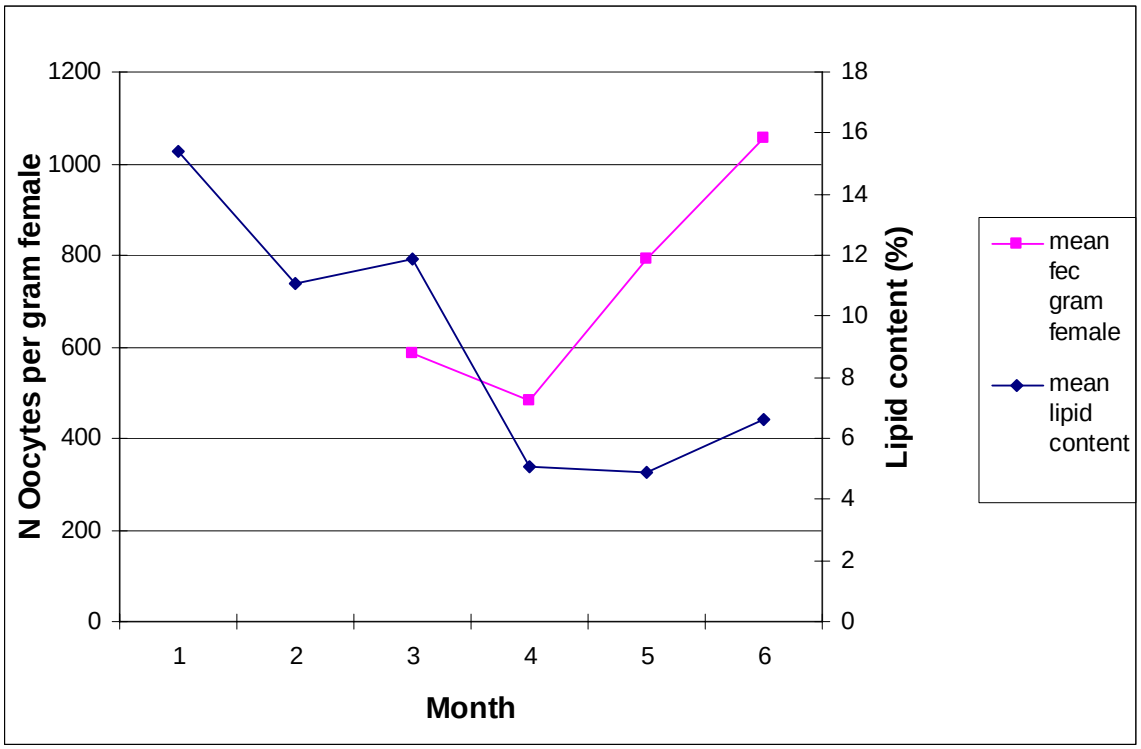

Figure 6.5.3: Horse mackerel lipid content and fecundity in the western area.

7 Southern horse mackerel: 2001 egg survey results

7.1 Spatial distribution of Stage I horse mackerel eggs

Distribution maps of daily stage I egg production per m^2 surface are given for the all survey periods in Figures 6.1.1a-e. The offshore limit of horse mackerel egg distribution was located at the shelf-break. Furthermore, the highest densities were usually found at the most coastal survey rectangles. In the Portuguese area, where sampling was particularly intense during periods 1 and 2, no high egg productions were found except in a single survey rectangle (38,25º N; 8,75º W) in the second period. The highest egg productions were estimated in the Cantabrian Sea during periods 3 and 4, with the peak of spawning in period 4. During these periods, important production values were found off the north Galician coast. In period 5, the daily egg production decreased considerably.

7.2 Stage I egg production of southern horse mackerel

The mean daily stage I egg production estimates for each survey period (Table 7.2.1) are plotted against the mid-cruise dates to provide egg production curve for horse mackerel (Figure 7.2.1). Total egg production values for survey periods and interpolated periods are given in Table 7.2.2. In 2004, the total annual egg production was estimated at 0.2484×10^{15} (s.e. 0.1208×10^{15}), that was approximately 45% higher than in 2001 (Table 7.2.3).

The start of spawning for horse mackerel was assumed to be on the 15 January, two days earlier than in previous years. This was based on recordings of stage I eggs found off the Portuguese coast during period 1. The end of the spawning was assumed to be the 17 July, as used previously.

Following the changes in the agreed boundary between the southern and western horse mackerel stocks (at 43º N latitude), mean daily egg production for each survey period is separately presented for Divisions VIIIc (western area) and IXa (southern area) (Table 7.2.4). As the survey was not designed for the new boundaries, there was coverage in both areas only period 3 and a part of period 4.

Table 7.2.1: Southern horse mackerel mean daily stage I egg production in 2004.

PERIOD	DATES	PRODUCTION(X 10^{-12})	SE
1	15 – 26/01	0.18	0.08
2	19/02 – 02/03	0.25	0.19
3	07/03 – 10/04	2.59	1.27
4	12/04 – 06/05	4.42	3.39
5	21 – 27/05	0.20	0.18

Table 7.2.2: Horse mackerel total stage I egg production estimates by time period for 2004 in southern area.

DATES	PERIOD	Nº OF DAYS	ANNUAL STAGE I EGG PRODUCTION (X 10^{-13})
15 January – 26 January	1	12	0.22
27 January – 18 February	*	23	0.49
19 February – 2 March	2	13	0.32
3 March – 6 March	*	4	0.38
7 March – 10 April	3	35	9.05
11 April	*	1	0.36
12 April – 6 May	4	25	11.04
7 May – 20 May	*	14	2.35
21 May –27 May	5	7	0.14
28 May – 17 July	*	51	0.48
	Total	185	24.84
	Se		12.08
	CV		0.49

Table 7.2.3: Total horse mackerel egg production in the southern area (VIIIc + IXa) from 1998 to 2004.

YEAR	ANNUAL STAGE I EGG PRODUCTION (X10–13)	
	estimate	se
1998	17.85	7.77
2001	17.13	6.16
2004	24.84	12.08

Table 7.2.4: Horse mackerel mean daily stage I egg production (x 10^{-12}) in ICES Division VIIIc and IXa in 2004.

PERIOD	DATES	VIIIC	SE	IXA	SE
1	15 – 26/01	Not sampled		0.18	0.08
2	19/02 – 02/03	Not sampled		0.25	0.19
3	07/03 – 10/04	2.15	1.26	0.44	0.19
4	12/04 – 06/05	4.34	3.39	0.06 *	0.06
5	21 – 27/05	0.20	0.18	Not sampled	

*** Sampled only from 42º to 43º N**

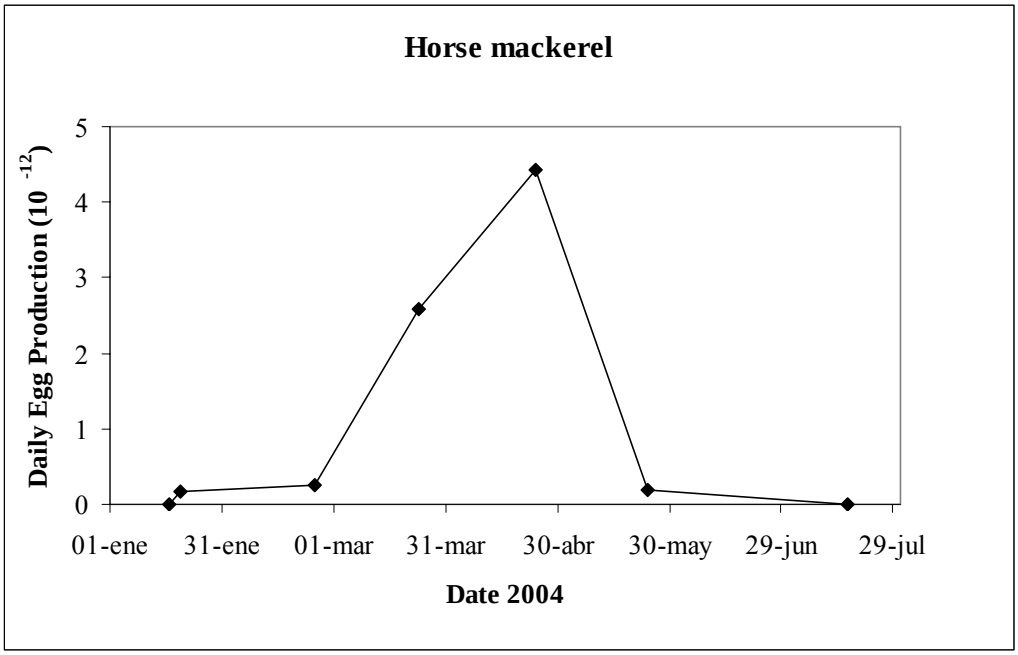

Figure 7.2.1: Annual egg production curve for southern horse mackerel.

7.3 Total fecundity of southern horse mackerel in 2001

No atresia sampling was carried out for horse mackerel in the southern area following the decisions of WGMEGS 2000.

Ovary samples for total fecundity determination from Portugal were examined using the histometric method (Emerson *et al*., 1990) and ovaries collected by Spain were examined using the auto-diametric method (Thorsen and Kjesbu, 2001).

The total fecundity was estimated by both Spain and Portugal based on 110 pre-spawning microscopic stage 3 ovary samples. A fecundity of 1619 oocytes/g (CV = 38.3 %) was estimated for Divisions VIIIc+IXa. This fecundity is 3 % higher than the value obtained in 2001 (Costa *et al.*, WD 2004) and is consistent with previous data. For Division IXa a fecundity of 1392 oocytes/g (CV = 43 %) has been estimated.

The monthly results of fecundity over the spawning season indicate that the fecundity increased over time (Figure 7.3.1).

Table 7.3.1 shows the evolution of total fecundity, annual egg production and respective CV's for Divisions VIIIc and IXa between 1995 and 2004.

Table 7.3.1: The evolution of total fecundity, annual egg production and respective CV's for Divisions VIIIc and IXa between 1995 and 2004.

YEAR	TOTAL EGGS X 10^{13}	CV	TOTAL FECUNDITY (EGGS/G)	CV
1995	17.54	?	1526	0.256
1998	17.85	0.422	1245	0.268
2001	17.13	0.360	1578	0.194
2004	24.84	0.490	1619	0.383

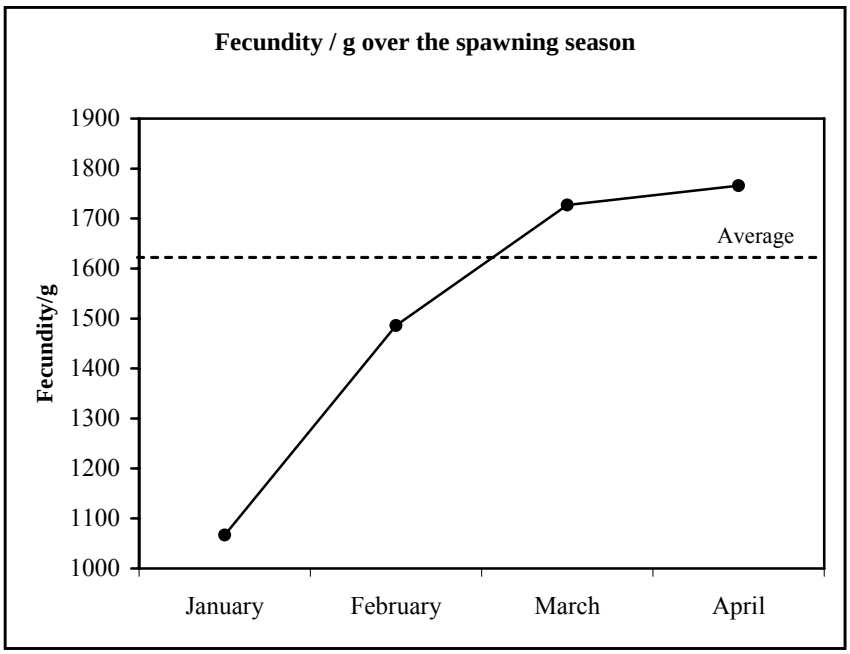

Figure 7.3.1: Monthly fecundity over the spawning season for southern horse mackerel for Divisions VIIIc and IXa.

8 Implications of new west/south division in horse mackerel

8.1 Revised egg production time series for the old and new southern and western horse mackerel stocks

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 2004 egg surveys were planned using the old definition of the southern horse mackerel stock corresponding to Divisions VIIIc and IXa (from Gibraltar to the French border). So the sampling strategy was not optimised for the new stock area. This was most noticeable for the coverage during sampling period 4 (April). This period corresponds to the maximum daily egg production in the full Iberian region; however, the surveys only included the west Galicia area.

Egg production data have been therefore been recalculated separately for the different areas affected by this change. This involved the calculation of a new egg production curve for Division VIIIc alone, in addition to the values given above for the old western and southern units. This was done for all surveys for the period 1995–2004 (in 1992 the survey used DEPM methodology and was not suitable for recalculation). The new southern area (Division IXa) total annual egg production was calculated by subtracting the value for division VIIIc from the old southern area (Divisions VIIIc + IXa) value.

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 total annual egg production estimates are given separately in the Table 8.1.1.

It should be noted that the survey coverage in the new southern area was incomplete, particularly in periods 4 and 5 in 2004 (April and May). Therefore the values are likely to be underestimates.

Total egg production for Division IXa represents 15–25% of the production for the area 0VIIIc + IXa, except in 1995 where very high production has been observed in Division IXa. This high 1995 egg production in Division IXa is consistent with the high horse mackerel recruitment observed in 1996 (ICES, CM 2005/ACFM:08).

Total egg production for Division VIIIc represents 75–85% of the production for the former southern stock and 12–25% for the former western stock, except in 1995 when low values were observed. This may be related to possible migrations during the spawning season. In 2004 western stock egg production has been increased by 32 % with the addition of the Division VIIIc.

8.2 Proposals for changes in egg production estimation in southern area

Taking into account the strong evidence that horse mackerel is an indeterminate spawner a daily egg production methodology must be apply in the future to the spawning stock biomass evaluation.

Since 2004 a new southern area has been adopted corresponding to the ICES Division IXa (ICES, CM 2005/ACFM: 08), see Figure 8.2.1.

In order to adapt eggs and adults sampling strategy to the new area and DEPM methodology, a 30–35 days survey during February-March is proposed covering adequately the eggs distribu-

tion area of figure below. Eggs and adults samples will be also collected for the AEPM mackerel evaluation.

Table 8.1.1: Total annual egg production figures based on both new and old definitions of the western and southern horse mackerel stocks.

AREA	TOTAL EGG PRODUCTION X 10^{-13}			
	1995	1998	2001	2004
Division IXa (new southern area)	13.29	4.58	3.45	3.19
Division VIIIc	4.25	13.27	13.68	21.65
Divisions VIIIc+IXa (old southern area)	17.54	17.85	17.13	24.84
Western area + VIIIc (new western area)	126.45	113.57	82.08	88.90
VIa +VIIbcefghjk+VIIIabde (old western area)	122.20	100.30	68.40	67.25

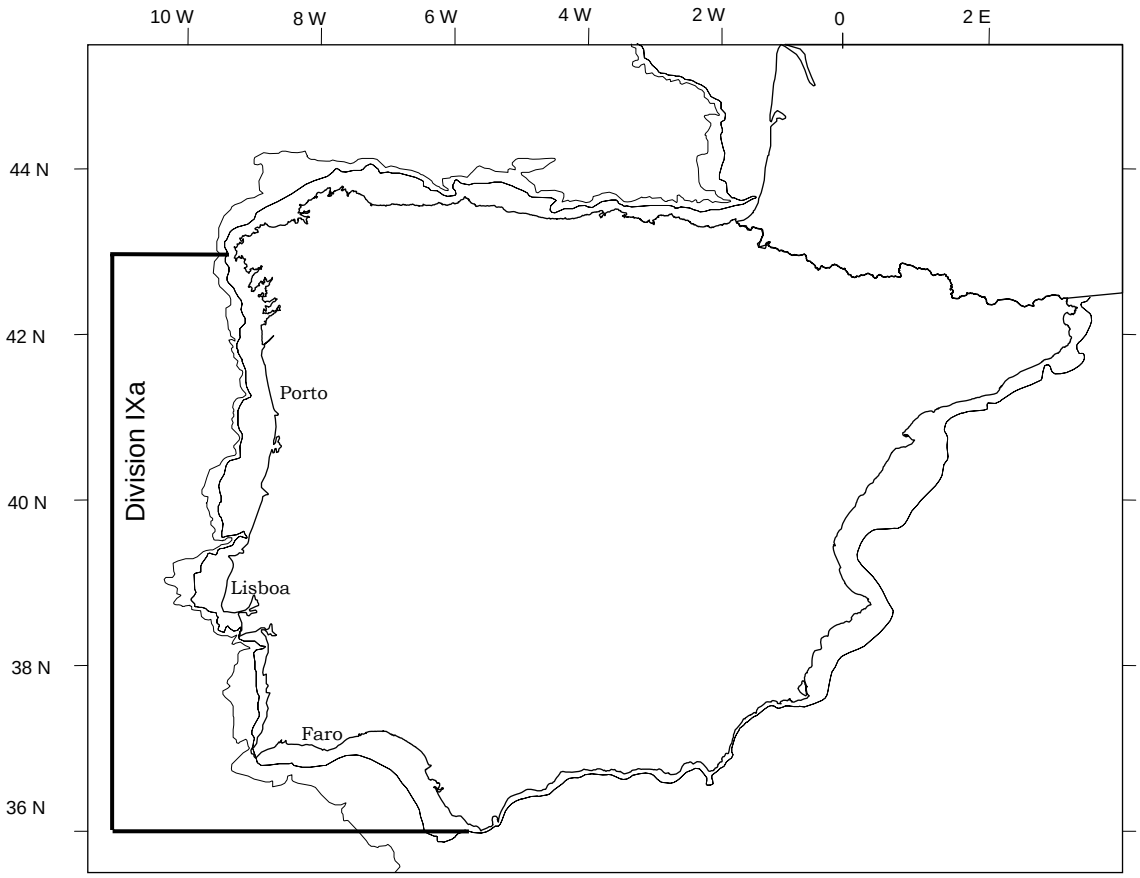

Figure 8.2.1: Map illustrating the area occupied by the new southern horse mackerel stock.

9 Variance and sources of variance

9.1 Review of method for traditional variance

The estimation of variance for the annual egg production using the Traditional Method has been described in previous reports (ref.). Estimations rely on the assumption that within a period each sampled rectangle has a different mean daily egg production but a constant coefficient of variation. Coefficient of variation can be estimated by two different approaches described below.

The first attempt uses the Analysis of Variance (ANOVA) on log-transformed values of daily egg production ($y = \log_e(D)$) over all replicated rectangles and periods.

$$\text{Mean Square Error} = \frac{\sum_{P}\sum_{R}\sum_{h}(y_i - \bar{y})^2}{\sum_{P}\sum_{R} n_{PR} - n_R} \cong (CV_D)^2 \qquad (1)$$

Where:

P = Period number

R = Rectangle number

h = haul number

$\bar{y}$ =Mean of log-transformed Daily egg production per rectangle

y_i = log-transformed Daily egg production per rectangle

n_{PR} = Number of rectangles by period

n_R = Number of hauls on rectangle

CV_D = *Coefficient of variation for Daily egg production per period*

It should be noted that samples with zero counts have to be excluded from the analysis. The mean square error output from the ANOVA is then assumed to be equivalent to that of the coefficient of variation squared per period (CV_D^2).

Currently coefficient of variation is implemented as a constant value of 1.24 for mackerel and 1.54 for horse mackerel (ICES, 1996). Early studies suggested that this value was rather constant and close to the value of 1 for both species (Fryer *et al.*, 1993). Coefficient of variation is then used to compute the overall annual variance as follows:

$$\sigma^2(\text{TAEP}) = \sum_{P} \lambda_p^2 \sigma^2(D_p) \qquad (2)$$

$\lambda_P =$ Length in days by Period

$D_p =$ Daily egg production by Period

P = Period

$\sigma^2(\text{TAEP})$=Variance of Total Annual Egg Production

Where variance of Daily egg production in Period ($\sigma^2(\mathrm{D_P})$) is the sum of the sampled rectangle variances:

$$\sigma^2(\mathrm{D_P}) = \sum^{R} \sigma^2(\overline{D}_R) \qquad (3)$$

$\overline{D}_R$ = Mean Daily egg production on rectangle

Variance of Mean Daily egg production on rectangle ($\sigma^2(\overline{\mathrm{D}}_\mathrm{R})$) is of the form:

$$\sigma^2(\overline{D}_R) = \frac{\left[\mathrm{A} * (\overline{\mathrm{D}}_\mathrm{R}) * \mathrm{CV_D}\right]^2}{\mathrm{n_R}} \qquad (4)$$

A = Rectangle area

$\mathrm{CV_D}$= Coefficient of variation of Daily egg production on rectangle

It has become apparent that after a threshold value (0.7), the assumption of coefficient of variation of non-transformed values equivalent to variance of transformed values is no longer valid. Above 0.7 coefficient of variation is systematically underestimated (Aitchinson and Brown, 1957). Pope and Woolner (1984) described an alternative way of estimate coefficient of variation on non-transformed values.

$$\mathrm{CV}_D = \sqrt{\mathrm{e}^{\sigma_y^2} - 1} \qquad (5)$$

y = log-transformed Daily egg production (D) on rectangle.

σ_y^2 = Variance of y

Estimated values of CV for the Southern were much higher than that of the western areas for both species when using the traditional approximation (values of 1.78 in period 1 to 3 and 2.03 in period 4 and 5 for mackerel and 1.99 in period 1 to 3 and 2.25 in period 4 and 5 for horse mackerel). We recommend the use of the second approximation (equations 2 to 5) for the estimates of variance in the southern area.

9.2 Wide scale review of sources, scale and direction of variance and its estimation

The egg survey estimate of total annual egg production (TAEP) would be improved if the variance of the estimator could be calculated reliably. Currently the TAEP is supplied with an estimate of variance and standard deviation. This variance is calculated in a rather crude manner from the egg survey data alone, i.e., directly from estimates of eggs m-2d-1. Other potential sources of variability are not considered which suggests that WGMEGs' estimates of variance on the egg survey data may be too low. The actual number used during the stock assessment process is the spawning stock biomass which is only some direct function of TAEP. The calculation of biomass from the egg survey data is complicated and involves many stages, each of which is subject to multifarious potential errors. The different types of variance can be summarised here as those which involve the egg survey itself and those that rely on data collected on individual fish, e.g., total fecundity, which are ultimately used in the spawning stock biomass assessment. The purpose of this section is to describe the potential sources of vari-

ability discussed at the working group and start the process of considering whether our variance estimates can be improved in the future.

Sources of variability on the egg survey:

1) The survey design itself may contribute to variance in our estimates of TAEP. It is always difficult to know whether all spawning activity is covered adequately in space and time. In the last survey (2004) for example, there was a large amount of relatively unusual spawning activity by both mackerel and horse mackerel in the Celtic Sea. Additionally, the current survey design and its constraints mean that only one observation per station is available and this makes the estimation of variance inherently difficult.

2) The estimation 'model'. The particular protocol used to estimate TAEP from the data will contribute to the variance. A geostatistical estimator for example, will better chart the spatial dynamic of the egg production process. This will result in an estimate of variance which is much lower than one given by the traditional procedure where spatial dependence is 'modelled' using simple linear interpolation. Different models have been tried by WGMEGs in the past (e.g., regression models, geostatistical models) but the traditional estimator which aggregates the data into the predefined 'periods' and interpolates linearly into unto unsampled rectangles has been found to be the most robust.

3) The TAEP estimation procedure uses a pair of stochastic models to help estimate the number of stage I eggs produced every 24 hours. These models were originally fitted to raw experimental data and have a standard error associated with them. It is straightforward to incorporate this source of error into the TAEP estimate but this is not currently attempted.

4) The age of the mackerel and horse mackerel eggs are approximated using a staging scheme; mackerel have 5 stages and horse mackerel have 4. It is possible, therefore, that some eggs are not assigned to the correct stage.

5) The mortality experienced by each egg between the moment it is spawned and the moment it is caught is not currently taken into consideration. Egg mortality is not only difficult to measure accurately but is also likely to be highly variable. Recent attempts to tackle this problem have found that only approximately 50% of stage I eggs survive to stage II. This means that more eggs are actually spawned than are recorded and that the estimate of TAEP we make is probably far too low. [Note: The estimates of mortality, however, are not sufficiently variable in space and time to seriously affect the long-term trend in TAEP which means that stock assessments for mackerel are unaffected]. Recent work by Enrique Portilla has indicated that stage I egg mortality (using birth-death models) and its associated variance (by resampling) can be estimated. This information could, in future, be supplied to WGMEGs and incorporated into the overall variance estimate.

6) The temperature history experienced by each egg and therefore its stage duration are not well known. Currently the temperature measured at 20m is inserted into the stage development equations. Egg development is actually quite sensitive to ambient temperature which can vary in the sea over surprisingly small scales. A better understanding of the range of temperatures experienced by each egg would be useful in variance estimation.

7) It was noted that different institutes might operate their sampling gear in different ways. In theory the water column should be sampled using a double oblique tow with the gear falling and being hauled at identical rates. In practice this is difficult to achieve due to varying winch efficiencies, monitoring and the unpredictable nature of currents, winds and tides. It is known that eggs aggregate at depth horizons. If, therefore, the sampler spends variable amounts of time at these important places, egg density might be over or under-estimated. It is difficult to know how variability due to this problem might be incorporated. The best solution is probably to improve monitoring of the sampling gear while deployed.

8) It is possible that not every egg is removed from each sample. Spray techniques have been developed to ameliorate this potential problem but they are not used by all participating institutes. Studies on the efficiency of the spray techniques have

been presented to WGMEGs and the conclusions are that well over 90% of eggs are removed. This means, however, that some are lost. The variance (and indeed bias) due to this could be estimated and incorporated into our variance estimate.

Sources of variability on subsequent estimate of spawning stock biomass:

1) In order to estimate spawning stock biomass (SSB) from the TAEP it is necessary to find out the relationship between egg numbers and individual female weights. This can vary spatially and temporally although it is well established that the quantity of eggs per gram of female flesh is constant with respect to total weight. Fecundities enter the SSB calculation as averages without concomitant variance estimation. This could be done in future.

2) Similarly it is essential to gauge how many eggs are resorbed by each female, a process which occurs sporadically in response to periods of starvation. This process is known as atresia and is variable, not only in the number of oocytes resorbed but in the number of fish that are resorbing them. In some populations/times/locations there is very little 'atretic loss' while in others it is much more prevalent. The rate of atretric loss also enters the SSB calculation as an average without variance and it could be done in future.

3) Sex ratio in mackerel is assumed to be 1:1 which may or may not be true. Clearly if it is incorrect it will cause bias. As far as variance estimation is concerned sex ratios could be calculated from various sources (research survey trawl data, assessment data etc.) and its variance then estimated which could be inserted into the SSB calculation.

In summary, while there is some attempt to estimate variance on the estimate of TAEP, none are attempted for many of the other potentially important parameters.

9.3 Development equations

Artificial fertilizations were carried out using ripe mackerel caught on hand-lines during March 2004 of the coast of San Sebastian (43º 47´– 1º 50´, Basque country, Spain). Mackerel eggs were incubated at 5 temperatures from 8 to 18 ºC. Development rate experiments were performed in 175 litres tanks in the San Sebastian Aquarium. At the same time, a study on egg mortality was made using a sample of 30 eggs placed individually into small tubes with 35 ml of sea water. The relationships between time to reach the end of each development stage and at each temperature were estimated and compared with those obtained by Lockwood *et al.* (1977). For stage IA and Stage 1B (Table 9.3.1) the egg development rates (b values) were statistically different at 99% and 95% of confidence level respectively. The equation obtained for stage IB was: Ln time (hours) = 6.902 – 1.314 Ln (Temp). Using this new development equation and at 10–12ºC temperature range, egg production would increase about 10–12%.

It was estimated that egg mortality would be in the range 9–14%, independent of temperature. I was observed that the mortality increased at the end point of stage V, just prior to hatching (see Figure 9.3.1). At this point, mortality was much higher at the lowest temperature.

Table 9.3.1: Parameters of the linear regression: "Ln time = a + b Ln temperature" for each development stage obtained by Lockwood 1997 and AZTI 2004. ANCOVAs comparing slopes and intercepts for each pair of regressions were made. * and **denotes differences at the 99% and 95% confidence level respectively.**

	Lockwood 1977		AZTI, 2004	
Stage	a	b	a	b
Stage 1A	7,206	-1.600***	5,036	-0.740
Stage 1B	7.759***	-1.613**	6,902	-1,314
Stage II	7.578***	-1.454	8,276	-1.588
Stag III	8.938***	-1.682	8,327	-1.513
Stage IV	8.987***	-1,647	8,497	-1.493
Stage V	8,738	-1.553	8,671	-1.511

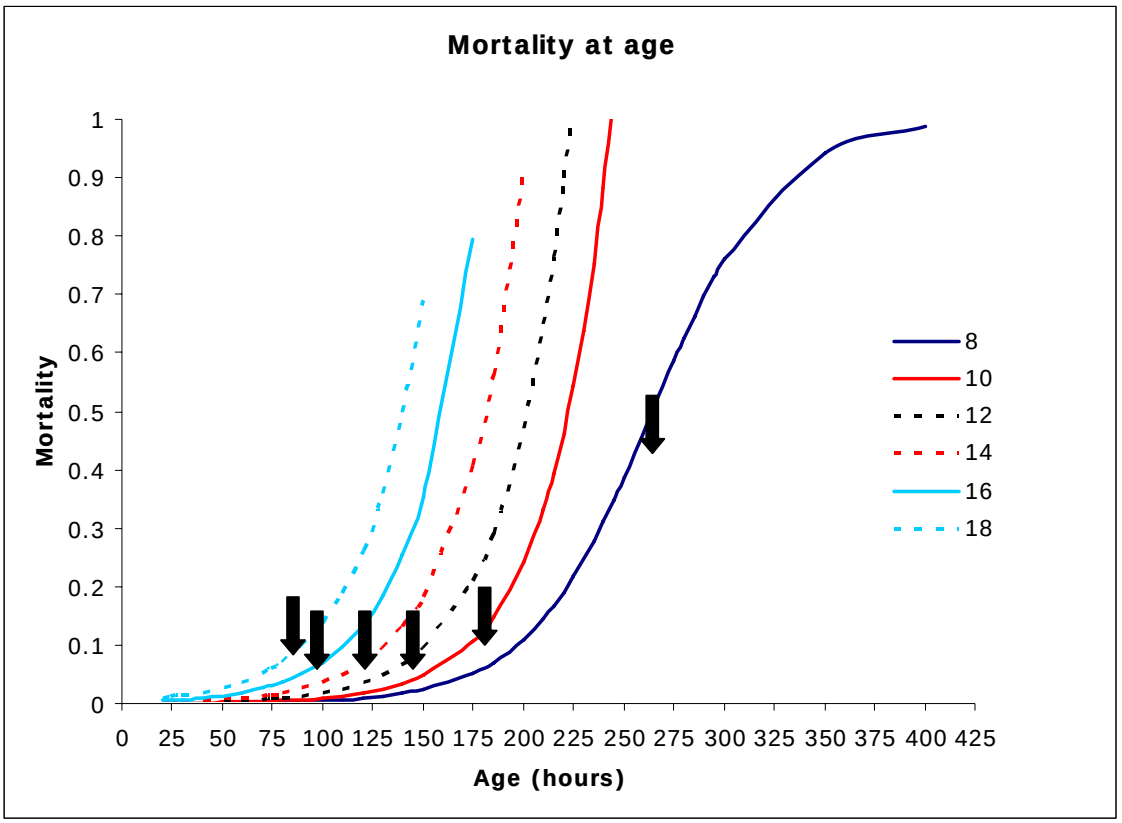

Figure 9.3.1: Curve of the logistic model between mortality rate at age (hours) for six theoretical temperature values. Arrows indicate the hatching time at each temperature interval.

10 Deficiencies and Recommendations

10.1 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 TACs 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.

Areas for concern highlighted by research or questions from WGMHMSA, include uncertainty in the calculation of some adult parameters, principally in supporting evidence for fecundity changes in mackerel, and whether horse mackerel is a determinate or indeterminate spawner. Both these questions have been addressed by the WG and research is in place to clarify these issues. The changes in mackerel fecundity observed between 1995 and 1998, have since been confirmed by later observations. In addition, a coincident change in the condition factor of mackerel in the previous autumn in the Norwegian Sea may provide a biological explanation for the change. The question of determinacy in horse mackerel is the subject of ongoing research. This is firstly, to definitively resolve the question of whether horse mackerel is a determinate or indeterminate spawner. Secondly, research is being carried out to explore the validity of using the annual egg production estimate as a biomass index.

There is also the potential for problems in the process of egg identification to species and then staging those eggs. This may impact on both accuracy and precision. WGMEGS have put in place a series of workshops, held immediately before the surveys to address these issues. The most recent workshops were held at Lowestoft in 2000 and 2003. The outcome produced an improvement in agreement between egg readers and a consistency and standardisation of approach. These workshops need to be held routinely before surveys to ensure the quality of the sample processing.

A review of the sampling gear and deployment methods 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. Also, the new proposed mechanical sorting method, the “spray-method”, which was introduced and validated on the 2003 Lowestoft workshop, was adopted differently by the participants. While none of these issues are believed to have major significance, standardisation of methods will be subject to review at the 2006 planning meeting of WGMEGS, in preparation for the 2007 egg survey. It is also recommended that the survey manual is redrafted at the 2006 planning meeting.

The temporal and spatial coverage of the sampling area was adequate during most of the spawning season in both the western and southern areas. However, there is some concern regarding the coverage at the beginning and the end of spawning in the western area. There was no coverage in February, although significant and widespread spawning was apparent by March (period 3). In period 7 it was only possible to survey the central part of the area due to lack of ship time, and it is clear that some spawning would be expected both north and south of this area. **It is recommended that participating institutes make every effort to provide vessel resources for these periods in the 2007 survey**. The only other major weaknesses in the coverage were in period 3 on the Portuguese coast where weather curtailed survey effort, and in period 6 where an unusual extension of spawning into the Celtic Sea was not fully surveyed.

10.2 Recommendations

WGMEGS recommends:

- The Working Group recommends that its next meeting, for the planning of the proposed 2007 Mackerel and Horse Mackerel Egg Survey, should be held from 3–7 April 2006 in Vigo, Spain. The Working Group nominated Dr. Paula Alvarez (AZTI, Spain) as its new Chair. The above recommendation and nomination will be sent to the ICES Living Resources Committee for consideration at the Annual Science Conference in September 2005. Proposed Terms of Reference for the meeting are provided below (Section 10.3).
- To arrange the routine workshop (WKMHMES) immediately before the surveys to address the problems with species identification and egg staging
- That further inter-calibration work concerning fecundity studies should be carried out and that each Institute should provide an Auto-diametric model to determine fecundity. This workshop should be held just after the egg identification and staging workshop (WKMHMES). (Section 2.2.3 of the report)
- To redraft and update the survey manual at the 2006 planning meeting.
- That participating institutes make every effort to provide vessel resources for February and July in the 2007 survey. Usually these periods are poorly or not covered at all.
- If there are surveys in western part of the North Sea (between 54°N–58°N) in May-early June 2005 it is recommended that they should try to provide samples for potential fecundity studies of North Sea mackerel. These samples will be worked by IMR, Bergen.
- To apply the 'Spray technique' as the primary method for removing eggs from plankton samples during the tri-ennial surveys (as recommended by WKMHMES).
- RIVO, IMR Norway, IEO and the Marine Institute Galway should standardise their estimates of fecundity based on the slides listed in Table 1 of Annex 3 to this report.
- The data from the samples in table 1 should be re-analysed using a standardised stereometric estimate of fecundity to recalculate values shown in Tables 1 and 2 of Annex 3 to this report.
- Oocytes measured in whole mount should be sectioned to identify at what size cortical alveoli start to accumulate around the nucleus.
- If the fecundity from the Stereometric method exceeds the Gravimetric fecundity then the further gravimetric samples should be analysed measuring oocytes larger than indicated by the results the recommendation above.

10.3 Proposed Terms of Reference for 2006

The **Working Group on Mackerel and Horse Mackerel Egg Surveys** [WGMEGS] (Chair: Paula Alvarez*, Basque Country, Spain) will meet in Vigo, Spain 3–7 April 2006 to:

a) Coordinate the timing and planning of the 2007 Mackerel/Horse Mackerel Egg Survey in the ICES Sub-areas VI to IX,

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

c) Report on current and potential future variance calculation procedures, and provide information on the scale and direction of any bias or variance in the biomass estimation procedure

d) Review procedures for egg sample sorting, species ID, staging and fecundity and atresia estimation. Based on workshop in late 2006.

e) Analyse and evaluate the results of the 2005 mackerel egg survey in the North sea;

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

11 Working documents presented to the Working Group

1) **The development of mackerel eggs at different temperatures and preliminary results of egg mortality at age and temperature.** Paula Alvarez[1] and Diego Mendiola[1]

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Abstract

Artificial fertilizations were carried out using ripe mackerel caught on hand-lines during March 2004 of the coast of San Sebastian (43º 47´– 1º 50´, Basque country, Spain). Mackerel eggs were incubated at 5 temperatures from 8 to 18 ºC. Development rate experiments were performed in 175 litres tanks in the San Sebastian Aquarium. At the same time, a study on egg mortality was made using a sample of 30 eggs placed individually into small tubes with 35 ml of sea water. The relationships between time to reach the end of each development stage and at each temperature were estimated and compared with those obtained by Lockwood *et al.* (1977). For stage IA and Stage 1B the egg development rates (b values) were statistically different at 99% and 95% of confidence level respectively. The equation obtained for stage IB was: Ln time (hours) = 6.902 – 1.314 Ln (Temp). Using this new development equation and at 10–12ºC temperature range, egg production would increase about 10–12%.

It was estimated that egg mortality would be in the range 9–14%, independent of temperature. I was observed that the mortality increased at the end point of stage V, just prior to hatching. At this point, mortality was much higher at the lowest temperature.

2) **Estimating inter-stage egg mortality in mackerel and horse-mackerel: results from a 24h intensive sampling program.** Enrique Portilla [1,2], Doug Beare [1], Eddie McKenzie [2], I. Gibbs [1], F. Burns [1], Dave Reid [1]

[1]FRS-Marine Laboratory, PO Box 101, Victoria Rd, Aberdeen AB11 9DB. Scotland.

[2]Department of Statistics and Modelling Science, University of Strathclyde, Livingstone Tower, Glasgow G1 1XT Scotland.

Correspondence to Enrique Portilla: tel: +44 (0) 1224 295316 email: e.portilla@marlab.ac.uk.

Abstract

Recent work on mortality rates using egg survey data collected on standard survey designs using birth-death models has suggested, that mortality rates are considerable important to the estimation of the Total Annual Egg Production. In order to enhance our confidence in the mortality rates estimates, an intensive period of sampling during the 2004 egg survey was undertaken. Sampling took place over 24 hours in one location at high rate following the same sampling strategy as standard survey. Using these data it was possible to quantify mortality rates more accurately with average values for mackerel of 1.12 d^{-1}. Mortality rates showed a diel variation within the 24h period possibly related to sunlight variation and average age of the samples. Moreover, estimates of egg mortality done in the traditional manner provided lower estimates of mortality (0.4 d^{-1}) than obtained with birth-death models. For horse mackerel, egg mortality using birth death models was computed by bootstrapping the available samples. Estimate of mortality was higher (1.63 d^{-1}) than that obtained by the traditional method (1.17 d^{-1}). Finally, conclusions about diel patterns observed might have to be taken with precaution because of the change on location during sampling period.

3) **Estimation of inter-stage mortality using linear birth death model for mackerel and horse mackerel in the North East Atlantic.** Enrique Portilla [1,2], Eddie McKenzie [2], Doug Beare [1], Dave Reid [1]

[1]FRS-Marine Laboratory, PO Box 101, Victoria Rd, Aberdeen AB11 9DB. Scotland.

[2]Department of Statistics and Modelling Science, University of Strathclyde, Livingstone Tower, Glasgow G1 1XT Scotland.

Correspondence to Enrique Portilla tel: +44(0)1224295316

Abstract

Egg mortality is a key parameter to understand early life history of fish because might cause large differences in final abundance. Assumption of constant mortality rate during a life period as eggs might be misleading. In this study we show how to estimate mortality for each stage of eggs using Birth-Death Process for mackerel and horse-mackerel. We analysed data from the ICES Triennial mackerel and horse mackerel egg surveys since 1977. The results include spatial and temporal dependencies on both inter-stage mortality proportions and mortality rates. Nevertheless, overall average of mortality daily rates estimated for both mackerel and Horse mackerel was similar (0.56 and 0.54 per day). We also show that mortality rates may be related to the level of egg production of both species, showing inter and intraspecific interaction. Although it was not possible to directly estimate Stage 1 mortality, results lead to suggest that high mortality in early stage might underestimate egg production for both species by 30%.

4) **Evaluation of the "Spray method": Exercise carried out by AZTI during the 2004 Triennial eggs surveys.** Paula Álvarez

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Abstract

157 Bongo 40 samples collected during the Triennial surveys were analysed to assess the effectiveness of "Spray method" in sorting eggs from plankton samples. Three consecutives spraying were performed and the number and specie of eggs were recorded. The eggs remaining in the plankton were counted and identified as well. The target species were: mackerel, horse mackerel, sardine, anchovy and other eggs. Data of cumulative number of eggs removed at each spraying and the total eggs found in the sample were fitted to linear regressions and the slopes were used to asses the effectiveness of this method. After three sprayings more than 99% of the eggs collected in the plankton samples were removed. Some difference was observed during sardine eggs extraction, where 14% of the eggs remaining in the plankton sample at the end of the process. Differences in the sardine eggs extraction between cruises suggest a human factor as responsible for this discrepancy. As these eggs tend to break easier than other ones, an inadequate execution of the method which could cause a lot of damage in sardine eggs. Our results support the used of this method to sorting eggs from plankton samples and highlight the importance of a good execution of this to avoid damages. Moreover, taking into account the percentage of eggs removed we suggest eliminating the checking of the eggs remaining in the plankton because the improvement of the number of eggs extracted is not significant.

5) **Evaluation of the spray technique in 2004 IEO Triennial mackerel & horse mackerel egg surveys.** Francisco Baldó, P. Cubero, A. Lago de Lanzós and C. Franco

Instituto Español de Oceanografía, IEO, Madrid (Spain)

Abstract

The Spray Technique to sort fish eggs from plankton samples was satisfactorily checked during the IEO 2004 Triennial Egg Survey. After three spraying processes, more than 99 % of the total eggs, as well of the eggs of each key-studied species (mackerel, horse mackerel, sardine and anchovy), were removed and errors higher than 10 % in the estimation of the collected eggs of each studied species were only occurred in less than 5 % of the samples.

6) **An investigation of mackerel and horse mackerel egg sizes from the ICES tri-ennial egg survey, 2004.** S.P. Milligan and N. Taylor.

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Abstract

At the last meeting of WKMHMES (Oct 2003, ICES, 2004) it was recommended that all participants make measurements of egg and oil globule diameters from as many preserved mackerel and horse mackerel eggs as possible, collected as part of the 2004 tri-ennial survey. Each country removed fish eggs from the plankton samples collected on their survey(s), utilising the 'Spray technique' as described in WKMHMES, ICES, 2004. The egg and oil globule diameters were measured using the standard techniques employed by each participating laboratory. The resultant data was input into an Access database and then combined into 50µm size ranges. Summary tables and graphs were then produced to compare and contrast the egg and oil globule diameters of the two species to aid future identification.

More than 15,000 egg measurements (9,400 mackerel and 5,500 horse mackerel) were made by all the participants who surveyed in periods 3 to 7. The results show a slight decrease in egg diameters through the spawning season. The mean egg size for mackerel decreases from 1.20mm in period 3 to 1.11mm in period 7. For horse mackerel the decrease is from 0.96mm in period 3 to 0.89mm in period 7. Mean oil globule sizes for both species do not appear to change very much through the period surveyed. There did not appear to be any differences in egg or oil globule size across the latitudinal range of the surveys. Reassuringly, there was also little difference between the egg and oil globule sizes recorded by each participant. It was also apparent that the egg and oil globule sizes did not change with egg development.

The results help to reinforce the information provided by Russell (1976) and other workers referenced in ICES, (2004). These data also demonstrate minimal overlap in the egg size ranges of mackerel and horse mackerel, which will help analysts in the future when attempting to distinguish between the eggs of these species.

7) **Horsemackerel fecundity in relation to body condition.** Cindy van Damme[1], Leonie Dransfeld[2], Maria Krüger-Johnsen[3], Jose Ramon Perez[4], Jens Ulleweit[5], Guus Eltink[1] and Peter Witthames[6]

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Abstract

Fecundity and lipid content of horse mackerel were estimated during the 2004 triennial egg survey. Fecundity estimates and measured oocytes diameter showed great variation between and within different institutes showing a great need for a stricter protocol. Fecundity is rising from the onset on spawning as was shown in the earlier egg surveys.

Although horse mackerel in the western area is considered as one population lipid content, is lower in the southern part. Lipid content is rapidly decreasing before the onset of spawning and remains at the same level during the spawning period. Lipid content before spawning may give an indication of fecundity but sampling needs to be done before the spawning starts. Stomach analyses showed no evidence for horse mackerel feeding during spawning.

8) **Mackerel fecundity and atresia.** Witthames, P. R. Greenwood, L. N.

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Abstract

A comparison of fecundity and atresia data was presented in Power Point showing the latitudinal coverage of sampling and comparing the results from each Institute (CEFAS, IEO IMR and the Marine Lab Aberdeen. Fecundity data varied according to Institute with CEFAS IMR and the Marine Lab more closely clustered together than IEO where the results were lower. The overall potential fecundity for the Western mackerel spawning component was 1190 eggs per g female. Atresia analysis was carried out by CEFAS and included the samples assigned to the Marine Laboratory and this was compared to results from IMR. Intensity and prevalence of atresia means were much smaller from IMR compared to CEFAS at 12.4 and 36.1 (prevalence) and 27 and 33 (relative intensity) respectively.

9) **Determinate versus indeterminate fecundity in horse mackerel.** L. S. Gordo[1], A. Costa[2], P. Abaunza3, P. Lucio[4], A.T.G.W. Eltink[5]

[1]Faculdade de Ciências de Lisboa – Departamento de Zoologia e Antropologia

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Abstract

The samples for these fecundity studies were collected during HOMSIR project in 2001 and 2002 in the northeast Atlantic and the western Mediterranean. The estimated standing stock of vitellogenic oocytes (= residual fecundity) represents a certain time point in the spawning season. The residual fecundity has been estimated separately for ovaries without signs of spawning, for ovaries with migrating nucleus and hydrated oocyte stage (imminent spawning) and for ovaries with postovulatory follicles (recently spent). The increase in residual fecundity from ovaries without signs of spawning to imminent spawning ovaries and the decrease again from imminent to spent ovaries indicate the occurrence of *de novo* vitellogenesis and therefore indeterminate fecundity. This phenomenon is observed all sampled areas. These differences in

residual fecundity roughly agree to the batch size. They indicate that a new batch of pre-vitellogenic oocytes is developped to vitellogenic oocytes during the short period of migrating nucleus and hydrated oocyte stage.

10) **Southern Horse Mackerel Fecundity Estimate – 2004.** Costa, A.M.[1]; Pérez, J.R.[2] and Pissarra, J.L.[1]

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[2]Instituto Español de Oceanografia, IEO (Spain)

Abstract

A fecundity estimation of southern horse mackerel (ICES Divisions VIIIc and IXa) from the 2004 egg surveys as well as data on total lipid content is presented.

The total fecundity was estimated based on 110 pre-spawning microscopic stage 3 ovaries collected by Portugal and Spain, in the area between Gibraltar and the French-Spanish border.

- A fecundity of 1619 eggs/g was estimated with a C.V. of 38.3 %.
- This fecundity is 3 % higher than the value obtained in 2001 (Costa *et al.*, 2002) and is consistent with previous data.

Fecundity has also been estimate over the whole period of spawning (January to April). The results indicated that the fecundity increased over time

Total lipid content was determined by Spain from 166 samples collected from October 2003 to May 2004.

11) **Mackerel and Horse mackerel Egg Production in ICES Divisions VIIIc and IXa in 2004.** Concha Franco [1], Lago de Lanzós, A.[1], Baldó, F.[1], Cubero, P. [1], Vendrell, C.[2], Farinha, A.[2], Pissarra, J.[2] and Álvarez, P.[3]

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Abstract

From 15 January to 27 May 2004, mackerel and horse mackerel in ICES Divisions VIIIc and IXa were investigated by ichthyoplankton research, in order to apply the Annual Egg Production Method (AEPM) to estimate the southern component of the North Atlantic mackerel stock and the southern stock of the horse mackerel. The spawning season was split into 5 periods and surveys were carried out by Portugal, Spain (IEO and AZTI), and The Netherlands. The annual mackerel stage I egg production was estimated at 12.56×10^{13} (s.e. 2.35×10^{13}). The egg production fell by 56% from 2001 to 2004. On the other hand, the annual horse mackerel stage I egg production in ICES Divisions VIIIc and IXa was estimated at 24.84×10^{13} (s.e. 12.08×10^{13}), that was 45 % higher than in 2001. Mean daily egg production for each survey period is also separately presented in Divisions VIIIc and IXa, according to the new boundary of the southern and western horse mackerel stock.

12) **Horse mackerel Daily Egg Prodution in Division VIIIc from 1992 to 2004.** Ana Lago de Lanzós, C. Franco and F. Baldó

Instituto Español de Oceanografía, IEO, Madrid (Spain)

Abstract

As a result of a horse mackerel stock structure study carried out by the EU funded project HOMSIR (QLK5-Ct1999-01438), it was proposed to move the actual boundary of the “Southern” and “Western” stocks from Cape Breton Canyon (southeast of Bay of Biscay) to the

northwest of Iberian Peninsula (43º N latitude) (ICES CM 2005/ACFM: 08). This paper presents horse mackerel daily egg production from 1992 to 2004 in the northwest of Iberian Peninsula from 43º N (ICES Division VIIIc).

13) **Review of calculation to estimation of variance for annual egg production in the southern area by traditional method.** Gersom Costas[1], M. Bernal[2], C. Franco[3], A. Lago de Lanzós[3] and F. Baldó[3]

[1]Instituto Español de Oceanografía, C.O. Vigo (Spain); [2] Instituto Español de Oceanografía, Unidad de Cádiz (Spain); [3] Instituto Español de Oceanografía, Madrid (Spain).

Abstract

A confuse methodology has been found in previous WGMEGS reports about the calculation of the variance for Total Annual Egg Production (TAEP). The objective of this paper is to understand the procedures performed to calculate the estimation of variances for the TAEP.

In estimation of variances for the annual egg production Method is used coefficient of variation of Daily egg production on replicated sample rectangles.

Moreover, this working document presents the coefficients of variation for Mackerel and Horse mackerel for 2001 and 2004 surveys in the southern area.

I would like highlight that in estimation of coefficient of variation is currently implemented by an approximation. But this approximation only holds for coefficient of variation below 0.7. That means that the variance estimate for the annual egg production by this approximation would be underestimated.

14) **Postovulatory follicles (POFs) ageing in *Trachurus trachurus*.** Gonçalves, P., Costa, A.M., Cunha, E., Vendrell, C., Pissarra, J.

IPIMAR Instituto Nacional de Investigação Agrária e das Pescas -IPIMAR, Av. Brasília, s/n, 1449–006 Lisboa, Portugal patricia@ipimar.pt.

Abstract

The postovulatory method may be used to estimate the spawning fraction of females spawning per day, which is an important reproductive variable of indeterminate spawners.

Adult females of horse mackerel (*Trachurus trachurus)* were collected during the "Triennial egg surveys" onboard of the Portuguese RV Capricornio, from January to March 2004. The histological slides of the mature ovaries were observed in order to assign females to the day of spawning according to the morphology of postovulatory follicles (POFs).

The aim of this work was to develop a stage scale to age the POFs. This scale describes very accurately the POFs involution process of horse mackerel in a three daily classes; the ageing is relatively to hauling time.

15) **Egg and larvae distribution of seven fish species in the north-east Atlantic waters.** Leire Ibaibarriaga [1], Xabier Irigoien [1], Maria Santos [1], Lorenzo Motos [1], Julie Fives [2], Concha Franco [3], Ana Lago de Lanzós [3], Silvana Acevedo [2], Miguel Bernal [3], Nicolas Bez [4], Guus Eltink [5], Anabela Farinha [6], Cornelius Hammer [7], Svein Iversen [8], Steve Milligan [9], and Dave Reid [10].

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Abstract

The distribution of egg and larvae of mackerel, horse mackerel, sardine, hake, megrim, blue whiting and anchovy along the European Atlantic waters (south Portugal to Scotland) during 1998 is described. Time of the year, sea surface temperature and bottom depth are used to define the spawning habitat of the different species. Mackerel, horse mackerel and sardine eggs and larvae presented the widest distribution, whereas megrim and anchovy showed a limited distribution, restricted to the Celtic Sea and the Bay of Biscay respectively. Correspondingly mackerel, horse mackerel and sardine showed the highest aggregation indices. Blue whiting spawned at the lowest temperatures, whereas anchovy were found in the warmest waters. The analysis is a basis for judgement of evaluation of upcoming or ongoing changes in the oceanographic regime of the north east Atlantic.

16) **Development of Fecundity methodology used during the 2004 survey to determine mackerel and horse mackerel potential fecundity.** Witthames, P.R. Greenwood, L.N.

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Abstract

Development of fecundity methodology during the 2004 survey was given as a Power Point presentation. This included details of the ovary sub-sample weight precision of taken by the sampling pipettes from 25 to 100 µl and development of the Auto-diametric method A comparison of the results from the Auto-diametric produced by the Marine Laboratory indicated that the Pipette gravimetric samples gave more reliable results showing lower variance and mean eggs per g female. Recommendations were made to 1) use a standard image for training to measure follicles prior to the start of assessment 2) weigh sample tubes to check performance of the pipette operators at sea and consider different wand configurations to aid the selection of the smallest vitellogenic oocytes.

17) **Feasibility to carry out experiments on horse mackerel reproduction with a case study concerning Herring on fecundity regulation in relation to food availability.** Witthames P.R.[1], Kjesbu O.S[2]., Hansen, T.[3]

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Abstract

Results were presented showing that it is feasible to capture Horse Mackerel and mackerel close to Matre Field Station and subsequently wean and feed them on to pellet food. An illustration of the value of this experiment to study ovary and fecundity was provided by referring to a cased study on herring reproduction.

18) **Potential scale of benefits from an additional egg survey between traditional survey years.** Marco Kienzle and John Simmonds.

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Abstract

The triennial Spawning Stock Biomass (SSB) survey of the North East Atlantic (NEA) mackerel stock provides an essential source of information to estimate the current level of stock biomass and fishing mortality with the Integrated Catch at Age method (ICA). The Mackerel horse mackerel sardine and anchovy working group (WGMHMSA) acknowledges that a three years time interval between the surveys has implications on the precision with which the status of the stock is estimated: the accuracy of the estimation of the fundamental variables used for management is best during the year of the SSB survey and decreases the further away from this measurement the stock assessment is performed.

An additional survey to be performed in years other than the triennial sequence currently employed should assist this situation and improve the assessment of this stock, in particular in years when no survey data are available. To help designing this additional survey, we conducted a simulation study to determine the most beneficial timing for an additional survey as well as to investigate the range of precision on the estimation of the SSB index of mackerel that would provide improved assessment of the stock.

The results show that an additional survey (AS) with better or slightly poorer precision will improve our knowledge of the status of the stock in all years and particularly in the year of the AS survey. The benefit for stock assessment of gathering additional information on the NEA mackerel SSB using an AS with poorer precision depends on timing of the assessment. Availability of such information during the year the further away from the current survey (CS), when no other SSB estimation is available (1 gap year between surveys), provides a more precise characterisation of the stock: a noisy source of information, up to 4.5 time as uncertain as the CS, provides better estimation of fishing mortality, SSB and TSB than no information at all. The other cases presented in this article, namely the assessment of the stock performed the year after the CS or the year of the CS, showed that large variability of the AS produces more uncertain estimation of the stock status unless the precision of the additional survey is known and incorporated into the model.

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Annex 2: Working document for the ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS)

Not to be cited without prior reference to the author.

Bergen, Norway, 4 – 8 April 2005

An investigation of mackerel and horse mackerel egg sizes from the ICES tri-ennial egg survey, 2004

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Introduction

One of the products from the last mackerel and horse mackerel egg staging and identification workshop (WKMHMES, ICES, 2004) was a description of fish eggs which are similar to those of mackerel and horse mackerel. The characteristics of five species (mackerel, horse mackerel, megrim, hake and snipefish) were described, and numerous references were provided to help plankton analysts identify these eggs correctly. All of these species have eggs of a comparable size and contain a single oil globule. These species are also known to spawn at a similar time and in the same geographical region as both mackerel and horse mackerel. It was apparent from the compiled reference list that much of the information was based on the measurements of a limited number of eggs and that much of this work was conducted many years ago. It was therefore recommended by WKMHMES that all participants make measurements of egg and oil globule diameters from as many preserved mackerel and horse mackerel eggs as possible, collected as part of the 2004 tri-ennial survey. These data would then provide both temporal and spatial resolution to the egg sizes of these species for the first time, and aid the identification of these eggs on future surveys.

Method

Each country removed fish eggs from the plankton samples collected on their survey(s), utilising the 'Spray technique' as described in WKMHMES, ICES, 2004. Preliminary identification and staging of the eggs was carried out at sea. However, most participants re-sorted the samples on return to the laboratory, where the eggs were all identified, staged and counted. At the request of WKMHMES, each participating laboratory then measured a sample of mackerel and horse mackerel eggs from a number of selected stations on each cruise. Stations containing most mackerel and horse mackerel eggs were selected from each degree of latitude to provide a wide geographical coverage of egg sizes.

The egg and oil globule diameters were measured using the standard techniques employed by each participating laboratory. This was usually by using stereo-zoom microscopes fitted with eyepiece graticules. However, three countries (Scotland, Germany and Ireland) supplied egg measurements from image-analysis equipment, which provided greater resolution to their data.

Most data was input to a standard spreadsheet table (provided by Dr G Eltink, RIVO, Netherlands) and sent to CEFAS, Lowestoft for compilation. The data was then transferred to an Access database to allow easier data manipulation. Unfortunately the resolution of the data produced by each country varied considerably (Table 1). In order to standardise and combine the results, various size ranges were chosen in which to sum the egg and oil globule sizes. It was eventually decided that combining the measurements into 50μm size ranges produced the most reasonable egg size distributions. Summary tables and graphs were then produced to compare and contrast the egg and oil globule diameters of the two species. The results also provided information on any changes in egg size both temporally and spatially by species. In addition, some countries staged the eggs before measuring and these results are also presented below.

Results

By survey period

A summary of the numbers of mackerel and horse mackerel eggs measured by each country in each time period is given in Table 1. This shows that over 15,000 egg measurements and slightly fewer oil globule measurements were made by all the participants who surveyed in periods 3 to 7. Approximately 9,400 of these measurements were made on mackerel eggs and over 5,500 were from horse mackerel eggs. The majority of the eggs were measured from samples collected during periods 3-5. Fewer eggs were measured from periods 6 and 7, which reflected the limited number of countries surveying during these time periods.

Figure 1 shows the size distribution of mackerel and horse mackerel egg and oil globule sizes by time period. Both species show a slight decrease in egg diameter towards the end of the spawning season. The mean egg size for mackerel decreases from 1.20mm in period 3 to 1.11mm in period 7. For horse mackerel the decrease is from 0.96mm in period 3 to 0.89mm in period 7. Mean oil globule sizes for both species do not appear to change very much through the period surveyed. Table 2 shows the modal and mean egg and oil globule sizes for both species by each time period. This table also shows the size range of eggs and oil globules measured. Only 1.1% of mackerel eggs and 1.5% of horse mackerel egg diameters measured were outside the size range given by Russell (1976).

By geographical regions

Figures 2 and 3 show the sampling positions from which eggs were selected for measuring from the 2004 tri-ennial survey. It is clear that eggs were selected from the major part of the spawning area of both species with most eggs being measured from areas where there was greatest spawning activity. Figure 4 shows the size distribution of mackerel and horse mackerel egg and oil globule sizes by geographical region. There is no apparent difference in the size of the eggs or oil globules at different latitudes.

By country

Table 3 shows the numbers of eggs and oil globules measured by each country participating in the surveys during periods 3 to 7. Very few eggs measured were outside the size ranges given by Russell, (1976). Most of the eggs identified as either mackerel or horse mackerel, and falling outside the published range, were found by Germany. 3.4% of their mackerel eggs and 5.8% of their horse mackerel eggs were outside the expected size ranges. There was no apparent difference in the egg or oil globule size distribution by country despite the difference in the measuring techniques and resolution of the data (Figure 5). Only in the data from the Netherlands can bi-modal size frequencies still be seen, which may reflect the lower resolution of their measurements.

By stage

Most countries (except the English and the Scots) measured the eggs by development stage. The size frequencies of eggs and oil globule diameters by development stage are presented in figure 6. This shows that there is no apparent difference in egg size by development stage.

Comparison of egg and oil globule sizes

Comparisons were made between the egg and oil globule diameters by country to determine whether there were significant differences in this relationship between the two species. The results are presented in figure 7. There do not appear to be significant differences in the egg to oil globule relationship between the two species but it is clear from figure 7 that there is a significant difference in the egg diameters of the two species as described by previous studies.

Discussion

A large quantity of data has been collated during the course of this egg measurement exercise. Only basic preliminary results are presented above and no attempt has been made to look at cumulative or combined effects of time, region, development stage or country, on the size frequency distributions. However, these data have helped to reinforce the information provided by Russell (1976) and other workers referenced in ICES, (2004). These data also demonstrate minimal overlap in the egg size ranges of mackerel and horse mackerel, which will help analysts in the future when attempting to distinguish between the eggs of these species. It must be noted that the data provided by both Norway and Ireland seems to indicate that egg size was used as a primary identification feature, as there was no overlap in the size of mackerel and horse mackerel eggs recorded by each of these participants. Ireland appeared to use a size threshold of 1.00mm and Norway a threshold of 1.05mm. Above these sizes eggs were designated as mackerel and eggs below these sizes were identified as horse mackerel. All other participants appeared to use the dark segmented yolk of horse mackerel as the primary identification feature, as some degree of size overlap was recorded.

The results have shown a slight decrease in egg size through the spawning season for both species, which is not entirely unexpected. However, there does not appear to be any difference in egg size throughout the spawning range covered by the tri-ennial surveys.

There was minimal effect on egg size recorded by each country, which showed that the techniques employed but each institute were sound. However, there were differences between the resolutions of the egg measurement data provided by each country. Those countries, which used an image analysis system, were able to provide data to at least 1µm resolution. Most other countries were able to provide data between 20 and 25µm resolution with the Netherlands providing data to 31µm resolution. It was decided to use size categories of 50µm range in which to sum the measurement data. This size range gave the smoothest histograms without losing too much resolution from the original data. It would be preferable, in any future exercises such as this, for every country to attempt to measure the eggs and oil globules to the same resolution. It would therefore be useful if consideration were given to using image analysis systems to measure eggs and oil globules in any future exercise.

It must be noted that the measurements made during this exercise were on eggs identified by experienced analysts from mixed plankton samples. It would have been preferable to measure eggs of known parentage where the species identification would have not been in doubt. Participants of WKMHMES were asked to carry out artificial fertilisations of as many species as possible during the 2004 survey to help with the identification of eggs caught in the wild. To date very few artificial fertilisations have been possible but it is recommended that all participants help in the provision of such samples to enable these to be available for the next meeting of WKMHMES scheduled for 2006.

References

ICES. 2004. Workshop on Mackerel and Horse Mackerel egg staging and identification. ICES CM 2004/G:13.

Russell, F.S. 1976. The eggs and planktonic stages of British marine fishes. Acedemic Press. ISBN: 0-12 604050-8.

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Table 1. Summary of egg and oil globule measurements from the 2004 tri-ennial egg survey

			Mackerel			Horse mackerel		
Country	Period	Resolution of measurements (um)	Nos. of eggs measured	% of period total	Nos.of eggs without oil measurement	Nos. of eggs measured	% of period total	Nos.of eggs without oil measurement
Spain, IEO	3	25	533	18	0	641	34	0
Spain, AZTI	3	20	1084	37	0	672	36	20
Germany	3	1	1297	45	0	561	30	0
Total			2914	100		1874	100	
Scotland	4	1	400	13	0	0	0	0
Spain, IEO	4	25	437	15	0	203	19	0
Germany	4	1	653	22	0	40	4	0
Ireland	4	1	700	23	0	0	0	0
Spain, AZTI	4	20	304	10	1	332	31	0
England	4	20	500	17	0	500	47	0
Total			2994	100		1075	100	
Netherlands	5	31	960	57	56	727	54	93
Spain, AZTI	5	20	132	8	1	328	25	10
England	5	20	500	30	0	183	14	0
Norway	5	23	100	6	0	100	7	1
Total			1692	100		1338	100	
Netherlands	6	31	742	65	82	744	100	124
Scotland	6	1	401	35	0	0	0	0
Total			1143	100		744	100	
Ireland	7	1	658	100	0	589	100	0
Total			658	100		589	100	
Seasonal total			9401			5620		

Table 2. The mode, mean, range and standard deviation of egg and oil globule sizes

Species	Period	No. of observati	Egg Ø (mm)					Oil Ø (mm)				
			Mode	Mean	Min	Max	Std Dev	Mode	Mean	Min	Max	Std Dev
MAC	3	2914	1.225	1.20	0.88	1.50	0.070	0.325	0.32	0.18	0.44	0.029
	4	2994	1.175	1.18	0.93	1.41	0.059	0.325	0.31	0.24	0.46	0.030
	5	1692	1.175	1.19	0.88	1.38	0.055	0.325	0.31	0.22	0.40	0.025
	6	1143	1.175	1.13	0.88	1.33	0.053	0.325	0.30	0.06	0.41	0.035
	7	658	1.125	1.11	1.00	1.29	0.043	0.275	0.30	0.22	0.38	0.020
	Total	9401	1.175	1.18	0.88	1.50	0.067	0.325	0.31	0.06	0.46	0.030
HOM	3	1874	0.975	0.96	0.82	1.16	0.038	0.225	0.24	0.17	0.38	0.024
	4	1075	0.975	0.94	0.84	1.10	0.034	0.225	0.25	0.18	0.40	0.028
	5	1338	0.925	0.96	0.78	1.25	0.045	0.275	0.26	0.13	0.38	0.033
	6	744	0.925	0.92	0.81	1.16	0.038	0.275	0.24	0.16	0.34	0.034
	7	589	0.875	0.89	0.76	1.00	0.040	0.225	0.23	0.15	0.38	0.026
	Total	5620	0.925	0.95	0.76	0.13	0.045	0.225	0.25	0.13	0.40	0.029

Species		Total measured	No. outside Russell's sizes	%. outside Russell's sizes
MAC	Egg	9401	100	1.1
	Oil	9261	1356	14.6
HOM	Egg	5620	86	1.5
	Oil	5372	436	8.1

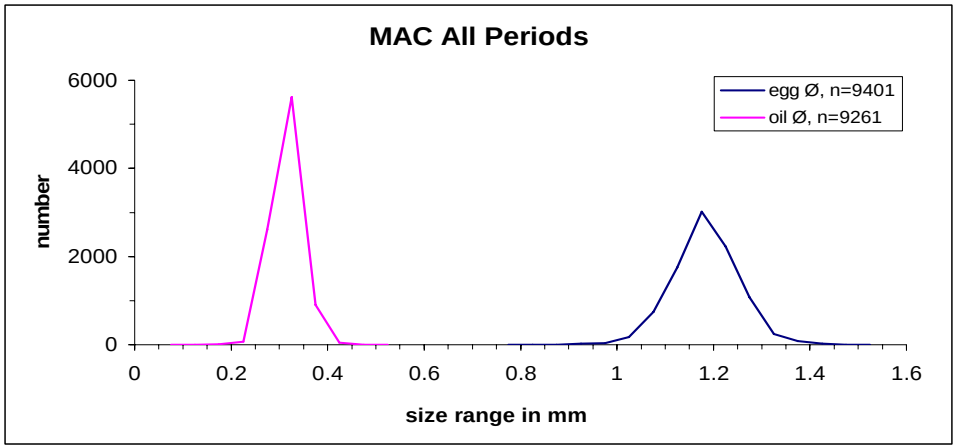
Table 3a. Summary of the numbers of mackerel eggs in each size class by country

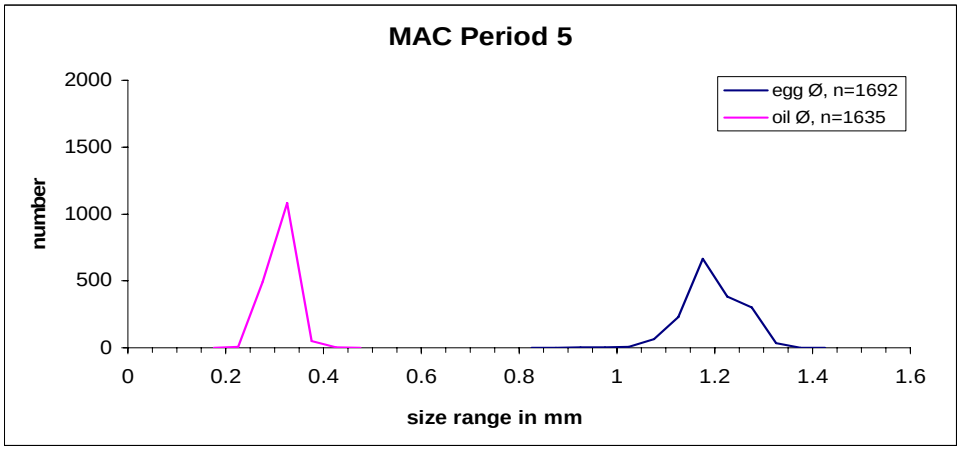
Size classes (microns)	Mackerel egg and oil globule sizes							
	England	Netherlands	Spain, AZTI	Germany	Norway	Ireland	Spain, IEO	Scotland
50: 99		1						
100:149		0						
150:199		5		4				
200:249	5	27		26		1		6
250:299	465	671	210	387	13	558	188	123
300:349	517	825	1224	1146	72	736	593	503
350:399	13	34	84	361	13	63	177	164
400:449		1		24	2		12	4
450:499				2				1
500:549								
550:599								
600:649								
650:699								
700:749								
750: 799								
800: 849								
850: 899		3		1				
900: 949		7		12				
950: 999		3		28				5
1000:1049	9	42	11	29		57	2	28
1050:1099	42	305	10	43	7	268	13	50
1100:1149	274	307	234	188	25	400	161	164
1150:1199	369	581	529	493	42	349	395	264
1200:1249	286	151	525	553	18	210	276	209
1250:1299	19	272	176	367	7	58	110	66
1300:1349	1	30	33	139	1	14	13	14
1350:1399		1	1	72		2		1
1400:1449			0	21				
1450:1499			0	4				
1500:1549			1					
Total oil measures	1000	1564	1518	1950	100	1358	970	801
Total egg measures	1000	1702	1520	1950	100	1358	970	801
Total egg measurements outside Russell's range	0	13	1	66	0	0	0	5
% outside range	0.0	0.8	0.1	3.4	0.0	0.0	0.0	0.6
Total oil measurements outside Russell's range	18	68	84	417	15	64	189	175
% outside range	1.8	4.3	5.5	21.4	15.0	4.7	19.5	21.8
	Size range given by Russell, 1976. Mackerel egg 1.0 - 1.38mm. Oil globule 0.28 - 0.35mm							

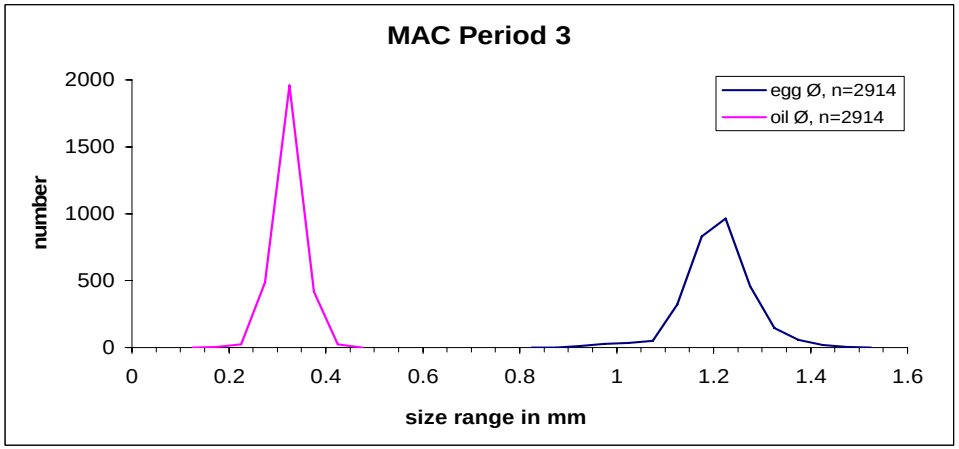
Table 3b. Summary of the numbers of horse mackerel eggs in each size class by country

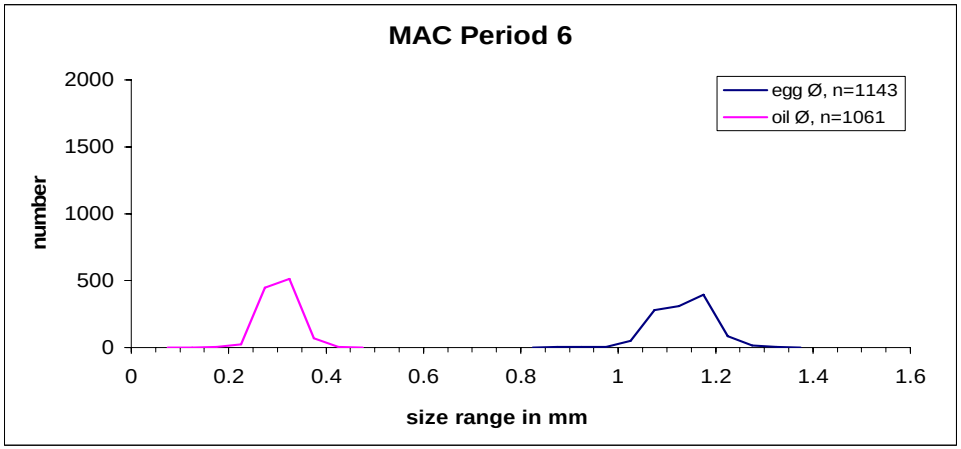
Size classes (microns)	Horse mackerel egg and oil globule sizes						
	England	Netherlands	Spain, AZTI	Germany	Norway	Ireland	Spain, IEO
50: 99							
100:149		1				1	
150:199	2	67	2	7	1	50	5
200:249	410	317	687	392	29	377	259
250:299	264	679	609	201	63	157	466
300:349	7	184	4	1	6	3	93
350:399		6				1	16
400:449							5
450:499							
500:549							
550:599							
600:649							
650:699							
700:749							
750: 799		1				3	
800: 849	2	41	2	5		86	
850: 899	46	130	45	33		238	8
900: 949	374	698	420	212	25	203	171
950: 999	244	204	627	253	61	59	477
1000:1049	15	371	232	63	14		182
1050:1099	1	21	6	29			6
1100:1149	1	2		5			
1150:1199		2		1			
1200:1249		0					
1250:1299		1					
1300:1349							
1350:1399							
1400:1449							
1450:1499							
1500:1549							
Total oil measures	683	1254	1302	601	99	589	844
Total egg measures	683	1471	1332	601	100	589	844
Total egg measurements outside Russell's range	2	27	6	35	0	3	6
% outside range	0.3	1.8	0.5	5.8	0.0	0.5	0.7
Total oil measurements outside Russell's range	9	258	6	8	7	55	119
% outside range	0.0	0.2	0.0	0.0	0.1	0.1	0.1
	Size range given by Russell, 1976. Horse mackerel egg 0.81 - 1.04mm. Oil globule 0.19 - 0.28mm						

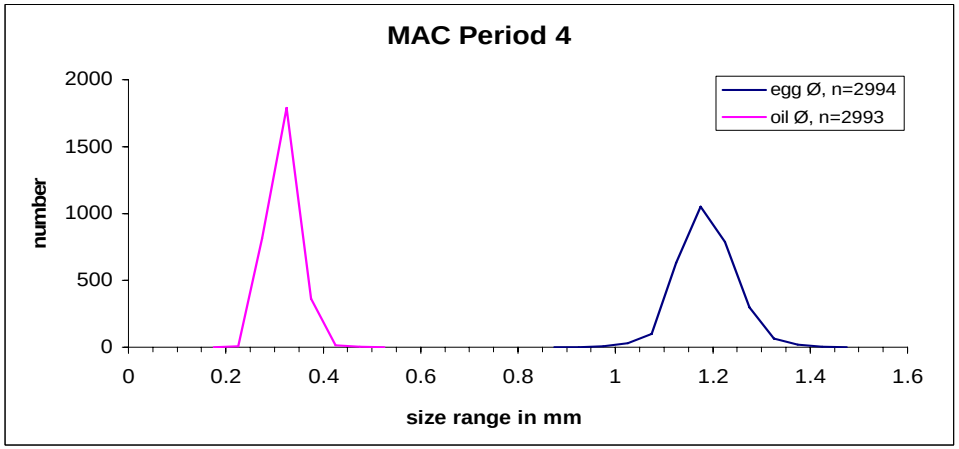
Figure 1a. The size distribution of mackerel egg and oil globules by time period 2004.











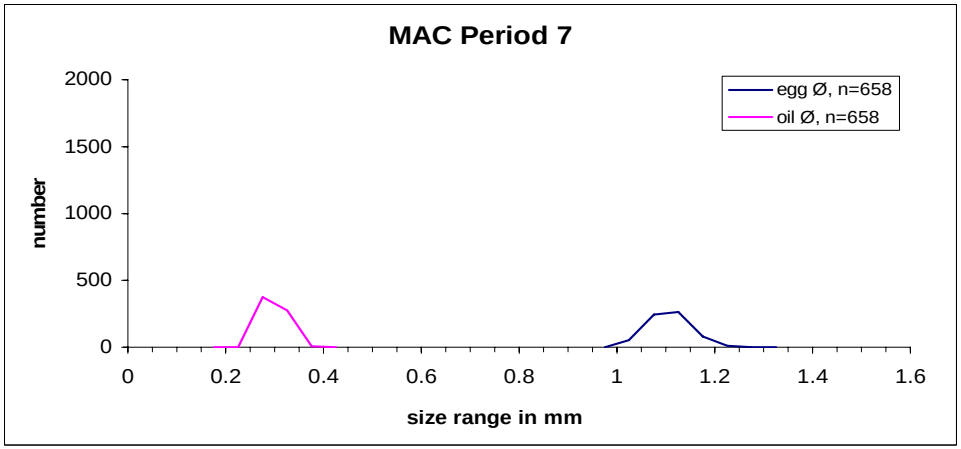
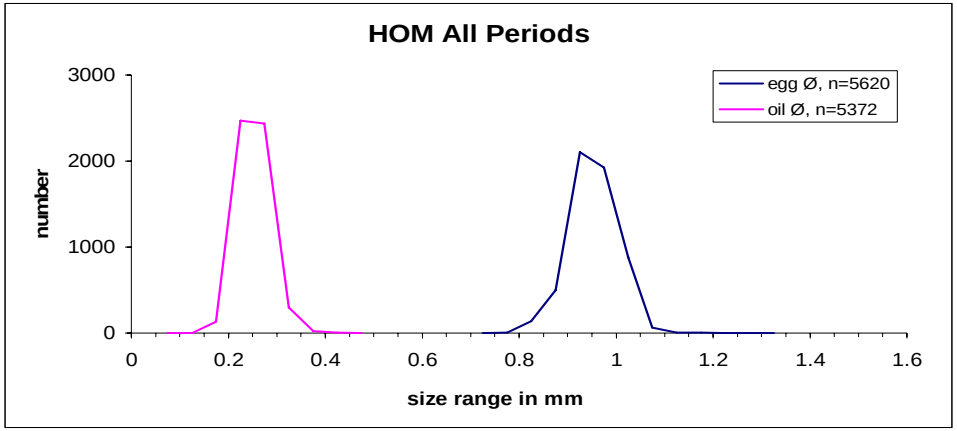
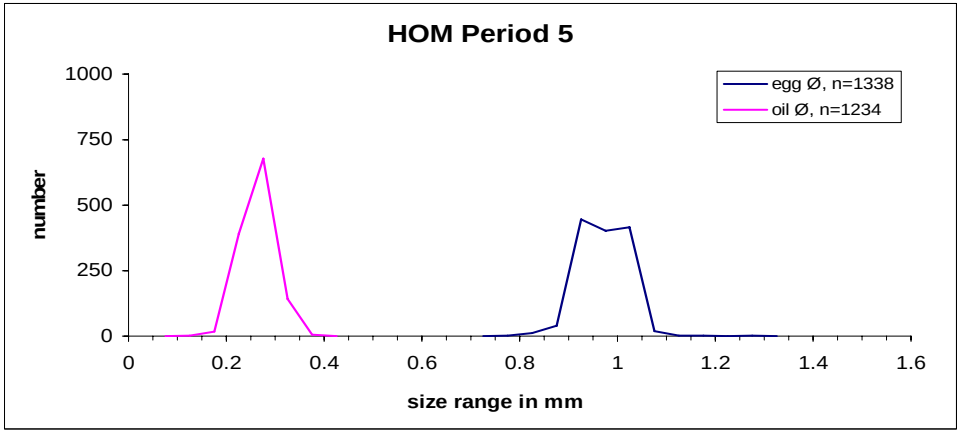
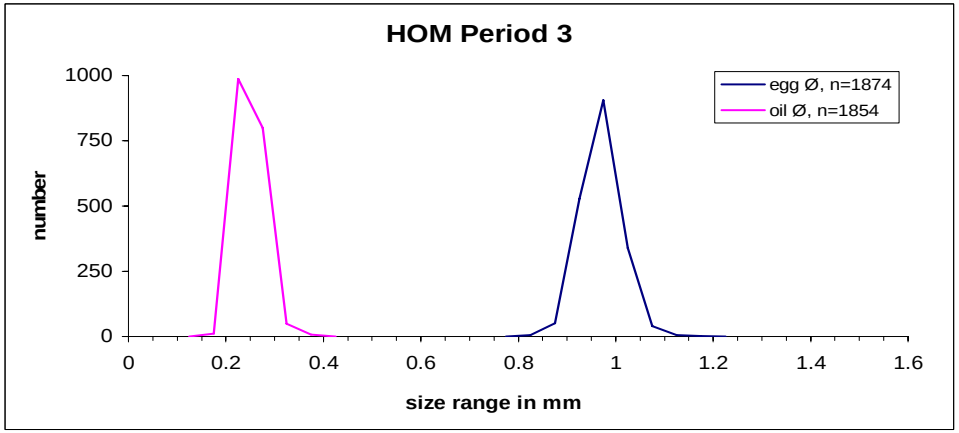
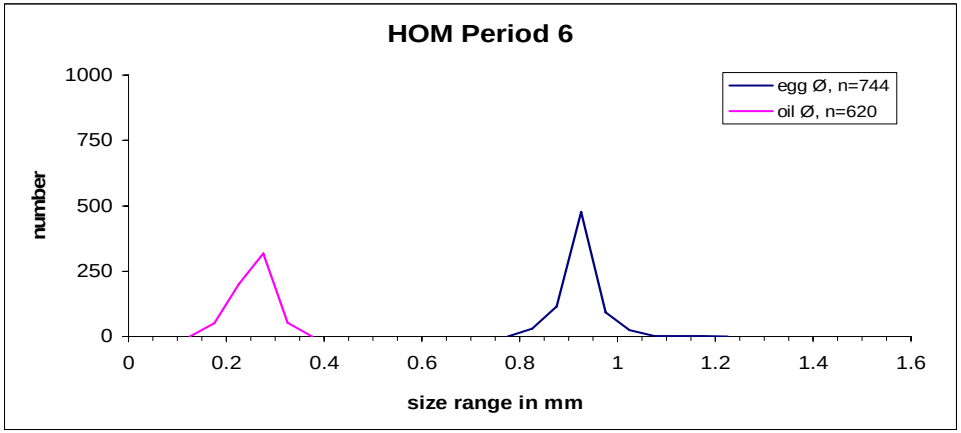


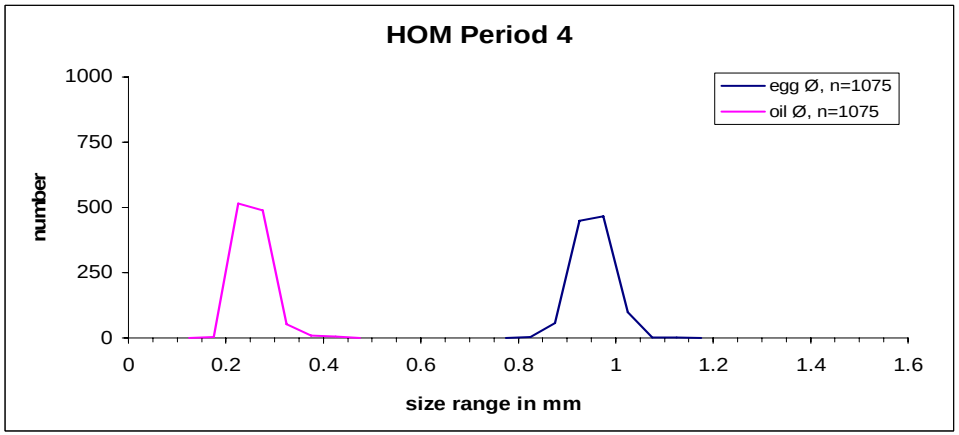
Figure 1b. The size distribution of horse mackerel egg and oil globules by time period 2004.











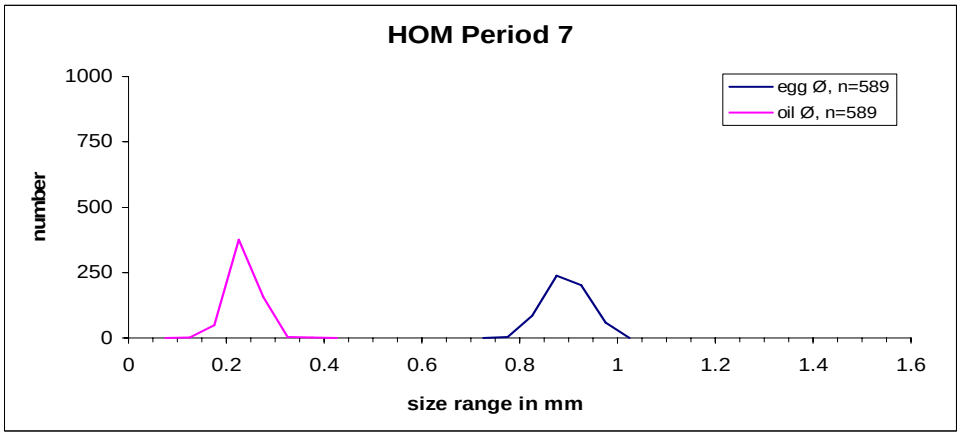


Figure 2. Station positions and numbers of mackerel eggs selected for measuring.

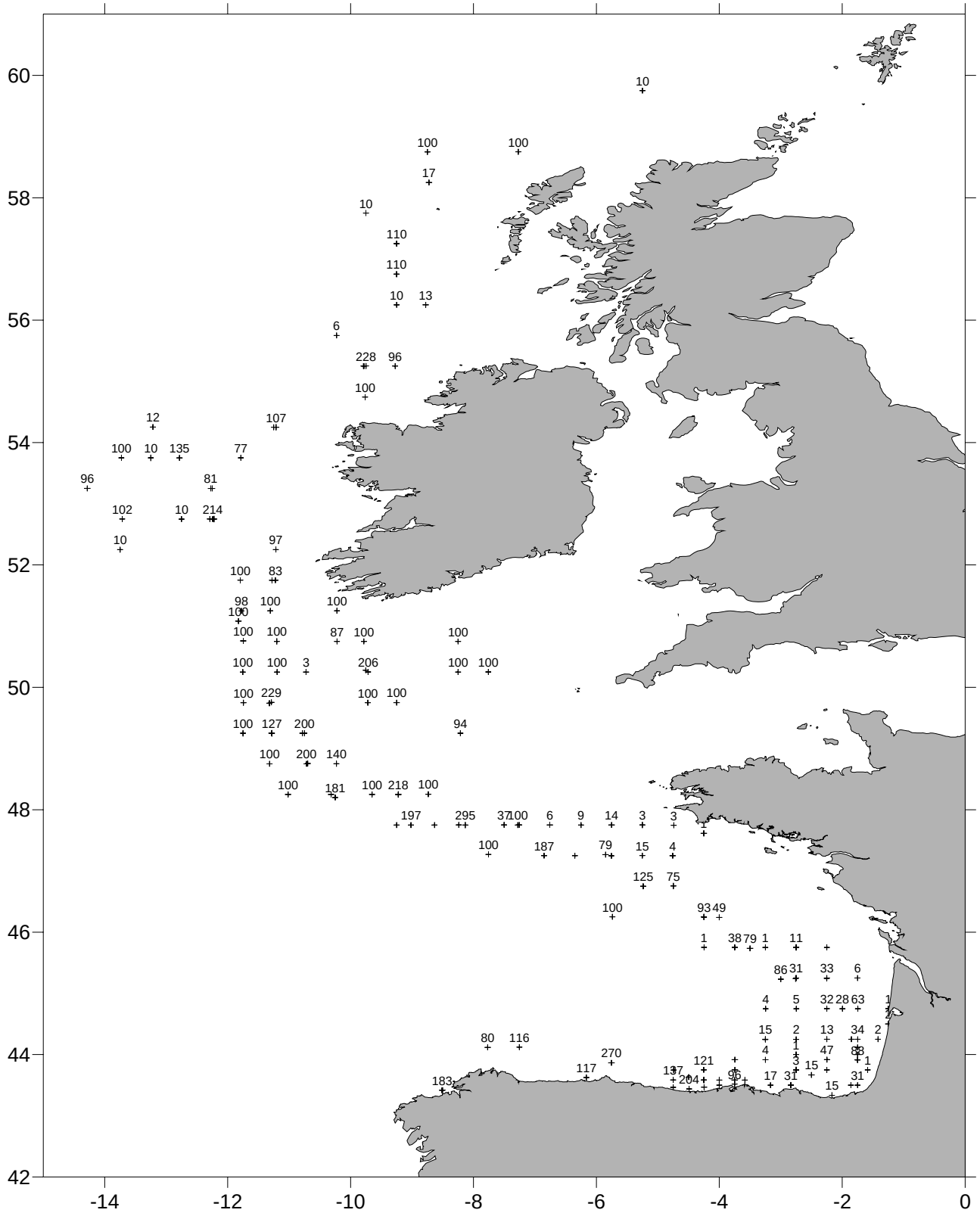


Figure 3. Station positions and numbers of horse mackerel eggs selected for measuring.

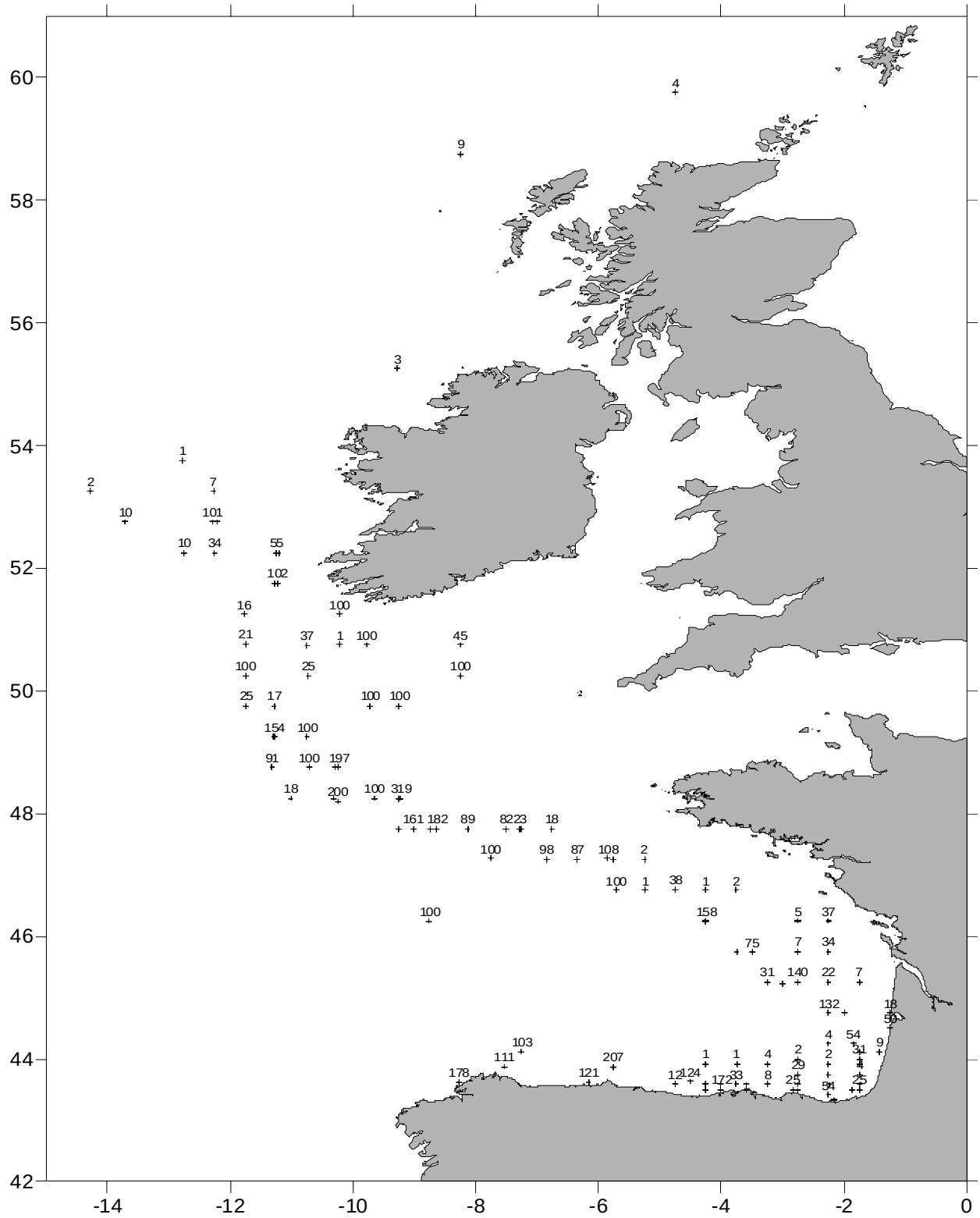
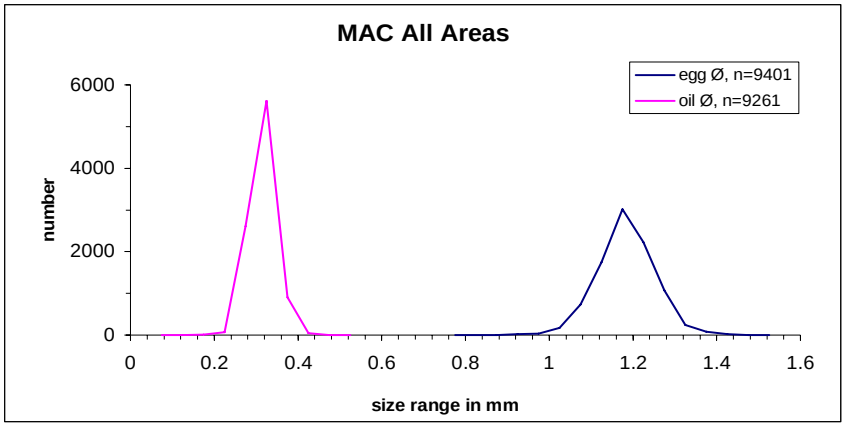
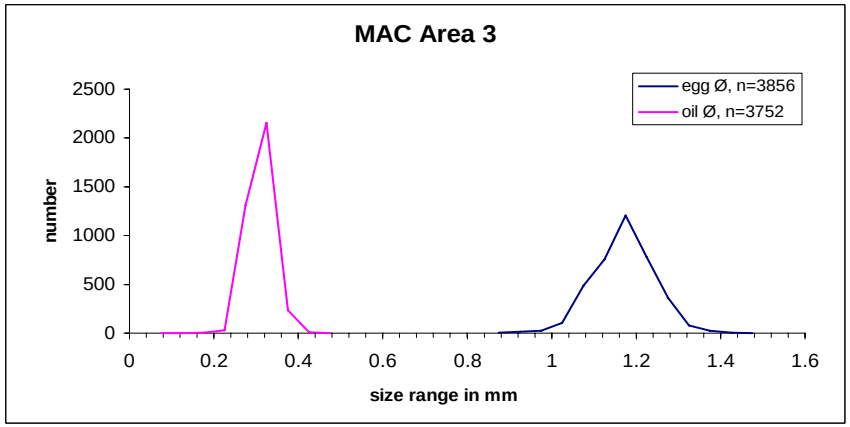
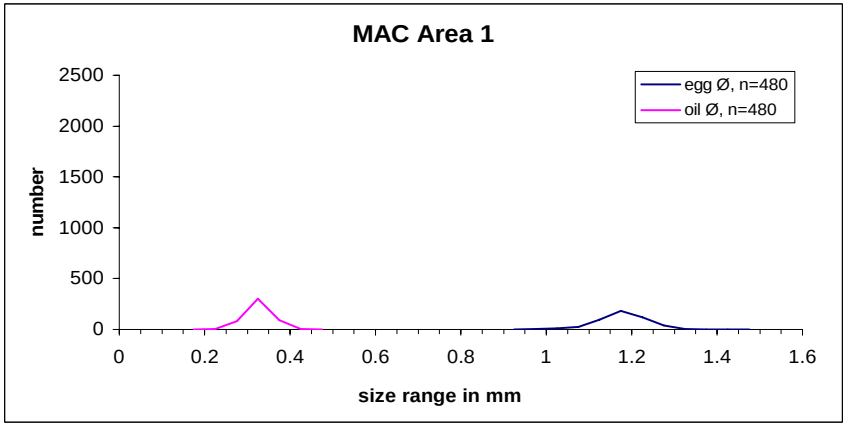
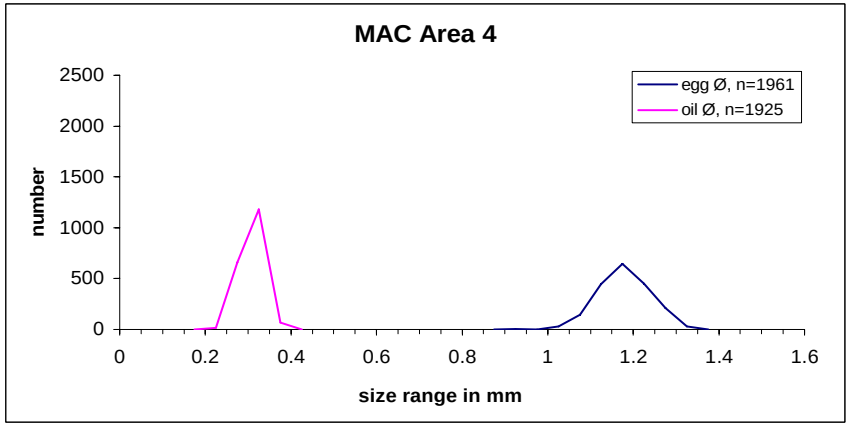


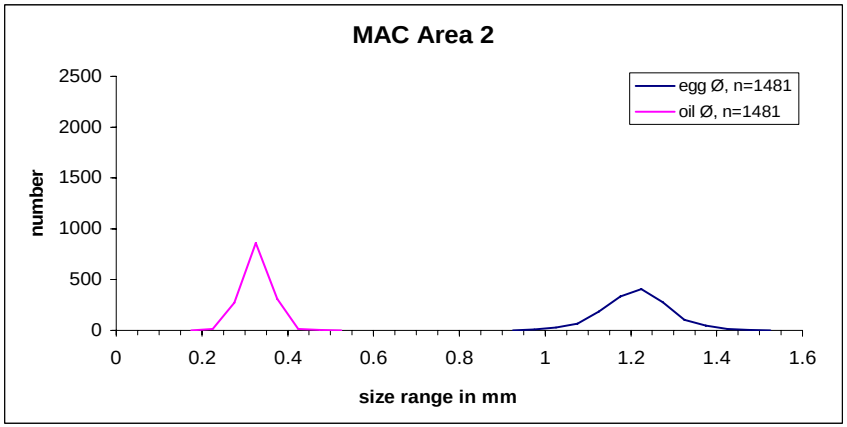
Figure 4a. The size distribution of mackerel eggs and oil globules by region.

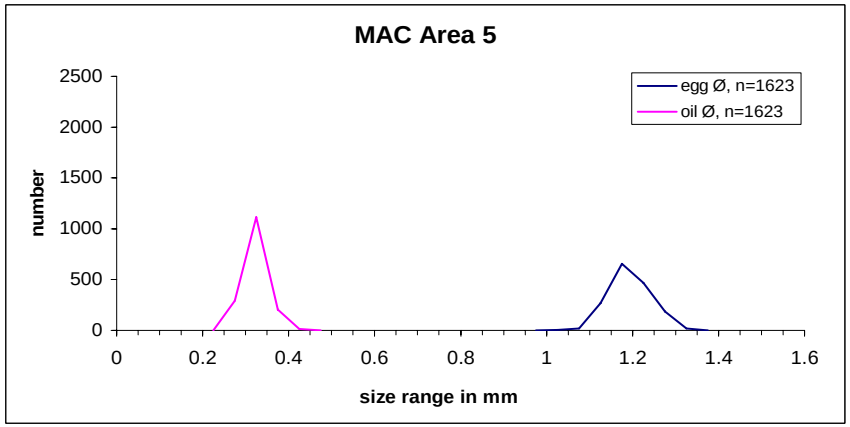






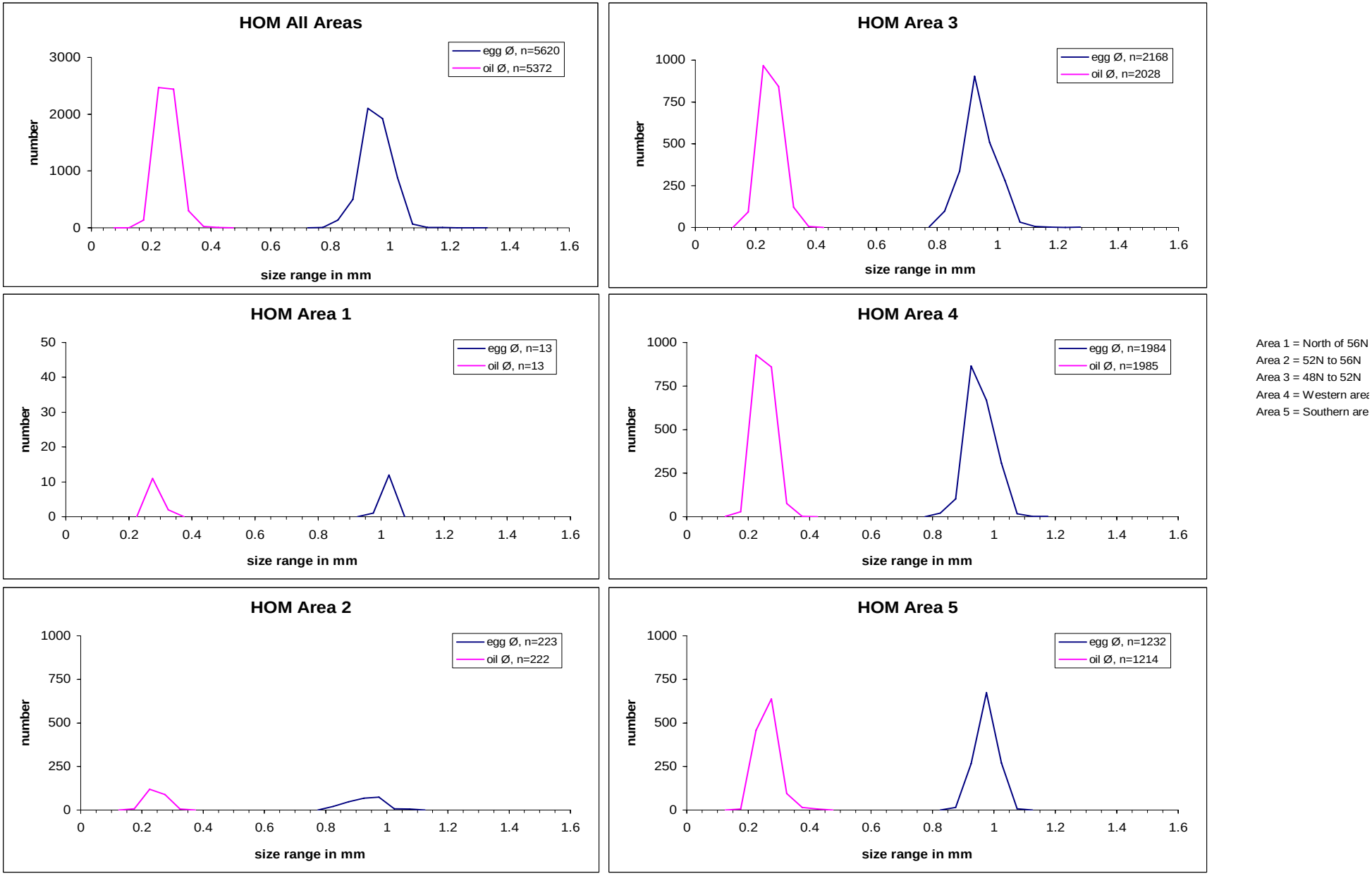






Area 1 = North of 56N
Area 2 = 52N to 56N
Area 3 = 48N to 52N
Area 4 = Western area
Area 5 = Southern are

Figure 4b. The size distribution of horse mackerel eggs and oil globules by region.



Area 1 = North of 56N
Area 2 = 52N to 56N
Area 3 = 48N to 52N
Area 4 = Western area
Area 5 = Southern are

Figure 5a. The size distribution of mackerel egg and oil globules by country.

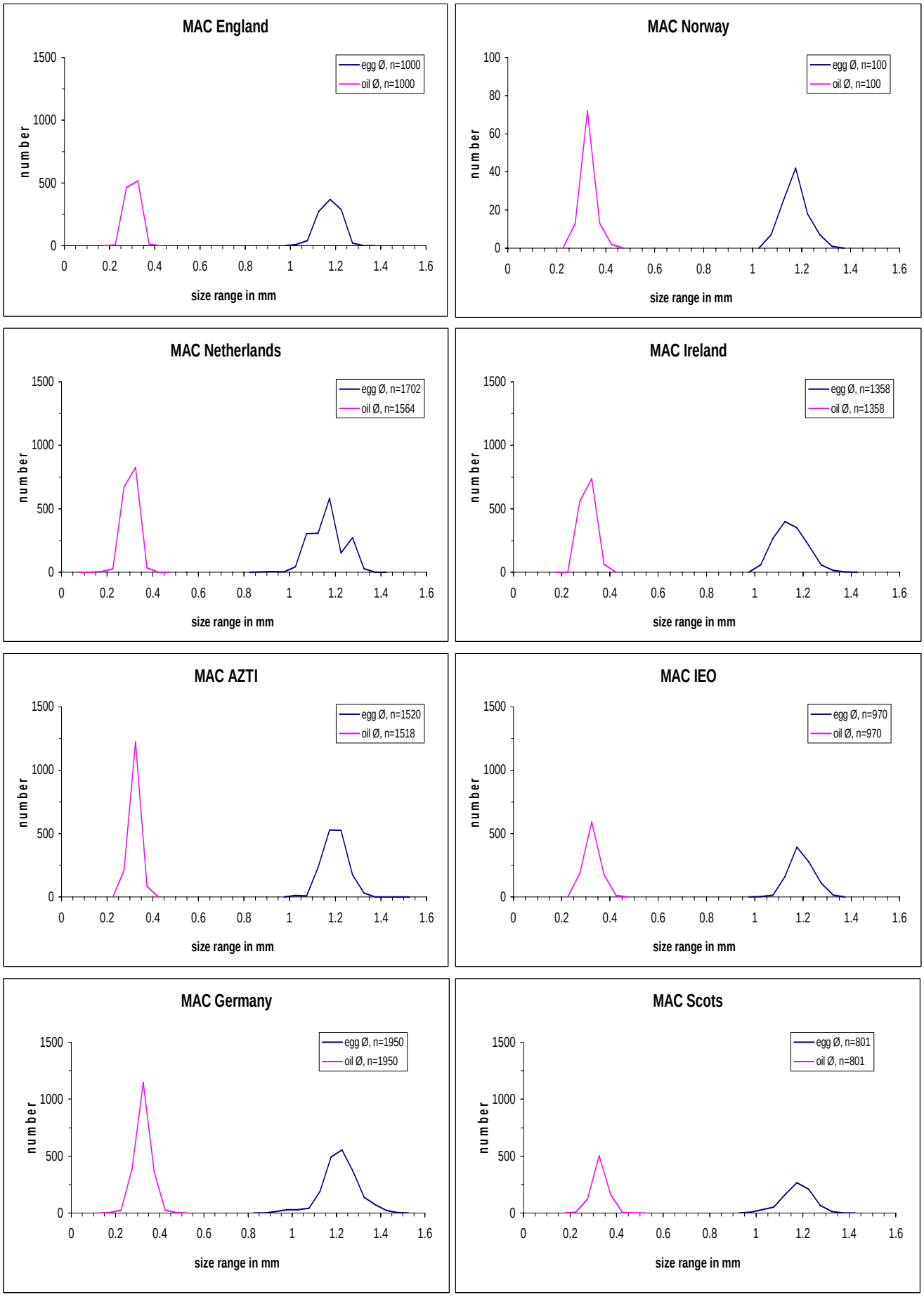
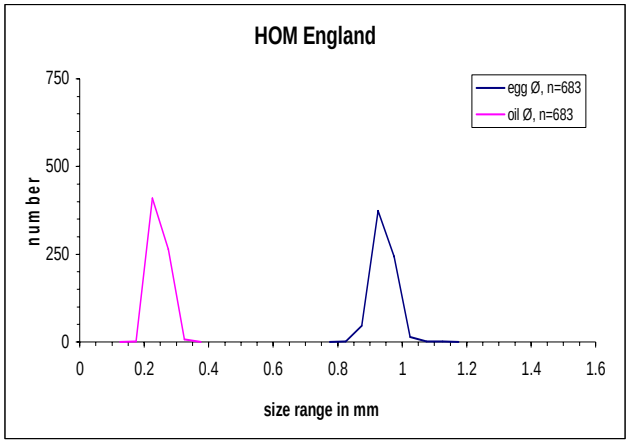
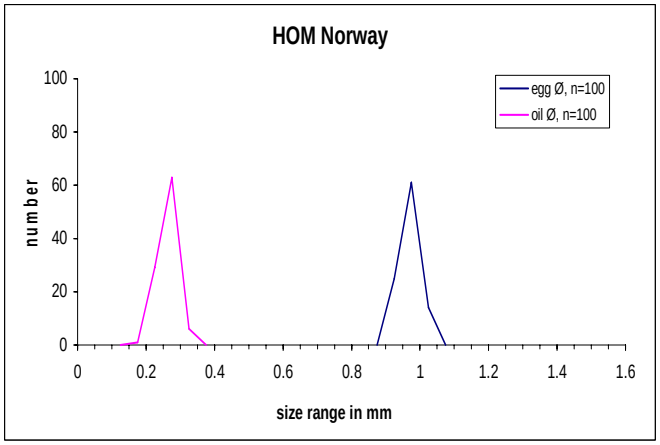
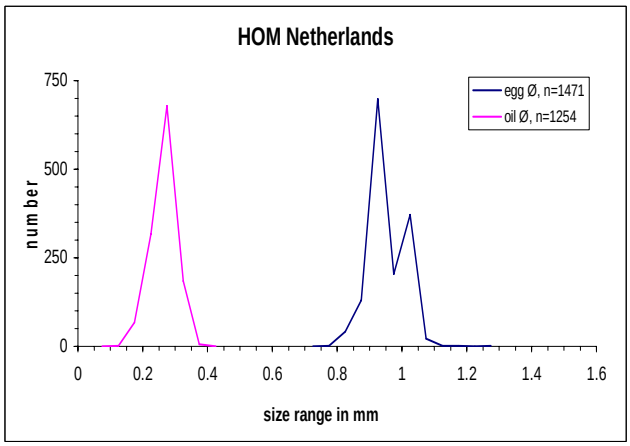
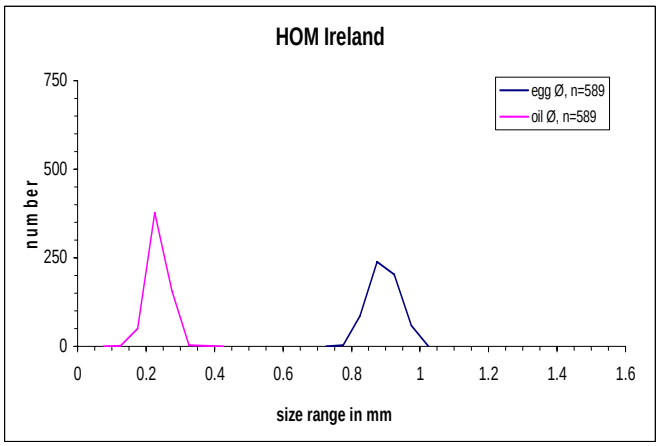


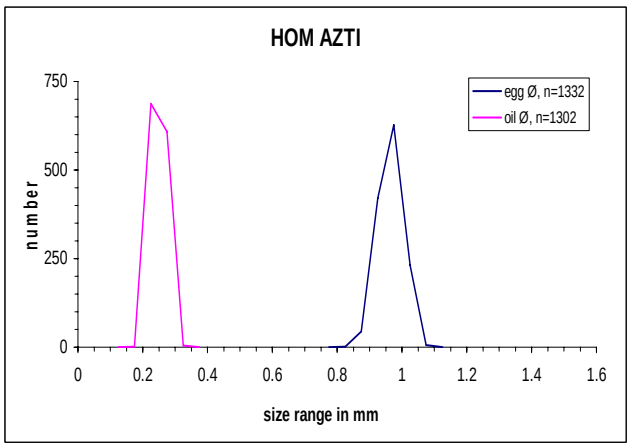
Figure 5b. The size distribution of horse mackerel egg and oil globules by country.

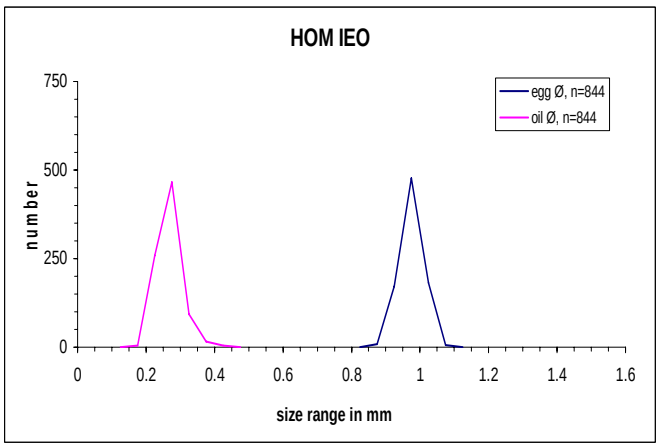












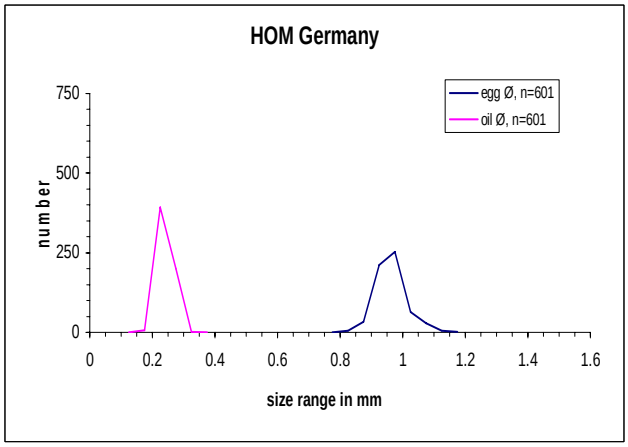


Figure 6. Size frequencies of mackerel and horse mackerel eggs and oil globules by development stage.

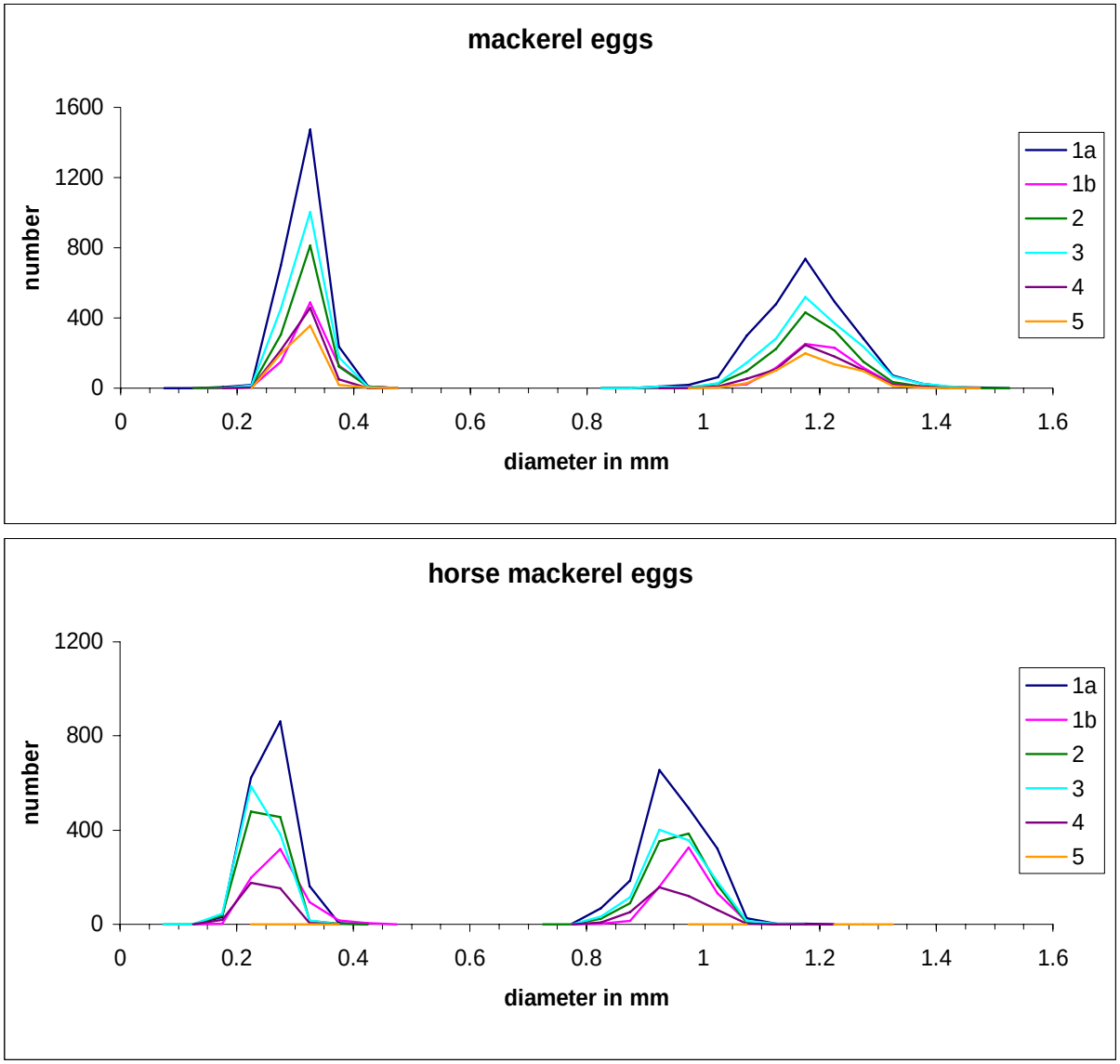
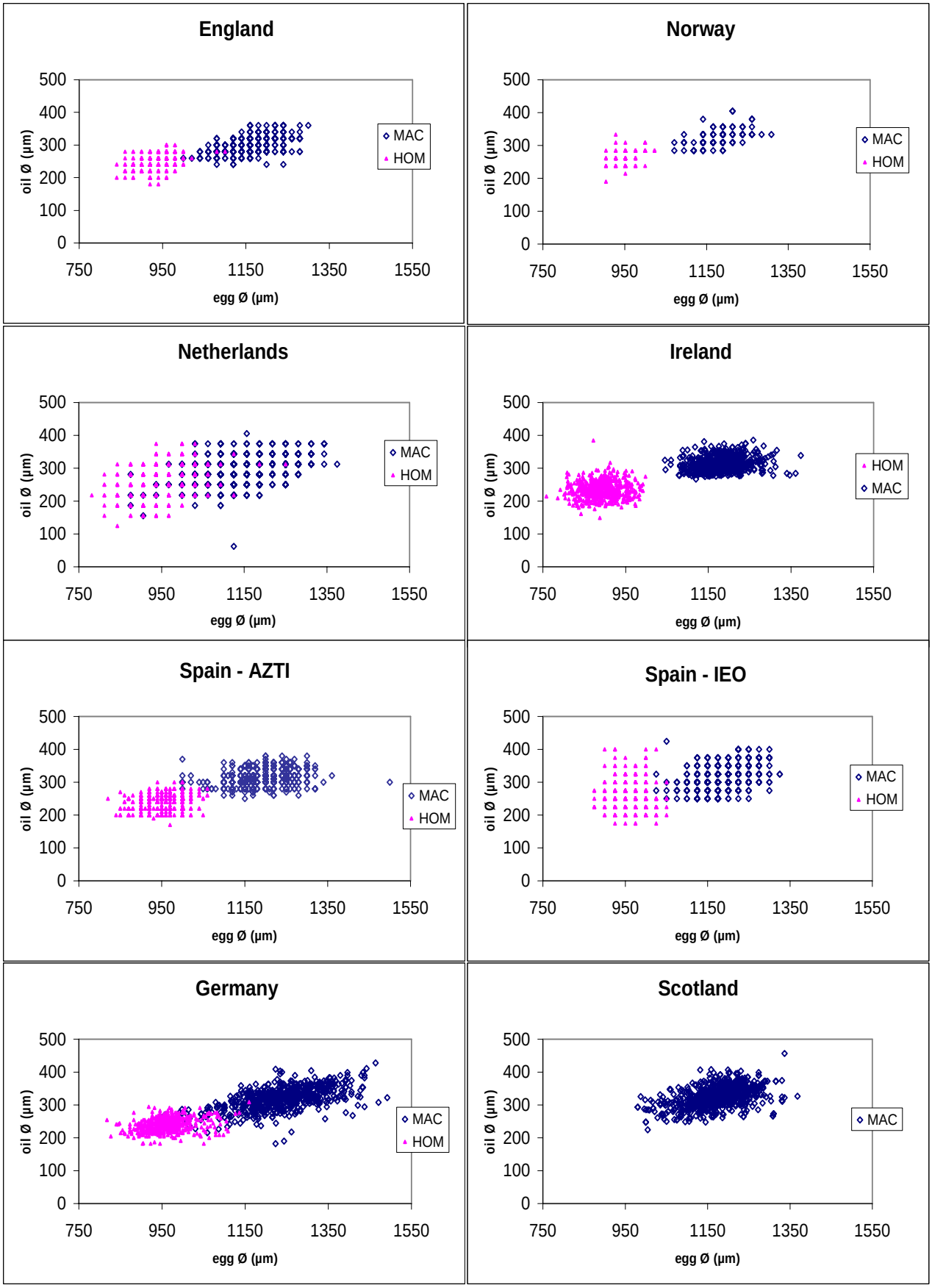


Figure 7. Relationship between mackerel and horse mackerel egg and oil globule diameters by country.



Annex 3: Report of Fecundity Analysis Workshop. CEFAS Lowestoft October 2003

Terms Of Reference

- Demonstrate the application of image analysis, ovary sampling and (GFA) software in fecundity analysis
- Intercalibrate the stereometric and gravimetric fecundity methods for horse mackerel.
- Develop the Auto-diametric fecundity method for horse mackerel
- Provide a manual and equipment list.

Participants

Course Date	Participants	Institute
20-22 Oct[1]	Josè Ramon Pérez Isabel Bruno	IEO Vigo
20–21 Oct	Selene Hoey Hans Gerritsen	ML Gallway
23-24 Oct	Ana Maria Costa Patricia de Jesus Gonçalves	IPIMAR
27-28 Oct	Cindy van Damme Findlay Burns	RIVO FRS MLA

[1] The extra day is for participants from Vigo to learn the procedures for producing slides from Technovit resin.

Summary

The Workshop included training to apply the Gravimetric and image analysis methods to determine fecundity and to identify previtellogemnic, vitellogenic, atretic and post ovulatory follicles in dispersed samples of ovary tissue (whole mounts). The work to intercalibrate the Stereometric and Gravimetric methods was not completed because the Stereometric fecundity data from the IEO Vigo and MI Galway gave different results and standardisation of the Stereometric method is recommended prior completing the analysis with the present data or new samples.

Introduction

Prior to the 2004 Triennial surveys mackerel fecundity was determined by a gravimetric method using Gilson fixative (Simpson 1951). This fixative contains strong acids, ethanol and highly toxic mercuric chloride to separate oocytes from the ovarian lamellae but also damages the sample and slowly erodes the oocyte surface causing extensive and progressive shrinkage in oocyte size (ICES, 2002). Apart from the high toxicity it is also likely that atretic and post ovulatory follicles are lost during the fixation process and the samples are not suitable for histological analysis. A gravimetric method based on taking sub-samples of formaldehyde fixed tissue (Hunter and Macewizc 1989) has recently been shown to provide equivalent estimates of fecundity compared with data from Gilson fixed tissue if all oocytes greater than 185 µm are included in the count (ICES 2002 WD Witthames and Greenwood ICES, 2002). Recently image analysis methods (Thorsen and Kjesbu 2001) have been applied to estimate fecundity in formaldehyde fixed tissue and this would offer substantial saving of time or allow many more samples to be processed for the same effort.

In the case of horse mackerel it has become apparent that histological - sterological methods cannot provide a reliable classification of spawning status to determine fecundity before the annual spawning commences. Analysis of oocyte frequency in preparations of dispersed oocytes (whole mounts) however, offers the possibility to determine the standing stock of fecundity, batch fecundity and spawning status with much lower costs using image analysis methods above. In order to introduce this new method the Planning Workshop at Lisbon agreed that a training course should be held in Lowestoft to demonstrate the use of equipment and carry out intercalibration with horse mackerel fecundity previously estimated by the stereometric method.

Methods

Application of image analysis to the estimation of fecundity

The underlying principle of automatic image analysis is to segment an electronic image into dark objects (oocytes) with a low grey value from the light background (high grey value). Each electronic image is made up of an array of picture elements (pixels) that define the basic unit of measurement captured by a CCD (Charged Couple Device) camera attached to the third port on a binocular microscope. Software used in the following analysis produces live images where the resolution is better than 4µm per pixel or 2.1% of the smallest oocyte (184µm) included in the stereometric fecundity estimate. The image is updated at a rate of 15 frames per second and allows real time focusing to produce sharp definition for analysis. Oocytes that do not segment automatically can be measured manually defining the diameter by 2 points across the X feret (Figure 1).

Staining oocytes to improve segmentation

Mature vitellogenic oocytes are very opaque but the cortical alveoli and hydrated oocytes are nearly transparent (Figure 2). Staining the oocytes with Periodic acid followed by Schiff's reagent increases the oocyte absorbance especially in the green and red colour bands so that segmentation of the oocyte from the background is more efficient.

A standard operating procedure for image analysis

In order to provide reliable oocyte diameter frequency data from image analysis it is important to control and define the light intensity (grey levels) of each colour band making up the background light (Figure 3). The camera field should be evenly illuminated but this is not always possible and depends on each microscope. It is also important to use the same voltage across the bulb filament to maintain the same proportions of red green and blue light. The cold light source listed below uses a variable neutral density filter to give fine control of light levels and operates at a constant voltage. In order to focus on all oocytes (diameters 0.19 to 1 mm) comprising the fecundity in mackerel and horse mackerel the microscope should contain an iris adjusted to nearly closed to increase the depth of field.

Staining ovary samples

Chemicals purchased from Taab email [sales@taab.co.uk]

0.1% Periodic acid prepared from 99.5% periodic acid code P005.

15% Schiffs Reagent prepared from product number J/7300/PB08

Method

Fix tissue for a minimum of 14 days in 3.6% neutral buffered formaldehyde before processing.

- Place the ovary tissue (100 mg of cod and 25 mg of hake, mackerel or horse mackerel) into a Netwell and suspend the well in a dish containing purified water for a minimum of 15 minutes to remove the formaldehyde. Disperse the sample during this stage into single or small clumps of follicles by forcing it through a fine glass pipette nozzle.
- Lift the Netwell from the wash, blot its base and rinse the sample with a jet from a wash bottle containing purified water. Blot the base.
- Suspend the Netwell in a dish containing 0.5% periodic acid for 15 minutes.
- Lift the Netwell, blot the base and suspend it in purified water for 5 minutes.
- Remove the Netwell, blot its base and rinse with a jet from a wash bottle containing RO water. Blot the base.
- Suspend Newell in the 30% Schiffs solution.
- Lift the Netwell, blot the base and suspend the Newell in a dish of purified water for 10 minutes.
- Remove the Netwell, blot the base and rinse further with a jet from a wash bottle containing RO water and resuspend in water.

Examine the sample and break up clumps using a fine brush

The size of oocytes measured by image analysis after staining with this protocol is stable for at least 4 days storing them at 0-5^{o} C.

Procedures for image analysis

Calibration of image analyser

The equipment used at CEFAS is listed in the appendix at the end of the report

1) Disperse samples of stained ovary tissue containing hydrated and developing oocytes from several species e.g. cod, hake, mackerel and plaice, in purified water.

2) Select 4 oocytes of different sizes and stage of maturation covering the calibration range from 185 to 2000 µm and place them together in a multi well plate. Replicate 12 times until all the wells of the plate are full.

3) Push the oocytes to the centre of the well and measure each group manually and then follow the staining protocol (in the well) before re-measuring each group by image analysis. Pair the two sets of measurements and compare oocyte size (Figure 4).

4) The RGB grey level of the illumination can be adjusted to decrease the difference between manual and image analysis size measurements whilst maintaining a high success in automatic measurement.

5) Record the statistics of RGB grey levels and use these conditions to determine the relationship between Ln mean oocyte diameter and Ln Oocytes g^{-1} ovary for the auto-diametric calibration as below.

Measurement of oocyte frequency using GFA

1) Spread out the sample (Figure 5) in a well (10 mm or 3.5 mm wide and 70 mm long for cod and horse mackerel respectively) of a specially manufactured clear plastic tray (See equipment list). The width of the well should fill the height of the image on the PC VDU.

2) Fill the tray with water including a few drops of photographic wetting agent so that the well is completely immersed. Oocytes are measured as dark objects against the transmitted light background.

3) Adjust the size of the scale circle displayed on screen (300 and 185 µm in mature cod and horse mackerel respectively) so that the operator can select the minimum size of oocyte to be included in manual frequency distribution measurements.

Measurements acquired in Automatic mode are filtered to exclude oocytes smaller than the size of the scale circle.

4) A few follicles that are not properly segmented can be excluded from the data acquired in automatic mode and measured manually defining their diameter by 2 dots.

5) Atretic and post follicles can be counted or measured in the last two fields so that the numbers of all follicle types are include in the analysis.

Fecundity determination

Gravimetric method

The pipette (Figure 6) needs to be calibrated to determine the weight of tissue removed after filling to either the lower or upper line engraved on the pipette. This should be done with fresh ovary tissue.

F = N *o * s

Where F = standing stock of vitellogenic oocytes

N = number of oocytes defined as vitellogenic.

O = Total weight of both ovaries

S = Sub sample weight removed by the pipette

Auto-diametric method

The Auto-diametric calibration method uses follicles from pre spawning and spawning cod (Figure 7) but more data is required from mackerel and horse mackerel at a range of spawning stages.

The calibration formula to estimate the standing stock of vitellogenic and atretic follicles are as follows.

GFA calibration $Ng = 1.262 \times 10^{13} \times OD^{-3.321}$

Where Ng = oocytes g-1 ovary and OD = mean oocyte diameter in microns.

Fp = Ng x O

Where Fp = standing stock of vitellogenic and atretic follicles O = ovary weight g

Inter calibration between gravimetric and stereometric fecundity estimates

Gravimetric method

Duplicate gravimetric ovary samples were removed from formaldehyde preserved ovaries and all oocytes larger than 150 µm were measured. According to previous experience with mackerel and examining the whole mounts it was expected that the standing stock of oocytes larger than 150 µm would exceed the stereometric fecundity. However if the minimum size of oocyte included on the gravimetric count was raised in 5 µm intervals at some point the two fecundity estimates would be identical.

Results

Oocyte frequency

A typical oocyte frequency distribution for horse mackerel in the final stage of batch production is shown in Figure 8. There is no clear break in the size distributions of previtelligenic and vitellogenic oocytes but morphology and size can be used for identification in whole mount (Figure 9) and section (Figure 10). Oocyte diameter is distorted (-10 to -20%) if measured in section as a result of processing and because of the section plain through the oocyte.

Interpretation of whole mounts in relation to histology

It is possible to identify and count Post ovulatory (Figure 10) and atretic follicles (Figure 12) in whole mounts and some inter calibration work has already been completed as part of a method development (RASER Work package 1 (http://raser.imr.no) funded by the European Commission.

Inter calibration between Gravimetric and Stereometric fecundity estimates

A comparison between Gravimetric and the Stereometric fecundity estimates from the same ovary for the 2001 survey is shown in Table 1 and summarised in Table 2. In the case of the Marine Institute Galway the standing stock of oocytes larger than 150 µm determined using Gravimetric method was larger (except in one case) than the fecundity determined by the Stereometric method. Ignoring this sample the two methods gave equivalent values of fecundity if all oocytes larger than 184 (se 7) µm were included in the Gravimetric count and equated to 70 % of the standing stock of oocytes larger than 150µm. However for IEO the Stereometric fecundity was much higher (2.3 times on average) compared to the standing stock of oocytes larger than 150 µm determined from the Gravimetric method.

Discussion and conclusions

Comparisons of the stereometric fecundity estimates for horse mackerel in relation to the Gravimetric method applied to the same samples (Table 2) indicate there is a methodological difference in the fecundity data from IEO Vigo and MI Galway. The basis for this divergence is not clear but the morphology of oocytes in whole mounts suggests that cortical alveoli accumulate in oocytes larger than 150µm (Figure 8). In order to resolve the discrepancy further work is required as detailed in points 1–4 below. Based on the MI Galway samples oocytes in horse mackerel start to accumulate cortical alveoli at a size of 184 µm which is almost identical to the values for mackerel (185 µm WD Witthames and Greenwood ICES, 2002).

The workshop made the following recommendations

1) RIVO, IMR Norway, IEO and the Marine Institute Galway should standardise their estimates of fecundity based on the slides listed in Table 1.

2) The data from the samples in table 1 should be re-analysed using a standardised stereometric estimate of fecundity to recalculate values shown in Tables 1 and 2.

3) Oocytes measured in whole mount should be sectioned to identify at what size cortical alveoli start to accumulate around the nucleus.

4) If the fecundity from the Stereometric method exceeds the Gravimetric fecundity then the further gravimetric samples should be analysed measuring oocytes larger than indicated by the results from 3 above.

References

ICES. 2002 Report of the working group on mackerel and horse mackerel egg survey. ICES CM 2002/G:06 ref. D Living Resources Committee.

ICES. 2003 Report of the working group on mackerel and horse mackerel egg survey. ICES CM 2003/G:7 ref. D Living Resources Committee

Simpson, A.C. The fecundity of the plaice. Fishery Investigations. Ministry of Agriculture Fisheries and Food. ser. 2(17(5)):1-27, 1951.

Thorsen, A. and Kjesbu, O. S. 2001. A rapid method for the estimation of oocyte size and potential fecundity in Atlantic cod using computer-aided particle analysis system. *Journal of Sea Research* 46:295-

Tables

Table 1: List of the gravimetric samples analysed from IEO Vigo and MI Galway.

INSTITUTE	SAMPLE REFERENCE	OVARY WEIGHT (G)	GRAVIMETRIC SUB SAMPLE WEIGHT (G)	STEREOMETRIC FECUNDITY	STANDING STOCK OF OOCYTES <150 µM GRAVIMETRIC	MINIMUM OOCYTE SIZE INCLUDED IN GRAVIMETRIC FECUNDITY µM	RATIO STEREOMETRIC COUNT / GRA-VIMETRIC COUNT	COMMENTS [1]
IEO	12-5-Y	8.0	0.0238	254416	204034		1.25	GF > S
IEO	12-5-b		0.0238		161008		1.59	GF > S
IEO	13-7-Y	8.0	0.0264	398481	275455		1.45	GF > S
IEO	13-7-b		0.0261		199847		2.00	GF > S
IEO	17-3-Y	14.2	0.0273	254416	167487		1.62	GF > S
IEO	17-3-B		0.0263		139034		1.83	GF > S
IEO	J-9-1 y	13.4	0.0263	343889	175270		1.96	GF > S
IEO	J-9-1 b		0.0271		259272	150	0.91	
IEO	J-9-4 y	10.0	.0271	348163	188191		1.85	GF > S
IEO	J-9-4 b		0.0271		138007		2.52	GF > S
MI	128a	12.6	0.022	190750	212540	170	0.76	[1]see footnote
MI	128b		0.0234		267688	200	0.60	
MI	127a	11.11	0.0274	167577	203696	175	0.52	
MI	127b		0.0223		263168	200	0.75	
MI	52a	13.99	0.0249	180255	345043	230	0.68	
MI	52 B		0.024		331163	195	0.63	
MI	126a	9.43	0.0234	174380	176520	150	0.66	
MI	126b		0.0204		242164	185	0.68	
MI	155A	14.19	0.0236	728495	769697	155	0.68	
MI	155b		0.021		597385			GF > S
MI	62B	10.02	0.0254	209703	260840	180	0.75	
MI	137a	14.38	0.0234	162129	239829	215	0.68	
MI	137b		0.0238		239426	225	0.68	

[1]GF > S Gravimetric fecundity < Stereology fecundity.

Includes 7 % atresia

Table 2: Summary of results indicating the smallest oocyte to include in the Gravimetric Count so that Gravimetric and Stereometric fecundity estimates are equivalent.

INSTITUTE	DATA	TOTAL
IEO Vigo	Average minimum oocyte size to include in the gravimetric fecundity (µm)	<150
	se minimum oocyte size to include in the gravimetric fecundity	na
	Average ratio (Stereometric fecundity / gravimetric fecundity standing stock >150 µm)	1.70
	Standard Deviation of ratio above	0.44
	Number of samples analysed	10
MI Galway	Average smallest oocyte size to include in the gravimetric fecundity (µm)	184 µm
	se smallest oocyte size to include in the gravimetric fecundity	7 µm
	Average ratio (Stereometric fecundity / gravimetric fecundity standing stock >150 µm)	0.67
	Std Dev of ratio above	0.08
	Number of samples analysed	10

Figures

Figure 1: Illustration of the method to measure oocyte diameter defined by two points on the X ferret.

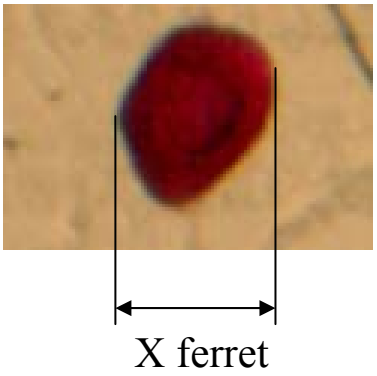
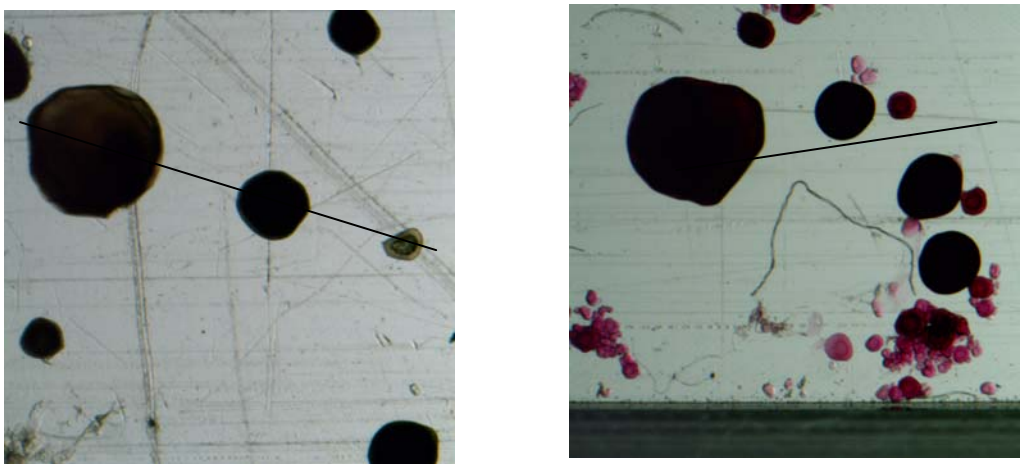


Figure 2: Images of oocytes after fixation in 3.6% formaldehyde (panel a) and after staining with Periodatic acid and Schiffs reagent (panel b). The grey levels along the transect lines displayed in Panels A and B are shown in panels c and d respectively.

Panel A

Panel B



Panel C

Panel D

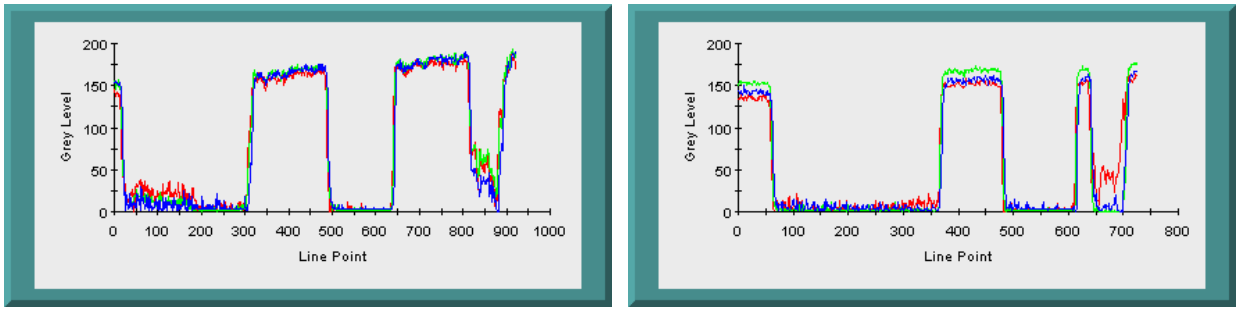
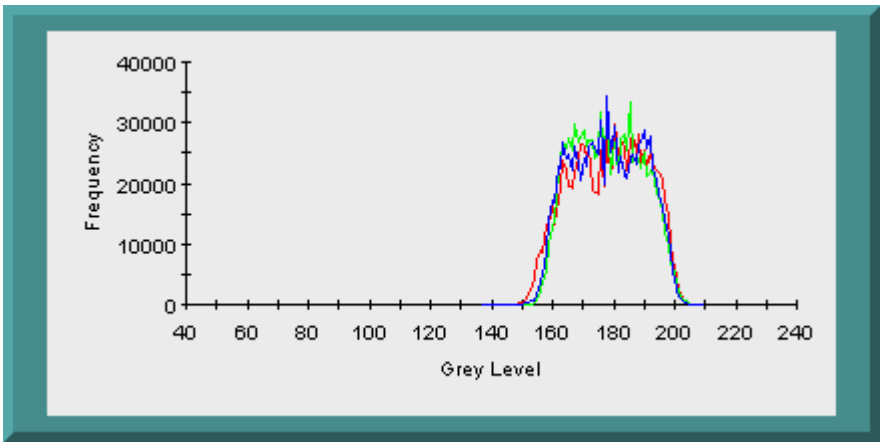


Figure 3: Two charts showing the grey level properties in the analysis field. Panel A shows the grey levels in each colour band after setting the white balance according to the camera operating instructions. Panel B shows a chart of average grey levels in each colour band in a line transect across the total field of view displayed on the monitor. On this equipment the overall variation in grey level across the field of view deviates ±8 % decreasing from left to right around a mean value of.180.

Panel A



Panel B

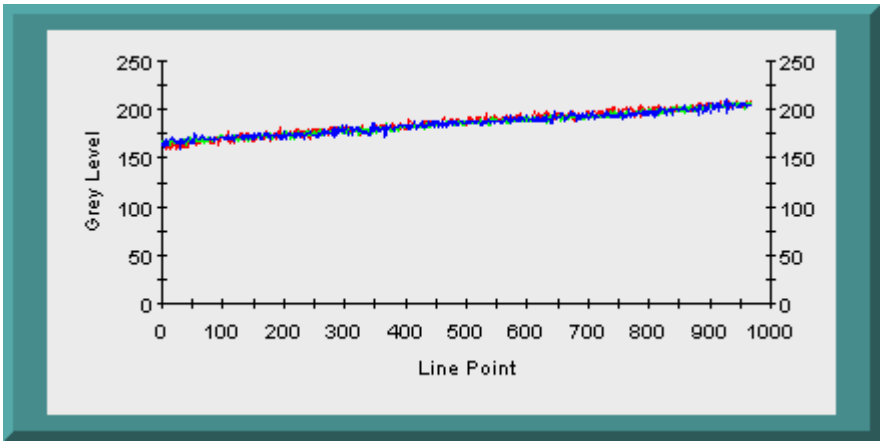


Figure 4

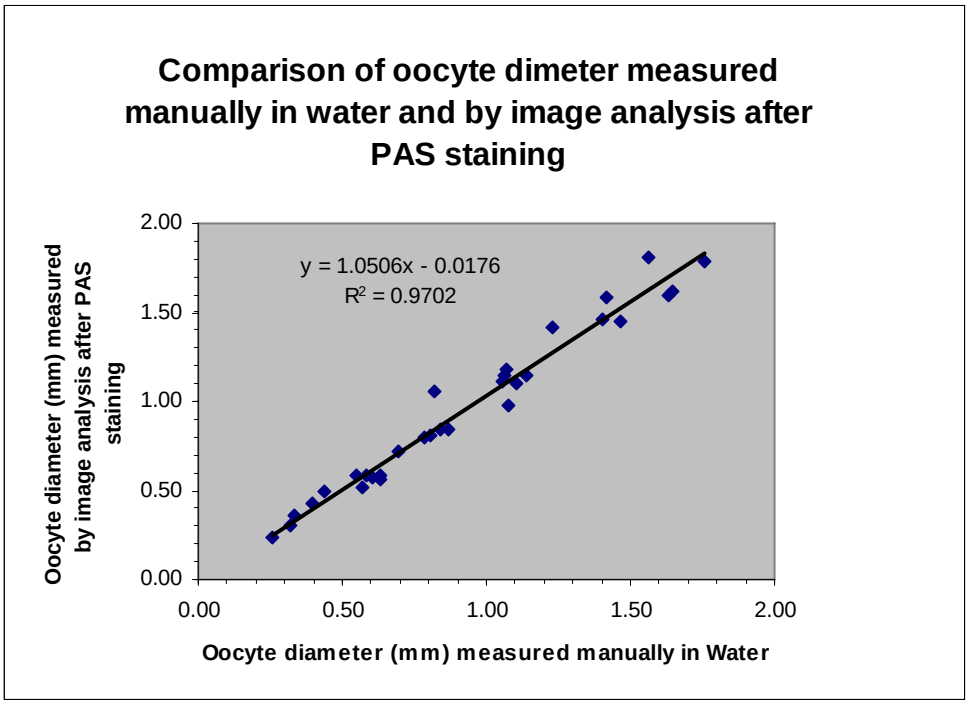
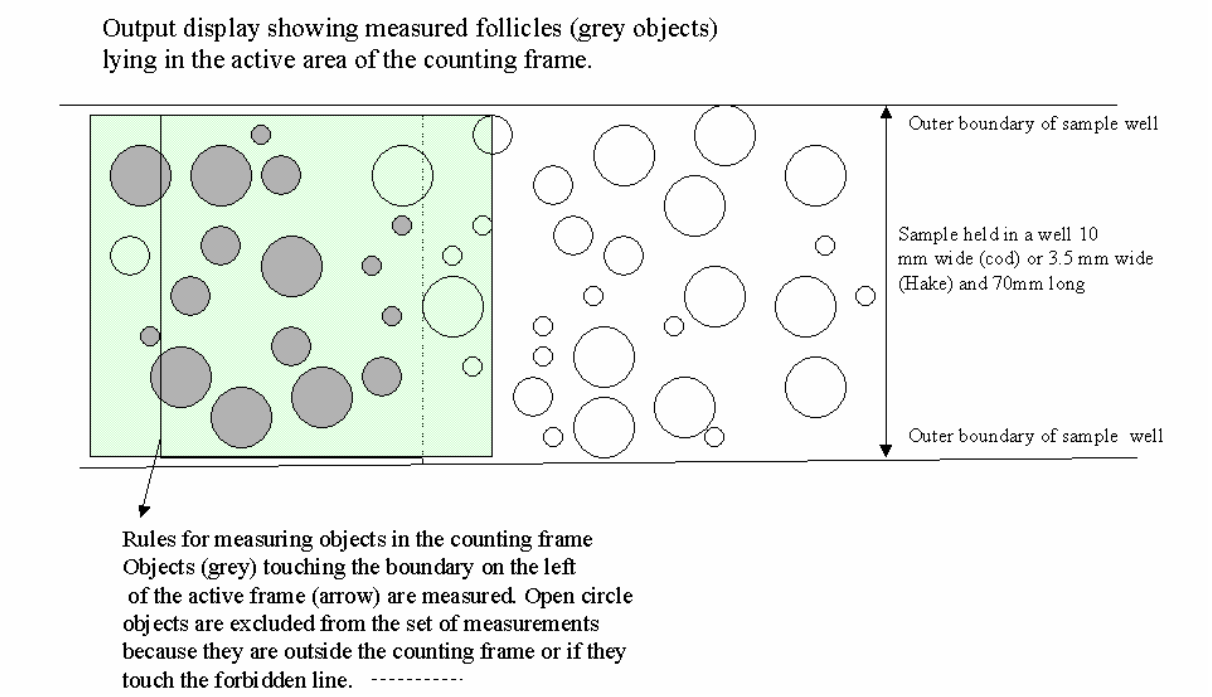

Comparison of oocyte dimeter measured manually in water and by image analysis after PAS staining
y = 1.0506x - 0.0176
R2 = 0.9702
Oocyte diameter (mm) measured by image analysis after PAS staining
Oocyte diameter (mm) measured manually in Water
0.00
0.50
1.00
1.50
2.00

Figure 5: GFA image analysis showing how the active frame within the larger field of view is used to measure all follicles in the sample without bias.



After selecting a new field the overlays on the right side of the active counting frame (4 in this case) move to the left into the non active field and are filled with hatched lines. The sample tray is shifted left until the previously counted oocytes lie under their displaced overlays. In this way the operator can easily see which objects have been previously measured. Follicles that do not segment properly can be deleted and measured manually by selecting pixels that span the x feret.

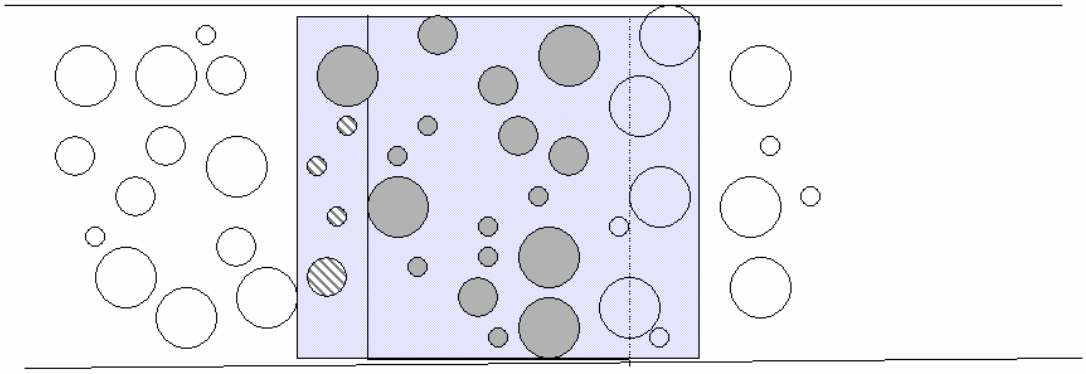


Figure 6: Illustration of the pipette use to remove Gravimetric samples from the ovary.

Push the plunger to the bottom of the glass tube and then push the pipette into a hole previously made in the ovary tunica. Pull up the plunger until the sample reaches the first blue line on the glass tube as below. This will provide a sample of 0.106 g with a CV 3%. Check there are no voids in the tissue sucked out of the ovary before expelling it into one of the sample tubes containing 3.6% formaldehyde.

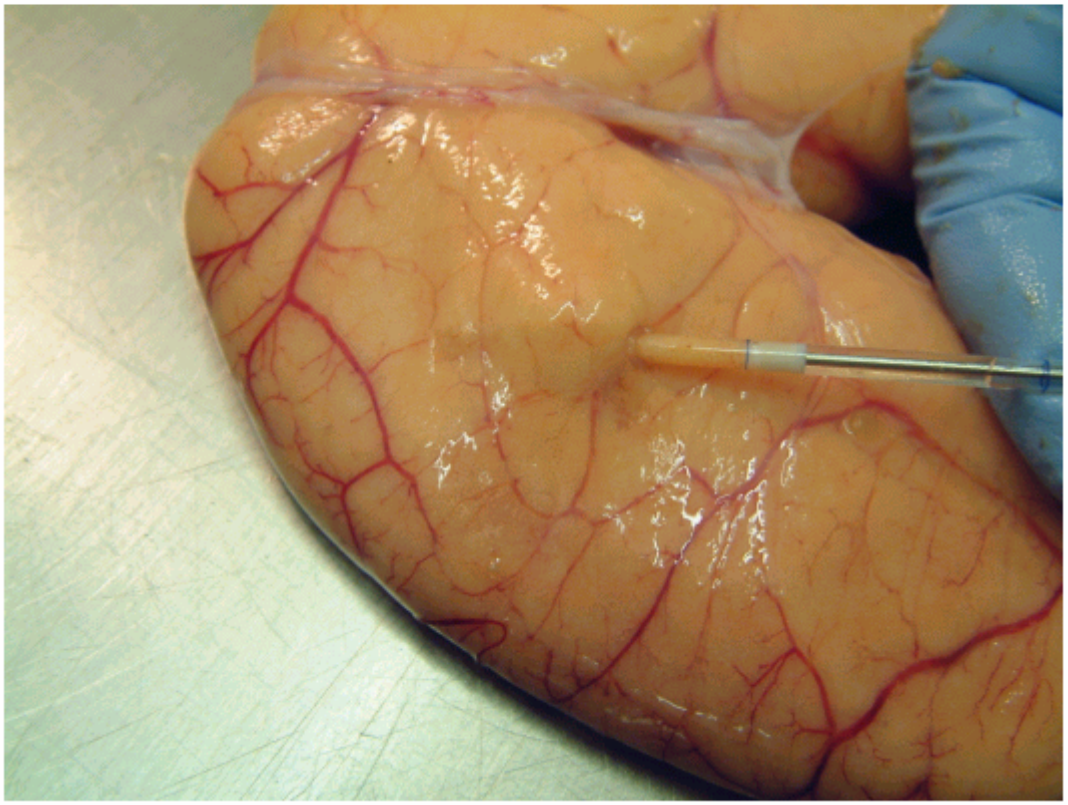


Figure 7: Auto-diametric calibration for pre and spawning cod showing the data points and the fitted line and regression parameters used to estimate oocytes g^{-1} ovary overlaid with a fitted line from data in Thorsen and Kjesbu 2001.

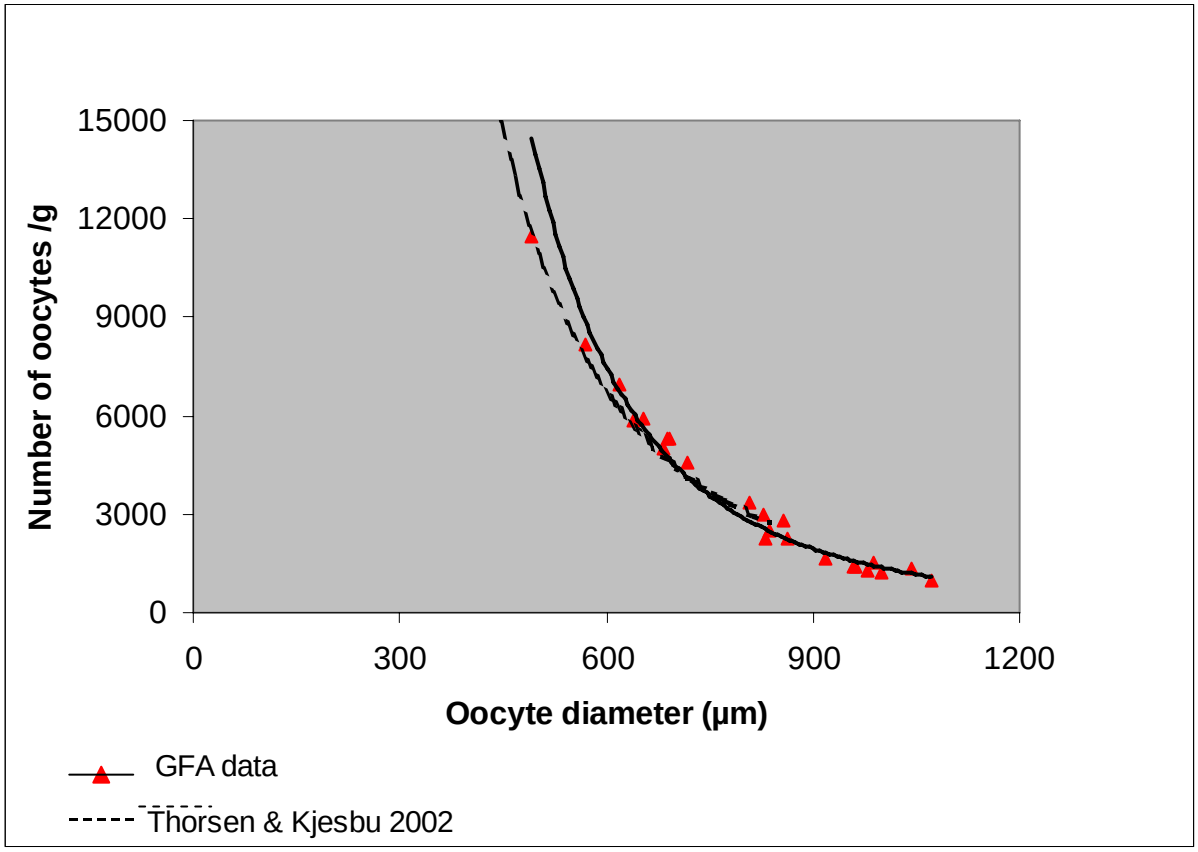


Figure 7 Continued: Horse mackerel oocyte frequency distribution measured using image analysis.

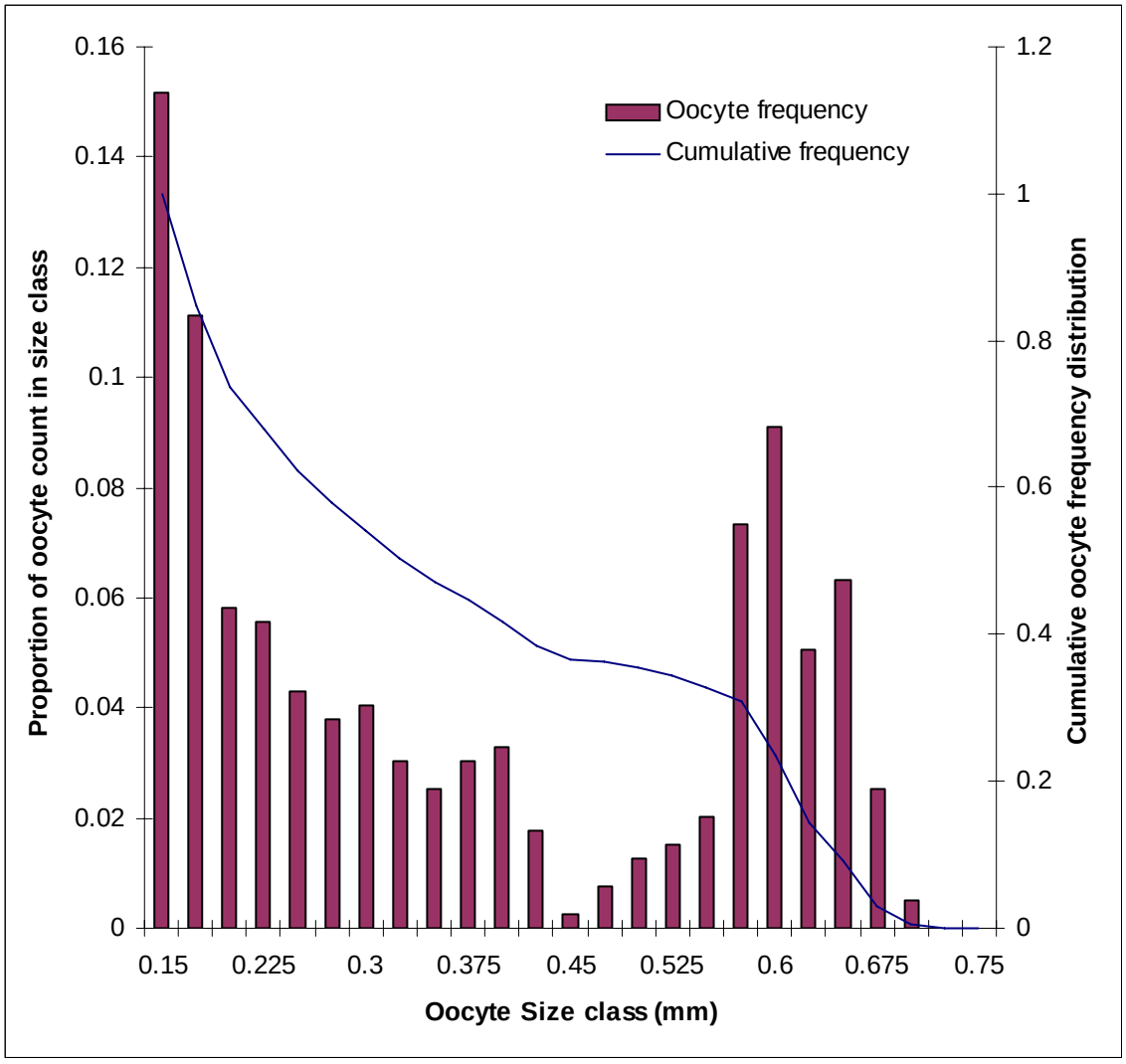


Figure 8: Oocytes in whole mount overlaid with a circle of 0.15 mm to indicate the minimum size class measured in the oocyte frequency distribution. Vacuoles in the cytoplasm are not present in previtellogenic oocytes (PVO) but appear as bubbles on top of the nucleus in the earliest stage of vitellogenic oocyte (VO) in the whole mount.

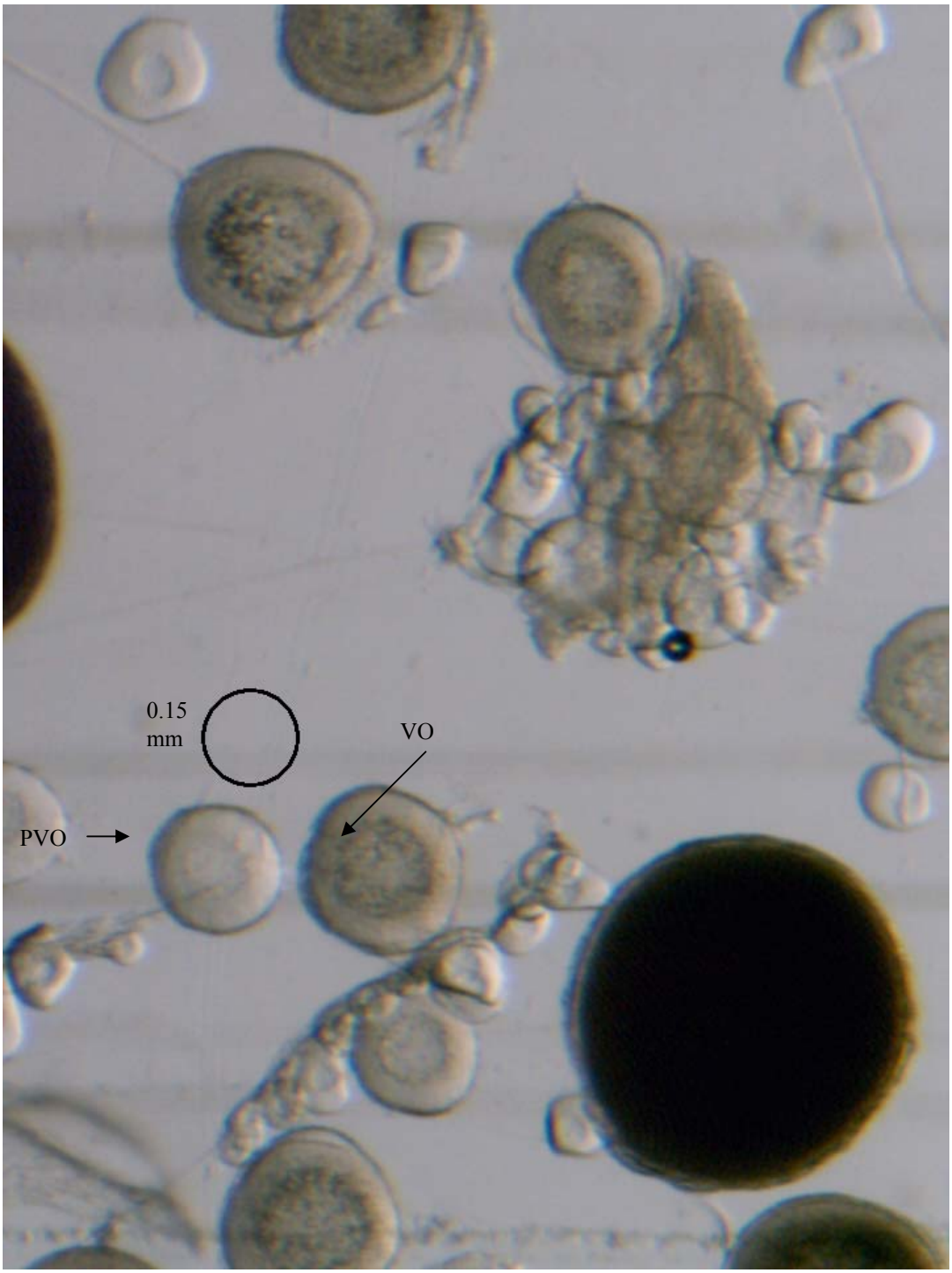


Figure 9: Resin section of a horse mackerel ovary stained with H&E showing previtellogenic oocytes (non vacuolated ctytoplasm) and early stage vitellogenic oocytes with vacuoles around the nucleus.

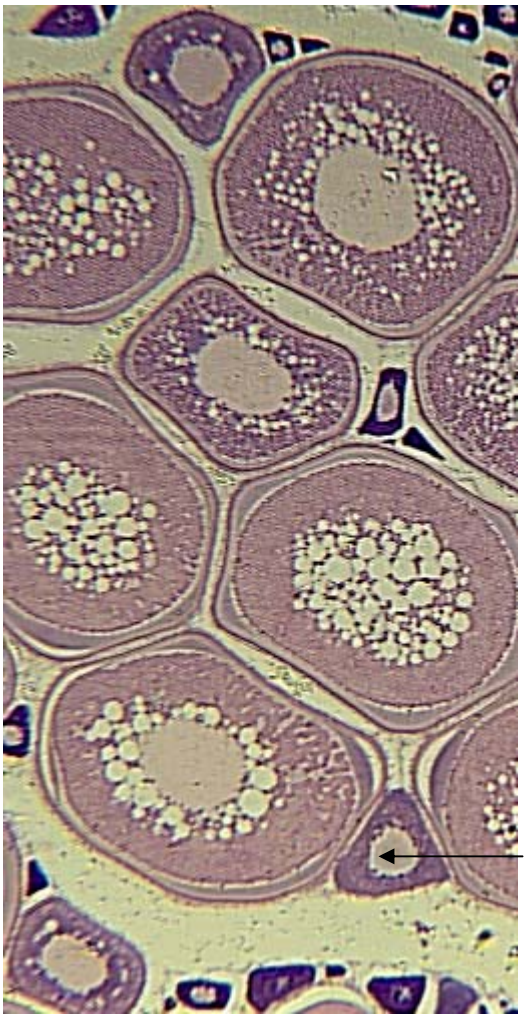
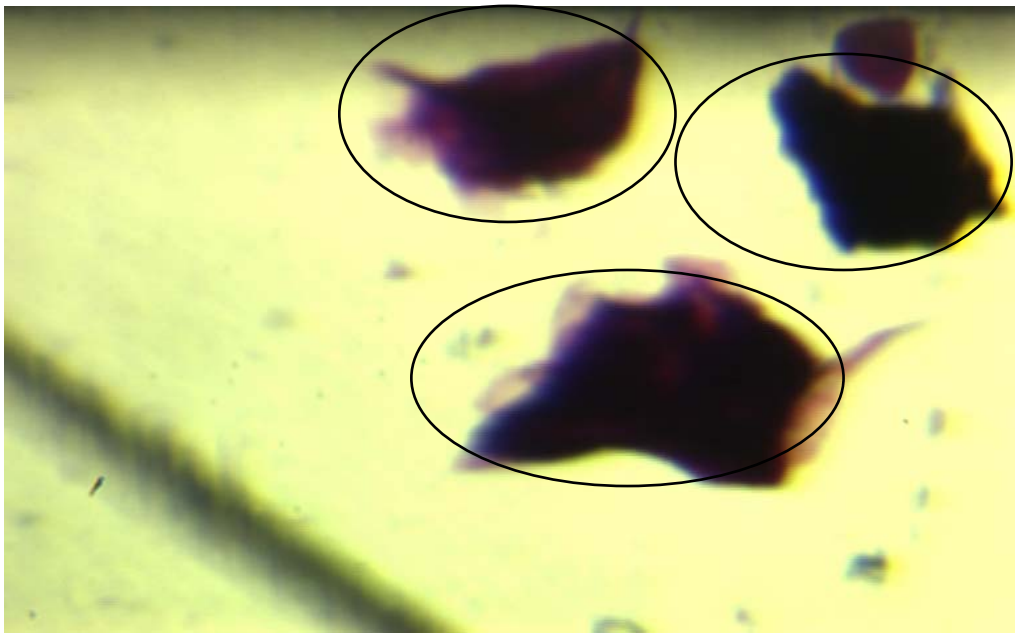


Figure 10: Images of post ovulatory follicles (circled) shown in whole mount (Panel a) and in section.

Panel b



Panel b

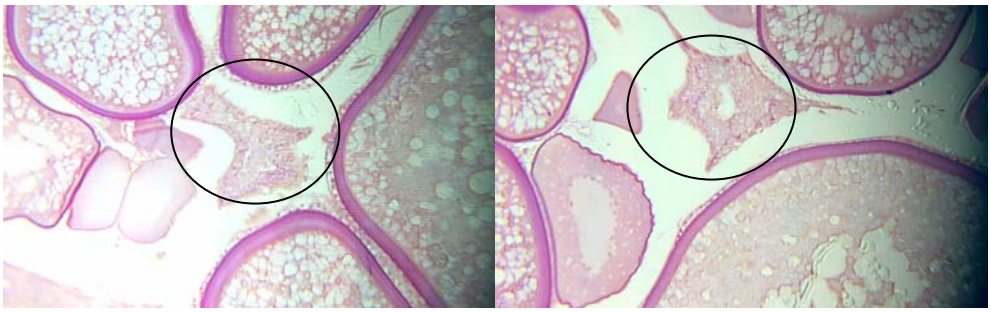
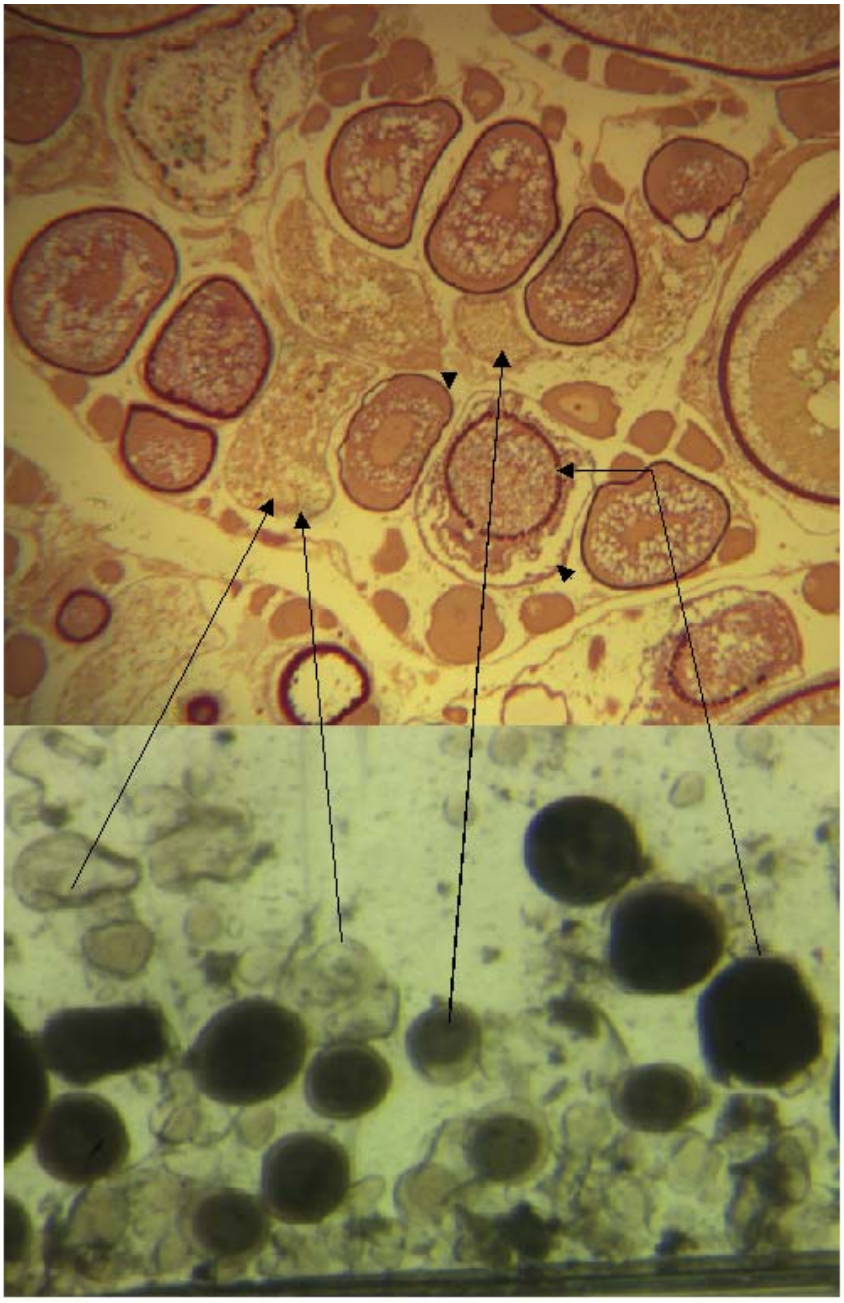


Figure 11: Comparison of a mackerel ovary containing a high proportion of atretic oocytes prepared as a section (top) and as a unstained whole mount (bottom). Arrows link atretic oocytes identified in the whole mount with their likely appearance in section. Note how the chorion becomes separated by a space from the follicle outer boundary as it becomes more fragmented and finally disappears.



Equipment and consumables

Ovary sampling

TASK	ITEM	MANUFACTURER	CODE
Image analysis	SZX12 Stereo microscope with f1.2 objective and SZX-ILLD light base including XY stage and SZX – BS beam splitter with 0.5x coupler and C mount. Price = £ 11,100 excl. vat	Olympus thowe@ olympus.uk.com	033710 033729
	High light 3100 source	Olympus	3100
	Dell precision work station 650 mini tower OS Windows XP Dual Xeon processors 2.4 GHz with 512 cache 21 inch LCD screen Price £2,900	Dell http://www1.euro.dell.com	
	Pulnix camera, Matrox meteor II Cl frame grabber cables, and image analysis software including installation Price = £ 9300	Pilkington Image Analysis Systems jpilkington(a)imageanalysis.fsnet.co.uk	
Sampling	Wiretrol pipette 25-50µml	Biohit Ltd PO Box 5163 Northampton NN5 5ZY www.biohit.co.uk	5-00-2050
	Rack	Axygen	
	Flip top tubes assorted colours	Axygen	MCT-200-A.
	Spot labels	Web Scientific	
	Water proof paper		
Staining	Net Well 74 µm net mesh + carriers and dishes	Costar Scientific Corporation Supplied in UK by Fisher	- Cat. ref. TKN –540 – 010L
	Schiffs stain	VWR	
	Perodic acid		
	Photographic wetting agent		
	Glass Pasteur pipettes		
	Staining dishes		
Image analysis	Sample trays plans available from CEFAS but the width of the sample track must be based on the camera field of view.	John Mayzes Lowestoft Tel. Int. 1502730058	
	Brushes		

[1] PIAS preferred option is that they install the software and frame store in a PC they supply.

Suppliers list

MANUFACTURER	ADDRESS	WEB SITE	TELEPHONE
BioHit			
VWR			
John Mayzes			
Plastic Workshop			