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REPORT OF THE WORKING GROUP ON MACKEREL AND HORSE MACKEREL EGG SURVEYS (WGMEGS)

27–31 MARCH 2006

VIGO, SPAIN

International Council for the Exploration of the Sea
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Contents

Executive summary

The Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS) met in Vigo, Spain in March 2006 to address eight Terms of Reference. These are detailed below.

Major highlights

- The 2007 egg survey in Portuguese waters in January/February will now be configured as a DEPM survey, rather than AEPM as is the case for all other individual surveys. This is targeted on the southern horse mackerel. The samples will still be useable for the NEA Mackerel AEPM, but means that this area will have one coverage, albeit more intense, rather than the tow in previous years. The remainder of the area will be covered in five periods from March to July, in a pattern similar to previous surveys.
- CEFAS have withdrawn from the survey. This entails the loss of one complete survey, and the loss of considerable experience in histological analysis. In addition CEFAS will no longer be able to provide adult mackerel at the start of the spawning season for fecundity estimation. The WG regret this decision, and hope that CEFAS may be able to review this at some point and return to the survey. The impact will be to decrease the accuracy of the survey and make it more vulnerable to operational exigencies.
- The group identified clear evidence of declining fecundity in horse mackerel over the last 25 years based on samples taken at the start of the spawning season. Samples taken during the spawning season since 1995 confirm this trend. This may be linked to the low levels of recruitment in this species and to the mismatch between landings based assessments and the surveys.
- The egg surveys in the North Sea in 2005 indicate a biomass for NS mackerel of 223,000 tonnes. This is equal to the highest in the time series (which was in 1983). It also confirms the increase seen in 2002. The distribution of spawning in recent years has concentrated along the UK coast, in contrast to the historical situation of spawning in the central part of the North Sea, west of Denmark. Combination of the NS with the main surveys in the west and south was examined and considered feasible.

Terms of Reference and outcomes

ToRs a) and b) referred to the planning of the next mackerel and horse mackerel egg survey in 2007.

The survey itself has been planned on the basis of five period coverage's. The first period is extended and covers the whole area up to early April. The change is due the adoption of a DEPM survey in Portuguese waters, targeting horse mackerel. This entire survey and those in The Cantabrian Sea and the western area have been combined in period 1. The remaining four periods cover April, May, June and July. Coverage is reduced in 2007 due to the withdrawal of CEFAS (detailed above).

The sampling and analysis for fecundity and atresia is an essential component of the survey. The WG followed the planning procedure and methodologies developed for the 2004 survey. Times and general locations for sample collection are provided to give the best spatial and temporal coverage. Samples will again be taken in triplicate. These will be analysed by a number of different institutes and include sampling in the southern area, allowing comparison between institutes. Sampling and analysis coordinators have been appointed to oversee the sampling and analysis programmes.

More detailed information on the survey and its design is presented in Section 2, and biological sampling in Section 3.

ToR c) referred to the ongoing examination of the issue of variance calculation.

The main focus in this area was in providing, for the first time, variance estimation for the North Sea mackerel egg surveys. This was to provide support for the process of including the NS estimates in the NEA estimates. Variance was found to be very high. This was due to the limited vessel coverage and time available. Significant amounts of interpolation were required, and some areas of potential spawning were not covered. In addition the spatial pattern of spawning seemed to be much more variable than in the west.

More detailed information on the calculation of variance for the North Sea is presented in Section 8 on combining NS and NEA estimates.

ToR d) referred to the standardization of handling and analyzing egg samples and for carrying out the histological work

The mackerel and horse mackerel egg survey is carried out only once every three years. This can lead to problems when analysis is carried out by many separate groups. WGMEGS has therefore set up pre-cruise workshops to ensure standard, agreed approaches are taken by all personnel involved. The first of these was prior to the 2001 survey, and the third is programmed for the autumn of 2006. The report includes details of the workshops for egg sample handling and sorting and for histological work.

The main aim for the egg workshop will be to retrain all participants in the sorting of egg samples to species and then identifying egg stages in the target species. This will be done using blind samples prepared prior to the meeting. The plan is to carry out one set of trials, analyse these, report back to the group, identify problems and then repeat the trials. The ultimate goal is to have consistent and accurate analyses by all participants.

The histological workshop will carry out the same function for the handling o and analysis of materials for the determination of fecundity and atresia. Again the target will be for consistency and accuracy.

The discussion also considers questions relating to the determination of atresia and ways to improve the quality of the estimation. A database entry system to allow consistent data entry and files is presented and will be used by participants in the 2007 survey.

More detailed information on the issues surrounding the workshops etc. is presented in Section 5.

ToR e) referred to the results of the mackerel egg survey in the North Sea in 2005.

The NS mackerel egg survey is carried out every three years, one year after the main triennial survey. The results were of the survey were reported. Two countries (Norway and Netherlands took part and were able to complete four survey periods June to July 2005. The survey appeared to cover the main period of spawning, and most of the major areas. Spawning was concentrated along the UK coast. Due to the resources available, the survey had some difficulties with definition of spawning areas, and was not able to cover the whole spawning period. There were substantial numbers of interpolated rectangles. Samples for fecundity analysis were collected for the first time since 1982. The estimated fecundity was 1359 oocytes.g-1 female compared to the previous figure of 1401. The outturn biomass estimate was 223,000 tonnes. This is equal to the highest in the time series (which was in 1983).

Variance estimates were also made for the first time for this survey in 2005. These were high due to the limited vessel coverage, significant amount of interpolation and the variable spatial pattern of spawning between periods.

More detailed information on the NS egg survey is presented in Section 6.

Tor f) referred to the issues of standardization and the survey manual.

The main standardization issue addressed at the meeting was centred on the choice and design of the samplers deployed. In the western area most countries now use the Gulf VII plankton sampler. This has an open body and a sharper nose cone than the original Gulf III. There may be potential bias between the two versions, with the Gulf VII operating more efficiently. A series of trials using paired samplers will be trialled in 2006, and if successful, relative efficiency tests carried out during the survey in 2007.

In the southern area, the standard sampler is the Bongo. QA checks revealed that there are differences in design between the nets used by IEO, AZTI and IPIMAR. In theory the system calibration and the method used to calculate eggs by volume should ensure compatibility. However, the group felt that a common standard specification would be appropriate. The relevant institutes will collaborate to set this up, and this will be discussed at WKMHMES in the autumn. .

The spray technique for separating eggs from plankton was used for the first time in anger in the 2004 egg survey and proved very successful. Since then some institutes have improved or modified the method, and it was agreed to standardize the system again, also at the WKMHMES.

More detailed information on progress on standardization of the sampling tools is presented in Section 7

ToR g) referred to the combination of North Sea and NE Atlantic mackerel survey data

WGMEGS was asked by WGMHSA to evaluate the possibility of combining the egg surveys for the western and southern areas with those for the North Sea. The Working Group examined the survey time series going back to 1980. In most years the surveys appeared to capture the peak of spawning but were unable to cover the full area or spawning period.

An important element to combining these data was to be able to describe the fecundity pattern in the different stock components. Combination of egg data would not be sensible without such data, and with them, a biomass combination would be feasible. Fecundity estimates for North Sea mackerel were produced for the first time in the 2005 survey. The estimated fecundity was 1359 oocytes.g^{-1} female compared to the previous figure of 1401. The most recent value for the western area was 1127. Atresia (prevalence and intensity) in the North Sea component was very low compared to that in the western area.

The variance in the North Sea estimate was also evaluated, and was substantially higher than in the western area for reasons discussed above.

In conclusion, the combination was considered feasible, but that the high variance and low abundance in the North Sea would suggest that there may be limited value in such combination. The appropriate data and analyses will be presented to WGMEGS

More detailed information on progress combination of North Se and NEAM survey estimates is presented in Section 8

ToR h) referred to an evaluation of potential causes of mismatch between recent survey estimates and assessment abundance trajectories for western horse mackerel

WGMEGS was asked by WGMHSA to evaluate possible causes for the change in q between the horse mackerel survey estimates and the assessment results.

The WG investigated the observed fecundity in both the western stock and the southern for comparison. It also evaluated the potential for systematic changes in the egg identification process and also in the variance in the survey results.

All available fecundity data for horse mackerel (west and south) was assembled. Fecundity measurements prior to spawning were found back to 1989. As horse mackerel was considered a determinate spawner at that time, no in season fecundity data were collected. From 1995 the WG also had access to fecundity data collected during the spawning season. In both case there was evidence of a systematic decline in fecundity over the observed period. The data since 1995 allowed maximum and minimum values to be placed on this. The steady decline in fecundity must be considered a clear candidate to explain the similarly systematic change in Q.

Horse mackerel eggs are smaller than mackerel and easier to confuse with other species. WGMEGS have carried out a series of exchanges and workshops to address this issue, and this provided information on the potential scales of error. Examination of possible errors or bias suggested that in most cases the scale of change in Q could not be explained by identification errors. One sample exchange did show substantial ID problems, however, the sample size was small, and the conditions of the exchange were not optimal. There was no evidence of any systematic trend in identification success.

Examination of the survey results for horse mackerel shows that there is a tendency for the surveys to produce one or two extremely large observations. The potential impact of encountering (or not) such egg concentrations was evaluated in detail for the most recent survey, and less detail for previous surveys. The analysis suggested that the horse mackerel survey data tend to be more skewed than for mackerel, and that this is probably a characteristic difference. However, the impact of including or not including the 10 largest observations showed that the scale of possible differences was similar to the scale of changes in Q. However, no systematic pattern was seen over the survey years. It was concluded that sampling problems related to skewness were not a candidate to explain the observed changes in Q.

In conclusion, the most likely candidate was changes in fecundity. The information will be presented to WGMHSA in the autumn.

More detailed information on horse mackerel survey estimates and potential candidates for changes in Q is presented in Section 9

Additional work carried out by the group included:

- a continued inventory of plankton samples from the triennial surveys held by participants;
- a proposal for new experiments on artificially fertilised eggs to examine the development rate equations used in the conversion of egg abundance to production.

1 Introduction

1.1 Terms of Reference

At the ICES Annual Science Conference in Aberdeen, Scotland, in September 2005 it was decided that (C.Res. 2005/2/LRC07) the Working Group on Mackerel and Horse Mackerel Egg Surveys [WGMEGS] (Chair: Dave Reid, Scotland, UK) would meet in Vigo, Spain, from 27–31 March 2006 to:

a) coordinate the timing and planning of the 2007 Mackerel/Horse Mackerel Egg Survey in the ICES Subareas 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;

g) evaluate and report on how to include the results from the North Sea mackerel egg surveys in the NE Atlantic Egg Survey time series, taking into account both the timing of the surveys and the precision of the surveys, in particularly for the earlier surveys in the North Sea. Consideration should be given to whether the distribution of the combined estimates is more or less precise than the current NEA survey and how much of the probability density functions is overlapping;

h) for Western horse mackerel knowledge of the magnitude of the variability in fecundity is necessary to evaluate the use of the egg survey as a proxy for SSB in the current assessment framework. Currently inclusion or exclusion of this survey can give rise to a factor of 4 difference of perception. The WGMEGS should give an estimate of precision for the relationship between the estimates egg abundance and its relationship to SSB in the context of resolving a factor of 4.

1.2 Participants

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

2 Planning of the 2007 mackerel and horse mackerel egg survey in the western and southern areas (referring to ToR "a")

2.1 Countries and ships participating

Germany, Ireland, Netherlands, Scotland, Portugal, Spain, Spain/Basque Country and Norway will participate in the mackerel/horse mackerel egg surveys in the western and southern area in 2007. The vessels and dates available for the survey are given in Table 2.1.1. CEFAS (UK) have withdrawn from the 2007 survey programme. The result of this is that the full survey area for all periods can no longer be sampled at the minimum required level of one station per sampling rectangle. Survey coverage of the western and southern area is given by area and period in Table 2.1.2. Detailed maps of the survey coverage by period are given in Figures 2.1.1 – 2.1.5. Both vessel availability and area assignments are provisional and will be finalised by the survey coordinator at the appropriate times.

The survey coordinator for the 2007 survey will be Finlay Burns, FRS Marine Laboratory, Aberdeen.

Table 2.1.1: Countries, vessels, areas assigned, dates and sampling periods for the 2007 survey.

COUNTRY	VESSEL	AREAS	DATES	PERIOD
Portugal	Noruega	Cadiz, Portugal and Galicia	20 February – 26 March	1
Spain (IEO)	Cornide de Saavedra	Cantabrian Sea	15 March – 5 April	1
		Biscay and Cantabrian Sea	10 April – 7 May	2
Germany	W. Herwig III	Celtic Sea	20 March – 6 April	1
			11 – 27 April	2
Netherlands	Tridens	Celtic Sea	7 – 27 May	3
			4 – 24 June	4
Spain (AZTI)	Visconde de Eza	Biscay	15 March – 5 April	1
		Biscay and Cantabrian Sea	7 – 27 May	3
Norway	GO Sars	West Ireland and West of Scotland	20 May – 10 June	3
Ireland	Celtic Explorer	West of Ireland and West Scotland	2 – 22 April	1
		Celtic Sea, West Ireland and West of Scotland	2 –22 July	5
Scotland	Scotia	West Ireland and West of Scotland	16April –7 May	2
		West Ireland and West of Scotland	4 –24 June	4

2.2 Sampling Areas and Sampling Effort

In contrast to previous years, the survey will be split into only five sampling periods. In previous years, the first two periods (approximately January and February) included surveys in ICES area IXa only, with fuller coverage starting in period 3 (March). In 2007 the survey effort in this area will be targeted on a DEPM survey for horse mackerel (see Section 2.3.) to be carried out in February/March. This survey along with those in the rest of the full survey area will constitute survey period 1. This period is broadly equivalent to period 3 in the 2004 survey. No surveys of area IXa will be made after period 1. In period 2 the survey will cover the full western area plus the Cantabrian Sea. From period 3 onwards coverage will only be of the western area. Some spawning is expected in the Cantabrian Sea during this period, and it has been surveyed at this time in previous years, but no vessels were available for 2007. Egg production in this area in period 3 will be assumed to be zero. In periods 4 and 5 the surveys are designed to identify a southern boundary of spawning and to survey all areas north of this boundary. The deployment of vessels to areas and periods is summarised in Table 2.1.1.

In the western area maximum deployment of effort is during the first, second and third sampling periods. These periods coincide with the expected peak spawning of both mackerel and horse mackerel in the area. The loss of the CEFAS (UK – ENG) April survey means that survey coverage for periods 2 and 3 is much reduced. Bearing this in mind, for the 2007 survey the emphasis will be based on area coverage, even more so than 2004, and if necessary occupation of alternate east/west transects. Cruise leaders have been asked to cover their entire assigned area using alternate transects and then use any remaining time to fill in the missed

transects. If time is short this should be concentrated in those areas identified as having high egg abundance on the first part of that vessels survey. Particular points to note are:

Period 1

In period 1, in contrast to previous years the entire western and southern area will be surveyed. This is to accommodate the changes made to the Portuguese survey which has been condensed from 3 surveys into a single extended (horse mackerel DEPM based – see Section 2.3) survey. Period 1 now reflects the calendar period traditionally covered by periods 1 – 3. For reasons which relate to the control of the period 2 survey it would be preferable for the German vessel to start and finish surveying at the southern boundary of her designated survey area (48°N) (Figure 2.1.1).

Period 2

There are 3 vessels available for period 2. The German vessel will commence sampling in Biscay along the southern boundary of the designated survey area (47°N). This will allow the Spanish vessel to complete the survey coverage in Biscay to the south of that covered by the German survey (46°30N – 47°N, 6°– 10°W). The west – east direction of the shelf break at this latitude requires careful sampling to avoid having large samples at the edge of the survey area. It is imperative that this area receives comprehensive coverage in order to define the edge of the spawning distribution. It should also be noted that the Spanish vessel will probably not have to survey in the area 45°N – 46°N, 5°– 10°W. This area is over deep water and very few eggs are normally found here. Given that the Spanish vessel will start its survey in Vigo, it is recommended that the survey be carried out as follows (Figure 2.1.2)

- Survey to the east through the Cantabrian Sea, occupying alternate north/south transects
- Move to 460 45’ N and complete that transect and then survey to the south, occupying all east/west transects
- Survey to the west through the Cantabrian Sea, occupying the remaining north/south transects

Period 3

In period 3 a similar situation exists as for period 2. There are three vessels available during this period to survey the western area. AZTI will be carrying out a targeted DEPM survey for anchovy in Biscay, although this provides mackerel and horse mackerel egg samples as well. The design of this survey is therefore constrained by that purpose. In 2004, this resulted in weak coverage along the shelf break between 45o 30’ and 47oN. AZTI have been requested by WGMEGS to take some additional samples in this area, to allow full coverage. The stations required will be advised to AZTI by the survey coordinator. The IMARES vessel will commence its survey north of 47°N and information from this transect will be used to advise AZTI. (Figure 2.1.3)

Period 4

In period 4, two vessels have to cover the entire area of spawning from northern Biscay to the West of Scotland. Alternate transects are recommended. The IMARES vessel covering the Biscay area will commence the survey along the southern boundary of the designated area although its exact latitude will depend on the results from period 3. The survey coordinator will advise the IMARES cruise leader prior to the survey. (Figure 2.1.4)

Period 5

In period 5, only one vessel will be available, and will have to cover the entire spawning area. This assignment has been given to Ireland who traditionally carries out this last survey. Again the southern boundary will be defined according to the results in period 4. Irrespective of this an alternate transect design will be necessary. (Figure 2.1.5)

Table 2.1.2: Periods and area assignments for vessels by week for the 2007 survey. Area assignments and dates are provisional.

WEEK	STARTS	AREA						PERIOD
		PORTUGAL, CADIZ AND GALICIA	CANT-ABRIAN SEA	BISCAY	CELTIC SEA	NORTH WEST IRELAND	WEST OF SCOTLAND	
1	19-Feb-07	PO1(DEPM)						1
2	26-Feb-07	PO1(DEPM)						1
3	5-Mar-07	PO1(DEPM)						1
4	12-Mar-07	PO1(DEPM)	IEO1	AZTI-1				1
5	19-Mar-07		IEO1	AZTI-1	GER			1
6	26-Mar-07		IEO1	AZTI-1	GER	IRL1	IRL1	1
7	2-Apr-07				GER	IRL1	IRL1	1
8	9-Apr-07		IEO2	GER		IRL1	IRL1	2
9	16-Apr-07			IEO2	GER	SCO1	SCO1	2
10	23-Apr-07			IEO2	GER	SCO1	SCO1	2
11	30-Apr-07		IEO2			SCO1	SCO1	2
12	7-May-07		AZTI-2 (DEPM)	IMARES1				3
13	14-May-07		AZTI-2 (DEPM)		IMARES1			3
14	21-May-07			AZTI-2 (DEPM)	IMARES 1	IMR	IMR	3
15	28-May-07					IMR	IMR	3
16	4-Jun-07			IMARES2		IMR	IMR	4
17	11-Jun-07				IMARES2	SC02	SC02	4
18	18-Jun-07				IMARES2	SC02	SC02	4
19	25-Jun-07					SC02	SC02	4
20	2-Jul-07				IRL2	IRL2	IRL2	5
21	9-Jul-07				IRL2	IRL2	IRL2	5
22	16-Jul-07				IRL2	IRL2	IRL2	5
23	23-Jul-07							5

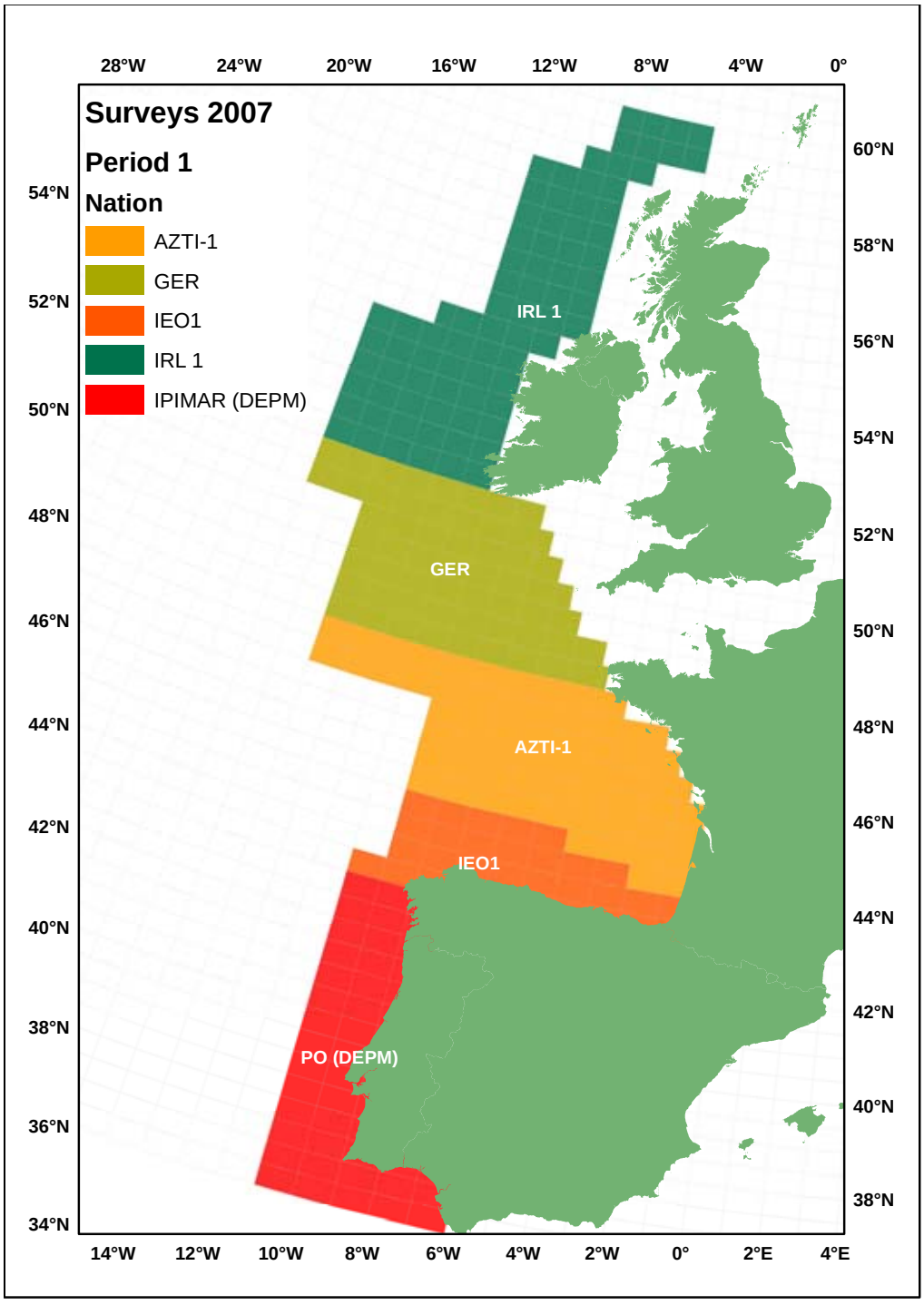


Figure 2.1.1: Survey plan for Period 1.

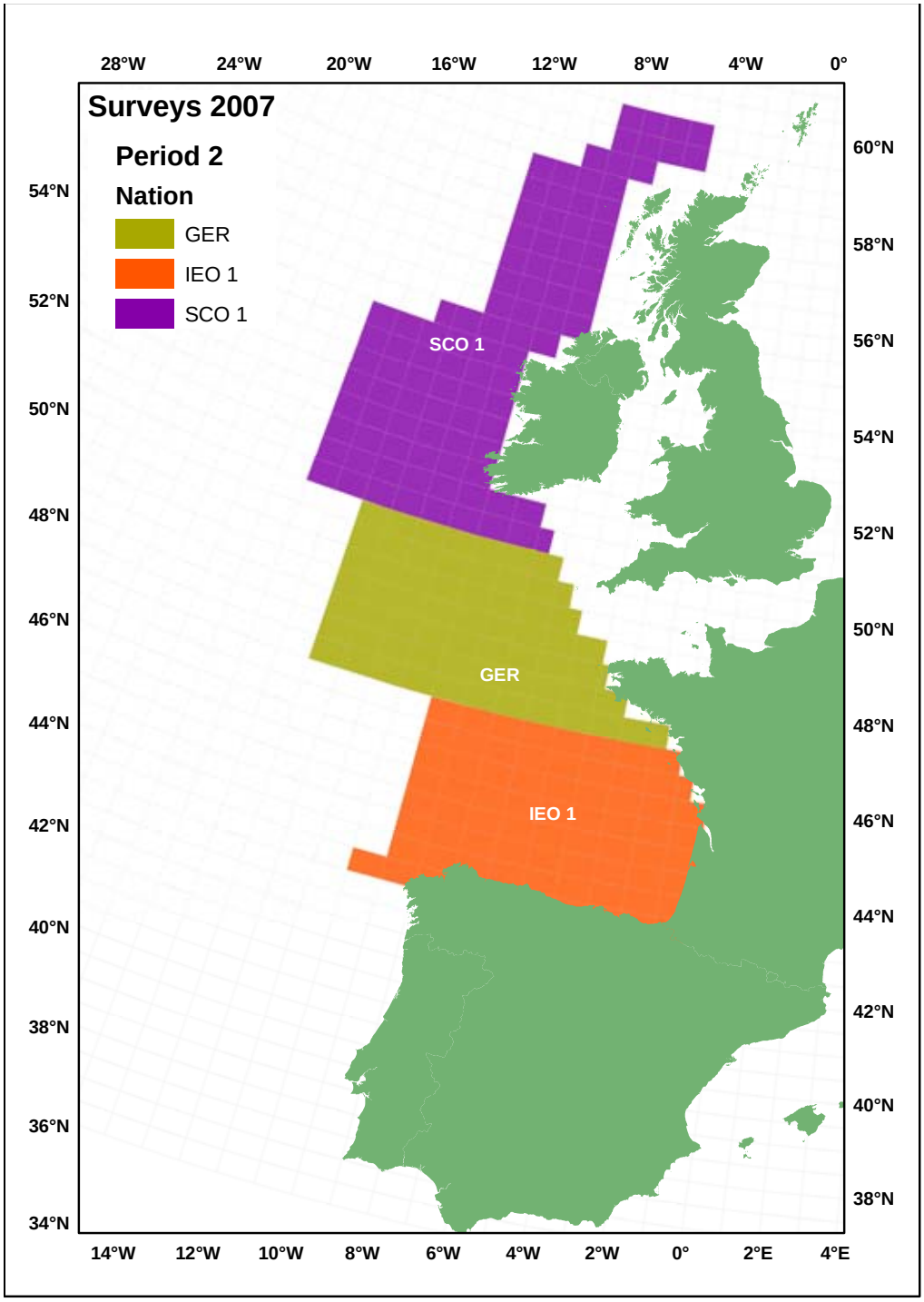


Figure 2.1.2: Survey plan for Period 2.

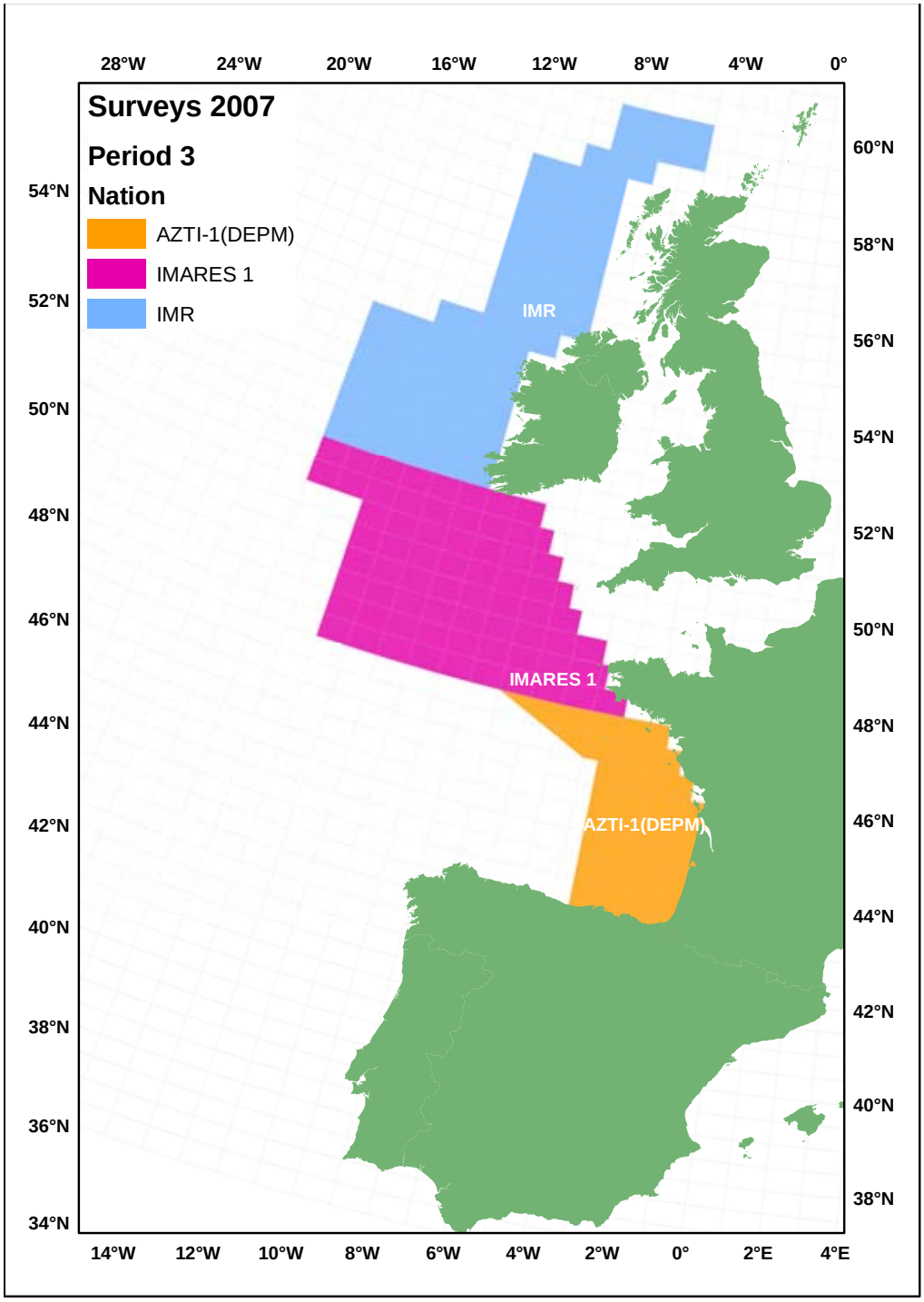


Figure 2.1.3: Survey plan for Period 3.

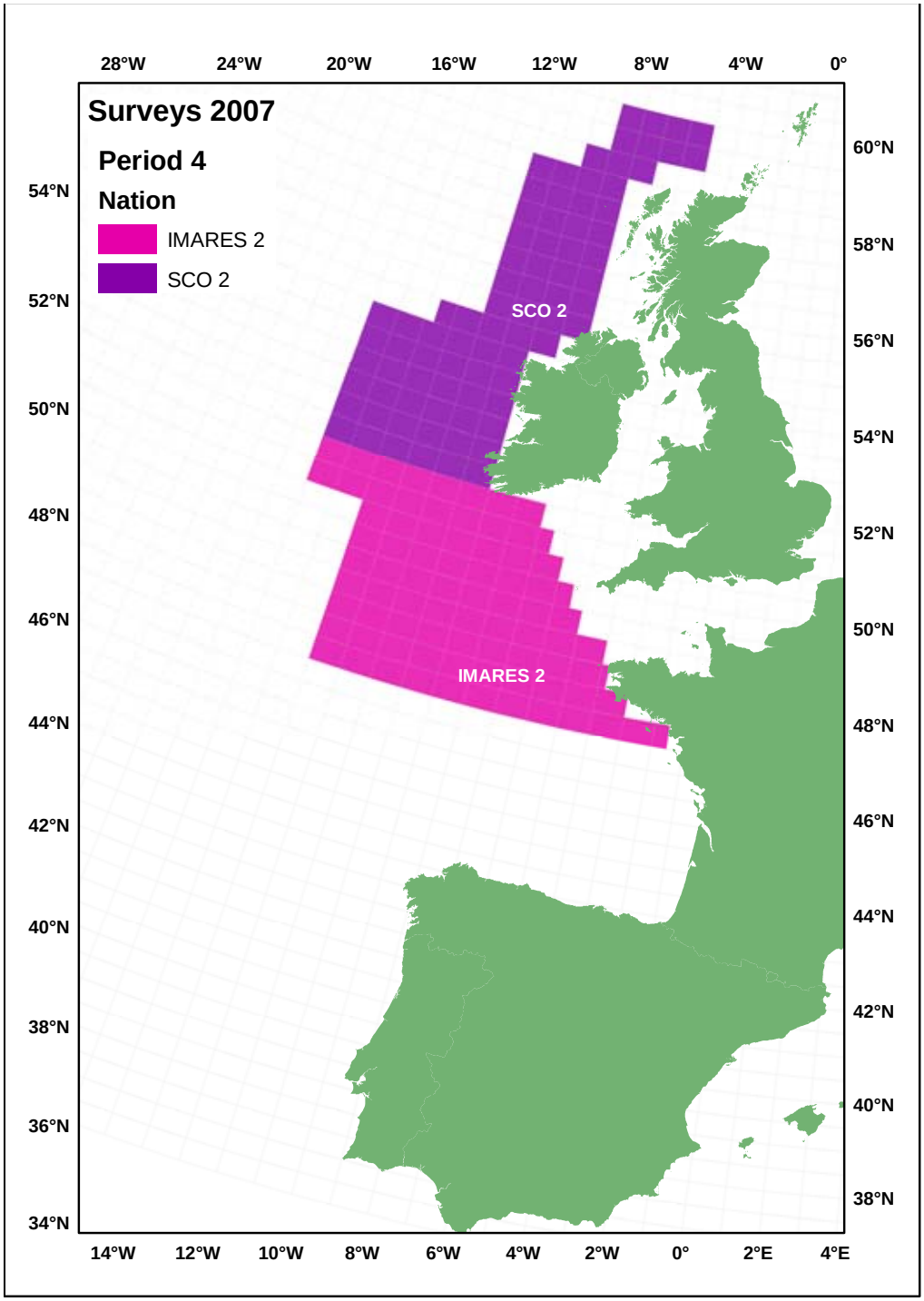


Figure 2.1.4: Survey plan for Period 4.

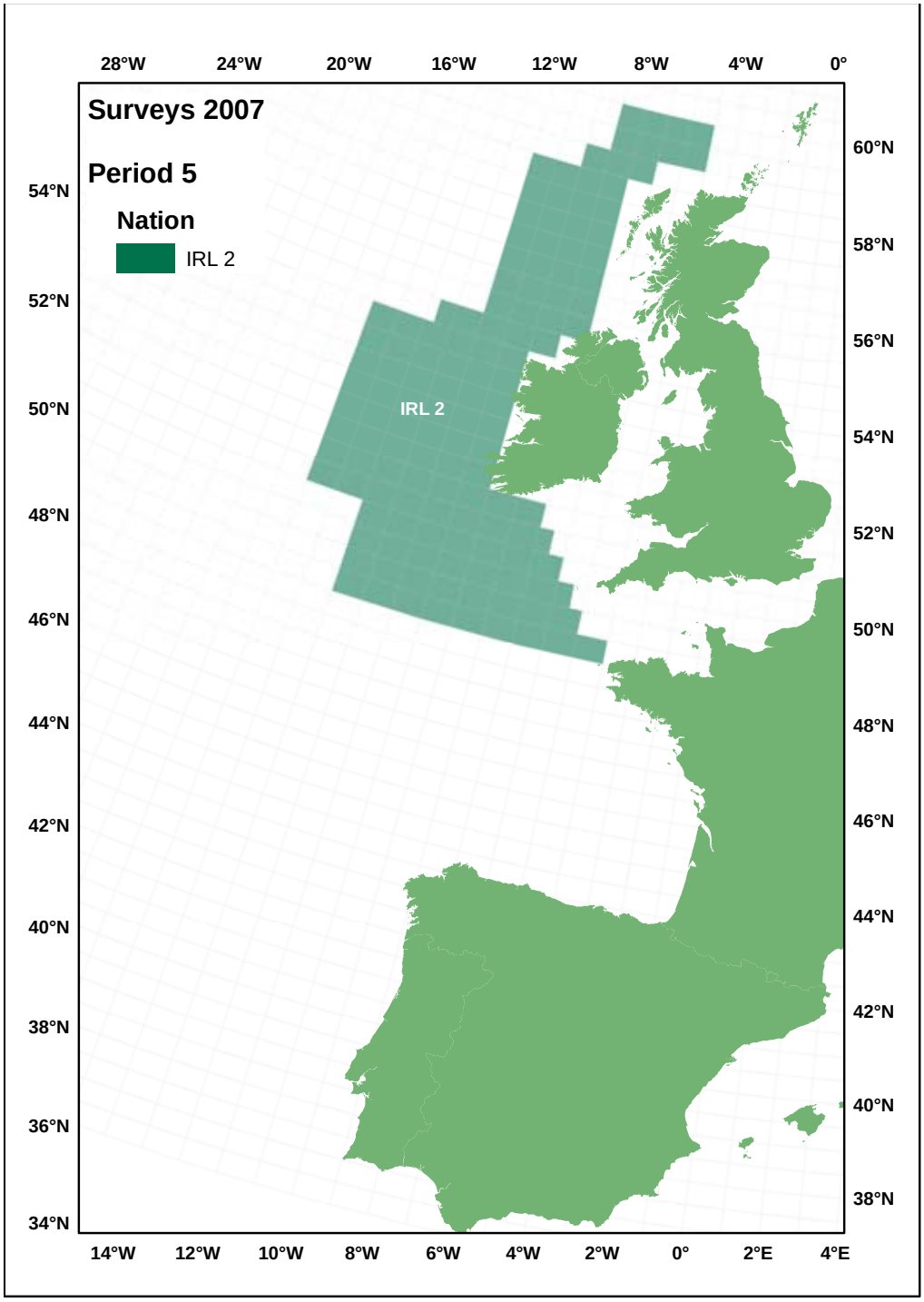


Figure 2.1.5: Survey plan for Period 5.

2.3 Horse mackerel DEPM survey in ICES Division IXa

Taking into account the strong and consistent evidence that horse mackerel is an indeterminate spawner (Abaunza, P. *et al*., 2003, ICES, 2003); southern horse mackerel stock spawning biomass will be assessed by Portugal and Spain during the spawning season by means of the Daily Egg Production Method (DEPM). This will cover the new defined stock area for southern horse mackerel corresponding to ICES Division IXa (36º to 43º N), from Gibraltar to Finisterre (WD Costa, A.M. *et al*., 2006).

Portugal/IPIMAR will perform from 20 February to 26 March 2007 a 35 days cruise with RV "Noruega", in order to collect egg samples and catch adult fishes (Figure 2.2.1).

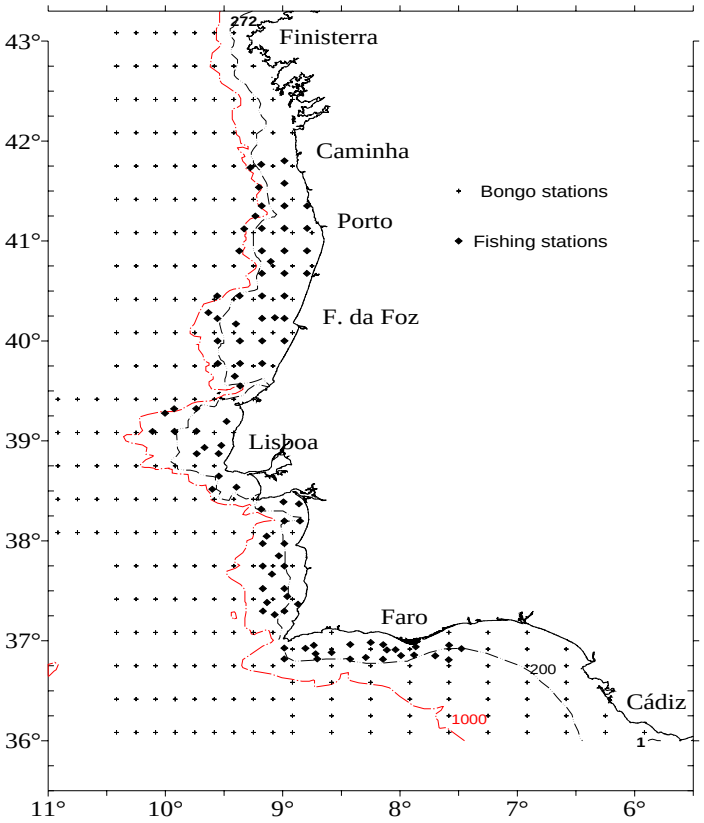


Figure 2.2.1: Bongo egg sampling and fishing stations.

272 double oblique hauls from surface to 200 meters depth with a 40 cm diameter Bongo net will be performed through a grid with ten minutes egg stations distance and twenty minutes between radials.

Fish sampling strategy is to perform two bottom-trawl hauls each day (60–70 hauls), located in selected places where horse mackerel is known to be usually present (Figure 2.2.1). From each positive trawl a simple random sample of at least 300 fishes and 100 gonads of maturity stages 3, 4 or 5 will be collected whenever possible.

3 Planning and sampling programme for mackerel and horse mackerel fecundity and mackerel atresia. (referring to ToR "b")

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

Following WGMEGS decision to use only formaldehyde fixative (ICES, 2003) it will be possible to provide a unified sampling scheme for fecundity and atresia for use in the 2007 survey. Following the experience of the 2004 survey the following changes have been recommended for the 2007 survey. In this context the Auto-diametric method, although useful where the fecundity sub-sample weight is not known produces more variable fecundity data compared to the Gravimetric method (Hunter *et al.*, 1989). The Working Group recommends that the latter technique is used for the 2007 survey

Table 3.1.1: Changes for 2007 compared to 2004.

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

Samples for estimation of mackerel potential fecundity and atresia will be mostly taken on vessels participating in the egg survey or from commercial fishing vessels by observers. Recognising the constraints of the egg survey cruise leaders should try to distribute trawl stations across the survey area aiming to complete a wide spread sampling regime for adults shown in Tables 3.1.2 a-b. The purpose of this table is not to exactly specify the time and location of trawl hauls but to give an impression of how trawl hauls should be dispersed in time and space and the numbers of required for the estimation of realised fecundity.

Tables 3.1.2a: Coverage of mackerel sampling in Southern spawning component.

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

MACKEREL

			Lon °											Lat °							
Week	Date	Period*	11W	10	9	8	7	6	5	4	3	2	1	36N	37	38	39	40	41	42	Total
8	19. Feb 07	**1**												10							10
9	26. Feb 07	**1**													10						10
10	05. Mrz 07	**1**															10				10
11	12. Mrz 07	**1**			20														10		30
12	19. Mrz 07	**1**						20													20
13	26. Mrz 07	**1**									20										20
14	02. Apr 07	**1**																			0
15	09. Apr 07	**1**	20																		20
16	16. Apr 07	**2**																			0
17	23. Apr 07	**2**																			0
18	30. Apr 07	**2**	20																		20
19	07. Mai 07	**3**										20									20
20	14. Mai 07	**3**											20								20
21	21. Mai 07	**3**																			0
22	28. Mai 07	**3**																			0
23	04. Jun 07	**4**																			0
24	11. Jun 07	**4**																			0
25	18. Jun 07	**4**																			0
26	25. Jun 07	**4**																			0
27	02. Jul 07	**5**																			0
28	09. Jul 07	**5**																			0
29	16. Jul 07	**5**																			0
30	23. Jul 07	**5**																			0
31	30. Jul 07	**5**																			0
																					180

* Note that period **1/2** is dominated by prespawning fish; in periods **3** to **5** = atresia sampling

	per period		
	1	2	3
IEO	80	20	
AZTI			40
IPIMAR	40		

Tables 3.1.2b: Coverage mackerel sampling in the Western spawning component survey area.

Fecundity sampling **Western Area**

MACKEREL Lat °

Week	Date	Period*	44N	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	Total
8	19. Feb 07	**1**																	0
9	26. Feb 07	**1**																	0
10	05. Mrz 07	**1**																	0
11	12. Mrz 07	**1**	20																20
12	19. Mrz 07	**1**		20	20		20												60
13	26. Mrz 07	**1**				20		20			20								60
14	02. Apr 07	**1**							20			20		20		20			80
15	09. Apr 07	**1**				20											20	20	60
16	16. Apr 07	**2**	20		20		20										20		80
17	23. Apr 07	**2**		20				20				20	20		20				100
18	30. Apr 07	**2**									20								20
19	07. Mai 07	**3**				20													20
20	14. Mai 07	**3**						20											20
21	21. Mai 07	**3**	20		20				20		20								80
22	28. Mai 07	**3**												20					20
23	04. Jun 07	**4**							20								20		40
24	11. Jun 07	**4**						20									20		40
25	18. Jun 07	**4**				20								20					40
26	25. Jun 07	**4**									20								20
27	02. Jul 07	**5**						20											20
28	09. Jul 07	**5**									20		20			20			60
29	16. Jul 07	**5**															20		20
30	23. Jul 07	**5**																	0
31	30. Jul 07	**5**																	0
																			860

* Note that period **1/2** is dominated by prespawning fish; in periods **3** to **5** = atresia sampling

	per period				
	1	2	3	4	5
AZTI	60		40		
BFA	80	40			
MI	120				100
FRS		100		60	
IMARES			60	60	
IMR			40	20	
IEO	20	60			

If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes in Table 3.1.3 below. In order not to concentrate the sampling on spawning fish it is preferable that trawling is not concentrated on the 200 metre depth contour but is adapted to fit in conveniently with the egg survey along the transects over the continental shelf. In 2007 CEFAS will not be contributing towards the collection and analysis of mackerel fecundity and atresia so the samples will be redistributed to Norway, Scotland, and Spain. Ireland has been requested to take over allocation of samples that were previously processed by Cefas. Details of preparation for fecundity sampling at sea are shown in Table 3.1.4.

Table 3.1.3: Weight classes for sampling females of maturity stages 2–6 (Walsh *et al.*, 1990) for Potential fecundity and atresia.

WEIGHT CATEGORY [G]	<250	251 – 400	401–550	>551	TOTAL
Number of fish	5	5	5	5	20

Table 3.1.4:

Protocol for processing and distribution of mackerel ovary sub-samples for either fecundity or atresia analysis
Prior to cruise departure Norway (Merete Fonn and Maria Kruger Johnsen) will coordinate the analysis of mackerel fecundity samples and assign tube reference numbers to cruise leaders for labelling the Eppendorf tubes used on their cruises Coordinators to assign unique codes to each participating cruise Procure Eppendorf type tubes and place in suitable racks (see Table 3.3.1 for details of suppliers). Attach a spot label to the Eppendorf lid and add 1.2 ml of 3.6% formaldehyde buffered with 0.1M sodium phosphate (referred to below as 'fixative') to each tube using a dispenser. The label should contain 3 alpha or numeric characters for a primary key in the fecundity database. Prepare 4 replicates for each tube label and colour the replicate white, red, blue and green respectively. Measure and record the weight of each tube including fixative (±0.0001 g) using the tube label code and colour for reference. Procure sample bottles for the remaining ovary tissue should have parallel walls and without a restricted neck opening (otherwise we cannot extract the ovary without cutting of the jar top). The largest ovaries will require 250 ml sample bottles but in many cases a 100 ml or smaller capacity jar will be adequate. Label the bottle with the Eppendorf code and cruise. Procure 25–50 µl capillary pipettes (Table 3.3.1) Test performance of the pipette by practice, taking 25 µl water samples and weighing the dispensed fluid

Procedures to follow at sea to collect samples and for sample analysis in the laboratory are shown in Tables 3.1.5. and 3.1.6 respectively. In order to compare estimates of fecundity made by each country 100 samples should be analyzed by all participants but, for the remainder, at least 2 of the quadruplicate samples should be analyzed. Overall targets for estimating realized fecundity are shown in Table 3.1.7. Provisional reporting of estimates for potential fecundity and atresia are required for the 2007 Mackerel Horse Mackerel Working Group in September and final results for WGMEGS in the spring of 2008. If the participants or the coordinator are not certain of the data quality it should be also passed on to the Working Group Coordinator (Findlay Burns FRS).

Table 3.1.5: Adult mackerel sampling programme – Flow diagram.

Mackerel and Horse Mackerel Egg Survey 2007

MACKEREL SAMPLING

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

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

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

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

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

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

(d) send on one of every parallel sample to Vigo, Aberdeen, Bergen, Galway → histological analysis at IEO, FRS, IMR, MI → potential fecundity, atresia, realised fecundity

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

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

(a) Collect a larger sample of mackerel

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

(c) Freeze 50 fish individually in plastic bags

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

Sample analysis targets for Ireland, Norway, Scotland and Spain participating in estimation of mackerel fecundity and atresia. Each country carrying out the various cruises listed in Table 3.1.2.a-b is responsible for distributing their sample collection alternately to the countries carrying out the fecundity analysis. Norway will coordinate mackerel fecundity sample analysis.

Table 3.1.6:

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

Table 3.1.7:

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

Determine atresia in mature fish identified as either spawning or spent above by Stereometric analysis using the protocol in the fecundity manual. Configure the macro used to process the atresia analysis results to complete all the columns in the histology table of the fecundity database.	All participating countries	

Table 3.1.8: Sampling targets for western and southern mackerel spawning components.

SPAWNING COMPONENT	TARGETS FOR POTENTIAL FECUNDITY ANALYSIS	TARGETS FOR ATRESIA ANALYSIS [1]
Southern	100	100
Western	300	300
Total	400	400

[1]The samples above suitable for atresia analysis will be selected from a much larger collection from the surveys detailed in the cruise sampling Table 3.1.2a-b.

3.2 Western Horse mackerel fecundity

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

Table 3.2.1: Changes for 2007 compared to 2004.

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

In the 2007 survey horse mackerel will be collected from the Southern and Western spawning components selecting fish in maturity stages 3–6 fish > 25 cm collected on trawl hauls shown in Table 3.1.2a-b. As in mackerel, the tables are only indicative of the range in temporal and spatial coverage to guide cruise leaders and are not in any way to be taken as a constraint on the timing in relation to spatial coverage of the plankton sampling grid. Details of the horse mackerel sampling over the spawning season (Table 3.2.2) showing the best case desired latitudinal collection of fish and fish processing are shown in the flow chart below (Table 3.2.2a-b). If one of the hauls fails to catch fish the number of fish taken can be doubled in the next trawl haul.

Table 3.2.2: Coverage horse mackerel sampling in the Western component survey area.

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

HORSE MACKEREL Lat ° Lon °

Week	Date	Period*	44N	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	11W	10	9	8	7	6	5	4	3	2	1	Total
8	19. Feb 07	**1**																												0
9	26. Feb 07	**1**																												0
10	05. Mrz 07	**1**																												0
11	12. Mrz 07	**1**	10																		10									20
12	19. Mrz 07	**1**		10	10		10																	10						40
13	26. Mrz 07	**1**				10		10			10																10			40
14	02. Apr 07	**1**							10			10		10		10														40
15	09. Apr 07	**1**				10											10	10						10						40
16	16. Apr 07	**2**	10		10		10										10													40
17	23. Apr 07	**2**		10				10				10	10		10															50
18	30. Apr 07	**2**									10													10						20
19	07. Mai 07	**3**				10																						10		20
20	14. Mai 07	**3**						10																					10	20
21	21. Mai 07	**3**	10		10				10		10																			40
22	28. Mai 07	**3**												10																10
23	04. Jun 07	**4**							10								10													20
24	11. Jun 07	**4**						10									10													20
25	18. Jun 07	**4**				10								10																20
26	25. Jun 07	**4**									10																			10
27	02. Jul 07	**5**						10																						10
28	09. Jul 07	**5**									10		10			10														30
29	16. Jul 07	**5**															10													10
30	23. Jul 07	**5**																												0
31	30. Jul 07	**5**																												0
																														500

AZTI
BFA
MI
FRS
IMARES
IMR
IEO

* Note that period **1/2** is dominated by prespawning fish; in periods **3** to **5** = atresia sampling

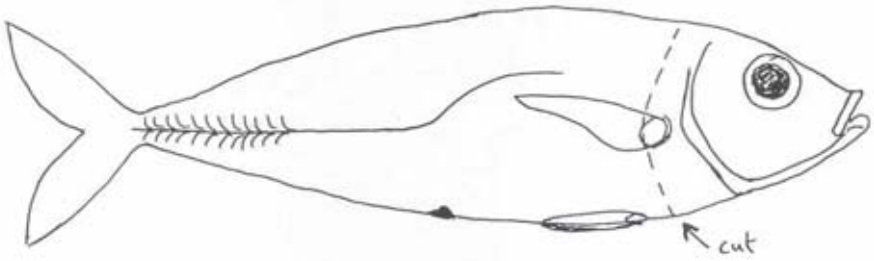
Protocols for horse mackerel sampling preparations, sampling at sea and analysis in the laboratory and analysis are shown in Tables 3.2.3–5 respectively. Cindy Van Damme from the Netherlands will coordinate the analysis of horse mackerel fecundity samples. 50 samples will be analysed by all 4 countries for quality assurance but at least 2 sub-samples should be analysed for all the remaining fish. A procedure shown in Figure 3.2.1 should be used to minimise damage whilst separating the ovary from the fish.

Table 3.2.3:

Protocol for processing and distribution of horse mackerel ovary sub-samples for either fecundity or atresia analysis
Prior to cruise departure **Cindy Van Damme (Netherlands)** will coordinate the analysis of horse mackerel fecundity sample and assign tube reference numbers to cruise leaders for labelling the Eppendorf tubes used on their cruises Procure Eppendorf type tubes and place in suitable racks (see Table 3.3.1 for details of suppliers). Attach a spot label to the Eppendorf lid and add 1.2 ml of 3.6% formaldehyde buffered with 0.1M sodium phosphate (referred to below as 'fixative') to each tube using a dispenser. The label should contain 3 alpha or numeric characters for a primary key in the fecundity database. Prepare 4 replicates for each tube label and colour the replicate red, blue and green respectively. Measure and record the weight of each tube including fixative (±0.0001 g) using the tube label code and colour for reference. Procure 25–50 µl capillary pipettes (Table 3.3.1) Test performance of the pipette by practice, taking 25 µl water samples and weighing the dispensed fluid

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

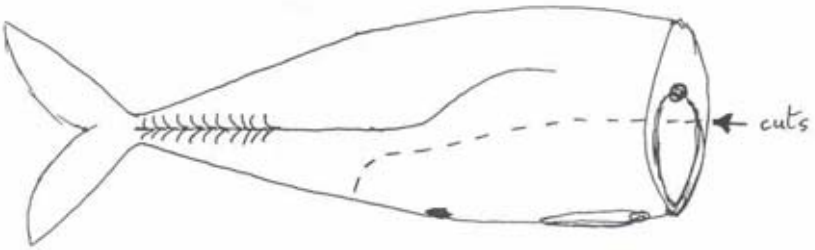
1) Measure and weigh the fish and make a temporary note of the information.
2) With a knife cut round the shoulders of the fish in a line just behind the base of the pectoral fins. Using blunt nosed scissors, join these cuts round the body cavity wall forward of the pelvic fins and sever the vertebral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



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

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

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



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

Figure 3.2.1: Method to remove undamaged ovaries from horse mackerel.

Table 3.2.3: Flow chart for selecting and processing horse mackerel samples.

Mackerel and Horse Mackerel Egg Survey 2007

HORSE MACKEREL SAMPLING

(1) Estimation of lipid content in pre-spawning fish

Market sampling

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

(a) obtain length distribution of larger sample ····► length distribution

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

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

or (depending on availability)*

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

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

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

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

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

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

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

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

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

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

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

or (depending on availability)*

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

* will be clarified Oct 2006 (Fecundity Analysis Workshop)

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

Table 3.2.3:

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

3.3 Methodology for taking samples from mackerel and horse mackerel ovaries

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

Table 3.4.6.1: Details of equipment and suppliers.

EQUIPMENT	CATALOGUE REFERENCE	SUPPLIER
Transferpettor capillary	307/5502/05	VWR International Dublin Critical Environment Business City west Business Campus Naas Road Dublin 22 Ireland Tel: ++3531 4660111 Fax: ++3531 4660380 The reference for the wiretrol pipette II is from Drummond scientific http://www.drummondsci.com/ catalogue number 5–000–2050
Transferpettor capillary	307/5502/15	VMX as above
Eppendorf type tubes	LA-MCT-200-C	Biohit Ltd, Unit 1 Barton Hill Torquay, Devon, TQ2 8JG England Tel. O800 685 4631 email sales@biohit.demon.co.uk
Racks for tubes	LL-9200–0	Biohit above
Laser tough spots, 0.375"	SPOT-1000	Web Scientific Ltd, Business and Technology, Centre Radway Green Venture Park, Radway Green, Crewe, Cheshire CW2 5PR Tel +44 (0) 1270 875172Fax +44 (0) 1270 878186 Website www.webscientific.co.uk

The capillary codes are for a 100 µl pipette and need revising to order a 25 µl pipette.

Method

The capillary pipette will remove an ovary sample of standard weight CV 3% from a stage 3 to 5 ovary but not stage 6. In the case of Stage 4 running ovaries squeeze out all the loose eggs

before taking the sample. In the case of stage 6 ovaries take a small piece with forceps from the centre of the ovary similar to that removed by the pipette. Repeat for each of the tube replicates.

Operation

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

Place the other unsampled ovary in a bottle for atresia estimation (mackerel only)

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

Push the plunger to the bottom of the glass tube and then push the tube into the hole previously made in the tunica. Pull up the plunger until the sample **reaches the lowest line** on the glass pipette (see picture). This will provide a sample of 26 mg of tissue. Ensure there are no air pockets in the sample sucked from the ovary and that it is expelled into the 3.6% formaldehyde solution held in the tube. Ovaries that are nearly spent will not readily provide samples and in these cases use forceps to remove a similar sized sample from the centre of the ovary. Before the cruise ensure operators are familiar with the pipette operation by dispensing water into a container weighed to ±0.0001g.

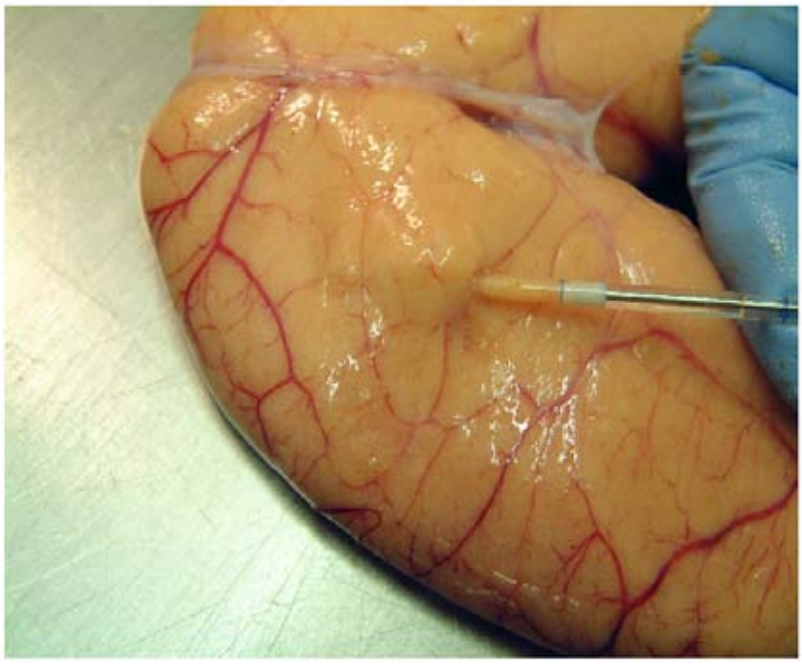


Figure 3.3.1: Method to use a capillary pipette to remove an ovary sample.

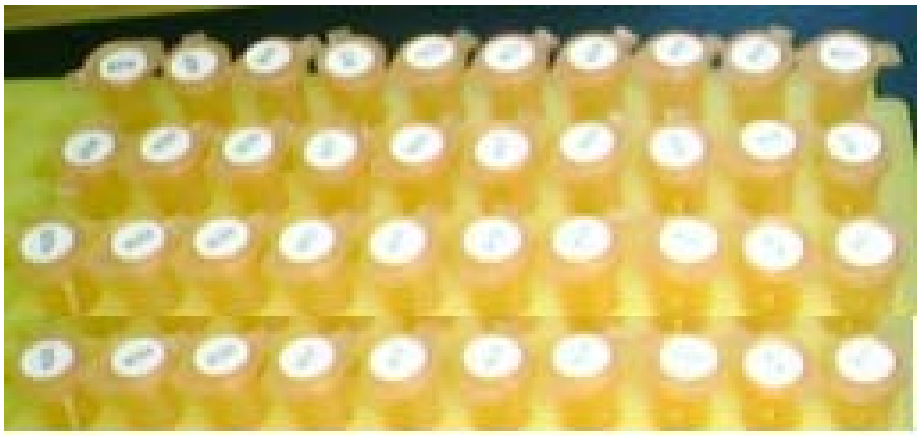


Figure 3.3.2: Picture of a rack holding Eppendorf like tubes for 10 fish with 3 replicates identified by spot labels on the lids. During storage a lid fits on top of the rack to keep the tubes in order during transport.

3.4 Collection of samples for genetic population analysis

IMR will apply for a national project to investigate the genetic structure of the different NEA mackerel spawning components. The egg survey in 2007 will be a useful opportunity to obtain samples for this project for the southern and western spawning components

WGMEGS recommends that whenever mackerel samples are collected for fecundity analysis or other purposes, a small piece of the mackerel (about 10*5*5 mm) should be cut and put in a 1.5 ml Eppendorf tube and preserved in absolute alcohol. If possible survey participants

should also freeze 50 fish individually in plastic bags to be analysed for parasites. All the samples should be sent to IMR in Bergen for further analysis.

3.5 DEPM horse mackerel adult sampling

In 2007 PIMAR and AZTI will undertake DEPM surveys within the context of the triennial survey. The Portuguese survey will be targeted on the southern horse mackerel stock. The AZTI survey will be targeted on anchovy in Biscay, however, the opportunity to test DEPM adult sampling and methods for horse mackerel will be taken on this survey. Table 3.5.1 summarizes the horse mackerel DEPM based adult sampling programme for IPIMAR and AZTI.

Table 3.5.1: DEPM horse mackerel adult sampling.

PARAMETER	AREA	COUNTRY	MONTH	N SAMPLES	INDIVIDUALS PER SAMPLE	TOTAL MATURE FEMALES	REMARKS
Batch fecundity	ICES Div. IXa	IPIMAR(Portugal)	2 – 3	40	300	150	Stage 4 gonads
	Bay of Biscay	AZTI (Spain)	5	30	150	150	
Spawning fraction	ICES Div. IXa	IPIMAR(Portugal)	2 –3	40	300	4000	Positive trawls (> 30 fishes)
	Bay of Biscay	AZTI (Spain)	5	30	100 –150	4000	
Weight	ICES Div.IXa	IPIMAR(Portugal)	2 – 3	40	300	4 000	Adult females
	Bay of Biscay	AZTI (Spain)	5	30	150	1500	

IPIMAR (Portugal):

For the application of DEPM methodology Portugal/IPIMAR will collect from each positive trawl, a simple random sample of at least 300 fishes. Each fish will be measured, weighted and opened. The sex, maturity stage, fat and stomach fullness will be recorded, and in case it is a mature female (maturity stages 3, 4 and 5) the gonad will be carefully removed, and preserved in 4% buffered formalin. The sampling process will continue until at least 100 gonads of maturity stages 3, 4 or 5 were collected. In the case that 100 gonads were collected before the sample size reached 300 individuals, the sampling process continues until 300 individuals are sampled.

Hauls with less than 30 fishes will only be sampled for batch fecundity and female total weight; therefore, if less than 30 fishes are caught all fish will be sampled, but only gonads in stage 4 (with hydrated oocytes) will be collected and preserved in formalin.

AZTI (Spain):

The objective is to estimate the spawning frequency and the batch fecundity of horse mackerel in the Bay of Biscay during May which can be considered the time and area for peak spawning for this species. This study is done in the context of the supposed indeterminate characteristic of the horse mackerel (Abaunza *et al.*, 2003).

In this way AZTI will achieve approximately 30 pelagic trawls spread through the survey area. From each trawl a minimum of 100 individuals will be taken randomly registering the

following biological parameters: total length, total weight, sex and maturity stage. In case it is a mature female the gonad will be removed, weighted and preserved in 4% formaldehyde. The objective is to obtain 50 mature females per trawl. When this objective is achieved, if the 100 individuals were not measured yet the sampling will continue until the 100 fish are measured, weighted, sexed and staged the maturity. Gonads won't be preserved except if hydrated females appear. These gonads will be kept for batch fecundity analysis.

When having sampled 100 individuals and the objective of 50 mature females hasn't been achieved another 25 fish will randomly be taken until a maximum of 50 is reached.

After the 50 mature females have been collected the rest of the haul will be targeted at hydrated females noting total length, total weight. Gonads should be preserved in 4% formaldehyde distinguishing these samples from those taken randomly.

4 Variance calculation procedures; (referring to ToR "c")

4.1 Variance estimation for the North Sea mackerel egg surveys

No new work was carried out for variance estimation in the western and southern surveys since the last report. The main work in this area was to develop the western area methodology to provide a variance estimate for the North Sea egg surveys. This work is detailed in Section 8.3.

5 Review procedures for egg sample sorting, species ID, staging and fecundity and atresia estimation. Based on workshop in late 2006; (referring to ToR "d")

5.1 Planning for egg sample sorting, species identification and staging workshop

It is recommended that each institute participating in the 2007 mackerel and horse mackerel egg survey has at least one scientist/technician at the egg workshop (WKMHMES) to be held at Cefas, Lowestoft between 23 and 27 October 2006. It is essential that this representative is the same person who will analyse the majority of their institute's plankton samples from the 2007 egg survey.

The workshop will attempt to standardise analytical procedures as far as possible. To help with this, the workshop will address each step of the plankton analysis, separately.

Sorting of eggs

An enhanced egg sorting protocol for the spray technique (WD Eltink) is given in Section 12, and it is recommended that each participant trials this procedure before the egg workshop. The procedure will be discussed, validated and possibly revised at the Lowestoft workshop. The 'spray method' will be validated against the normal procedures for egg sorting which utilise microscopes and magnifying lenses to enable the eggs to be seen and removed from the rest of the plankton. It is anticipated that the workshop will recommend a standard plankton sorting procedure which will utilise the spray technique to rapidly remove the majority of the eggs during each survey (which will facilitate adaptive sampling). A manual sorting for any remaining eggs will follow this.

Identification of eggs

Each institute has been asked to try to obtain artificially fertilised eggs of mackerel, horse mackerel and similar eggs of other species, which are regularly encountered in tri-ennial egg

survey samples. Some mackerel and horse mackerel eggs have already been collected from artificial fertilisations. In addition, naturally spawned eggs of horse mackerel will be obtained from captive fish held at IMR, Matre, Norway. The eggs of known species will be used for training and subsequent testing of participants' egg identification skills at the Cefas workshop. This is the first time that eggs of known species have been available for these workshops, which should help the participants to distinguish between them.

Staging of eggs

The allocation of eggs to each development stage will also be discussed at the Cefas workshop. The procedure will follow that of the 2003 egg workshop (ICES, 2003). Definitive mackerel and horse mackerel eggs, in all stages of development, will be provided. Each participant will stage each egg and the results will be input into a standard Excel spreadsheet for further analysis. The results will be discussed and differences between participants will be identified. Hopefully any staging difficulties will be resolved before the exercise is repeated to attempt to improve agreement in staging criteria amongst participants. Again, the freshly preserved eggs from both artificial fertilisations and natural spawning of captive fish should simulate actual survey samples more closely, thereby providing a better estimate of the errors involved in both egg identification and staging.

5.2 Planning for fecundity workshop

It is recommended that each institute participating in the 2007 mackerel and horse mackerel egg survey has at least one scientist/technician at the fecundity workshop (WKMHMES) to be held at CEFAS, Lowestoft between 30 October and 2 November 2006 (4 days inclusive). It is essential that this representative is the same person who will analyse the majority of their institute's fecundity samples from the 2007 egg survey.

The workshop will attempt to standardise analytical procedures as far as possible. To help with this, the workshop will focus on each step of the fecundity analysis listed under the bullet points below. Participants should bring a lap top with a CD or DVD drive because this will be used for scoring images prepared from horse mackerel and mackerel whole mounts and slides from mackerel. Norway and England will prepare slides stained by Toluidine blue and PAS Mallory respectively to compare and subsequently select and agree a staining method for mackerel atresia. The fecundity database will be circulated to all the participants who registered their intention to participate at the Workshop before the start of August 2006. Prior to the Workshop the image analysis and stereometric macros will be modified by the Workshop coordinator so that the data will input directly into the fecundity database tables.

- Weighing of Eppendorf tubes
- Fecundity sampling using the Wiretroll pipette
- Use of the fecundity database both at sea and to interface with the fecundity and stereometry macros
- Standardisation of whole mount and slide staining protocols to estimate fecundity and atresia respectively.
- Use of image analysis hardware and software to achieve reproducible data.
- Standardisation of whole mount interpretation to identify spawning markers and follicle measurement
- Standardisation of slide interpretation to estimate 3 classes of early alpha atresia (Yolk vesical, Yolk vesical /Yolk Granule and Yolk granule).
- Standardisation of threshold for horse mackerel fecundity.
- Update the fecundity manual.

5.3 Issues relating to Atresia and spawning duration and it's persistence

Methods of data analysis to discount the production of atretic follicles defined in ICES,

1996 rely critically on the duration of spawning (D) and the early alpha atretic atresia stage Ad referred to in the equation 3 below.

1

$$SSB = \frac{E}{Fr}$$

Where E = Population annual egg production
Fr = Realised fecundity (eggs / g female)

2

$$Fr = Fp - Ap$$

Where Fp = Potential fecundity (vitellogenic follicles / g measured just prior to spawning)
Ap = Atretic follicles (per g female produced over the spawning cycle of the average female

3

$$Ap = Ai \,.\, P \,.\, D \,.\, Ad$$

Where Ai = intensity of atresia (standing stock of atretic follicles per g female)
P = prevalence of atresia (proportion of spawning females containing atretic follicles)
D = Spawning duration of the average female
Ad = duration of the atretic follicle takes to regress

At present WGMEGS uses values of 7.5 and 60 days for early alpha atresia and spawning duration respectively (ICES, 1996) but these values are not supported by citations of strong experimental evidence and there is no variance term to include in the overall SSB variance. Recent work carried out in RASER (an EC funded Frame Work 5 project) reported the early alpha stage in cod is rather shorter (3.8 days se 0.8 n = 6) and this finding should be investigated in the context of mackerel fecundity regulation.

Ovaries collected during the 2004 WGMEGS survey where also analysed to investigate whether trends in ovary mass supports the estimate of spawning duration used by WGMEGS. Hydrated females contain the heaviest ovaries and are therefore likely to be the most prolific egg producers so this data can be interpreted to indicate the spawning intensity within the population (Figure 5.3.1.1). In this case the data can be considered as representative of the whole Western mackerel Spawning component because the collection was made from trawl hauls dispersed across a wide latitudinal range (Figure 5.3.1.2) of the whole Western Spawning Component. Spawning intensity varied over the season with higher levels at the start and towards the end of the survey period in weeks 22 to 25. Lower, declining intensity of spawning was observed from weeks 16 to 20 indicating a drop in the daily egg production. This data also indicated that the ovary mass index fell to the lowest levels at the end of the survey and corresponds to the progressive decline in egg production at the end of the last survey. Previous WGMEGS estimates of egg production based on GAM models (ICES, 1996) also suggest that egg production has some tendency to rise for a second peak of egg production towards the end of the surveys. It would be useful in the 2004 egg abundance could be modelled with a GAM to confirm whether this happened in 2004. In conclusion this data indicated that some females appeared with a low ovary mass (near spent) from weeks 16 to 20 and suggests that many females spawned for somewhat shorter period than 60 days. The second peak of spawning may have come from fish previously considered to be spent or from arrival of new spawners and this could be resolved by a study of the residual fecundity in the fish considered to be spent in periods 16 to 20. A study of spawning in captive mackerel

should also be carried out to provide further insight into the dynamics of egg production and fecundity down regulation in mackerel.

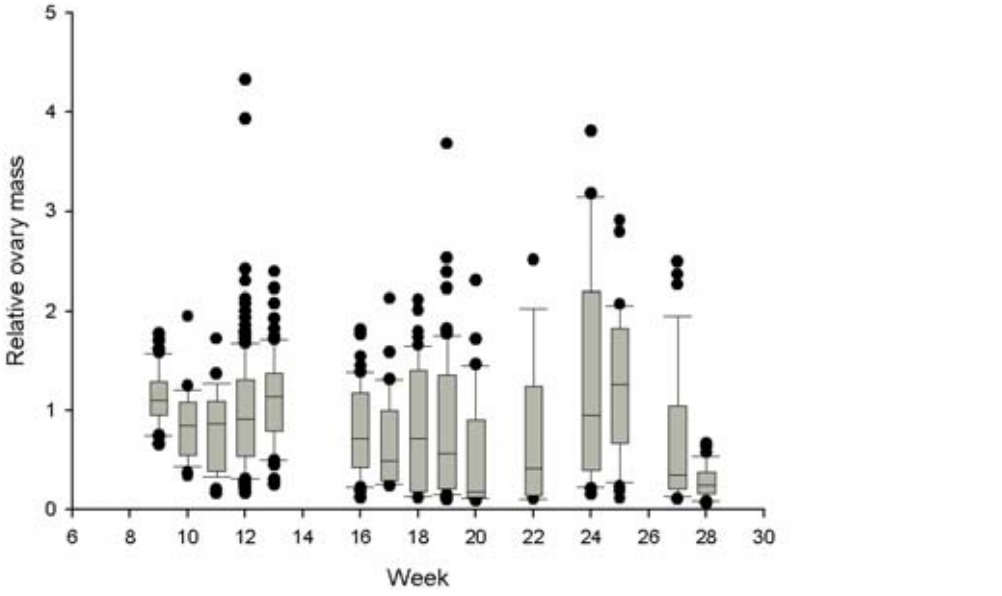


Figure 5.3.1.1: Box plot of relative ovary mass in mackerel collected from trawl hauls shown in Figure 5.3.1.2. Ovary mass was normalised for different sizes of fish by dividing the observed ovary mass of each fish in the collection by the predicted ovary mass from equation O=l.a+b where O =log ovary weight, l = log fish length. The reference fish were all pre-spawning maturity stage 3 females. Points are outliers to the 95% confidence limits shown by the upper and lower bars. The box represents bounds of 25 and 75 percentiles and the median value is shown by the horizontal line in the box.

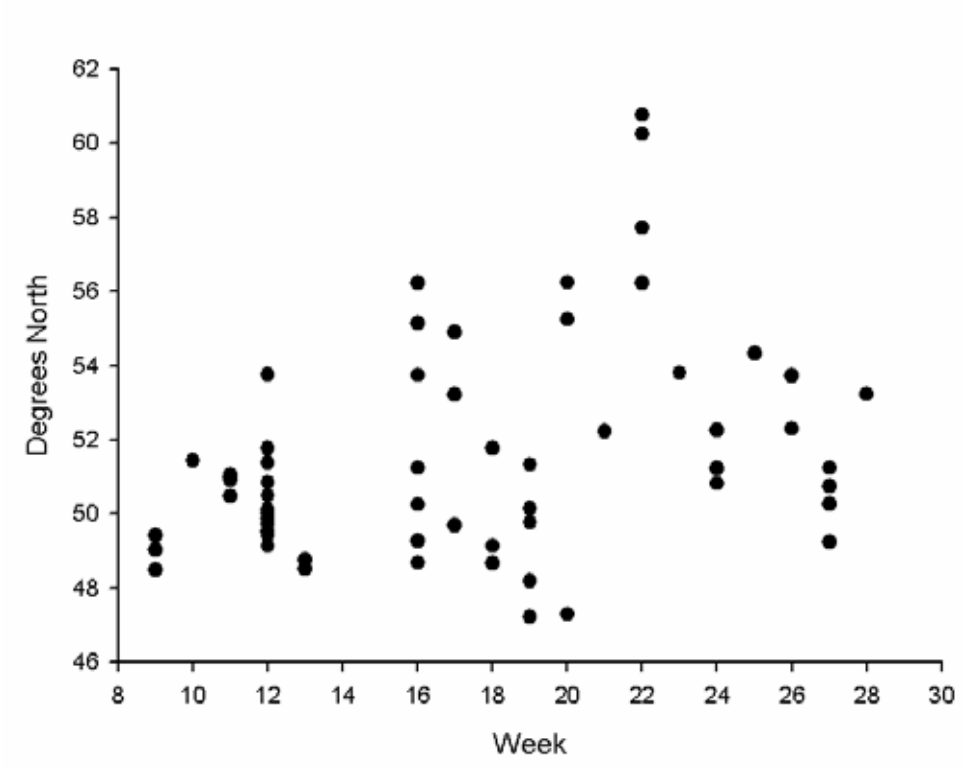


Figure 5.3.1.2: Latitudinal coverage of trawl hauls to collect mackerel fecundity and atresia samples.

5.4 Fecundity database

Previously WGMEGS has used Excel flat files for the storage of fecundity data and parameters associated with the trawl hauls and fish details. The data arriving from each country working on fecundity always takes some time to integrate into a data set for analysis of potential and realised fecundity. For the 2007 survey WGMEGS agreed to use a Microsoft Access 2 Data base containing several tables linked by a fish reference primary key and details of the station, cruise, and vessel. The tables are supported by several input forms accessed by the start form. Example forms are presented in Figures 5.4.1–5.

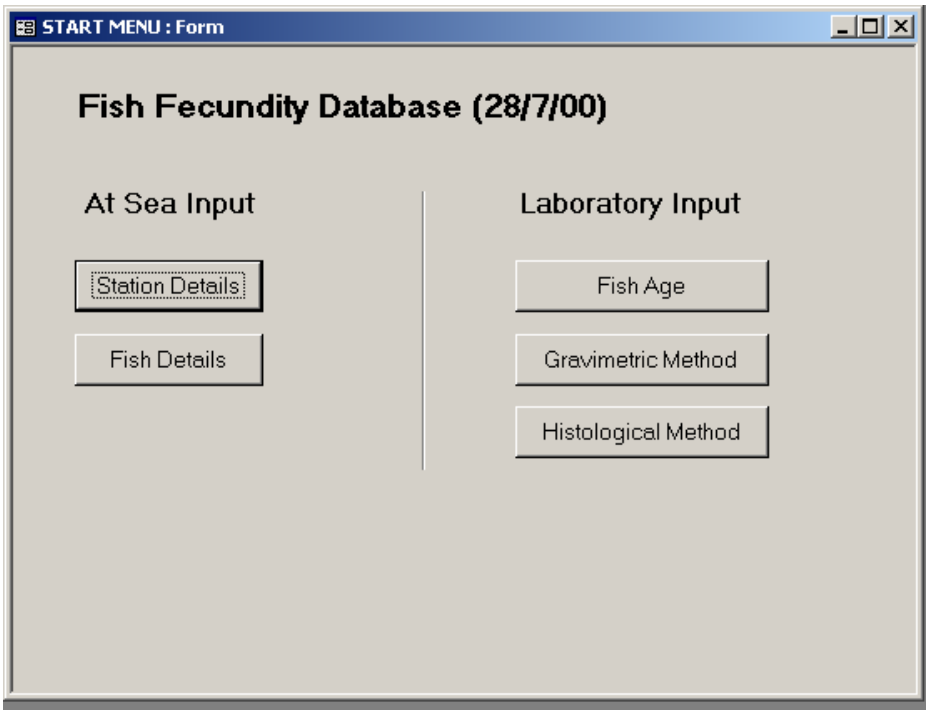


Figure 5.4.1: Start form.

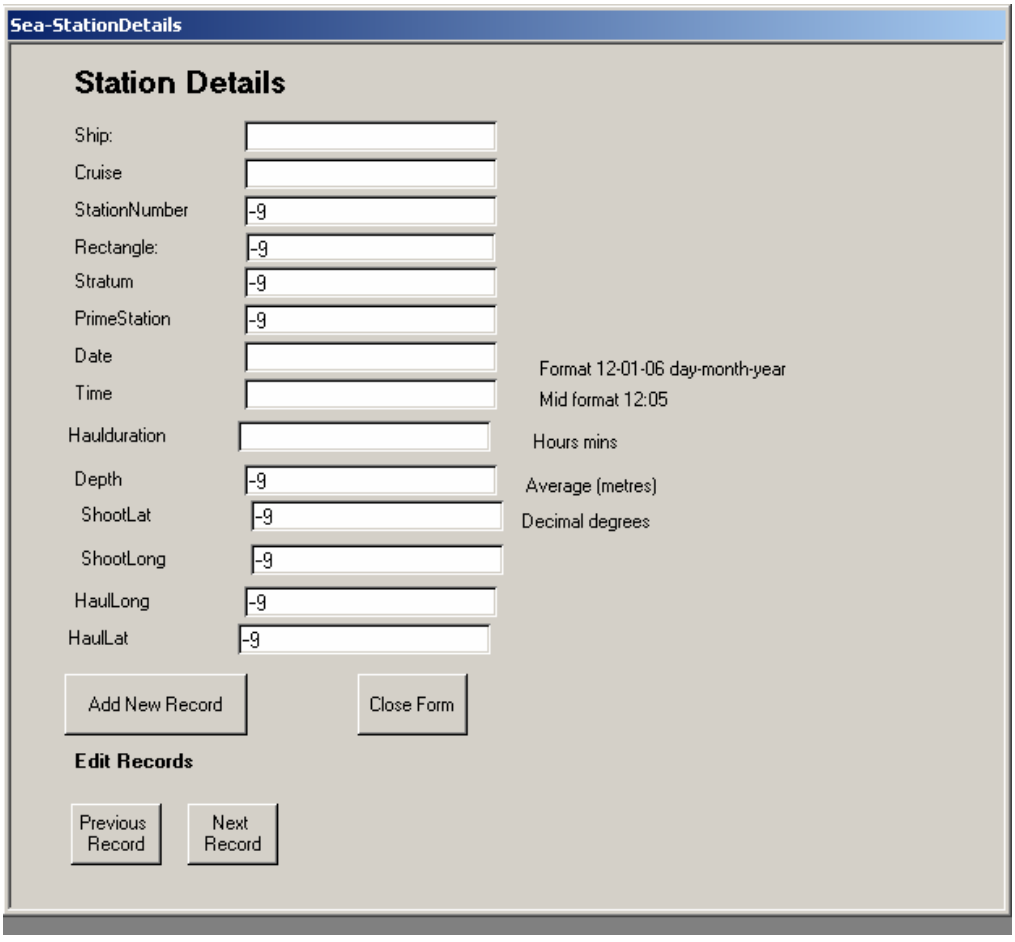
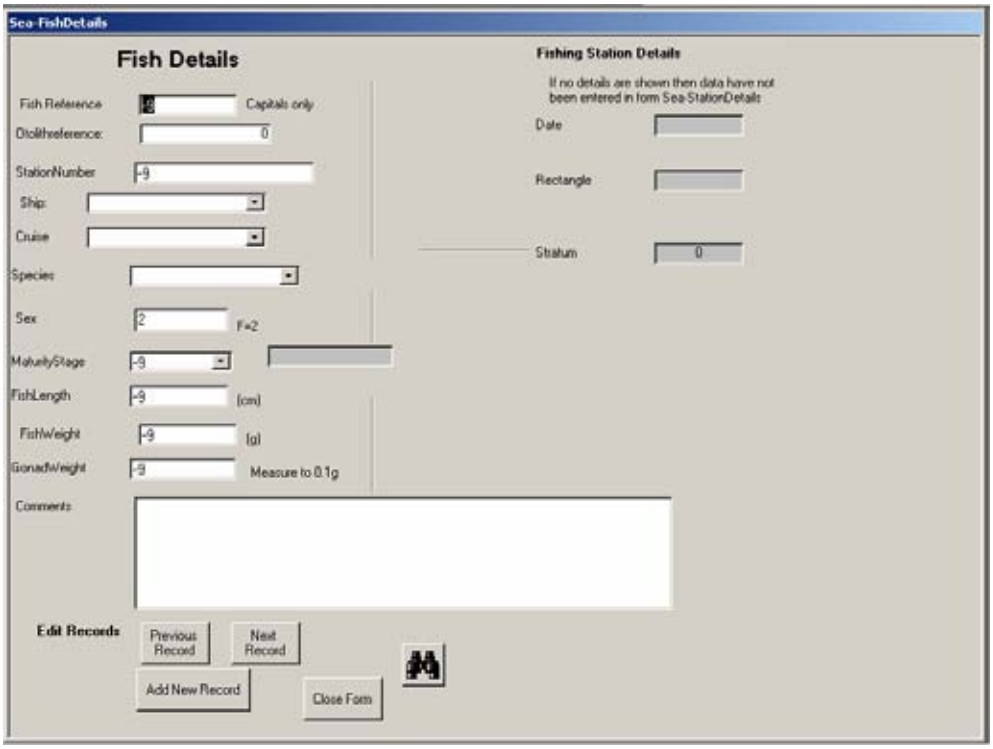
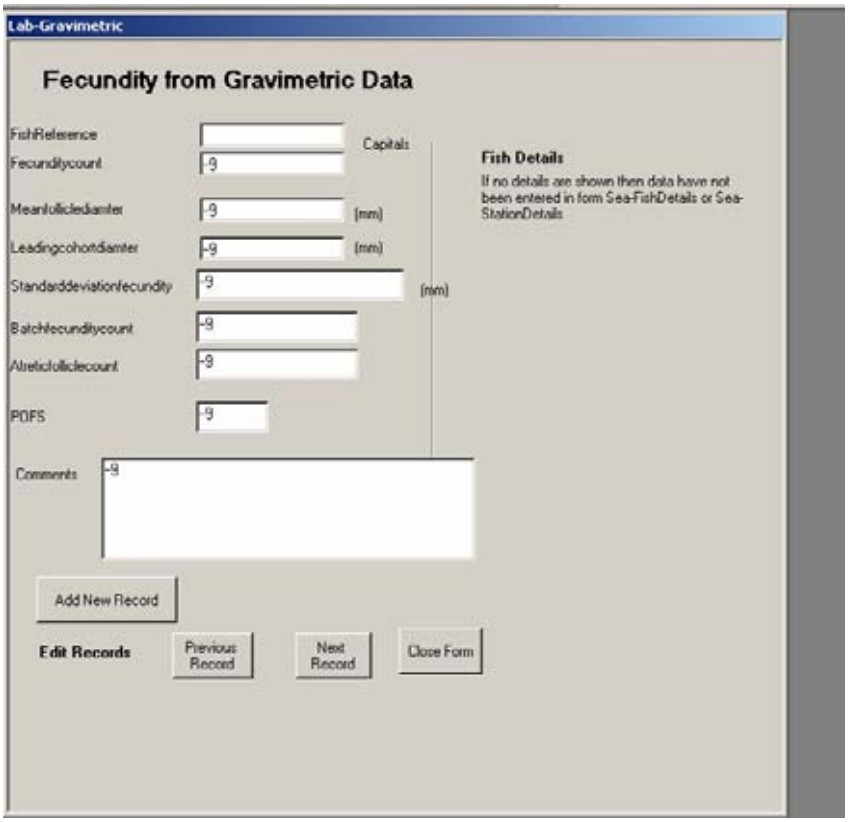


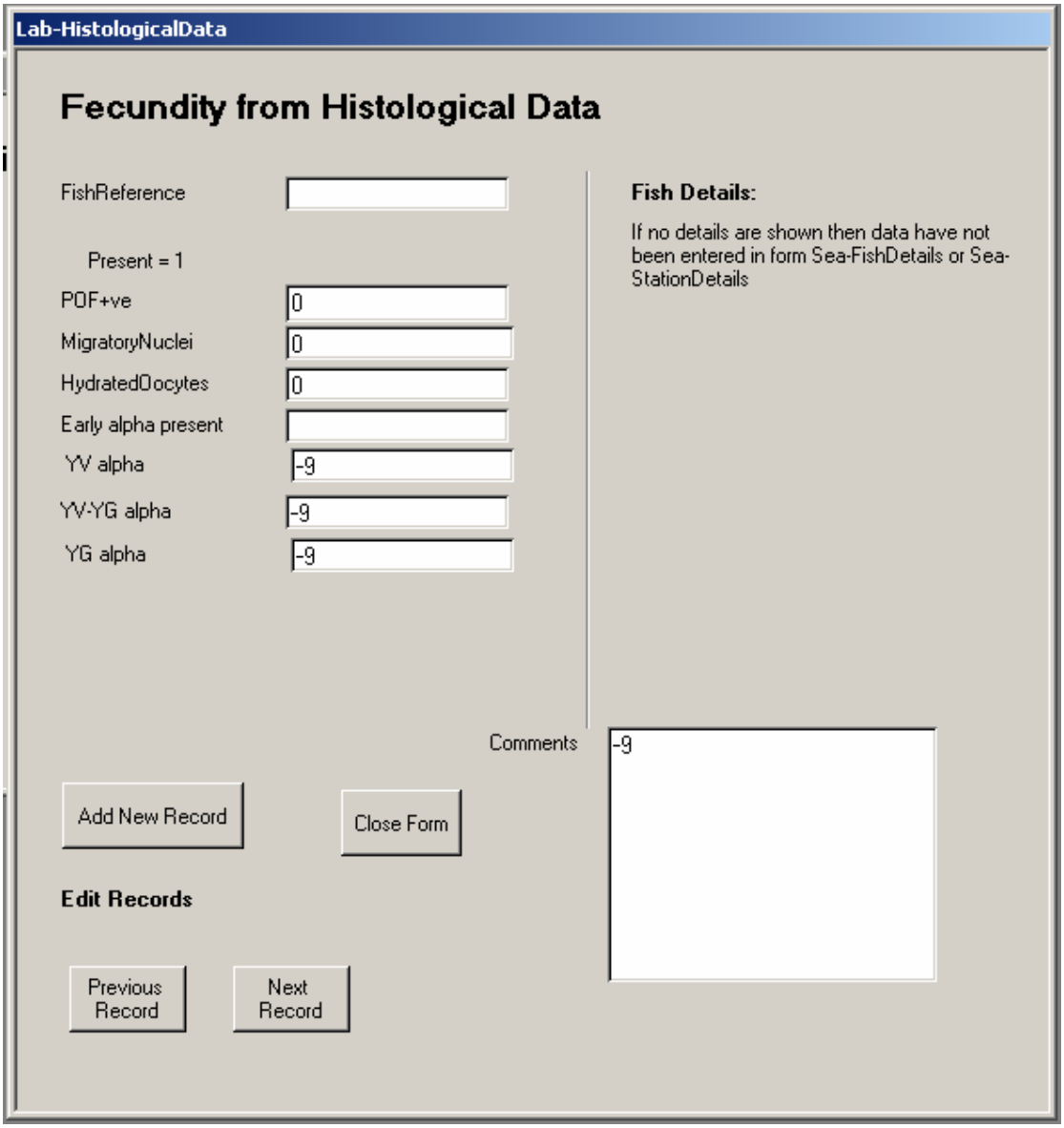
Figure 5.4.2: Station details.



5.4.3: Fish details from.



5.4.4: Gravimetric fecundity form.



5.4.5: Atresia – Histology form.

5.5 Experimental study of growth and reproduction in Atlantic horse mackerel

This project is conducted by the Institute of Marine Research (IMR) following recommendations by the ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS). It is the first time a study on horse mackerel in captivity has been conducted and will hopefully improve our understanding of horse mackerel reproductive biology. The experimental work is conducted at the IMR facilities in Matre (Matre Aquaculture Research Station, N-5984 Matredal). It was started in October 2005 and will be running through the spawning season until October 2006. One of the main objectives of the experiment is to clarify the question of determinacy/indeterminacy in horse mackerel. The experiment will provide data on the length of the spawning period/season. Energy allocation patterns (condition indices) will be monitored and, during the spawning period, eggs will be collected and staged.

In late September 2005, approximately 600 horse mackerel were caught by purse seine in the Masfjord. The fish were transported to the aquaculture station in Matre and distributed between two 5 m circular tanks. Water temperature will be increased in April 2006 to mimic the natural conditions. In both tanks the fish are fed to satiation three times a week with dry feed. Samples of five females from each tank are removed monthly, and more frequently around and during spawning. Otoliths are taken for age determination, total and fork length, total weight, as well as gonad, liver and intestinal weights are recorded for each fish. Half of the ovary is fixed in 4% buffered formaldehyde and the other half, together with liver, intestines and the rest of the fish are frozen for chemical analyses. During the spawning season egg production will be monitored by using egg collecting devices. So far the fish seem to have adapted well to their captive environment and mortality has been low. For more information please visit **http://www.horse-mackerel.imr.no**

6 Analysis and evaluation of the results of the 2005 mackerel egg survey in the North Sea (referring to ToR "e")

6.1 Spatial and temporal coverage

During the period 6 June–3 July 2005 the Netherlands and Norway carried out egg surveys in the North Sea to estimate the spawning stock biomass (SSB) of mackerel (WD 2006 Iversen *et al*.,). During this period the spawning area was covered four times. The last time egg surveys were carried out in the North Sea was 2002 (WD Iversen and Eltink, ICES 2002b). In 2002 and 2005 the Netherlands and Norway spent altogether respectively 40 and 38 survey days. In 2002 three coverage's were carried out and maximum egg production was observed during the last coverage. It is, therefore, not clear if the surveys covered the peak spawning period, i.e. egg production could have been higher after the surveys than during them. The derived egg production curve (Figure 6.5.1) should therefore be treated with caution. The survey strategy was changed in 2005 in order to achieve four coverage's with about the same amount of available survey days, and hopefully to include the period of peak spawning. The first and last coverage's were carried out by one vessel while the two vessels cooperated during coverage's two and three.

6.2 Sampling and data analysis

The data collecting and the handling of the samples were carried out according to ICES (1997/H:4). RV "Johan Hjort" carried out the survey with a Gulf VII working in double oblique hauls from the surface to 70 m or 5 m above the bottom. RV "Tridens" always sampled from surface to 5 m above the bottom. The timing and the results of the surveys are given in Table 6.2.1 except for the first and fourth coverages when the area was surveyed by

one vessel. The survey area was divided so that "Johan Hjort" worked mostly in the area north of 56o and "Tridens" mostly south of this latitude.

The eggs were sorted from each of the sampled stations using the spray method (WD Eltink) and their ages were estimated according to development stage and to the observed temperature in 5 m. The development stages used in the calculations were eggs in stage 1A and 1B. The staging of the eggs and their respective ages were calculated according to Lockwood *et.al*. (1981). The average number of eggs produced per day per m^2 was calculated for each statistical rectangle of 0.5^o latitude * 0.5^o longitude (Figures 6.3.1–6.3.4). The samples were taken in the middle of each rectangle. The spawning area was covered four times and the egg production was calculated for the total investigated area for each of the four periods (Table 6.2.1).

6.3 Mackerel egg distribution

The distribution of daily egg production per m^2 surface is shown for each of the coverage's in Figures 6.3.1–6.3.4. During the three first coverage's the highest egg production (333, 460 and 274 eggs.m^{-2}) was observed in the same rectangle ($54^o45'$N and $0^o45'$W, Figures 2.3.1–2.3.3). During these three coverage's 16%, 11% and 12% respectively of the total egg production were produced in this rectangle. The main impression of the four surveys relative to the spawning area was as follows:

- Survey 1 did not define the southern border
- Survey 2 generally seemed to cover the spawning area fairly well
- Survey 3 did not define the northern and southern border
- Survey 4 did not define the southern border

The surveys were not able to cover the total spawning area or period (Table 6.2.1). The survey was designed to capture the period of peak spawning but was not able to cover the early or late spawning periods. Some of the unsampled rectangles were allocated interpolated values following standard procedures (these are indicated as shadowed rectangles in Figures 6.3.1–6.3.4). The interpolated component of the egg production for the four coverage's was respectively 11%, 19%, 13% and 13%.

6.4 Potential fecundity and atresia of North Sea mackerel

Fecundity

Ovaries from 39 mackerel in maturity stage 3 and 4 (Walsh *et al*., 1990) were collected by Norway, England and the Netherlands in the period May-July 2005 (Tables 6.4.1, 6.4.2). Samples were sent to Norway to estimate fecundity. The Netherlands used the sampling protocol from ICES (2000a) and Norway and England applied the methodology described in ICES, (2003). This means that the Norwegian and English samples were taken by micropipette and the Dutch samples were preserved as small cuts of the ovaries. The majority of samples were inappropriate for determining potential fecundity because they showed evidence of past spawning activity (presence of hydrating eggs or Post Ovulatory Follicles – POFS). A criteria of using samples with < 5 POFS for the analysis was agreed upon based on the assumption that a sample would contain a higher proportion of POFS once spawning has commenced (pers. com. P. Witthames). This left 15 samples for analysis in which all but 3 contained POFS. The fish ranged between 30–35 cm and weighed 221–427 g. Plots of potential fecundity and relative fecundity in relation to length are shown in Figures 6.4.1 and 6.4.2 respectively. Potential fecundity ranged from 230 000 eggs for a 30 cm fish to 615 000 eggs for a 35 cm fish. Relative potential fecundity was weakly related to fish length and was estimated at a mean of 1359 oocytes.g^{-1} female. The relative fecundity of 1359 oocytes.g^{-1} female estimated in the present study is 3% less than that observed in 1982 of 1401 oocytes.g^{-1}

female (Iversen and Adoff, 1983). This would increase the SSB estimate by 3%. However, there is reason to believe that the fecundity calculation from 2005 is an underestimate due to the presence of POFS indicating spawning had already started. The 3 samples containing no POFS were also found to lie above the mean, which supports this conclusion. In light of this, it is recommended that the traditional weight fecundity relationship of 1401 oocytes / gram female be applied to estimate SSB also in 2005.

Atresia

67 samples were prepared for stereometric analysis following procedures described in the manual by Whittames, P.R., (WD Witthames, ICES 2001) to quantify prevalence (number of fish with atresia present in the ovary) and intensity of atresia (number of early alpha atretic oocytes.g^{-1} total weight). At the time of the WG meeting in 2006, 23 samples had been screened. The presence and intensity of atresia was very low in the analysed samples (Table 6.4.3) so no correction for atretic losses was done to calculate realised fecundity.

6.5 Mackerel egg production and spawning stock estimate

The egg production estimates (Table 6.5.1) are considered minimum estimates since the sampling was not carried out until zero values were obtained in all directions. Based on the four production estimates and the duration of spawning period (Table 6.2.1) the egg production curve was drawn (Figure 6.5.1). Particularly the production obtained during the first survey might be significantly underestimated due to the unsampled area south of the south western rectangle with high production. Therefore the peak of spawning might have occurred earlier than during the second coverage, which was the period with the best coverage of the spawning area. If so this would have been earlier than ever observed (Table 6.5.2). The increasing temperature in the North Sea over the later years could have influenced the spawning this way. Therefore this has to be taken into consideration when planning the next survey in 2008. However, since neither the northern nor southern border of the spawning area was defined during the third survey, the egg production of this period should also be considered as an underestimate.

By integrating the egg production curve, Figure 6.5.1, the total egg production was estimated at 156.3*1012 eggs. By applying the traditional weight fecundity relationship, 1401 eggs.g^{-1} female (Iversen and Adoff, 1983), the SSB was estimated at 223,000 tons. Since the egg production was considered as an underestimate, the SSB should also be considered as underestimated.

Table 6.5.1 gives the estimated egg production in the North Sea for the years with multiple surveys of the spawning area. The corresponding SSBs based on the traditionally used fecundity of 1401 eggs/g/female (Iversen and Adoff, 1983) are also given.

Both “Tridens” and “Johan Hjort” trawled for mackerel during the survey. The age composition obtained from samples by the two vessels is shown in Table 6.5.2. The combined age distribution was weighted according to the egg production north and south of 56^{o} N. Since 48% of the egg production was observed north of 56^{o}N, the “Johan Hjort” and “Tridens” samples were given equal weights when combined. Based on the average weight of the SSB, 335g, the numbers of North Sea spawners by age group were calculated (Table 6.5.2).

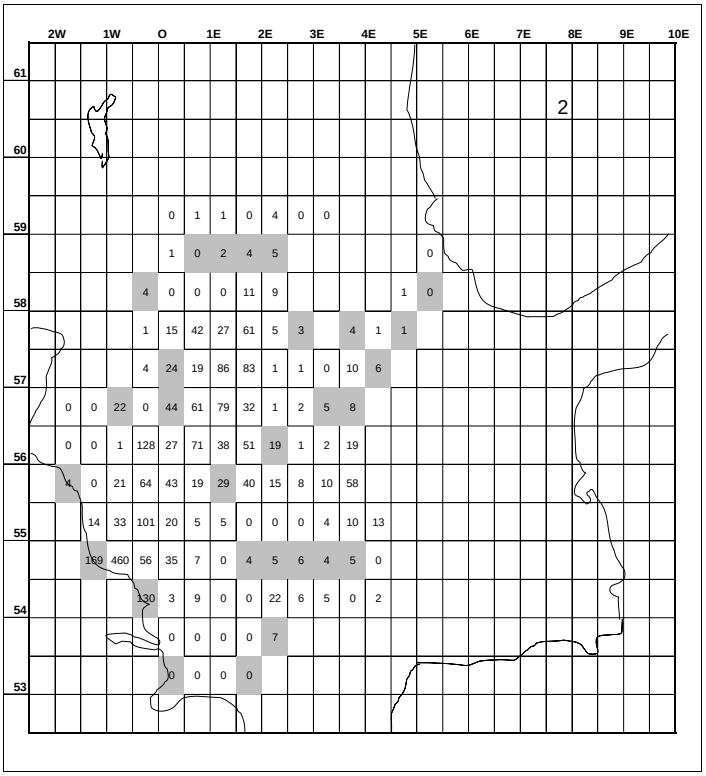


Figure 6.3.1: Daily production of mackerel eggs per m^2 during the first coverage (interpolated rectangles are shadowed).

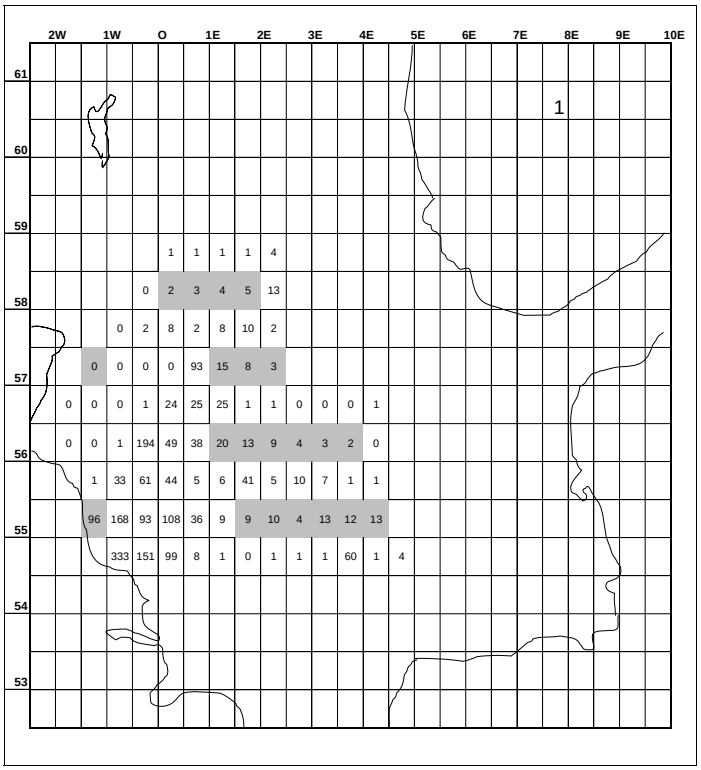


Figure 6.3.2: Daily production of mackerel eggs per m^2 during the second coverage (interpolated rectangles are shadowed).

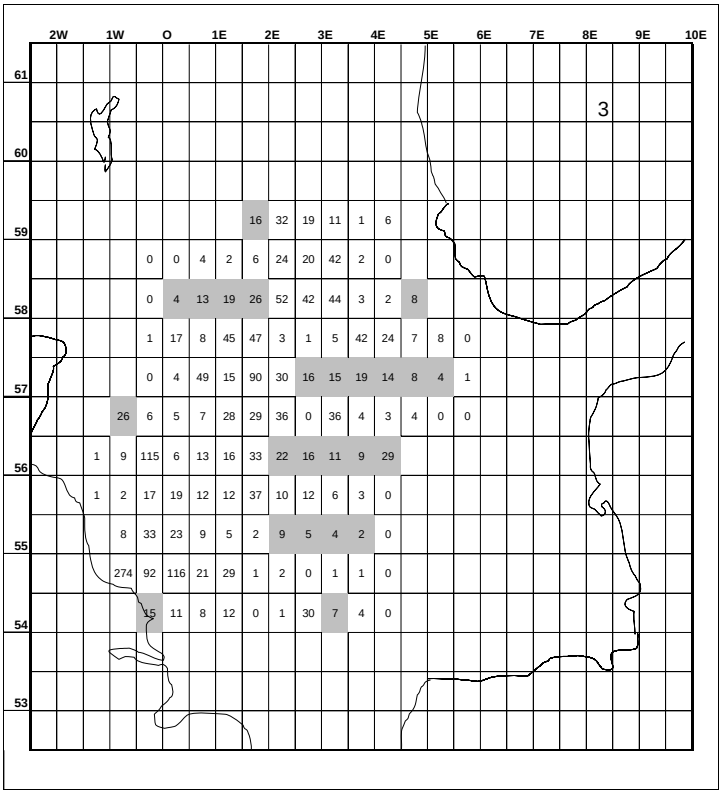


Figure 6.3.3: Daily production of mackerel eggs per m^2 during the third coverage (interpolated rectangles are shadowed).

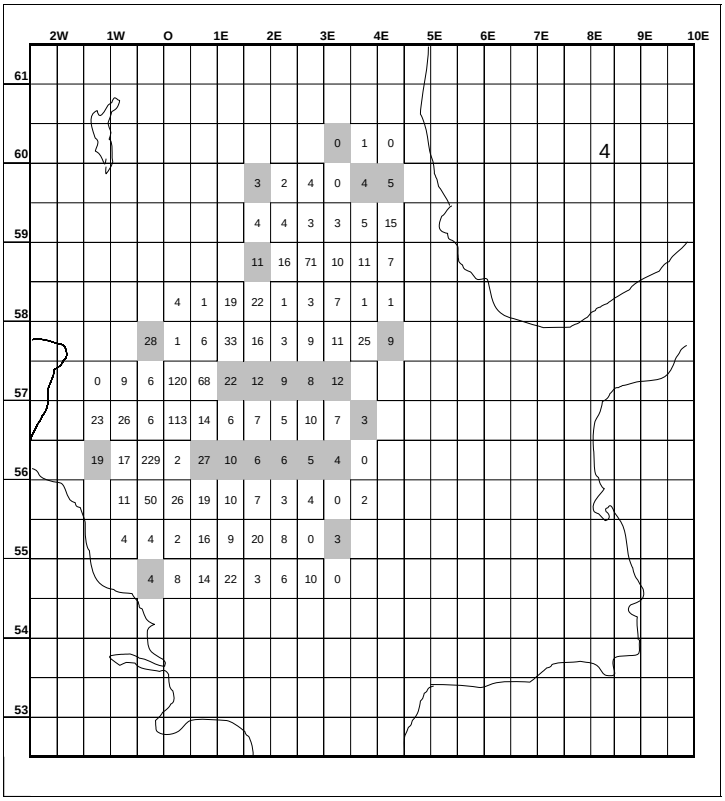


Figure 6.3.4: Daily production of mackerel eggs per m^2 during the fourth coverage (interpolated rectangles are shadowed).

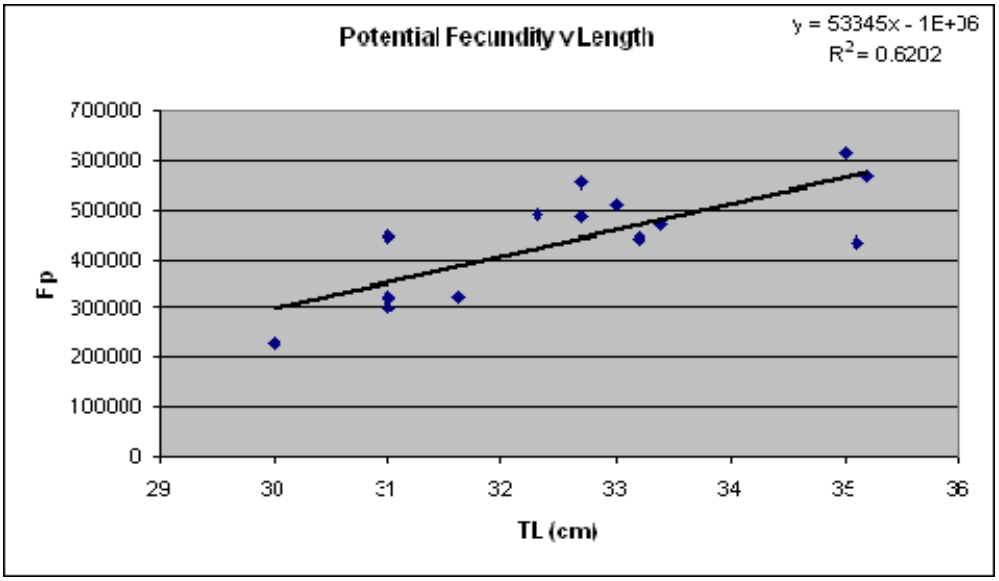


Figure 6.4.1: Potential annual fecundity of North Sea mackerel sampled June 2005.

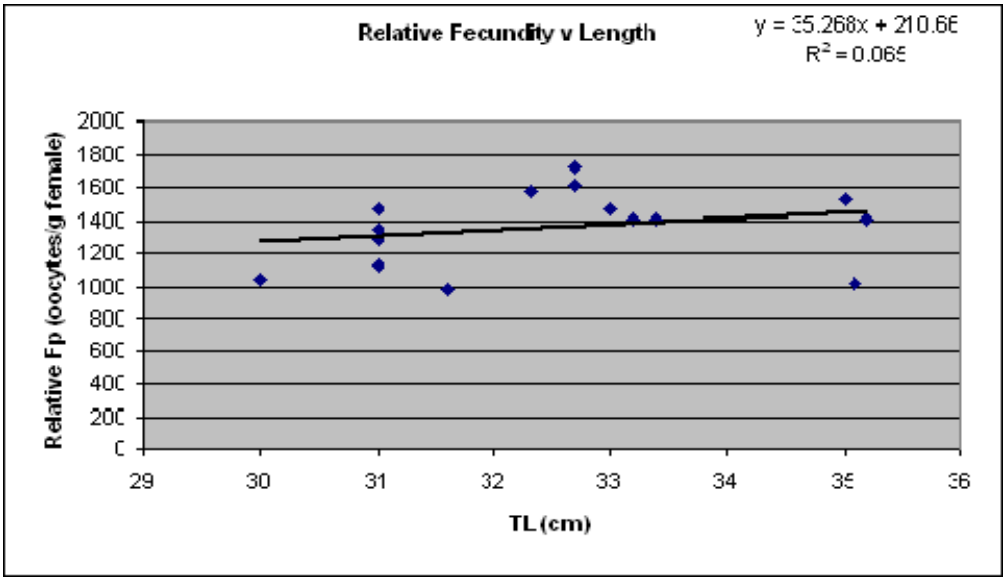


Figure 6.4.2: Relative fecundity versus weight for North Sea mackerel sampled June 2005.

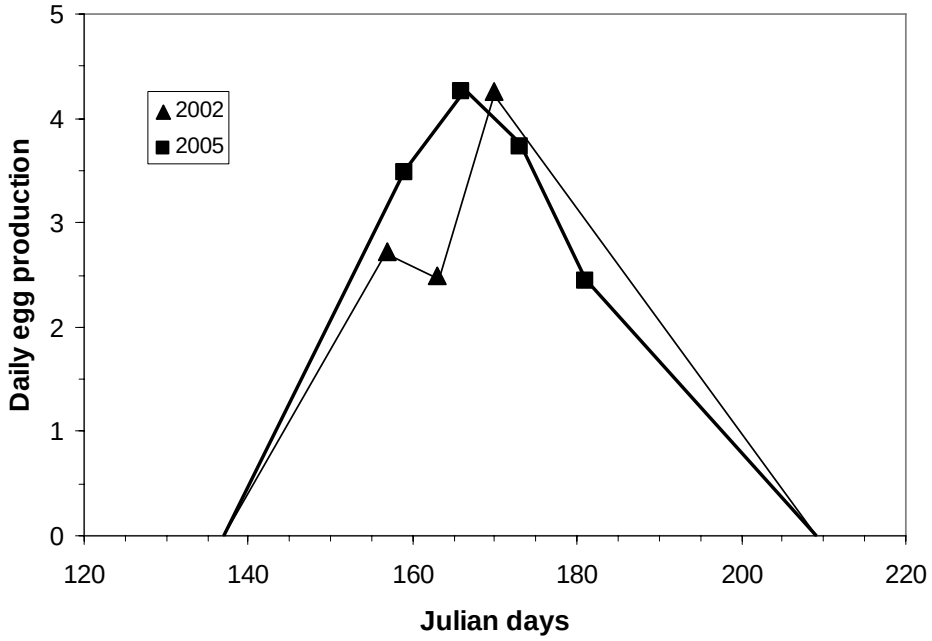


Figure 6.5.1: Daily egg production (eggs * 10–12) of North Sea mackerel during the 2002 and 2005 surveys.

Table 6.2.1: The 2005 mackerel egg survey in the North Sea.

COVERAGE	1	2	3	4
"Tridens"	6–10 June	13–16 June	20–24 June	-
"Johan Hjort"	-	13–19 June	20–25 June	26 June–3 July
Midpoint of survey Julian day	8 June 159	15 June 166	22 June 173	30 June 181
Total daily egg production x 10^{-12}	3.64	4.28	3.89	2.45
Interpolated daily egg production x 10^{-12}	0.39	0.81	0.49	0.32

Table 6.2.2: Parameters and formulas used in the egg production and SSB estimates.

PARAMETER	VALUE/FORMULA	REFERENCE
Age of stage 1A+1B eggs	$Age = Temp^{-1.61} * e^{7.76}$	Lockwood *et.al*. 1981
Fecundity North Sea	1401 eggs/g female	Iversen and Adoff, 1983
Sex Ratio	1 : 1	as in previous years
Spawning period (Julian days)	17 May – 27 July (137–208)	as in previous years, excl.1990
Number of spawning days	72	as in previous years, excl.1990

Table 6.4.1: Fecundity and atresia from ovaries obtained at the following pelagic trawl stations.

ST	VESSEL	LATITUDE	LONGITUDE
356	Johan Hjort	59.15	0.15 E
357	Johan Hjort	57.45	0.45 E
358	Johan Hjort	56.15	1.15 E
359	Johan Hjort	56.45	2.45 E
361	Johan Hjort	58.45	3.45 E
363	Johan Hjort	57.15	5.45 E
1	Tridens	56.15	0.45 W
2	Tridens	54.45	0.45 E
3	Tridens	55.15	0.15 E

Table 6.4.2: Number of fecundity and atresia samples by collecting vessel and institute. Samples in brackets not analysed by WG meeting 2006.

		TOTAL NUMBER OF SAMPLES	
VESSEL	DATE	FECUNDITY	ATRESIA
IMR RV Johan Hjort	13 – 25 June 2005	27	51 (44)
IMARES RV Tridens	6 – 24 June 2005	(3)	16
CEFAS RV Endeavour	May 2005	9	-

Table 6.4.3: Results of atresia screening of North Sea mackerel samples from June 2005.

SERIE NO.	SAMPLE ID	YV	YG	MIG NUC	HYD	POF	EARLY ALPHA	LATE ALPHA	BETA STAGE	COMMENTS
24302	18	x	x							
24302	30	x	x	x						
24303	10	x	x	x	x					start hyd.
24303	16	x	x							
24307	2	x	x	x	x	x				
24307	16	x	x							
24308	DP2	x	x	x	x					start hyd.
NE 401	2	x	x	x		x			x	1 atretic cell
NE 401	3	x	x	x		x				
NE 401	7	x	x	x		x				
NE 401	11	x	x	x		x				
NE 401	15	x	x	x		x				
NE 403	17	x	x	x		x				
NE 403	18	x	x	x		x				
NE 403	20	x	x	x		x			x	few atretic cells
NE 403	21	x	x	x		x	x	x	x	Massive atresia
NE 403	25	x	x	x	x	x				
NE 403	26	x	x	x	x	x				
NE 403	27	x	x	x	x	x				start hyd.
NE 403	28	x	x	x		x			x	1 atretic cell
NE 403	29	x	x	x	x	x				
NE 403	30	x	x							
NE 403	31	x								

Table 6.5.1: Egg production estimates from egg surveys in the North Sea and corresponding SSB based on a standard fecundity of 1401 eggs/g/female. [1]This was the first coverage in 1980. 2 Low egg production (0.02*1012) was observed in the south eastern part of the North Sea during 23.04–3.05, Iversen *et al.*, 1991).

YEAR	EGG PROD $*10^{-12}$	SSB$*10^{-3}$ TONS	OBSERVED PEAK OF SPAWNING (MIDPOINT OF SURVEY)
1980	60	86	(25 June?)[1]
1981	40	57	17 June
1982	126	180	23 June
1983	160	228	13 June
1984	78	111	12 June
1986	30	43	23 June
1988	25	36	20 June
1990[2]	53	76	24 June
1996	77	110	19 June
1999	48	68	-
2002	147 (118)	210 (168)	-
2005	156	223	22 June

Table 6.5.2: Age compositions obtained by "Johan Hjort" and "Tridens", the combined age distribution and the estimated numbers of North Sea spawners per age group.

	Johan Hjort		Tridens		Total stock		Mat	Spawning stock		
Age	%	W (g)	%	W (g)	%	W (g)	Ogive	%	W (g)	N (mill)
1	37.2	113	18	117	27.6	115	0	0.0	-	0.0
2	14.8	233	4	231	9.4	232	0.37	5.2	232	34.8
3	29.0	269	22	273	25.5	271	1	38.4	271	255.6
4	13.4	378	32	324	22.7	351	1	34.1	351	227.2
5	2.2	431	8	392	5.1	412	1	7.7	412	51.0
6	1.0	442	12	445	6.5	443	1	9.8	443	65.3
7	2.1	490	4	488	3.0	489	1	4.6	489	30.3
8	0.3	467	0	-	0.1	467	1	0.2	467	1.3
9	0.0	-	0	-	0.0	-	1	0.0	-	0.0
10	0.0	-	0	-	0.0	-	1	0.0	-	0.0
11	0.0	-	0	-	0.0	-	1	0.0	-	0.0
12+	0.1	857	0	-	0.1	857	1	0.1	857	0.6
Total	100	231		298		268			335	666.2
N(aged)	137		50							
N(length)	779		50							

7 Updates on the survey manual and standardization of sampling tools and survey gears (referring to ToR "f")

7.1 General overview

An update on the survey manual and standardization of sampling tools and survey gears is included in this report as Annex 2. Annex 2 will also detail recommendations and recent changes to the existing manual which was last presented in the 2003 report of this Working Group.

The two following sections are dealing with the use of different plankton sampling gears and the enhancement in the mechanical plankton sorting method, the "spray method".

7.2 Investigation into the bias between Gulf III and Gulf VII plankton samplers

Since 2004 the Netherlands has changed from using Gulf III to Gulf VII plankton samplers for all plankton surveys. The Gulf VII seems to perform better in that the oblique hauls show a sharp 'V' shape (even during bad weather) as opposed to the more 'U' shape profile produced by the old Gulf III sampler. This is probably due to the fact that the Gulf VII has an open frame instead of the net being enclosed. The open frame also decreases the sampler weight and, therefore, the Gulf VII is easier to handle on board the vessel. Other important differences between the two samplers concern the nosecone design. The Gulf III has both a steeper nosecone angle with a blunt leading edge whilst that of the Gulf VII is less steep with a sharper leading edge. It has been well documented (AIR3-CT94-1911, Arnold *et al.*, 1990) that plankton sampler nosecones with total enclosed angles of >30° (Gulf III) are less efficient than those with angles <30°.

As a result of this change of gear, the Netherlands was asked by PGHERS (ICES, 2006) to investigate any bias which might have occurred in the sampling of herring larvae. Unfortunately, no data are currently available to address this issue. Since this gear change (and any potential bias) is also important for WGMEGS, the proposed investigation will also be extended to the sampling of fish eggs.

Possible differences between the two samplers will be investigated by sampling simultaneously with the Gulf III and Gulf VII. A frame will be manufactured to enable deployment of both samplers at the same time. A pitch-and-roll sensor, altimeter, three flow meters (internal on the Gulf III, both internal and external on the Gulf VII) and a depressor will be mounted on this frame. This configuration will be tested and improved during a survey in May 2006 targeting horse mackerel eggs in the southern North Sea. If the tests prove successful, the apparatus will be also be used during a herring larvae survey in September 2006 and again during the mackerel and horse mackerel egg survey in 2007 to obtain a set of intercalibration hauls. The results will be examined to determine the difference in performance between the gears. Preliminary results will be presented at the next PGHERS in January 2007, and the full analysis will be available for the next WGMEGS meeting in 2008.

7.3 Current status of spray method

Refer to the last egg staging workshop report (ICES, 2004b) where an initial protocol and assessment of a new spray technique was described and tested. During the 2004 surveys some participants used this method exclusively for extracting eggs from plankton samples. Since then Guus Eltink has enhanced the technique and provided some validation in the form of a laboratory experiment conducted comparing the spray versus manual egg extraction techniques. The results of the experiment as well as a detailed description of the enhancements made to the spray technique are published in a working document. (WD Eltink) The WG endorses the enhancements made to the spray technique and recommends their use where appropriate in the 2007 surveys. In relation to the materials used in the spray setup a definitive list of key components and manufacturers will be compiled during the 2006 egg staging workshop in Lowestoft. Participants are urged to contribute to this by providing details of materials and manufacturers used.

7.4 Standardisation in design and use of Bongo sampler

Gear and Procedures

The Bongo net is a standard sampler acceptable for use in Mackerel and Horse Mackerel egg surveys. However subtle differences in the design of the nets used by the different labs have been detected (see Table 7.4.1). The WG recommends that a standardised design of Bongo sampler should be followed to minimise the risk of sampler bias and to ensure consistency between samples and surveys. This standardised design should be approved by the WG.

Standardisation in design and use of Bongo sampler

The aperture of the Bongo sampler should be 40 cm in diameter. A nylon mesh with an aperture between 250 and 280 microns is the recommended size for these surveys. This small mesh size allows the collection of plankton samples which can then become available for studies on plankton species composition, etc.

Bongo samplers should incorporate salinity, temperature and depth sensors (CTD's). These sensors log the environmental information and allow its downloading once the station has been completed. The Bongo sampler should also be fitted with mechanical flowmeters (e.g. General Oceanics 2030) to enable the calculation of the volume of water filtered on each deployment.

It is also critical that participants understand the importance of calibrating flowmeters and changes in flowmeter performance when they are mounted in the apertures of plankton samplers (EU AIR3 CT94 1911). The WG recommends that all participants review the performance of their flowmeters and regularly check their calibration at the beginning and at the end of each egg survey (i.e. within the sampling device).

Moreover, an agreement should be reached on the deployment procedure in order to minimise possible biases due to turbulences caused by the propeller (if deployment takes place over the stern and not over the side of the boat), make sure a smooth ascend and descend profiles are achieved, etc.

Table 7.4.1: Currently used specifications for Bongo samplers in Spain and Portugal.

COUNTRY	NET	DIAMETER (CM)	SHAPE	MESH SIZE (µM)	TOTAL LENGTH (CM)
Spain (IEO)	Bongo	40	Conical	250	248
Spain (AZTI)	Bongo	40	Cylinder-cone	335	284.3
Portugal (IPIMAR)	Bongo	40	Cylinder-cone	250	227

8 Combination of North Sea and NE Atlantic mackerel survey data gears (referring to ToR "g")

8.1 Time series of North Sea mackerel egg surveys

The estimated egg production and corresponding SSB for North Sea mackerel is given in section 6 and in the text table below. The egg production estimates are considered underestimates of the actual egg production because the survey effort has been limited over the years. The complete spawning period has never been surveyed and the total spawning area has seldom been covered by sampling to zero eggs in all directions. The spawning period has been defined as 17 May–27 July based on daily plankton samples obtained from stand-by vessels in the Cod and Ekofisk areas (Iversen and Eltink, 1983). The surveys have usually been carried out in June/July to cover the presumed peak of spawning. Since the egg productions are considered as underestimated, the corresponding SSBs should also be considered as underestimates.

The egg production and corresponding SSB applying a fecundity of 1401 egg.g female-1 (see Section 6.4):

YEAR	1980	1981	1982	1983	1984	1986	1988	1990	1996	1999	2002	2005
Egg* 10^{-12}	60	40	126	160	78	30	25	53	77	48	147	156
SSB 1000t	86	57	180	228	111	43	36	76	110	68	210	223

8.2 Differences in estimates of fecundity and atresia – North Sea and NEAM

Due to the relative low egg production in the North Sea the fecundity has only been investigated twice, in 1982 and in 2005 (Iversen and Adoff, 1983, Krüger-Johnsen, WD 2006). The fecundity was observed to be very similar; 1401 and 1359 eggs g^{-1} female respectively in 1982 and 2005 (Section 6.4). The fecundity obtained in 1982 has been applied to convert the egg production to SSB for all the years. During the same period the fecundity in the western area has changed as shown below:

YEAR	1977–1983	1986	1989	1992	1995	1998	2001	2004
Egg/g female	1315	1246	1282	1431	1302	1003	1033	1052

In 1977, 1980 and 1983 the same fecundity was applied, and for the other years the fecundity and atresia have been obtained from samples for each of the survey years. The major change between 1995 and 1998 can be seen in these data.

In 2005 there was a very low prevalence and intensity of atresia in the North Sea samples (Table 6.4.3). These samples were collected in June and are taken to represent the average for the spawning season. In the western component with samples taken throughout the spawning season, potential fecundity was reduced by 17%, 6% and 7% in 1998, 2001, 2004 respectively, due to atretic losses (ICES, 2005) showing that there might be a higher degree of down regulation by atresia in the western component compared to the North Sea mackerel.

8.3 Calculation of variance estimates for North Sea surveys

Traditional approaches to estimate the variance of egg production estimates are based in the assumption that each sampled rectangle has a different mean but a constant coefficient of variation (CV). The within rectangle CV can be estimated from replicated hauls within a period. Nevertheless, although most rectangles were sampled in each period, there are no haul replicates by rectangle in the North Sea survey and therefore to calculate this CV it was necessary to combine data from different periods. Examination of 2005 survey data by period suggested that periods 2 and 3 could be combined as they both resulted in similar estimates of total egg production and had similar spatial coverage. Then, a CV of 3.66 was calculated by combining hauls by rectangle of both periods using the approach described by Costas *et al.* (WD ICES 2005).

This procedure does not assume that CV is equivalent to the residual standard deviation from the analysis of variance of log-transformed egg production by rectangle (having excluded those rectangles with any zero value hauls, and those no-replicate rectangles from the calculations) as was usually assumed in Western and Southern areas. This is due to the fact that above a value of 0.7, the coefficient of variation is systematically underestimated (Aitchinson and Brown, 1957). This approach follows Pope and Woolner (WD ICES, 1984) that describes an alternative way of estimating the CV on non-transformed values:

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

y = log-transformed daily egg production on rectangle

$$\sigma_y^2 = \text{Variance of } y$$

The CV value obtained is quite high but this is to be expected since the data used precludes a more precise approach. This CV was applied to compute the 2005 variance of daily egg production in each period (Table 8.3-1) and of the total egg production according to the approach applied by Fryer *et al.* (WD ICES, 1993). The North Sea mackerel annual egg production has been estimated by this method at 1.75 1014 egg with a variance of 1.65 1028.

Table 8.3-1: Variance estimates of North Sea mackerel daily egg production (egg/day) by periods:

PERIOD	1	2	3	4
Daily egg production	$3.63\ 10^{12}$	$4.28\ 10^{12}$	$3.89\ 10^{12}$	$2.48\ 10^{12}$
Variance	$1.45\ 10^{25}$	$2.58\ 10^{25}$	$9.47\ 10^{24}$	$4.52\ 10^{24}$
S.e.	$3{,}81\ 10^{12}$	$5.08\ 10^{12}$	$3.08\ 10^{12}$	$2.13\ 10^{12}$
CV	105%	140%	85%	58%

The analysis of the data from the North Sea survey used to provide the variance estimation presented here indicated that it would be necessary to increase the spatial coverage and the replication of hauls (at least in the areas with highest egg densities) in order to obtain more accurate estimates of egg production. This recommendation also applies to the Western and Southern areas where the egg production estimates are also not very precise.

8.4 Combination of biomass data

The WG examined the estimates of annual egg production and associated variances available from the North Sea mackerel egg surveys and the NE Atlantic Egg Survey time series with the view of obtaining combined estimates for the entire NEA mackerel stock. Confidence limits for the North Sea egg abundances were estimated by Iversen and Westgard (1984) for the 1982 and 1983 surveys. Given assumptions of eggs' distribution they estimated confidence intervals by re-sampling survey stations with replacement. North Sea mackerel egg survey was carried out several times since 1980 (Sections 6.5 and 8.1) to estimate SSB. Since 1996 they have been carried out every 3rd year but a year after the western and southern egg surveys. The WG agreed to ignore the year lag and to perform the calculations assuming that both estimates correspond to the year when the western and southern egg surveys took place.

Moreover, although most rectangles were sampled in each period there are no haul replicates by rectangle in the North Sea therefore variances by rectangle (required to compute a total variance using the approach developed by Fryer (ICES, 1996) could not be computed unless the data from different periods were combined. Examination of 2004 survey data by period suggested that periods 2 and 3 could be combined as they both resulted in similar estimates of total egg abundance and had similar spatial coverage. Once the rectangle CVs were computed by combining hauls by rectangle those CVs were applied to all periods to compute the 2004 variance of total egg abundance according to Fryer's approach.

9 Evaluation of variability in index value estimation in horse mackerel. (referring to ToR "h")

9.1 Historical fecundity data – Western stock

Historic estimates of western horse mackerel fecundity from surveys were assembled to address ToR h). This followed a request from the WGMHSA to provide information on precision, trends over time and likely upper and lower limits for this parameter.

We use the term "potential fecundity" here to define the fecundity calculated for fish at or before the start of the spawning season. In the case of a determinate spawner, this would represent the maximum amount of eggs that the fish could produce during the spawning season. Recent findings (ICES, 2003) suggest that horse mackerel is an indeterminate spawner, and may be capable of de novo vitellogenesis during spawning. At his point in time we do not know how much potential fecundity is enhanced by de novo vitellogenesis during spawning, but we have retained the term here, because it was used in the historic reports cited in this study.

Estimates of potential fecundity over time are shown in Figure 9.1.1. From 1977 – 1989 not much research was done into horse mackerel fecundity and for these years potential fecundity was set at 1589 oocytes per gram female, based on Nazarov's study in 1977 (Nazarov 1977; ICES 1984; 1987; 1988). In 1989 the first horse mackerel fecundity study was carried out for the Working Group (Eltink and Vingerhoed, 1989). Fecundity was estimated using the volumetric and histological method. Potential fecundity was 1478 and 1655 oocytes per gram female for both methods respectively. The later was adopted by the Working Group. A new study was performed in 1992; estimated potential fecundity was 1454 oocytes per gram female (Table 9.1.1, ICES 1993).

In 1995 mean potential fecundity was estimated as 1291 oocytes per gram female. However, many fish showed signs of spawning and therefore the 1995 period 3 fecundity estimates were considered incorrect. Only a very few fish were sampled in the 4th period so, fecundity was re-estimated by combining the 1995 mean with results from previous years, resulting in a new estimate of 1557 eggs per gram (ICES, 1996a). From 1995 onwards horse mackerel fecundity was investigated during every survey (ICES 1999, 2002a, unpublished 2004 data).

A sharp decline in estimated potential fecundity can be seen since 1995. Potential fecundity in 2004 was estimated as almost 1/3 of that estimated in 1989 (Figure 9.1.1). This decline is seen in the potential fecundity (mean for periods 3 to 6) as well as in the minimum and maximum fecundity per period. For the years 2001 and 2004 variability in fecundity was larger than in earlier years. This is probably due to the fact that many more samples were collected in 2001 and 2004 and that these were collected throughout the spawning season (Table 9.1.1).

Figure 9.1.2 shows potential fecundity over time in pre-spawning horse mackerel (based on samples collected during at the start of the spawning season). This shows the same trend as mean potential fecundity, with fecundity almost 3 times lower in 2004 compared to 1995. This trend in estimated fecundity could be related to the gradual disappearance of the dominant 1982 year class with young fish becoming dominant in the population.

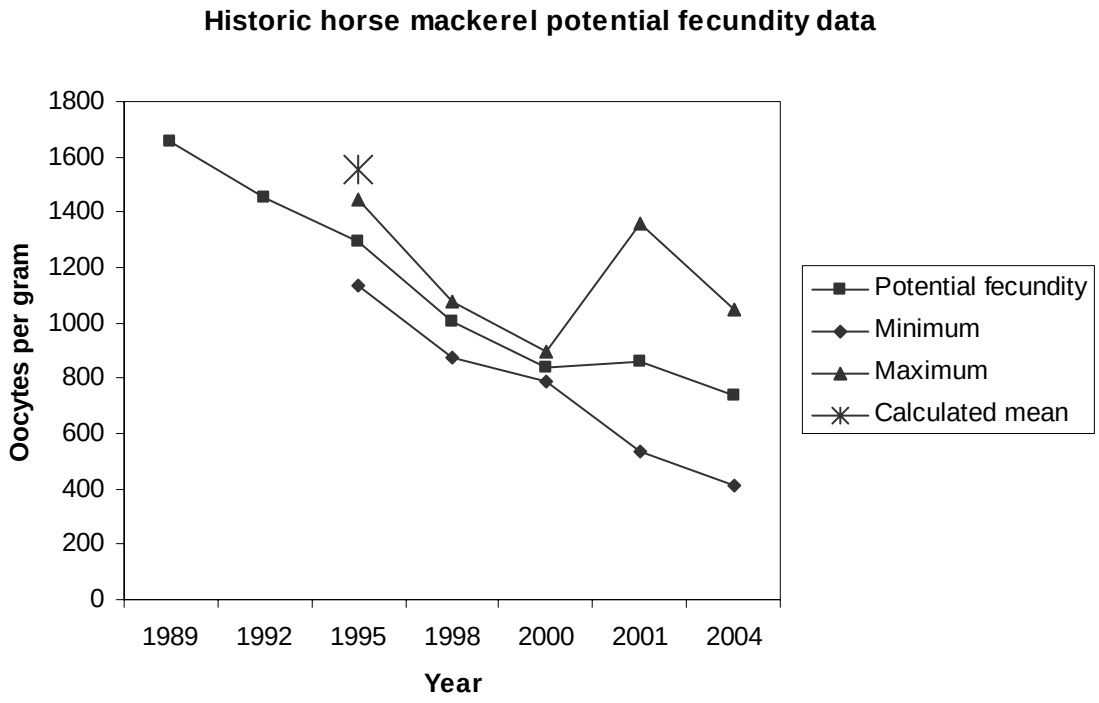


Figure 9.1.1: Historic western horse mackerel fecundity.

*** In 1995 WGMEGS decided to use a mean fecundity that was calculated from results from 1995, 1992 and 1989.**

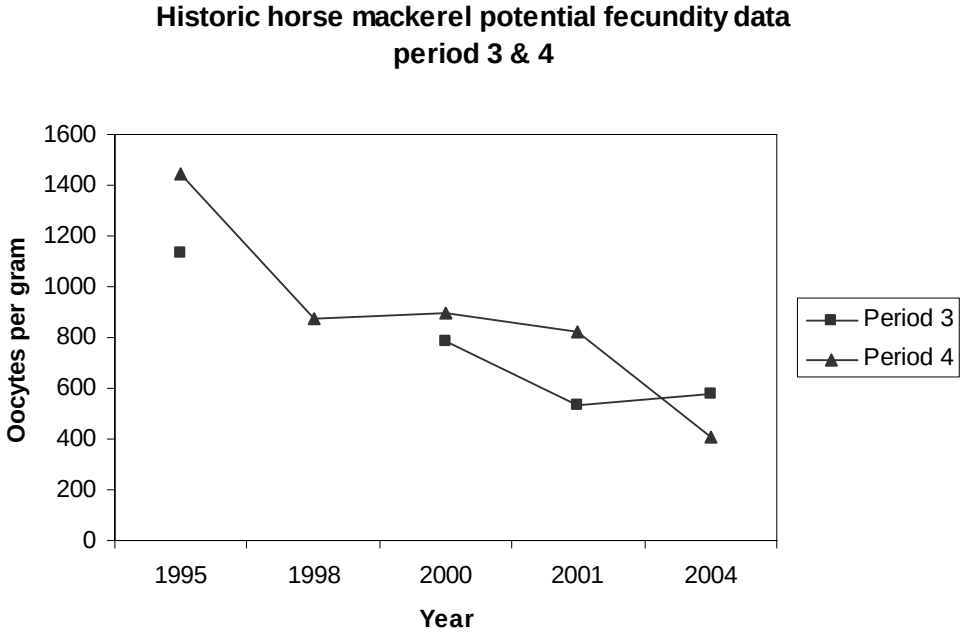


Figure 9.1.2: Historic western horse mackerel fecundity in periods 3 and 4.

Table 9.1.1: Historic western horse mackerel potential fecundity data.

YEAR	PERIOD	POTENTIAL FECUNDITY	NUMBER OF FISH
1989		1655	
1992		1454	31
1995 calculated mean[1]		1557	
1995	3	1136	12
1995	4	1446	18
1995	**mean**	**1291**	**30**
1998	4	872	4
1998	5	1078	5
1998	6	1071	2
1998	**mean**	**1007**	**11**
2000	3	785	10
2000	4	893	30
2000	**mean**	**839**	**40**
2001	3	532	71
2001	4	819	36
2001	5	721	21
2001	6	1361	43
2001	**mean**	**858**	**171**
2004	3	576	60
2004	4	409	57
2004	5	914	5
2004	6	1046	44
2004	**mean**	**736**	**166**

[1] In 1995 a mean was calculated based on data collected in 1987, 1988, 1992 and 1995.

9.2 Historical fecundity data –Southern stock

A set of adult parameter estimates from samples taken every third year from 1992 to 2004 and in 2002, were presented for the Southern horse mackerel (Table 9.2). Standing stock fecundity (Ft) corresponds to samples taken from pre-spawning females while batch fecundity was measured from maturity stage 4 females. Examination of changes in batch fecundity and their associated CVs suggests stability for that estimate during the period. Similar conclusion applies to standing stock fecundity. Spawning fraction appears to be more variable although the CVs are higher, so the estimates across years may not be significantly different from each other. Spawning fraction is likely to change over time depending on the population age structure. But high spatial variability is also expected during the period of a survey therefore a large number of samples are probably required to obtain precise estimates of that parameter.

Table 9.2: Southern horse mackerel. Estimates of standing stock fecundity (Ft, eggs/g), batch fecundity (Fbatch, eggs/g), spawning fraction (SF) and coefficients of variation (CV).

Year	Month	Ft	CV	Fbatch	CV	SF	CV	Area
1992	Feb			173	0.06	0.04	0.58	Portugal
1995	Jan-April	*526	0.03					Port+Spain
1998	Jan-April	*245	0.08					Port+Spain
2001	Jan-April	*578	0.02					Port+Spain
2002	Jan			172	0.10	0.10	0.21	Porugal
2004	Jan-April	*619	0.04					Port+Spain
2004	Jan	*060		169*	0.03	0.19	0.22	Portugal
2004	Feb	*500		165	0.10	0.33	0.06	Portugal
2004	Mar	*720		170	0.03	0.25	0.17	Portugal
2004	April	*171	0.04					Spain

***Weighted average (February and March).**

9.3 Scope for potential error in horse mackerel egg identification

A plankton sample exchange was initiated in 2001 to test the precision in egg sorting, identification and staging of the individual laboratories. The results from this sample exchange (WD Milligan and Shaw, ICES, 2004(b)) showed significant differences between the participants in the numbers of eggs retrieved from the plankton samples, the identification of those eggs to species and the staging (ageing) of the eggs. These differences caused real concern for members of WGMEGS. Consequently, the Working Group recommended (ICES, 2002a) that a further egg workshop (WKMHMES) be held at Cefas in 2003 (following a successful egg staging workshop in 2000 (ICES, 2001), but this time to evaluate egg sorting and identification as well as egg staging (ICES, 2004(b)).

The sample exchange in 2001 involved three samples being passed from institute to institute where the fish eggs were removed from the samples, identified, staged and counted. The eggs were then returned to the samples before being sent to the next institute. For the first two samples, the differences in the number of horse mackerel eggs identified, ranged from 83% to 127% of the mean numbers identified (478 and 440) by each of the 9 participants. The third sample caused even greater concern as the number of horse mackerel eggs identified ranged from 25% to 151% of the mean (547).

These results can be regarded as extreme estimates of the errors involved with horse mackerel egg identification. Inevitably, as the samples were passed from institute to institute, some eggs were damaged or lost. The number of eggs lost was difficult to quantify as even total fish egg numbers varied from one participant to the next. It was consequently, extremely difficult to separate sorting errors from identification errors in this analysis. When the samples were re-analysed at CEFAS, following completion of the sample exchange, it was noted that sample condition had deteriorated to the point where it was very difficult to be certain of species identification. This would make identification of the eggs much more difficult for the participants who received the samples towards the end of the exchange. This exchange therefore puts extreme limits on the mis-identification of horse mackerel eggs, as real survey samples would not be subject to either the loss or damage experienced by the exchanged samples.

The second workshop on mackerel and horse mackerel egg staging and identification (WKMHMES, ICES 2004(b)) was charged with addressing egg identification and sorting, as well as egg staging. It was much easier to assess egg identification, independently from sorting and staging, during this workshop. Agreement between participants for horse mackerel egg identification increased from 70% agreement to 88% agreement between the two rounds of analysis. Again, the levels of agreement in these results are probably lower than in the

analysis of real survey samples. Not only were the eggs old (preserved for >2 years), some of the validated eggs were unusual (little or no segmentation of the yolk; usually a main diagnostic feature of horse mackerel eggs). In addition, most of the eggs were from survey plankton samples (i.e. not of known parentage) and some of the eggs were accidentally moved from one well to another, again causing problems when the results were analysed.

It is hoped to address these problems in the forthcoming third WKMHMES workshop in 2006, by using validated, naturally spawned eggs, from captive fish held at IMR, Matre, Norway. These eggs will be removed gently from the holding tanks and preserved immediately in 4% buffered formaldehyde, minimising damage as far as practicable.

9.4 Scope of survey sampling variance

One potential source of variability in the annual egg production is the potential for single or small numbers of high abundance samples. To an extent skewed distributions are to be expected in all marine resource surveys, however there was a perception that it may provide a part of the explanation for the mismatch between the egg abundance and the assessment models. The impact of these high amplitude samples was examined in this context.

2004 survey

The 2004 survey in the western area was divided into five periods, periods 3 – 7. Table 9.4.1 shows the summed daily egg production values (stage 1eggs.day-1.m^{-2}) by period for the 2004 survey. It also shows the single highest daily egg production value in each period and as a percentage of the total. The next row shows the number of days in each survey period. The following row is the daily egg production multiplied by the number of days as a proxy for period egg production. The final row shows the period egg production calculated without the single highest value in each period.

Table 9.4.1: Influence of largest sample value on period egg production estimation.

PERIOD	P3	P4	P5	P6	P7
Total (eggs.m^{-2}.day^{-1})	714	1040	2623	6539	3281
Largest sample (eggs.m^{-2}.day^{-1})	176.5	143	917	2327	2082
as a percentage	25%	14%	35%	36%	63%
total minus largest sample	537.5	897	1706	4212	1199
days for the period	16	24.5	24	12.5	20.5
total * period days	11424	25480	62952	81737.5	67260.5
(Total – largest sample) * period days	8600	21976.5	40944	52650	24579.5

Summed total daily egg production multiplied by the period duration can then be used as a proxy for the actual total annual egg production. As such the egg production has not been raised to the rectangle area, and there was no period interpolation applied. However, this provides a good approximation for the egg production curve and is able to give an approximate indication of the scale of variability that might be introduced if these exceptional large values were missed by the survey. The two values for total annual egg production (proxy) would then be:

- Actual value – using all samples = 248854
- Reduced value – without largest samples = 148750
- Percentage difference = 40%

These values then suggest that if none of the exceptionally large samples were encountered the annual egg production estimate would be 40% lower. Broadly then, the survey result could vary by a factor of 2 depending on this small number of high values.

The study was extended over the last three egg surveys in 1998, 2001, and 2004, although in less detail. In this case the contribution to the total of the 10 largest samples was examined. Again the egg production values were totalled, this time for all periods combined. In addition the egg production contributed by the top 10 samples was calculated, and their percentage contribution determined. The same calculations were performed for mackerel in these surveys for comparison. The results are presented in Table 9.4.2 below.

Table 9.4.2: Total annual egg production for horse mackerel in 1998, 2001 and 2004 detailing contribution from the largest 10 samples.

YEAR	EGG PROD^N TOTAL	EGG PROD^N TOP 10	H. MACKEREL TOP 10 % CONTRIBUTION	MACKEREL TOP 10 % CONTRIBUTION
2004	14197	7932	52	18
2001	11976	3434	29	21
1998	16314	8167	50	23

To provide a perspective over the complete survey time series, the contribution of the top 10 samples for stage 1 horse mackerel eggs was calculated using sample values rather than egg production values. The perception for the three most recent surveys is very similar, with higher contributions in 1998 and 2004, than 2001. The top 10 contribution tended to be lower through the rest of the 1990s and 1980s, but increased substantially in 1980 and 1977. It is worth noting that the stock level was very low at that time, but also that the surveys were in an early stage of development as well. The results are presented in Figure 9.4.1

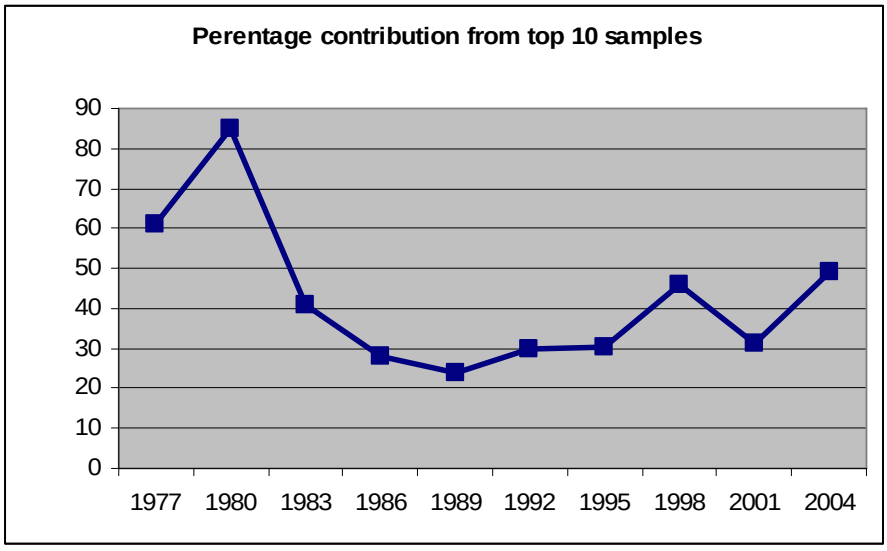


Figure 9.4.1: Percentage contribution of largest 10 samples to sample totals in all survey years.

Conclusion

The sampling for horse mackerel eggs is clearly more variable than that for mackerel, and the contribution by a few large values is proportionally greater. This may reflect the differences in SSB levels. Based on the 2004 data the egg production estimate could vary by a factor of two depending on encounter with such samples. The 2001 and 1998 surveys also indicated a

higher variability is sample values than for mackerel suggesting that this is a characteristic for the sampling of eggs for this species, at least in recent years, with a low SSB. It should be noted that in 2004 single large value samples were taken in all five periods, and that missing all of these would be unlikely. Using sample values only rather than daily egg production suggests that this variability has increased in recent years, was lower throughout the high biomass period when the 1982 year class dominated the stock, and was high again before that year class had recruited. There is no evidence of a trend over the period 1977 to 2004, although not surprisingly, variability was higher when SSB was low.

9.5 Implications for the use of the annual egg production estimate in the assessment

A benchmark assessment of Western horse mackerel was performed in 2005 by the WGMHSA using a number of VPA assessment models. Estimated spawning stock biomasses (SSB) from two of the models that fitted the catch-at-age data and the egg estimates from the triennial surveys generally followed the eggs' trend. However, the Integrated Catch Assessment Model (AMCI) was fit only to the catch-at-age data and did not include the egg production data as an index of SSB (Figure 9.5.1. – ICES, 2006). The relationship between the SSB and the egg estimates over time is illustrated by the ratio between those estimates and is shown Figure 9.5.2. An increasing trend from 1986 to 2001 following by a decline since then is suggested by the plot of the ratios. This effect could be the result of an increase in relative fecundity as the strong 1982 year class joined the spawning stock followed by a decline as it gradually disappeared. The time-series of catch numbers at age (Figure 9.5.3) suggests that the 1982 year class recruited to the fishery in 1982 but still represented a substantial fraction of the catch as a plus group in 1995. After that year their contribution to the fishery, and probably to the population, declined steadily.

A number of issues that contribute to the variability in the egg production estimate for horse mackerel were examined by the group (Sections 9.1–4) in an attempt to explain the change by a factor of 4 in the Eggs/SSB relationship in the period of the assessment. A sharp decline was observed in horse mackerel estimated standing stock fecundity since 1995 from both the overall mean fecundity as well as in the minimum and maxima estimates per period, see Section 9.1. So, it is possible that the decline in egg production estimates (Figure 9.5.1) was caused by a decrease in both egg production and relative fecundity as the 1982 year class declined and the population became dominated by younger fish. Little historic data showing trends in fecundity is available for similar stocks. Historic data from southern horse mackerel suggests that fecundity has been relatively stable for that stock since 1995 (Section 9.2).

There is at present large uncertainty about the absolute level of horse mackerel total realised fecundity (Ft). As the assessment results are heavily dependant on the egg survey estimates an average Ft across the assessment period would allow scaling the model estimates of SSB. The WG looked at historic data in an attempt of providing upper and lower bounds for that estimate. A frequency distribution of historic values of standing stock fecundity is shown in Figure 9.5.4 based on data presented in Section 9.1. Examination of Figure 9.5.4 suggests that it is possible that on average realised fecundity could lie within a range of 400–1800 oocytes/g female.

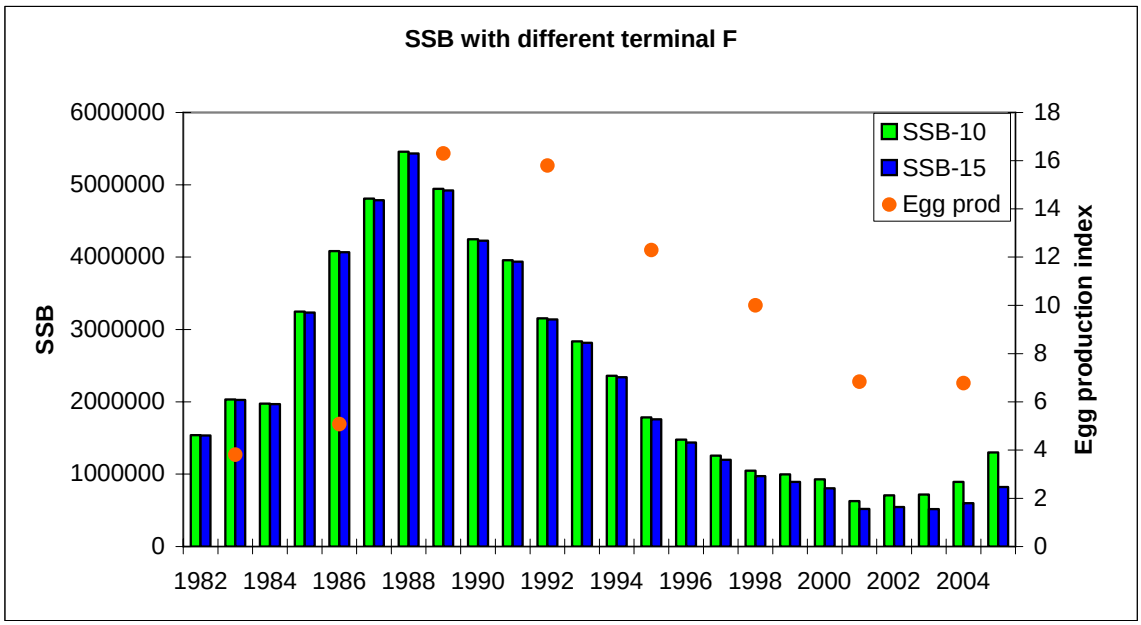


Figure 9.5.1: Western horse mackerel. Time-series of SSB as estimated by the WGMHSA using AMCI (using 0.1 and 0.15 as terminal fishing mortalities) and estimates of Egg Production from the triennial surveys.

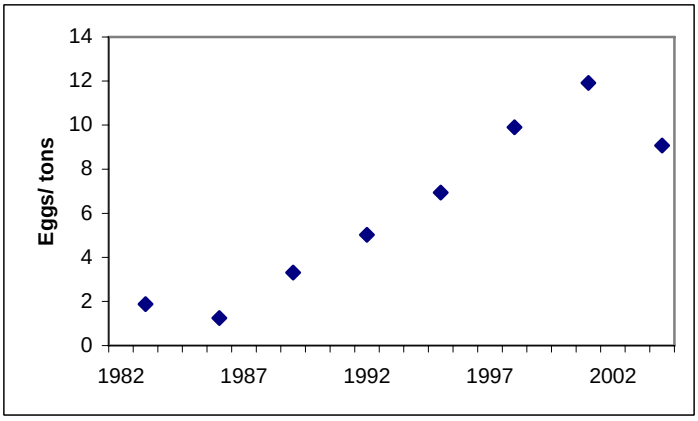


Figure 9.5.2: Western horse mackerel. Egg production estimates (times 108, from Triennial survey) to estimated SSB (from AMCI assessment model).

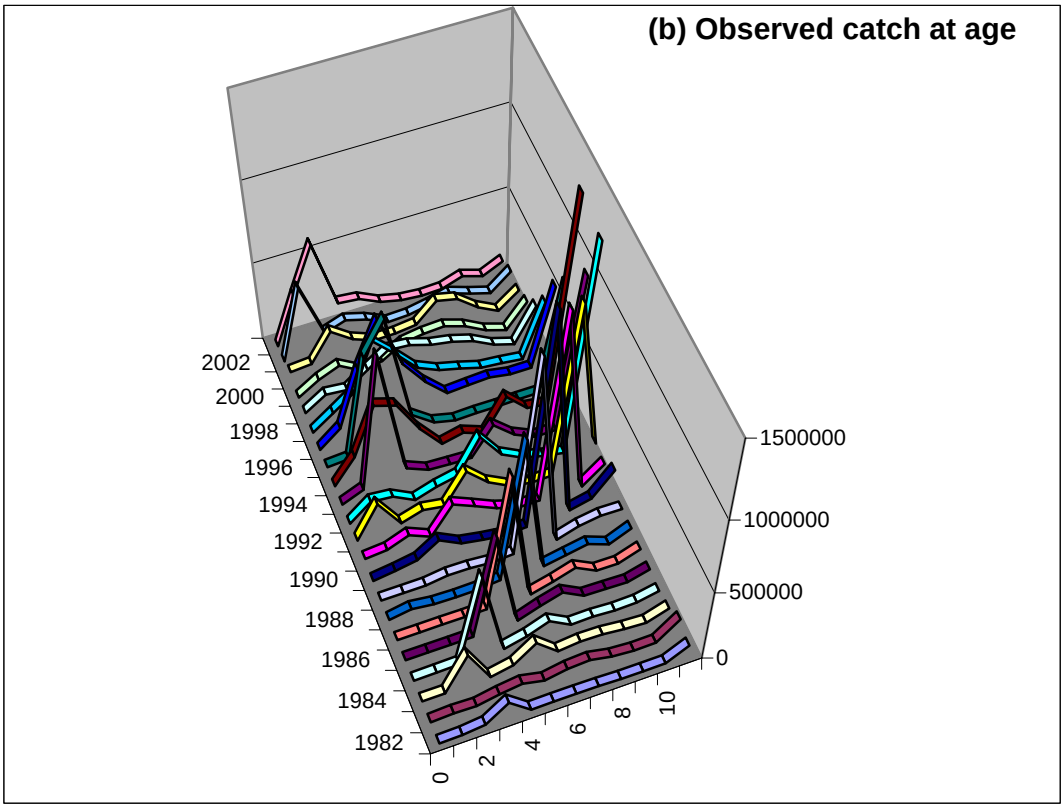


Figure 9.5.3: Western horse mackerel. Catch-at-age data used as input data in the 2005 stock assessment (WGMHSA 2005 Report). The highest pick in the 11 plus age-group corresponds to 1995.

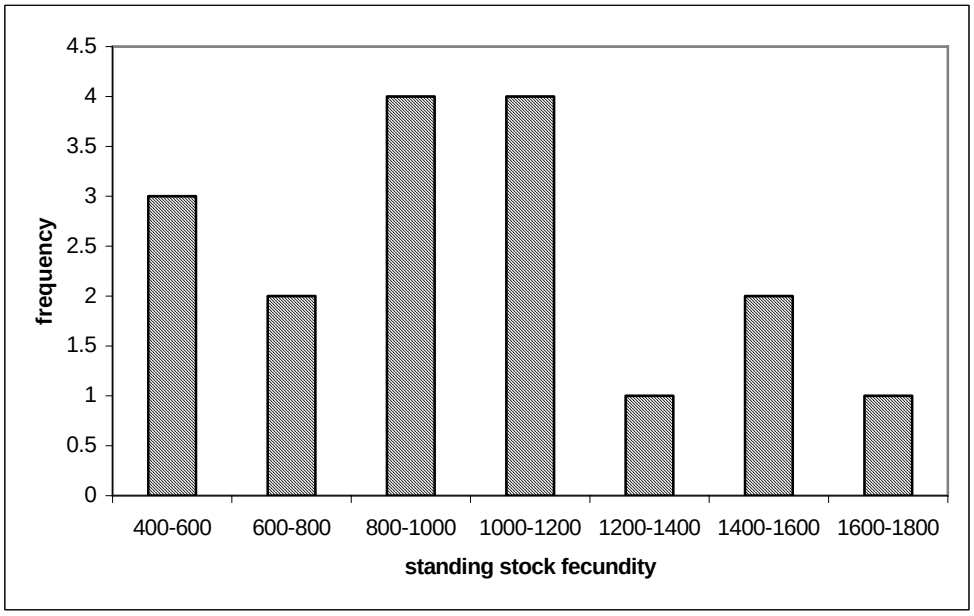


Figure 9.5.4: Frequency of standing stock fecundity (oocytes/g) from historic series (Section 9.1).

10 Scope for the use of the historical survey data for further analysis with respect to other species

10.1 Inventory of samples and data available

As recommended in the ICES WGMEGS report 2005, all egg samples from the triennial surveys combined were reanalyzed to obtain additional information.

AZTI (Spain) reanalyzed the following number of samples from the 2004 surveys: 100 samples from Netherland (IMARES), 65 from CEFAS (England) and 151 from AZTI (Spain). CEFAS reanalyzed their own 147 samples. Target species were, as recommended, mackerel and horse mackerel larvae as well as eggs and larvae of anchovy, sardine, hake, megrim and blue whiting. All the larvae were measured and the eggs were staged. At IEO (Spain) all larvae were extracted and anchovy and sardine eggs were identified from 2004 samples.

Data available and the analysis completed until now at AZTI, CEFAS, IMR (Norway) BFA (Germany) and IMARES (Nederland) are showed in Annex 1.

11 Equations for egg development rate with temperature

The equation describing the relationship between egg development and temperature is an important parameter for the Working Group of mackerel and horse mackerel egg surveys, (WGMEGS), as it is directly used to calculate the daily egg production for mackerel. Lockwood *et al.* (1977), presented data on the egg development times in relation to temperature for Northeast Atlantic Mackerel, and this model is used to calculate daily egg production by WGMEGS. In 1989, Nichols and Warnes (1993) repeated the experiments of Lockwood, increasing the precision in the determination of the age of the eggs particularly in the early stages of development. In 2004, a temperature controlled incubation experiment was carried out on mackerel eggs from the southern component (WD, Alvarez and Mendiola, ICES, 2005). The results were significantly different from those of Lockwood.

The application of a new temperature development equation to the calculation of the daily egg production may have strong consequences to the results and the subsequent estimation of the spawning stock biomass of mackerel. The Marine Institute, Galway, Ireland, intend to repeat the work of Alvarez with artificial fertilised eggs from mackerel caught in the western area. The results will be presented to WGMEGS in 2008, and incorporated into the working group report.

12 Deficiencies and Recommendations

12.1 Deficiencies

The major deficiency noted by the WG was the withdrawal of CEFAS from the 2007 survey. This will have the impact of reducing the coverage and accuracy of the survey. CEFAS also took a lead role in the histological analysis for fecundity and atresia, and were prominent in the evaluation of species ID and sample sorting. While the survey can be carried out without CEFAS it will undoubtedly affect the quality of the result. The WG regretted the withdrawal.

12.2 Recommendations

12.2.1 Fecundity estimation

WGMEGS recommends that for measuring fat content in horse mackerel a fatmeter should be used. The fatmeter is a quick method of measuring fat content. The cost of acquiring a fatmeter would be outweighed by the cost of personnel and environmental pollution of the method used in the previous survey.

WGMEGS recommends that in those periods where sampling of adult mackerel or horse mackerel is not possible during the egg survey, institutes should try and collect samples via their commercial sampling program in order to ensure enough fecundity and atresia samples are collected.

WGMEGS recommends that an experimental study be initiated to study reproductive potential of mackerel under different temperature and feeding regimes. The experiment can take place at the Matre Aquaculture Station, Norway.

12.3 Adoption of the agenda

13 References

This reference list includes all those used in this report of WGMEGS, and also a range of useful references in relation to the work of the group in recent years.

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14 Working documents presented to the Working Group

1. Egg production and spawning stock size of mackerel in the North Sea in 2005

Svein A. Iversen[1], Cindy van Damme[2] and Guus Eltink[2].

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Abstract

The mackerel spawning area in the North Sea has been surveyed since 1968. Since 1996 (Iversen and Stæhr, 1996) surveys have been carried out on a triennial basis. In 2002 (Iversen and Eltink, 2002) the Netherlands and Norway surveyed for 40 days between them, covering the spawning area three times, with the peak spawning observed during the last survey. This has implications for the reliability of the egg production curve (Figure 5). In 2005, the survey was again carried out by both countries, from 6 June to 3 July, totalling 38 days. During this survey the spawning area was covered four times, with the first and last surveys being carried out by one vessel, while the second and third were carried out by both vessels. The egg production is underestimated since the spawning area was not totally covered. SSB was estimated at 223,000 tons.

2. Spray technique for a fast and accurate automatic separation of fish eggs from plankton

Guus Eltink

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Abstract

A fast automatic method for the separation of fish eggs from plankton samples has been developed, because each egg sorting procedure requires less than 3 minutes. Therefore, a targeted high accuracy can be achieved by repeating the spray method. It appeared to be a factor 25, 60 and 110 faster than the traditional manual method when using samples with respectively low, medium and high plankton. It can successfully be applied onboard research vessels and it can cope with different plankton compositions. Its egg fractions contained less contamination by plankton particles than from the manual method. This new method is much less prone to human-errors and can be standardised. The accuracy and precision in egg sorting achieved in this study are not representative and are an example how well this method might

work. Therefore, it is an absolute requirement to estimate at regular intervals the achieved accuracy. Prior to the egg sorting it is recommended to set a targeted accuracy in egg sorting and to write out a working procedure how often to apply the spray technique per plankton sample. The accuracy can be estimated afterwards by thoroughly checking whether any eggs had been left. If necessary the working procedure can be adjusted to achieve the targeted accuracy.

3. Fecundity of North Sea mackerel in 2005

Maria Krüger-Johnsen

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Abstract

Since no new fecundity data has been obtained since 1982 (Iversen and Adoff, 1983), WGMEGS, in 2005, agreed to run a small scale fecundity and atresia study. Samples were collected during the egg survey in the North Sea in June and July 2005. In addition CEFAS provided samples from the Dogger Bank in May 2005. Samples were sent to IMR for analysis. From a sample size of 36 ovaries, only 15 were appropriate for fecundity analysis, and of these 15 samples all but three contained spawning markers. There is thus evidence suggesting that the fish had started spawning and that the results on potential and realised fecundity need to be interpreted with some caution. The relative fecundity of 1359 oocytes g^{-1} female estimated in the present study is comparable with that observed in 1982 of 1401 oocytes g^{-1} female.

4. Horse mackerel (*Trachurus trachurus*) southern stock evaluation by Daily Egg Production Method (DEPM)

Costa, A.M.[1], Vendrell,C.[1], Pissarra, J.[1], Murta, A.[1], Gonçalves, P.[1],.Farinha, A.[1], Franco, C.[2], Pérez, J.R.[2],Lago de Lanzós, A.[2] , Baldó, F.[2]

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Abstract

As a recommendation of the ICES Working Group on Mackerel and Horse Mackerel Egg Surveys (ICES 2005), horse mackerel southern stock spawning biomass will be assessed by Portugal and Spain during the 2007 spawning season by means of the Daily Egg Production Method (DEPM).

Spatial distribution and abundance estimates of fish eggs in the horse mackerel southern stock (ICES Division IXa) will be obtained during a 35 days cruise on February/ March 2007.

Adult fish parameters (mature female mean weight, batch fecundity, spawning fraction and sex ratio) will be simultaneously estimated trough fishing stations in order to apply the DEPM to evaluate the horse mackerel spawning-stock biomass in the area.

The present document is intended to describe the proposed methodology of DEPM sampling and estimation for horse mackerel in the southern stock.

An application of this methodology to the horse mackerel southern stock with data from 2002 triennial DEPM for sardine and anchovy egg survey and 2004 triennial AEPM for mackerel and horse mackerel egg survey is presented.

5. Horse mackerel (*Trachurus trachurus*) southern stock 2007 cruise planning

Pissarra, J.[1], Costa, A.M.[1], Vendrell, C.[1], Franco, C.[2], Pérez, J.R.[21] *Instituto Nacional de Investigação Agrária e das Pescas – IPIMAR (Portugal)*

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Abstract

2007 southern horse mackerel egg surveys will cover the new defined stock area corresponding to ICES Division IXa (36º to 43º N).

Also horse mackerel southern stock spawning biomass will be assessed by Portugal and Spain during 2007 spawning season by means of the Daily Egg Production Method (DEPM).

Portugal/IPIMAR will perform in February-March 2007 a 35 days cruise with RV "Noruega", from Gibraltar to Finisterre, in order to collect egg samples and catch adult fishes.

6. Experimental study of growth and reproduction in Atlantic horse mackerel

Maria Krüger-Johnsen

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Abstract

A presentation was given on the status of the experiment running in Matre Aquaculture Station, N-5984 Matredal from October 2005 – October 2006. The objectives as well as the sampling methods were presented. So far the fish seem to have adapted well to their captive environment and natural mortality has been low. For more information please visit http://www.horse-mackerel.imr.no

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Annex 2: WGMEGS Terms of Reference 2006

The **Working Group on Mackerel and Horse Mackerel Egg Surveys** [WGMEGS] (Chair: P. Alvarez*, Spain) will work by correspondence in 2006/2007 to:

a) examine the results of the Lowestoft workshops (October 2006) on mackerel and horse mackerel egg staging and identification and histology, and incorporate these into the Survey Manual for the 2007 survey;

b) fine-tune survey execution in 2007.

WGMEGS will report by 1 June 2007 for the attention of the Living Resources and the Resource Management Committees.

Supporting Information

PRIORITY:	Essential. Terms of Reference are set up to provide ACFM with the information required for responding to requests for advice/information from NEAFC and EC DGXIV.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	Action Plan No: 1. The egg survey provides the only fishery-independent stock estimate for north-east Atlantic mackerel and for both the western and the southern horse mackerel stocks. The surveys provide the most essential indices for the tuning of the VPAs. The survey is based on a time series since 1977. The ToR for this year is largely routine, as the group does not meet in the year of a survey. Term of Reference a) WGMEGS has previously sponsored pre-survey Workshops in 2000 and 2003. These are essential to standardise many aspects of the survey protocol, but particularly egg sample collection, sorting, species ID and staging. In 2003 the workshop was expanded to provide the same standardisation for the histological work required for the survey estimates; fecundity and atresia. As the surveys are held only once every 3 years it is vital to have all participants working in concert. The workshop will make recommendations for survey procedures and analysis, and these will be assimilated into the survey manual and used for the 2007 survey Term of Reference b) The 2006 report of WGMEGS outlined the *provisional* plan for the 2007 surveys. The group will maintain a watching brief on how this transpires in practice. The main actions are to ensure that the best coverage is obtained for the survey in the five survey periods. Problems with weather, vessels etc must be taken account of. The group will also maintain oversight of the adult sampling aspects of the work, to ensure the best temporal and spatial coverage of these samples.
RESOURCE REQUIREMENTS:	None. The surveys are all part of the national programmes. The surveys and associated meetings are also partially funded under the EU data directive
PARTICIPANTS:	N, NL, P, ESP, UK (E), UK (Scot), D, IRL. Usually 25 – 30 participants
SECRETARIAT FACILITIES:	None.
FINANCIAL:	No financial implications.
LINKAGES TO ADVISORY COMMITTEES:	ACFM.
LINKAGES TO OTHER COMMITTEES OR GROUPS:	Reports to the Living Resources and the Resource Management Committees, as well as WGMHSA. Other less formal links with SGRESP, WKSAD, and WGACEGG
LINKAGES TO OTHER ORGANIZATIONS:	There are or have been a number of associated EU funded projects which make reports to the Group
SECRETARIAT MARGINAL COST SHARE:	ICES: 100%.

Annex 3: Recommendations

RECOMMENDATION	ACTION
1. All survey participants should collect biological material for genetic analysis from samples taken as part of the adult sampling programme for mackerel.	Survey teams to collect samples
2. That each institute participating in the 2007 mackerel and horse mackerel egg survey has at least one scientist/technician at the egg workshop (WKMHMES)	Survey teams to send at least one active person to WKMHMES
3. That a standardised design of Bongo sampler should be followed to minimise the risk of sampler bias and to ensure consistency between samples and surveys. This standardised design should be approved by the WG	Spanish and Portuguese institutes to produce a standard design and implement for the 2007 surveys
4. The WG recommends that all participants review the performance of their flowmeters and regularly check their calibration at the beginning and at the end of each egg survey (i.e. within the sampling device).	Survey teams to ensure calibration of samplers
5. That all participants update the inventory of samples and data available from the historical surveys	Survey teams to make inventory and forward to AZTI for archival