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Report of the

Working Group on Mackerel and Horse Mackerel Egg Survey

1–4 April 2003
Lisbon, Portugal

International Council for the Exploration of the Sea

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1 INTRODUCTION

1.1 Terms of reference

At the ICES Annual Science Conference in Copenhagen, Denmark, in September 2002 it was decided that (C.Res. 1999/2G01) the Working Group on Mackerel and Horse Mackerel Egg surveys [WGMEGS] (Chair Dave Reid, Scotland, UK) will meet in Lisbon, Portugal 01-05 April 2003 to:

a) coordinate the timing and planning of the 2004 Mackerel/Horse Mackerel Egg Survey in the ICES Sub-areas VI to IX, and of the 2005 Mackerel Egg Survey in ICES Sub-area IV, for estimating the spawning stock size;
b) coordinate the planning and sampling for fecundity and atresia taking into account the recommendations of the WGMHSA on whether or not any changes should be made to the 2001 data set.
c) Review research on the determination of fecundity in mackerel
d) Examine current and potential future variance calculation procedures
e) Review procedures for egg sample sorting, species ID and staging
f) review the procedures and methodologies for the conversion of total annual egg production to biomass for horse mackerel in the light of the problems identified in the 2001 survey.
g) review the results of the 2002 North Sea Egg Survey;
h) develop protocols and criteria to ensure standardization of all sampling tools and survey gears.

1.2 Participants

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2 NORTH SEA EGG SURVEY IN 2002 (REFERRING TO TOR "G")

2.1 Spatial and temporal coverage

During the period 3-24 June 2002 the Netherlands and Norway carried out egg surveys in the North Sea to estimate the spawning stock biomass (SSB) of mackerel (Iversen and Eltink WD WGMHSA 2002). During this period the main part of the spawning area was covered three times. The last time egg surveys were carried out in the North Sea were in 1999 and 1996.

2.2 Sampling and Data Analysis

The handling of the samples and data were done according to ICES (1997/H:4), while the sampling deviated from this. The Netherlands carried out their survey with a Gulf III working in double oblique hauls from the surface to 5m above the bottom. Norway carried out their survey with a 20 cm Bongo towed for 5 minutes in each of the depths 20m, 15m, 10m, 5m and in the surface. Norway has applied this method since 1980. In the North Sea most of the mackerel eggs are distributed in the surface layer. It is therefore important not to over sample this layer. Norway has experienced problems with double oblique hauls while the Netherlands are confident with their sampling.

The timing and the results of the surveys are given in Table 2.4.1. The "G.O. Sars" and "Tridens" worked respectively mainly the area north and south of 56^{o} N. The eggs were sorted from each of the sampled stations and their age were estimated according to development stage and the observed temperature at 5 m. The development stages used in the calculations are eggs in stages 1A+1B (Lockwood et.al., 1981).

2.3 Mackerel egg distribution

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 2.3.1-3). The samples were obtained in the middle of each of the rectangles. The egg production was calculated for the total investigated area for each of the three periods (Table 2.4.1). During all three coverages very high egg production (197-753 eggs/m^2) was observed in the western part of the spawning area. About 20, 30 and 40% of the total egg production during the three respective coverages came from these rectangles.

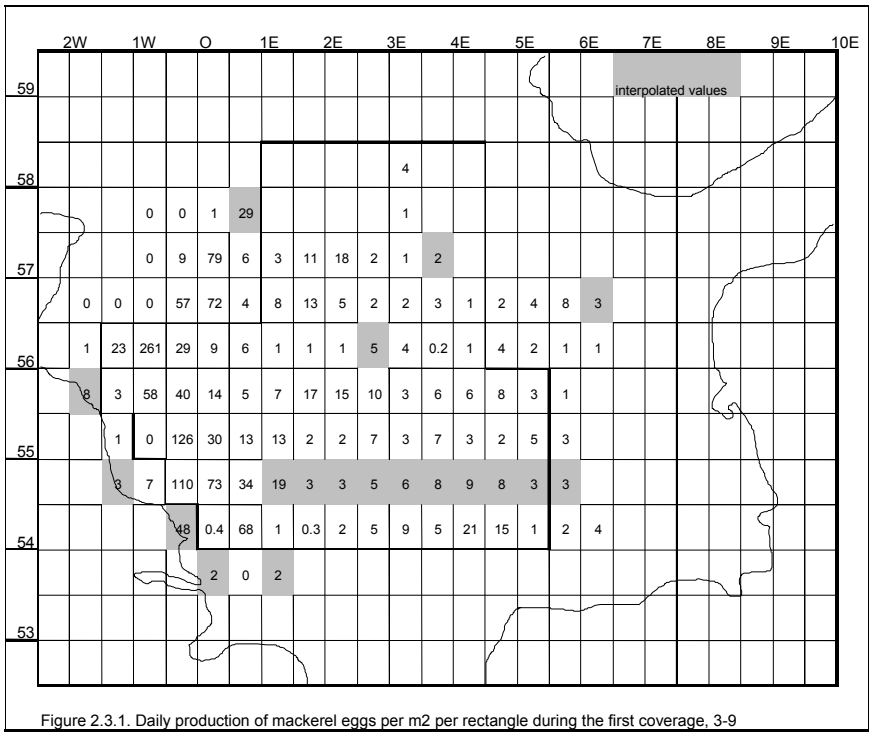


Figure 2.3.1. Daily production of mackerel eggs per m2 per rectangle during the first coverage, 3-9

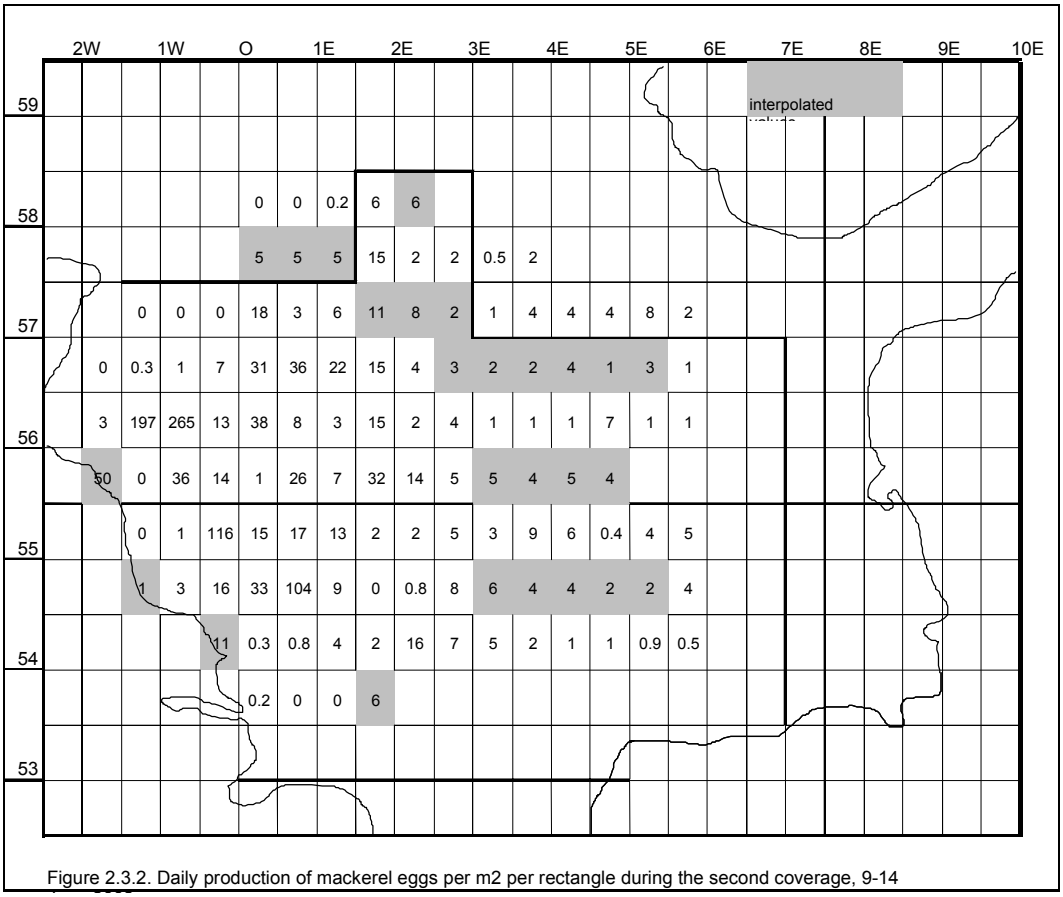


Figure 2.3.2. Daily production of mackerel eggs per m2 per rectangle during the second coverage, 9-14

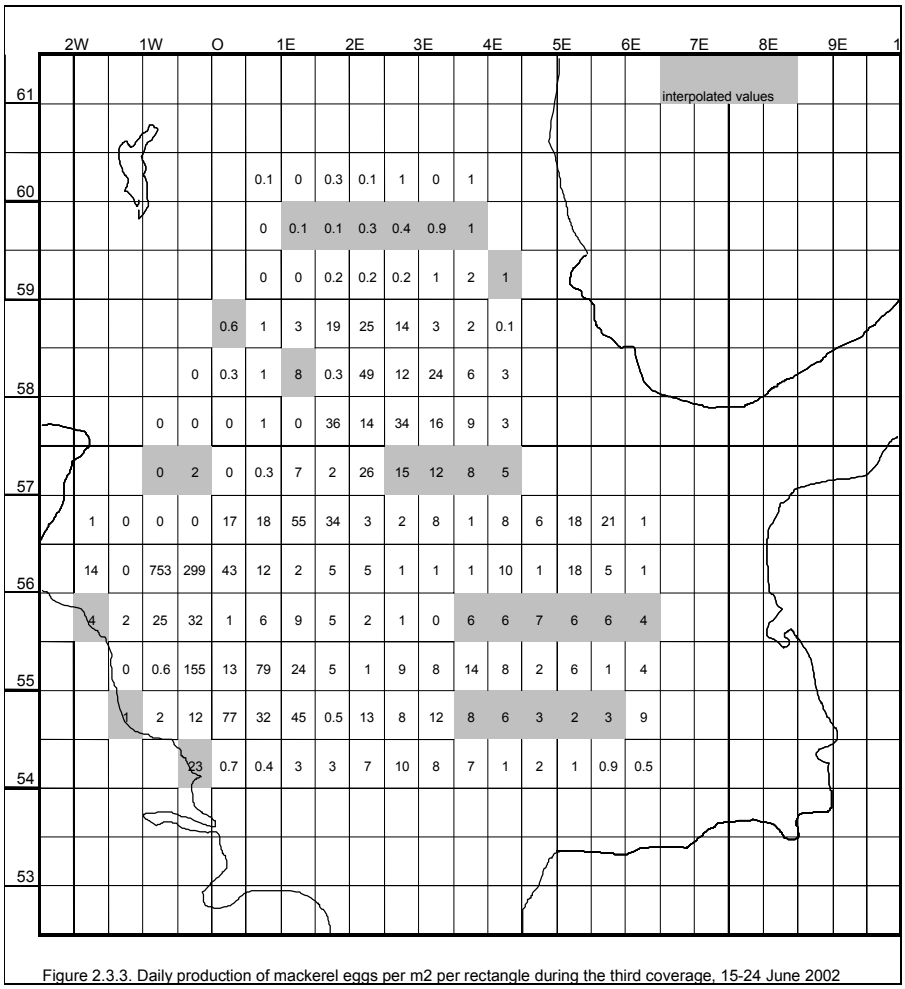


Figure 2.3.3. Daily production of mackerel eggs per m2 per rectangle during the third coverage, 15-24 June 2002

2.4 Mackerel egg production and spawning stock size estimate

The surveys did not cover the total spawning area and period. Some of the unsampled rectangles were given interpolated values according the standard interpolation rule. The contribution of the interpolated egg production was about 10% of the total production estimates during the two first coverages and about 5% during the third coverage. The parameters necessary for drawing the egg production curve and calculation of the egg production and SSB are given in Table 2.4.2. Based on the three production estimates the spawning curve was drawn (Figure 2.4.1). The three production estimates are considered minimum estimates since the sampling was not carried out until zero values were obtained in all directions.

Table 2.4.1. Mackerel egg surveys in the North Sea in 2002.

Coverage	1	2	3
"Tridens"	3-6 June	10-14 June	17-21 June
"G.O. Sars"	3-9 June	9-14 June	15-24 June
Midpoint of survey	6 June	12 June	19 June
Julian day	157	163	170
Total daily egg x 10^{-12}	2.72	2.50	4.26
Interpolated daily egg x 10^{-12}	0.27	0.24	0.20

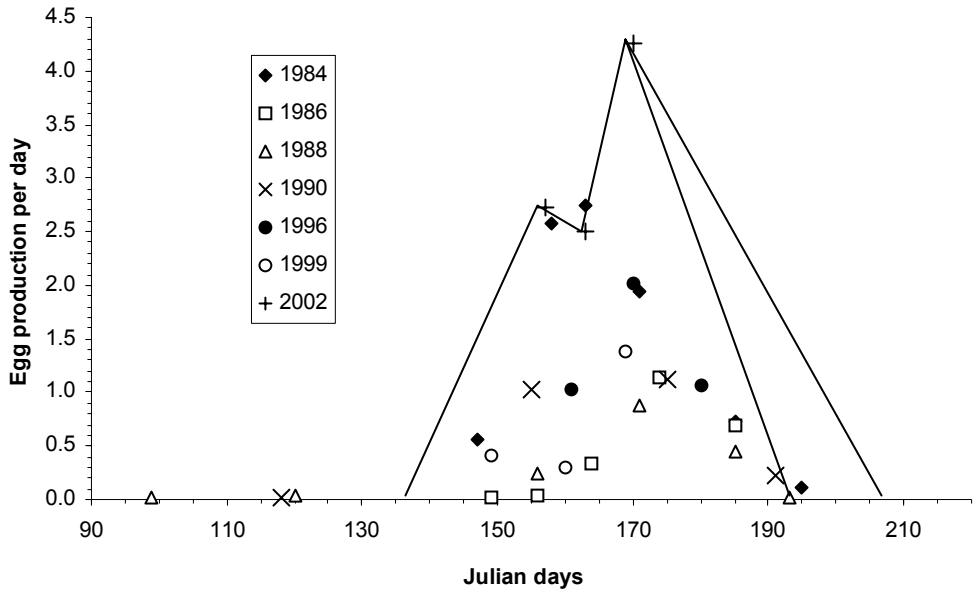


Figure 2.4.1. Daily egg production (eggs x 10^{-12}) of North Sea mackerel during the different surveys since 1984. The production curve for 2002 is given as two alternatives.

The highest egg production was obtained during the last coverage. If the third survey was carried out before the peak of spawning in 2002, the egg production might be underestimated. In years with adequate sampling for defining peak spawning, this period occurred within 12-24 June (Table 2.4.3). Therefore it is unlikely that the egg production obtained during the third coverage in 2002 was a serious underestimate of the peak production. The egg production curve might be drawn as a straight line from this point to the end of spawning or as a steeper line as indicated in Figure 2.4.1.

Table 2.4.2. 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	$Fec. = 560 * weight(g)^{1.14}$ (i.e. 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

By integrating the maximum egg production curve in Figure 2.4.1 the total egg production was estimated at $147*10^{12}$ eggs. The weight fecundity relationship 1401 eggs/g/female (Iversen and Adoff, 1983) corresponds to a SSB of 210,000 tons. However by applying the alternative line from peak of spawning (Figure 2.4.1) the egg production and the SSB is reduced by 20% ($118*10^{12}$ eggs and 168,000 tons).

There are no new fecundity data from the North Sea since 1982 (Iversen and Adoff, 1983). So far atresia in ovaries from North Sea spawners have not been studied. For mackerel spawning in the western area such data are available from several years. Both in 1998 and 2001 the realized fecundity in the western area was rather low (about 1000 eggs per g female) (ICES 2002/G:6). If the same weight fecundity relation is applied for the North Sea survey the SSB estimate is increased by about 40%.

Due to the uncertainties in the SSB estimate in 2002 because of limited temporal and spatial coverage of the spawning area and the applied standard fecundity the WG considers 210,000 tons as an approximate estimate of the SSB of North Sea mackerel in 2002.

Table 2.4.3 gives the estimated egg production in the North Sea for the years with multiple surveys of the spawning area. The corresponding SSBs given in the table are based on a standard fecundity of 1401 eggs/g/female (Iversen and Adoff, 1983). Thereby both the egg production and the SSB have tripled since 1999. According samples obtained by "Tridens", "G.O.Sars" and a rented Norwegian purse seiner equipped for trawling the SSB in the North Sea was dominated by the 1999 year class in 2002 (Table 2.4.4).

Table 2.4.3. Egg production estimates from egg surveys in the North Sea and corresponding SSB based on a standard fecundity of 1401 eggs/g/female.

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	53	76	24 June
1996	77	110	19 June
1999	48	68	-
2002	147 (118)	210 (168)	-

[1]This was the first coverage in 1980.

Table 2.4.4. Age compositions obtained by the different vessels, the suggested age distribution and the estimated numbers of North Sea spawners per age group.

	G. O. SARS		ENDRE DYRØY		TRIDENS		TOTAL		Mat.	SPAWNING STOCK	
Age	%	W (g)	%	W (g)	%	W (g)	%	W (g)	ogive	W (g)	N (millions)
0	0		0		0	0	0	0	0		0.00
1	10.60	116.8	0.50	128.3	6.00	122.0	5.78	119.8	0.00	119.8	0.00
2	12.60	234.0	7.80	247.0	12.00	184.0	11.10	209.3	0.37	209.3	29.76
3	51.20	310.4	47.10	248.4	48.00	310.6	48.58	295.5	1.00	295.5	351.98
4	10.20	360.0	13.10	288.0	8.00	373.5	9.83	341.5	1.00	341.5	71.19
5	10.60	396.0	16.40	383.0	12.00	336.3	12.75	363.7	1.00	363.7	92.39
6	2.60	373.0	6.50	341.0	8.00	486.5	6.28	437.1	1.00	437.1	45.47
7	0.30	397.0	1.80	411.0	2.00	462.0	1.53	443.8	1.00	443.8	11.05
8	0.90	410.0	2.00	437.0	0.00	-	0.73	428.6	1.00	428.6	5.25
9	0.80	454.0	1.30	543.0	0.00	-	0.53	509.1	1.00	509.1	3.80
10	0.00	-	1.20	541.0	2.00	626.0	1.30	606.4	1.00	606.4	9.42
11	0.00	-	1.30	643.0	0.00	-	0.33	643.0	1.00	643.0	2.35
12	0.00	-	1.00	643.0	0.00	-	0.25	643.0	1.00	643.0	1.81
13	0.24	899.0	0.00	-	0.00	-	0.06	899.0	1.00	899.0	0.43
14	0.00	-	0.20	665.0	2.00	500.0	1.05	507.9	1.00	507.9	7.61
12+							1.36	550.0	1.00	550.0	9.85
Total		299.7		304.80		319.00		310.80		332.00	632.53

3 PLANNING OF THE 2004 MACKEREL AND HORSE MACKEREL EGG SURVEY IN THE WESTERN AND SOUTHERN AREAS (REFERRING TO TOR: "A")

3.1 Countries and Ships Participating

England, 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 2004. The vessels and dates available for the survey are given in table 3.1. The survey coverage of the western and southern area is given by area and period in table 3.2. Both vessel availability and area assignments are provisional and will be finalised by the area coordinators at the appropriate times.

Table 3.1. Countries, vessels, areas assigned, dates and sampling periods for the 2004 survey.

Country	Vessel	Areas	Dates	Period
Portugal	Capricorn	Cadiz, Portugal and Galicia	6-21 Jan	1
			3-18 Feb	2
			2-24 Mar	3
Spain (IEO)	Cornide de Saavedra	Cantabrian Sea	15 Mar - 5 Apr	3
			9-30 Apr	3/4
Germany	W. Herwig III	Biscay (N), Celtic Sea & NW Ireland	16 Mar - 23 Apr	3/4
Netherlands	Tridens	Biscay and Celtic Sea	10 – 27 May	5
			8 – 28 June	6
Spain (AZTI)	Investigador	Cantabrian Sea & Biscay	20 Mar - 10 Apr	3
			15-31 May	5
UK (CEFAS)	CEFAS Endeavour	N. Biscay and Celtic Sea	22 Apr - 19 May	4/5
Norway	GO Sars	North west Ireland & West of Scotland	23 May - 15 June	5
Ireland	Celtic Explorer	Celtic Sea	13 Apr - 3 May	4
	Celtic Voyager	Biscay, Celtic Sea, North west Ireland & West of Scotland	6-20 July	7
Scotland	Scotia	North west Ireland & West of Scotland	6 –26 Apr	4
		Celtic Sea, North west Ireland & West of Scotland	15 Jun - 5 July	6

As in previous years, the survey will be split into seven sampling periods, allowing full coverage of the expected spawning area in the south (periods 1-5) and in the western area (periods 3-7) (see Table 3.1). The widest area cover is provided during the third sampling period when the distribution of mackerel and horse mackerel spawning is at it's most widespread in the southern and western area.

In the western area maximum deployment of effort is during the fourth, fifth and sixth sampling periods. These periods coinciding with the expected peak spawning of both mackerel and horse mackerel in the area. For the 2004 survey, the emphasis will be based on area coverage, if necessary requiring occupation of alternate east/west transects. Cruise leaders have been asked to cover their entire assigned area using alternate transects and then use the remaining time to fill in the missed transects. If time is short sampling effort should be concentrated in those area identified as having high egg abundance on the first part of that vessels survey. Particular points to note are:

- In periods 1 & 2 only the western and southern (Cadiz) seaboard of the Iberian Peninsula will be surveyed. This is line with the practice on previous surveys and of the timing of spawning in this area

- In period 3 it is planned to cover the entire area of spawning from the Gulf of Cadiz and north. The German vessel will have to cover the entire area of expected spawning north from the middle of Biscay (North of the area surveyed by AZTI). This will require an alternate transect strategy

- In period 4 there are 4 vessels available which should allow detailed coverage from the Cantabrian Sea (IEO) to the north of Scotland

- In period 5, two vessels will have to cover the bulk of the spawning area, although the AZTI survey will cover the area south of 47°N. Again alternate transects are recommended.

- In period 6, again two vessels will have to cover the entire area of spawning. Again alternate transects are recommended. Both vessels availability are provisional at the time of writing and may be altered. The assignment of the RIVO vessel to this period is at the specific request of the Working Group

- In period 7, only one vessel will be available, and will have to cover the entire spawning area. This assignment has been given to Ireland who traditionally carry out this last survey, although in 2004 they are also providing valuable effort in period 4. Given that the *Celtic Explorer* will not be available, the Working Group would encourage Ireland to use a commercial pelagic vessel during this period following the success of the *Atlantean* survey in 2002

Table 3.2. Periods and area assignments for vessels by week for the 2004 survey. Area assignments and dates are provisional

		Area							
week	Starts	Portugal, Cadiz & Galicia	Cantabrian Sea	Biscay	Celtic Sea	CS2	North west Ireland	West of Scotland	Period
1	30-Dec-03								1
2	6-Jan-04	PO1							1
3	13-Jan-04	PO1							1
4	20-Jan-04								1
5	27-Jan-04								2
6	3-Feb-04	PO2							2
7	10-Feb-04	PO2							2
8	17-Feb-04								2
9	24-Feb-04								2
10	2-Mar-04	PO3							3
11	9-Mar-04	PO3							3
12	16-Mar-04	PO3	IEO1		GER				3
13	23-Mar-04		IEO1	AZTI-1	GER				3
14	30-Mar-04		IEO1	AZTI-1	GER				3
15	6-Apr-04		IEO2	AZTI-1	GER				3
16	13-Apr-04		IEO2		GER		SCO1	SCO1	4
17	20-Apr-04		IEO2	ENG	IRL1	ENG	SCO1	SCO1	4
18	27-Apr-04			ENG	IRL1	ENG	SCO1	SCO1	4
19	4-May-04			ENG	IRL1	ENG			4
20	11-May-04				RIVO1	ENG			5
21	18-May-04			AZTI-2	RIVO1		NO	NO	5
22	25-May-04			AZTI-2	RIVO1		NO	NO	5
23	1-Jun-04						NO	NO	5
24	8-Jun-04			RIVO2	RIVO2				6
25	15-Jun-04			RIVO2	RIVO2		SCO2	SCO2	6
26	22-Jun-04			RIVO2	RIVO2		SCO2	SCO2	6
27	29-Jun-04						SCO2	SCO2	6
28	6-Jul-04			IRL2	IRL2	IRL2	IRL2	IRL2	7
29	13-Jul-04			IRL2	IRL2	IRL2	IRL2	IRL2	7
30	20-Jul-04								7
31	27-Jul-04								7

3.2 Sampling Areas and Sampling Effort

As in previous years it was decided that the spatial and temporal distribution of sampling would be designed to ensure an adequate coverage of both mackerel and horse mackerel spawning and that estimates of stage 1 egg production would be made for both species.

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

Based on the expansion of the "standard area" since the start of the Triennial surveys, the Working Group agreed at the Dublin meeting to reconsider it's use. It was agreed that the existing "standard area" should be retained only as a guide to the core survey area for cruise leaders, and that the extent of coverage should be decided based on finding the edges of the egg distribution only. I.e. boundaries should be set based on the adaptive sampling guidelines given below (section 3.3.). The core areas for the western and southern surveys together, are presented in Figure 3.2.1 The sampling area in the south has been modified from the design used in 2001 and previously. The stations have been placed closer together in the onshore/offshore direction and further apart in the alongshore direction (Figure 3.2.2). As stated above the limits of the survey in both areas should be established on the basis of two consecutive zero samples, and not by the boundaries on this map.

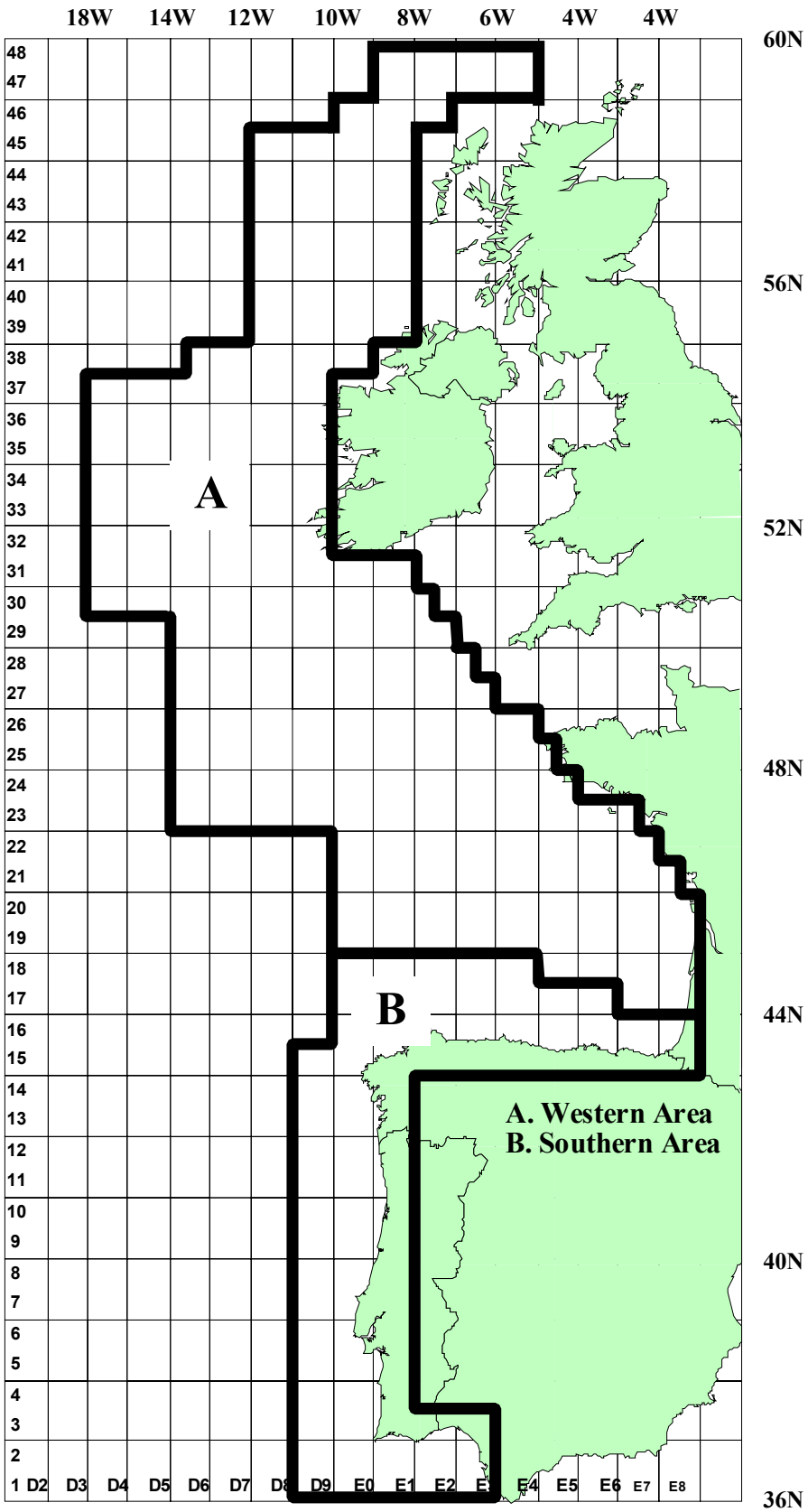


Figure 3.2.1. Core sampling areas for mackerel and horse mackerel eggs in the western and southern areas for 2004. Sampling will be continued outside these limits on surveys based on the adaptive sampling guidelines (Section 3.3).

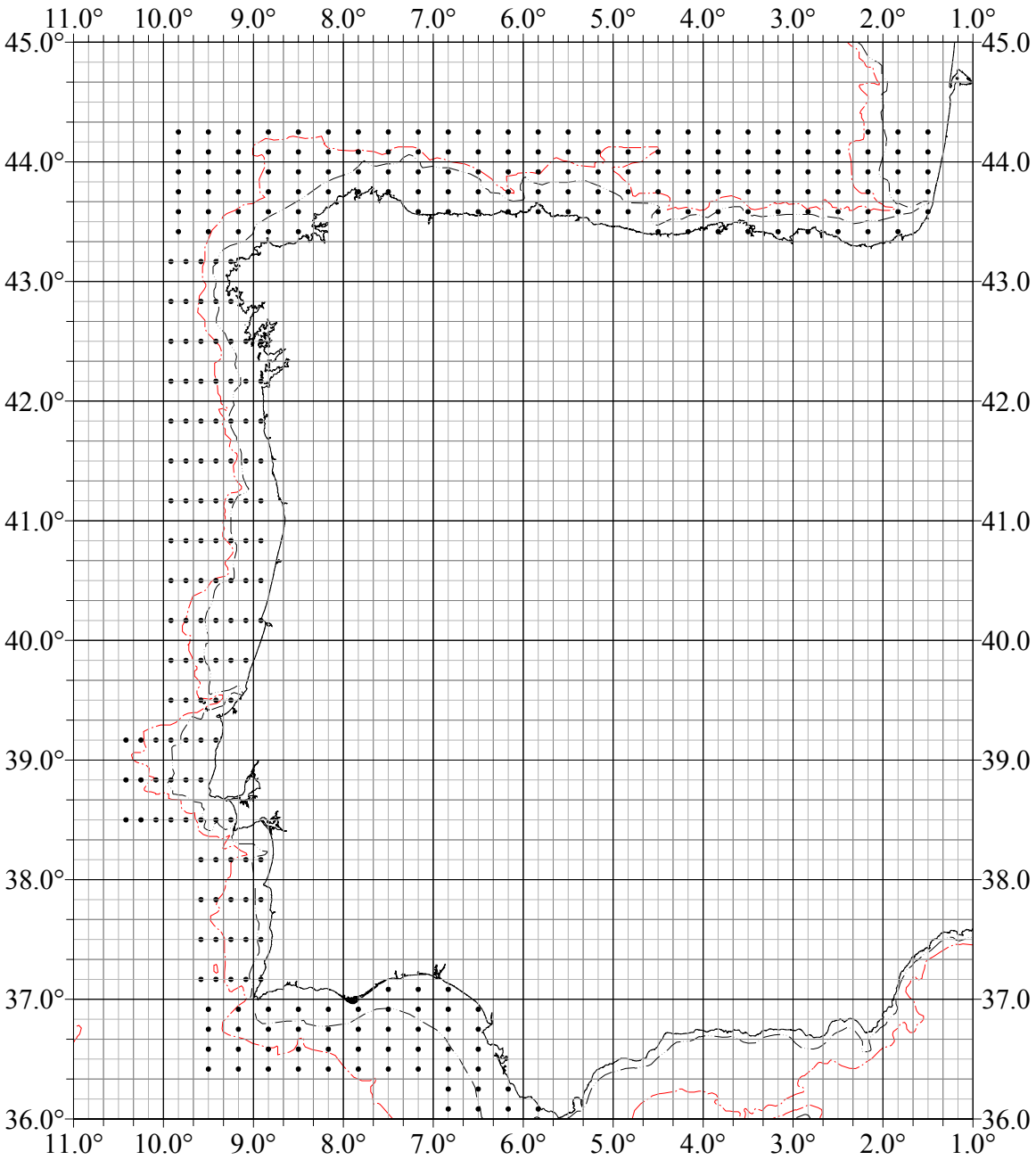


Figure 3.3.2 Provisional station location for mackerel and horse mackerel egg surveys in the southern area in 2004. Offshore boundaries will be based on two consecutive zero rectangles.

3.3 Sampling Strategy, Gear and Procedures

A manual for the conduct of egg surveys, targeted at the AEPM, is given in Section 8 of the Report of the Mackerel/Horse Mackerel Egg Production Workshop (ICES, 1994). The instructions given there are repeated in the following Sections 5.3.1 to 5.3.8. Any alterations from the 1994-Report, changes, additions or clarifications, have been underlined in this report.

3.3.1 Develop protocols and criteria to ensure standardization of all sampling tools and survey gears (referring to ToR:"h")

In pursuance of this Term of Reference the working group carried out an extensive review of the tools, survey gears and survey methodologies used in the surveys. The report includes, under section 3.3. a detailed description of gears used and the protocols for both plankton and adult sample collection. During the current meeting the working group held a workshop where all participants went through the current practices used on the surveys. Each participant detailed their procedures in each area, and where appropriate deviations from these.

Standardisation of survey design

It was recognized that there were subtle differences in practice in different geographical areas of the combined survey. For example, the station placing and transect design is different in the waters around the Iberian peninsula from those used in the western area. This is based on the different topography in this area, with a narrow shelf and very sharp shelf break falling rapidly to substantial depths. The working group agreed that these differences in design should be retained, as one survey design was not appropriate across the whole survey area. Protocols have been successfully harmonised in the western area in recent surveys and the survey design is complied with by all nations. Integration is handled by the survey coordinators, one each for west and south.

Standardisation of survey gears

The standard plankton samplers for use on these surveys are national variants of the Gulf III or Bongo 'high-speed' samplers (section 5.3.1). These samplers generally incorporate conductivity, temperature and depth probes (CTD's) and either contain mechanical or electronic Flowmeters to enable the volume of water filtered on each deployment to be calculated. These sensors either relay 'real-time' environmental data back to a shipboard computer display or log the information, ready for downloading once the station has been completed.

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

The estimation of volume of water filtered by each sampler is critical in the calculation of egg production. Again, the suggestions provided by Nash *et al* (1998), and Coombs et al (1996) provide an acceptable standard. It is recommended that participants follow these standards as closely as possible. It is also critical that participants understand the importance of calibrating Flowmeters and changes in Flowmeter performance when they are mounted in the apertures of plankton samplers (EU AIR3 CT94 1911). It is recommended that all participants review the performance of their Flowmeters and regularly their check their calibration *in-situ* (i.e. within the sampling device).

Standardisation of adult sampling – data collection and analysis

The working group has prepared a new protocol for the collection and analysis of adult parameters; fecundity, atresia and parameters for condition and feeding in the case of horse mackerel. These are detailed in sections 3.4 to 3.6. The analysis of these samples, particularly with reference to fecundity estimation, the use of the Auto-diametric approach and oocyte diameter determination, will be standardised at the Lowestoft workshop to be held in October 2003.

Standardisation of plankton sample collection

The protocols for the use of samplers and initial handling of plankton samples are detailed in the report and also in the section on standardisation of survey gears (see above). Procedures for a standard approach to sample sorting using a mechanical, rather than a manual, technique will be trialed and documented at the Lowestoft workshop in October 2003.

In general, all samples are collected using oblique tows at standard speeds for the nets used (GULF III or Bongo). However, due to the particular situation in the North Sea – small numbers of eggs, often in the surface layers – a different design is adopted on the Norwegian surveys in this area. This involves the use of a stepped tow, with the sampler held in 5m depth layers for a fixed period. This differs from the more standard design followed by the Netherlands vessel in the same area. For the present, the working group agreed that these differences were acceptable, given the lack of the desired full coverage. However, development of a harmonised design should be a matter of priority, particularly if other nations were to join the survey.

Standardisation of plankton sample analysis – species ID and egg staging

This is a key area for standardization and has been the subject of considerable attention by the working group. Egg staging was the subject of a detailed workshop held in Lowestoft in 2000 (ICES 2001). This workshop produced a detailed manual on plankton sample handling and analysis, which is used by all survey participants. A follow up exchange programme on plankton sorting, species ID AND staging revealed some deficiencies, mainly in the species ID. It should be noted that this was a small scale exercise, and was mainly intended to highlight areas for further work rather than as an analysis exercise in itself. Based on these findings a further workshop is planned for October 2003 in Lowestoft, to include all key survey practitioners. This will include:

- Sample sorting, particularly validation and standardization of the mechanical sorting methods
- Species ID, particularly for the extraction of mackerel and horse mackerel eggs
- Egg staging, particularly for differentiation of stage I from stage II eggs, as the former are used in the biomass estimation. The workshop will also deal with the differentiation of stage Ia from Ib as this could be a valuable cross validation tool for future surveys.

Standardisation of data analysis

Detailed procedures for the post analysis of egg abundance data to produce daily and, finally, annual egg production estimates are given in section 3.7. This analysis is carried out by the two data coordinators only, using data submitted in a standard format, and is subject to examination and approval by the full working group. This approach ensures a standard approach and methodology.

3.3.2 Sampling Gear

The standard samplers acceptable for use on the 2001 surveys are national variants of the Gulf III or towed Bongo samplers (Nash *et al.* 1998). The Gulf III sampler is deployed on a double oblique tow, at 5 knots, from the surface to sampling depth and return, and the Bongo sampler at 2-3 knots. The aim is for an even, not stepped, dive profile filtering the same volume of water from each depth band. Portugal has used a 60 cm Bongo, Norway uses the Gulf III but deploys a Bongo in the North Sea, IEO and AZTI have used 40 cm Bongo throughout the period of the triennials For reference, Coombs et al (1996) provided a good overall description of Bongos as well as a user's guide. An ICES Manual on Zooplankton sampling is available but does not refer to the instruments used in the triennial surveys. It was decided that a detailed description of the national samplers in relation to the sampler described by Nash *et al.* 1998 or for Bongos – Coombs et al (1996) should be provided by all participants for the 2005 meeting.

The current Flowmeters used in the survey are largely considered as state-of-the-art, however, new developments are being made in non-intrusive Flow meters. It is recommended that participants investigate the utility and cost-benefits of these and report back to WGMEGS as appropriate.

Different mouth openings for Bongos deployed do not seem to make a difference in sampling efficiency or performance, although 60 cm nets (vs 40 cm) are apparently more prone to clogging. At present, Portugal uses 60 cm, and moving to 40 cm would be expensive. At present, Portugal also uses a CalVET (with similar features to the Bongo

and a mouth aperture of 30cm) for other surveys, therefore it was suggested that they deploy their CalVET in oblique hauls for the Triennial surveys and this was agreed.

Although a mesh size of 500 micron aperture is adequate for sampling mackerel and horse mackerel eggs, a nylon mesh with an aperture between 250 and 280 microns is the recommended size for these surveys. This allows the plankton samples to be more widely used for investigations on other species and taxa. In the North Sea surveys where clogging is a problem a 500 micron aperture mesh is used.

The aperture on the Gulf III type sampler should be 20 cm in diameter in order to ensure that an adequate volume of water is filtered to quantitatively sample the eggs of other species, in particular hake, which may be present at lower densities than the target species. The aperture of the Bongo samplers should be either 40 cm or 60 cm diameter. It is recommended that no *ad hoc* changes take place.

In relation to deployment rate, a recommendation can be found in an earlier WGMEGS Rep 19?? The requirement is for an even, 'V' shaped dive profile, filtering the same volume of water per unit of depth. The aim is to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band (ICES CM 2001/G:01).

3.3.3 Target Species

The sampling programme for 2004 will be targeted at mackerel and horse mackerel. Other species' eggs and larvae should be extracted whenever possible (Indices EU Project 97/017). An egg production estimate will be calculated for both species in both areas. In addition, an egg production estimate for mackerel will be calculated for the combined North East Atlantic area.

3.3.4 Standard Sampling Area

The sampling area is defined in every survey year according to the most recent information on the distribution of mackerel and horse mackerel eggs. A standard sampling area was defined in the past but this concept seems no longer necessary as an adaptive strategy is employed whilst sampling is taking place.

3.3.5 Sampling Strategy

The sampling strategy in the western and southern areas in 2004 will be targeted at the AEPM only. From analyses of 1992 egg survey data presented to the 1994 Egg Production Workshop (ICES, 1994) and from knowledge of previous years distributions, it is clear that egg distributions in all survey periods conform to a characteristic spatial pattern which can be modelled. These analyses indicate that changes in the distribution of sampling effort, coupled with the use of a model based approach, could lead to significant improvements in estimates of egg production in future. From the point of view of sampling effort the analysis indicated that two important factors needed to be considered when planning the survey strategy.

Firstly, a set of rules must be established in order to decide when to stop sampling along a given transect, in order to ensure that the whole area of egg distribution is sampled with no effort wasted outside the spawning area.

Secondly, some guide-lines need to be provided to cruise leaders on the number and spacing of transects which may be omitted in order to best match available effort to the size of the area to be surveyed. This approach was adopted for the 1995, 1998 & 2001 surveys and it is proposed that the same flexible approach be adopted for the 2004 surveys. This will permit alternative analyses of the data set using GAM (WGMEGS 2000) or Geostatistics (section 4.1) .

As a first guide to planning the distribution of sampling effort in the western and southern areas in 2004, historic egg distributions are provided in Figures 5.1.1a-f for mackerel and 6.1.1a-d for horse mackerel in ICES (1999). The core distributional areas, identified for each of the different sampling periods, should always be sampled to the north/south and east/west limits although individual transects may be omitted. When sampling along transects, shipboard enumeration of results should be undertaken several rectangles before the limit of the core area is reached. Sampling should be completed either after one zero (or near zero) value or two consecutive low values, *i.e.* less than about 20 stage I eggs of either species are present in the sample. In practice eggs do not become visible until an hour or so after fixation – roughly the steaming time between stations – so that one extra station after a zero or 2 low values will always be necessary before steaming to the next transect. In some cases it will be necessary to sample beyond the core area limits and even beyond the standard survey area limits.

With regard to the spacing and omission of sampling transects this will depend on the size of the area to be covered and the amount of ship time available. During periods when several ships are available it should be possible to sample all transects while at other times it may be necessary to omit several, at least during the first pass over the designated sampling area. No more than one consecutive transect should ever be omitted. Given that the area to be covered is more or less known, as is ship time, cruise leaders should be able to estimate fairly accurately the number of the full transects they will be able to make. **It is strongly recommended that, where practical, and even where total coverage is expected, a first pass over the area be made on alternate transects. The intervening transect should be sampled on the return leg**. If time is limited, on the return leg sampling should concentrate where high densities were observed in the first pass. The cruise leader should be aware of edge definition problems where the contours run east-west. In this way weather problems, equipment failure and vessel breakdown need not seriously prejudice results. Such a strategy, furthermore, enables better evaluation of distributional change with time, which is likely to be important in modelling the results. An example of an appropriate sampling strategy where one in two transects is fully sampled is given in Fig. 6.16 in ICES (1994).

Additional sampling should be carried out in areas where high densities of either mackerel or horse mackerel eggs can be expected. As guidance to the areas where high densities are likely to occur, cruise leaders should refer to the charts showing the maximum contribution to egg production of either species in each time period in the previous reports of this WG. Otherwise, additional sampling will be based on results from a first pass, used as a pilot survey, over the area.

3.3.6 Sampling Depth

Maximum sampling depth is to **200m** or to within **5m of the bottom** where the bottom is less than 200m. In the presence of a thermocline greater than **2.5°C in 10m depth**, sampling can be confined to a maximum depth of **20m below the base of the thermocline**. Specifically for the North Sea, a stepwise oblique profile dive is implemented. Details of any compromise on gear between Norway and the Netherlands will be reported later.

Some research about the relation between the sampling depth and other covariates like bottom depth and filtered volume has been carried out within the EC project 97/097 "Evaluation and development of spatio-temporal models and survey designs for efficient assessment of mackerel and horse mackerel". As a result, some possible problems related to the depth measurements were found for the 1992 data set. These problems are shown by a large range of filtered volumes for depths of approximately 200m, and also by a large number of samples taken with exactly 200 meters maximum depth. Similar features, although less marked, can be observed in the 1995 survey.

The high frequency of samples taken at exactly the recommended maximum depth can only be achieved by vessels with automatic devices controlling the sampling depth of the or by vessels with real-time bathymeters. Otherwise, these features can indicate some bias in the depth measures. As a result, and because depth is an important parameter to calculate egg densities, the working group recommends the depth measurements to be taken more carefully, and also to carry exploratory analysis of the data related to the net deployment in order to detect possible problems. The WG recommends the use of real-time depth, Flowmeter and temperature monitoring systems.

3.3.7 Sample Fixation

The standard fixative for use on these surveys is a 4% solution of buffered (sodium acetate is the standard buffer, details in ICES CM 2001/G:01) formaldehyde in either distilled or freshwater. This solution is approximately iso-osmotic with sea water and should be used in preference to a 4% formaldehyde solution in sea water in order to minimise the problem of distortion of the eggs. The sample should be directly fixed with the addition of the 4% formaldehyde solution and should not come into contact with formaldehyde strength in excess of 4%.

The 4% solution should be made up as follows; 40 % formaldehyde as purchased, 1 part; distilled or freshwater, 9 parts, plus an appropriate buffer to pH 7-8.

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

3.3.8 Sample Sorting, Egg Identification, Staging and Ageing

Whenever practicable the whole sample should be sorted in order to remove all the eggs of non target species such as hake and sardine, which may be present in lower densities than the target species. All sorted eggs should be kept in tubes, in fixative, inside the sample container for future reference and use. Only the eggs of mackerel and horse mackerel need be identified to species. A minimum of 100 eggs of each of the target species must be staged from the sorted sample or sub-sample. Standards for sampling will be reviewed after the Workshop in Lowestoft in October 2003 (see section 5).

The eggs of mackerel and horse mackerel should be classified into one of six morphological stages (Ia, Ib, II, III, IV and V) (Lockwood *et al*., 1981) following the descriptions in ICES 2001. For horse mackerel the description of stages is the same with the exception of stage V which does not exist. Horse mackerel larvae hatch at the end of egg stage IV (Pipe and Walker, 1987).

For the estimation of the daily egg production for both species only the counts of stage I eggs are used. This is recognised as a conservative estimate of the total spawned because some mortality probably occurs during development.

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

The formula for calculating the duration of **stage I mackerel eggs** from the sea temperature (T°C) is

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

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

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

Work aimed at reviewing the existing calculation to estimate the rate of development is taking place. The temperature at 20 m depth (5m for the North Sea) should be used for the calculation of egg stage duration. If that is not available then the sub-surface temperature (ca. 3m) should be used.

3.3.9 Rectangle Sampling

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

On some occasions and in particular where multiple observations are made within a rectangle sampling positions may fall on a dividing line between rectangles. When this occurs the sample is allocated to the rectangle to the north of the line of latitude and to the west of the line of longitude.

3.4 Review of research on the determination of fecundity in mackerel (referring to ToR: "c")

3.4.1 Definition of Terms

Table 3.4.1.1 Definition of terms

Term	Definition

Previtellogenic oocyte	A precursor oocyte stage that develops into a vitellogenic oocyte
Vitellogenic oocyte (VO)	Oocytes that comprise the annual potential fecundity
De novo vitellogenesis	The process of producing vitellogenic oocytes from previtellogenic oocytes; used especially in relation to determinate / indeterminate fecundity and spawning strategy.
Determinate / Indeterminate fecundity	A fish is described as 'determinate' when the annual potential fecundity is either the same as, or more than the number of eggs shed during the spawning season. This is a basic assumption of the annual egg production based mackerel stock assessment. If additional oocytes mature to enhance the potential fecundity during the spawning season the spawning strategy is described as 'indeterminate'.
Annual potential fecundity	The number of vitellogenic oocytes in a female just before the start of spawning and often expressed as the relative potential fecundity (oocytes per g female)
Migratory nucleus stage oocyte	Oocytes in the final stage of maturation which are about to hydrate prior to ovulation and spawning.
Hydrated oocyte	Fully mature oocytes ready for ovulation but still held in a follicle and part of the ovary tissue.
Ovulated oocyte	Loose oocytes ready for spawning, found in 'running' females.
Realised fecundity	Number of ovulated oocytes spawned in a year by a female.
Batch fecundity	The number of eggs released during a spawning event. Species like horse mackerel and mackerel have multiple spawning events during the spawning season and the realised fecundity is the sum of the number of batches released times the batch fecundity.
Standing stock of fecundity	The number of vitellogenic oocytes present in the ovary when the fish was sampled. A key issue is the minimum size of oocyte considered to be vitellogenic which in ICES assessment equates to 185 μm in whole mounts and all oocytes containing lipid vesicals in horse mackerel. Hunter et al consider only advanced yolk oocytes larger than 400 μm comprise the potential fecundity.
Post ovulated follicle	A structure marking the site in the ovary where an oocyte grew to maturity. They quickly collapse and disappear after ovulation and are used as indicators of previous spawning activity
Spawning frequency	The proportion of female spawning events in the population per unit time. Measured by identifying the proportion of females containing a spawning marker (post ovulatory follicle, migratory nucleus stage oocyte or undergoing batch hydration). Data on the duration of the spawning marker is also required to calculate this parameter.
Atresia stage duration	The early alpha atresia stage has been estimated to last 7.5 days in mackerel.
Prevalence of atresia	The proportion of fish with one or more early alpha atretic oocytes present in a section of the ovary.
Relative intensity of atresia	The number of early alpha stage atretic oocytes found in the ovary estimated by stereological analysis (expressed as the number per g. female).
Condition indices	Weight of whole body, carcass or organ such as the liver of guts / length ^3

3.4.2 Methodological advances in fecundity determination for mackerel and horse mackerel

The use of a highly toxic Gilson fixative containing mercury in the protocol for fecundity determination is increasingly considered by WGMEGS to be unacceptable because of safety, environmental impact and cost of disposal. Ovaries fixed in Gilson solution are also not suitable for histology and therefore a duplicate sample fixed in formaldehyde is required to exclude spawning fish from the fecundity estimate. In the protocol using Gilson fixed tissue to estimate fecundity all oocytes larger than 130 μm were included in the count (Walsh et al 1991) and this was found to correspond to oocytes of 185 μm in formaldehyde (Witthames and Greenwood WD in ICES 2002). Accordingly WGMEGS agreed that the use of Gilson fixative to estimate fecundity will be discontinued and in 2004 formaldehyde fixative would be used for both fecundity and atresia estimation. An added advantage of using a single fixative is that all fish collected through out the survey period can be used for estimation of fecundity and atresia according to the spawning status of the ovary.

The Auto-diametric method (Thorsen and Kjesbu 2001) has been used to estimate potential fecundity in pre-spawning cod and in combination with image analysis, offers potential advantages over the Gravimetric method (Hunter et al 1989). These advantages include the possibility to automate the analysis and provide a single method to determine

spawning status, batch fecundity and the standing stock of fecundity. Recent work (Witthames and Greenwood WD) illustrated that the method would work with ovaries showing very different oocyte frequency distributions (plaice and mackerel) and spawning status (Figures 3.4.2.1 & 2). Although further validation and inter-calibration is required comparing the output with the stereometric method (Emerson et al 1991) the method should also be applicable to horse mackerel.

Figure 3.4.2.1 Examples of oocyte size frequency distributions found in ripe pre-spawning mackerel and plaice.

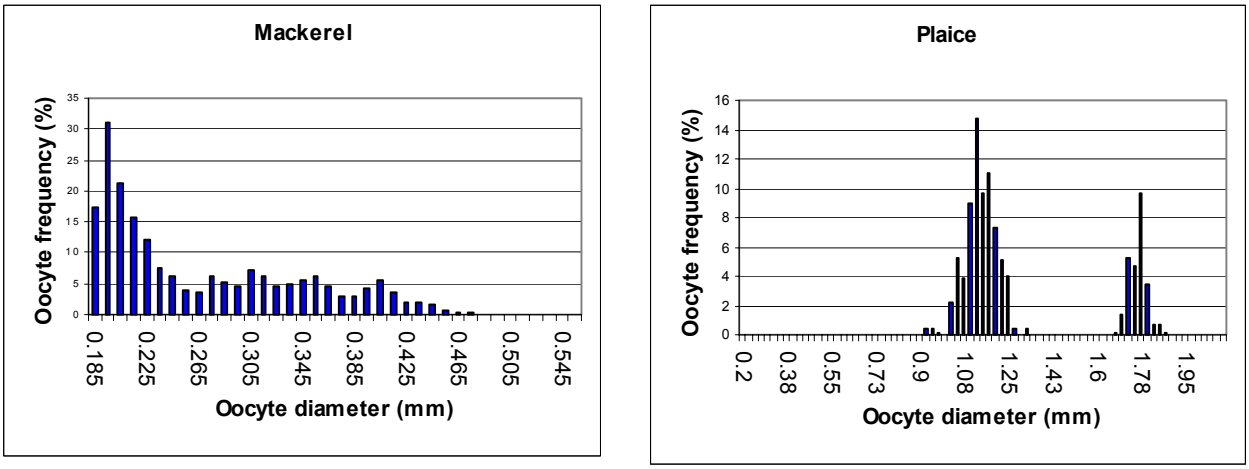
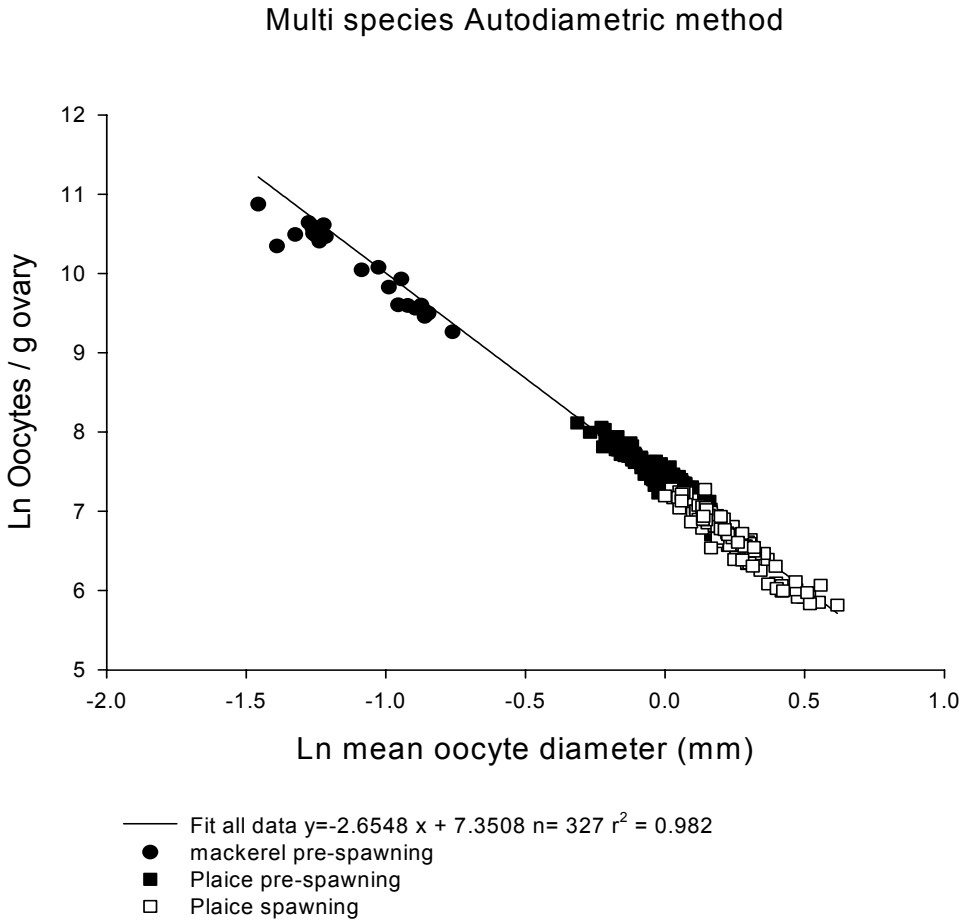


Figure 3.4.2.2. Plot of log oocytes per g ovary in relation to log mean oocyte diameter prepared from samples of mackerel and plaice ovaries



Estimation of realised fecundity in batch spawning fish such as mackerel and horse mackerel following either the AEPM (Lockwood et al 1981) or DEPM (ICES 1990 and 1993) have previously been carried out by direct estimation of potential fecundity and atresia or batch fecundity and spawning fraction respectively. The dynamics of atretic oocyte or batch production are not well documented but further information will be available in an EU Framework V project 'RASER, reporting in 2005. An alternative approach, that may be more efficient on resources, is to develop indices of realised fecundity (WD Witthames and Greenwood) but their basis is dependent on spawning strategy (determinate v indeterminate).

3.4.2.1 Mackerel (a determinate spawning strategy)

In this species spawning is very dependent on body reserves and food consumption is low in the spawning season (Figure 3.4.2.4), and an index based on body condition (Weight of ovary plus carcass / length^3) may have predictive power in relation to it's realised fecundity. It seems that higher condition factors in the autumn correspond with higher fecundity the following spawning season (Figure 3.4.2.3.). A detailed analysis of this relationship will be carried out for the WGMHSA meeting in September 2003.

Figure3.4.2.3 The condition factor obtained from Norwegian commercial purse seine catches in October 1990-2000 compared with realised fecundity the following spawning season.

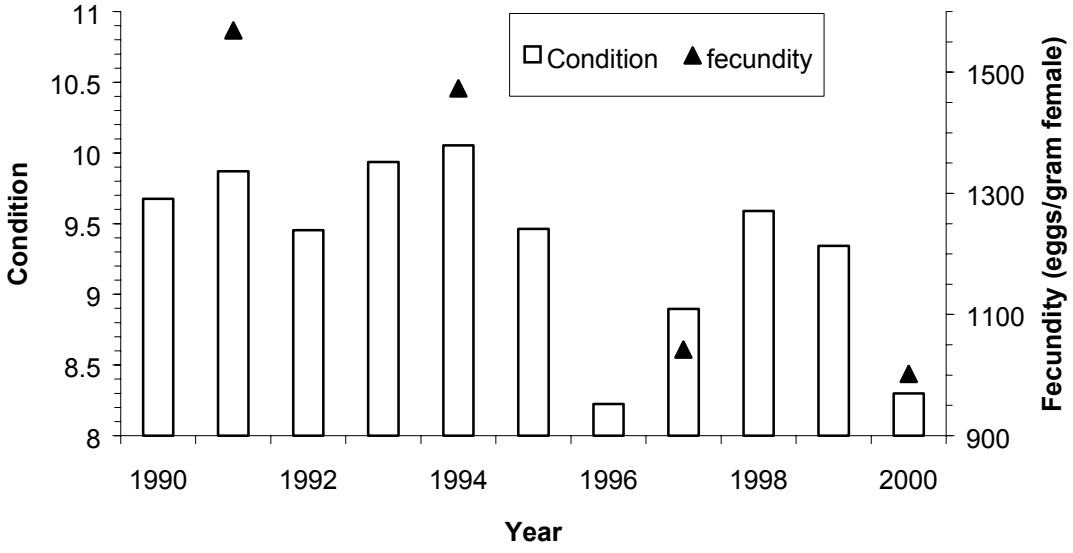
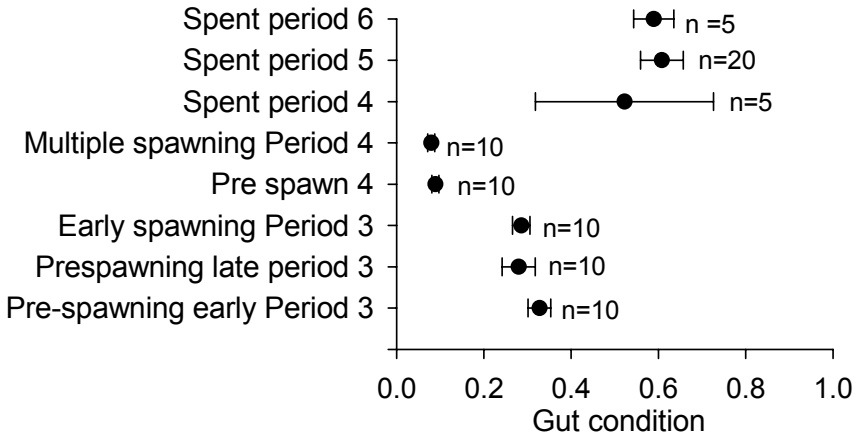
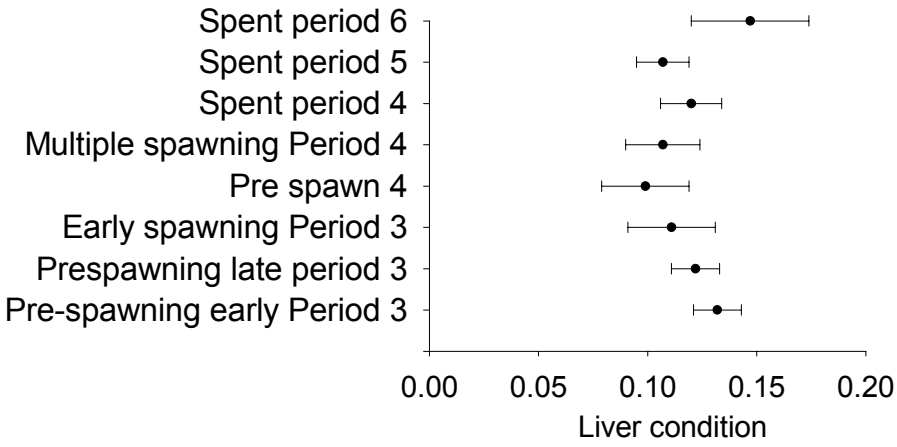
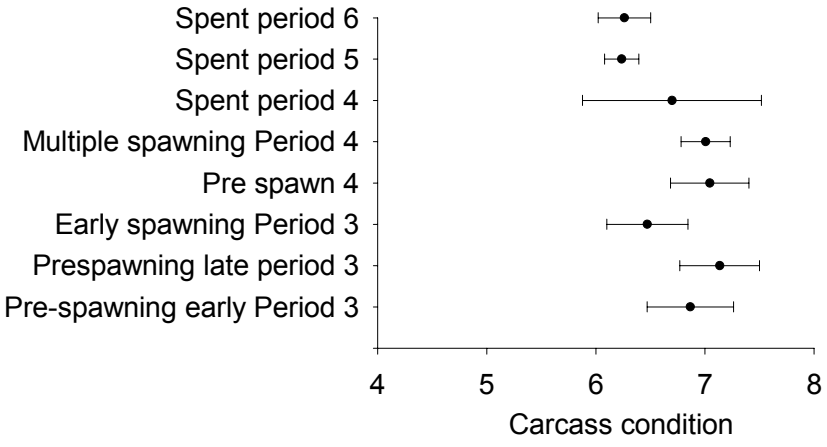


Figure 3.4.2.4

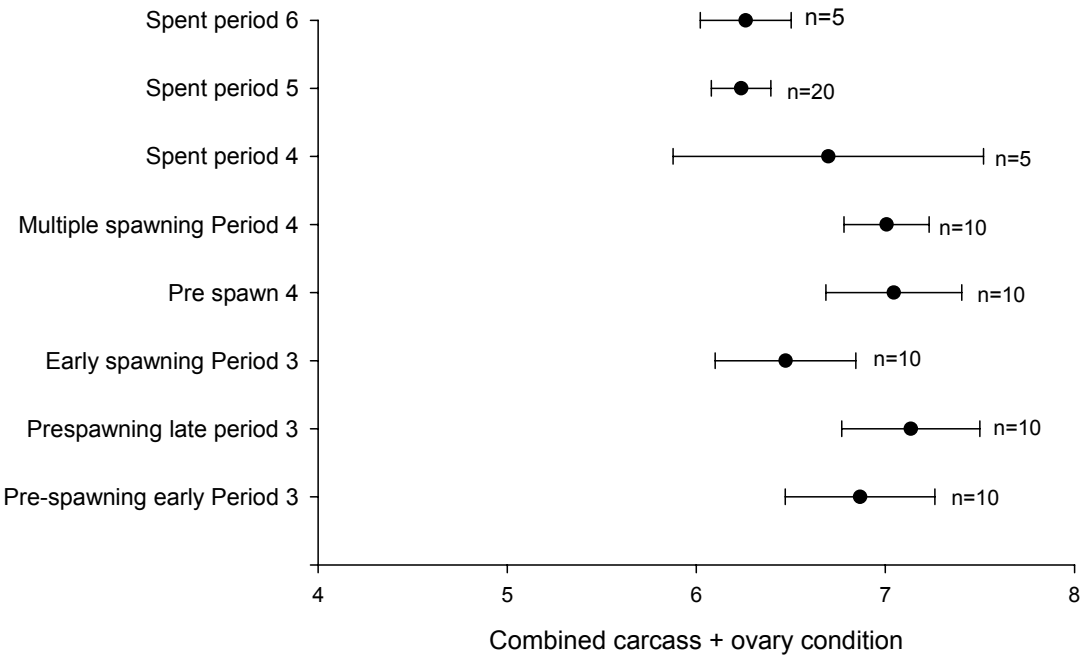
Mean condition indices for the gut (including contents), liver, and carcass for mackerel collected on the 2001 Triennial survey grouped according to the period of collection and spawning status. The error bars show two standard errors around the mean and the sample number is shown in the top panel to the right of the error bar.







Mean condition indices for carcass and ovary combined from mackerel caught on the 2001Triennial survey grouped according to the period of capture and spawning status. The error bars show two standard errors from the mean and the nunber of fish in each group is shown to the left of the error bar.



3.4.2.2 Horse mackerel (an indeterminate spawning strategy?)- (referring to ToR: "f")

Two WDs presented at this meeting (Gonçalves and Karlou-Riga) concluded that horse mackerel probably has an indeterminate spawning strategy. At the previous meeting (Dublin 2002) WGMEGS considered the evidence from the adult sampling in 1998 and 2001 (ICES 2002 section 6.3). Observed potential fecundity for horse mackerel appeared to rise from a relatively low level of around 200 eggs/g to close to 1400 by the time of peak spawning. While this may be the result of immigration of new spawners to the survey area, the most likely cause is *de novo* vitellogenisis. This would again suggest that horse mackerel is an indeterminate spawner.

In the previous report (ICES 2002) the WG suggested that to resolve this question, tank experiments be carried out with captive fish populations. The WG was given a presentation on the facilities available in Matre, Norway for the maintenance of wild fish populations and monitoring their reproductive biology. This facility has been previously used mainly for salmon fecundity research, but is now being used for marine fish such as cod and mackerel. Olav Kjesbu of IMR offered the use of this facility to carry out a pilot study on deteminacy in horse mackerel. The study would be in 3 parts:

- Capture and transfer of wild fish to the facility – wild horse mackerel usually enter the fjord where the facility is sited in August. These will be captured using a fishing vessel and transferred to holding tanks.

- The fish will be maintained in the tanks and fed initially on live food (krill) and then, hopefully transferred to pellet food. This has been done successfully for other marine species.

- Based on the success of the first two parts, the fish will be held throughout the spawning season in 2004. Food uptake and egg production will be monitored. Individual groups fish will be periodically sacrificed and their fecundity assessed.

If the pilot is successful, it should be able to definitively answer the question of determinacy in this species. Further work on fecundity in relation to feeding and water temperature may be possible but this will depend on collaborative

funding. The WG would like to express it's appreciation of this offer from IMR and would like to thank Olav Kjesbu in particular for his help.

In the 2002 report, the WG also recommended that, assuming indeterminacy, alternative methods of using the annual egg production estimate should be investigated. One possibility considered was to use the survey to carry out a Daily Egg Production Method (DEPM) analysis. However, the examination of the utility of a DEPM made during the 1992 survey indicated that the collection of data on adult parameters was difficult for this species, particularly the spawning fraction. The variability in this was very high, and the calculated error bounds for the survey biomass estimate included zero. Given the more limited resources in the current surveys this was deemed impractical.

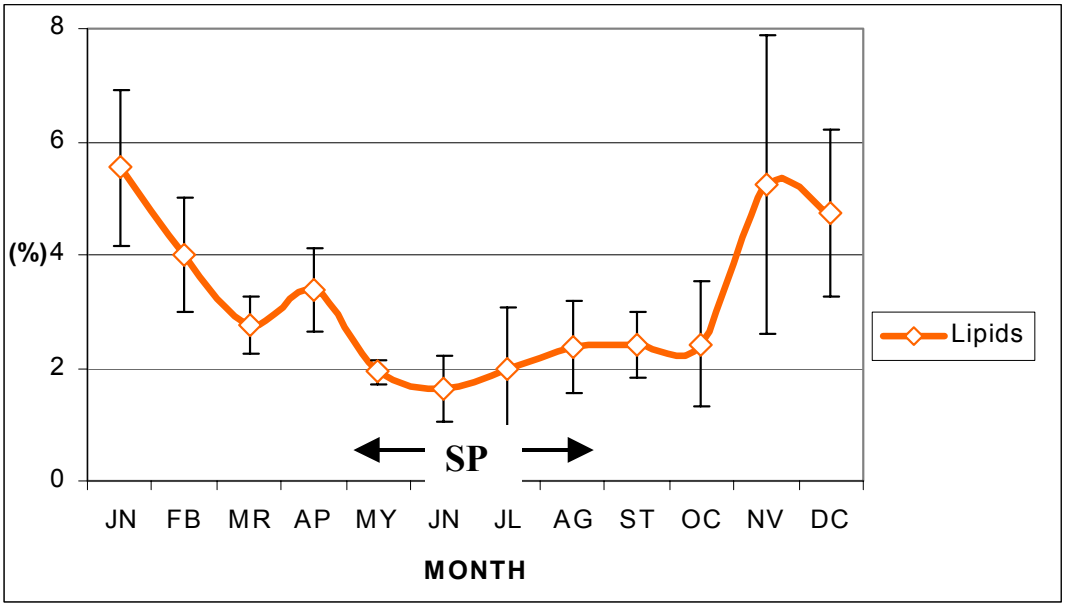
Currently, in the face of these problems the WGMHSA (ICES 2003) has opted for the use of the TAEP for the tuning of the assessment. As there is no fecundity component in the TAEP, this approach is vulnerable to the type of fecundity changes observed in many fish e.g. mackerel (ICES 2002). If we are to use the TAEP there is a clear need for some measure of fecundity to be incorporated in the tuning, possibly in the form of an index. There are two possible approaches. In the first case a spatial and temporal mean index of batch fecundity and spawning fraction could be used to tune TAEP. This approach would require extensive fishing effort to be carried out throughout the 2004 Triennial survey. As stated above, in the 1992 DEPM study, the variance on the spawning fraction was so large that the lower bounds of the SSB estimate reached zero, despite high levels of adult sampling. Given the more limited resources available in 2004 and for the foreseeable future, this was agreed to be impractical. An alternative approach would be based on the following:

WGMEGS assumes that two factors determine the realised fecundity in horse mackerel:

a) The energy indicated by lipid content Figure 3.4.2.5. and dry weight fraction prior to the onset of spawning (Lucio WD).

b) The energy taken in as food during spawning.

Figure 3.4.2.5. Seasonal evolution of lipids content for horse mackerel (length >30 cm) in the Bay of Biscay (1987-1993). For each month the mean value and the CI is shown. The spawning period (SP) for horse mackerel in the bay of Biscay is marked.



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

In 2002 the assessment of western horse mackerel was tuned to the total egg production estimates of 1983, 1989, 1992, 1995, 1998 and 2001 (ICES, 2003 ACFM:07), because horse mackerel was regarded to be an indeterminate spawner. Total egg productions can be used for tuning because of a linear relationship between fish weight and fecundity albeit with a high uncertainty introduced by the interannual variability in the realised fecundity per g female. The combination of indices on lipid content and food availability are expected to provide information on inter-annual variations in fecundity and are therefore expected to improve the assessment based on tuning to total egg productions. In the course of the 2004 survey data on potential fecundity and the standing stock of fecundity will be collected prior to and during the spawning season in relation to lipid content and feeding success.

3.4.3 Sampling for mackerel and horse mackerel adult parameters in 2004: General issues (referring to ToR "b")

The 1999 WGMHMSA recommended (TOR d) that fecundity sampling for both species should be based on weight rather than length sampling targets (Darby WD in ICES 2000) and collected over a wide part of the spawning area. They also expressed the view that sampling should be greater in numerical terms. In 2001 Western mackerel potential fecundity was found to vary according to when the fish were collected in time and space. Accordingly sampling for the 2004 survey should again be spread out both in time and space throughout the Southern and Western spawning areas. Trawling (both bottom and pelagic) or purse-seining are recommended fishing methods for collecting samples. Trawling on the surface at night appears to be particularly successful during the May-July period. In general terms, spreading the trawling depth throughout the survey is recommended. Hand-lining seems to result in higher numbers of running fish which could bias the fecundity estimate and horse mackerel are also less likely to be caught so WGMEGS recommends that fish samples should be obtained from trawling or purse-seining if at all possible.

The WGMEGS has adopted the new method for estimation of fecundity in both species based on formaldehyde fixed ovaries using the gravimetric (Hunter *et al.* 1999) and the Auto-diametric method (Thorsen and Kjesbu 2001) following development of calibration curves . The special advantages of this new approach are:

- Discontinuing the use of a highly toxic fixative containing mercuric chloride (mackerel fecundity analysis only) to lessen the environmental impact of this work.

- A saving of at least 50% analysis time.

- The possibility of automating the analysis of fecundity determination by the use of image analysis.

The method is based on measurements of ovary weight and mean oocyte diameter to calculate fecundity. Further validation will be presented at the 2005 WGMEGS meeting for mackerel and horse mackerel

Details of the sample collection protocols and illustrations are provided in Section 3.4.6.

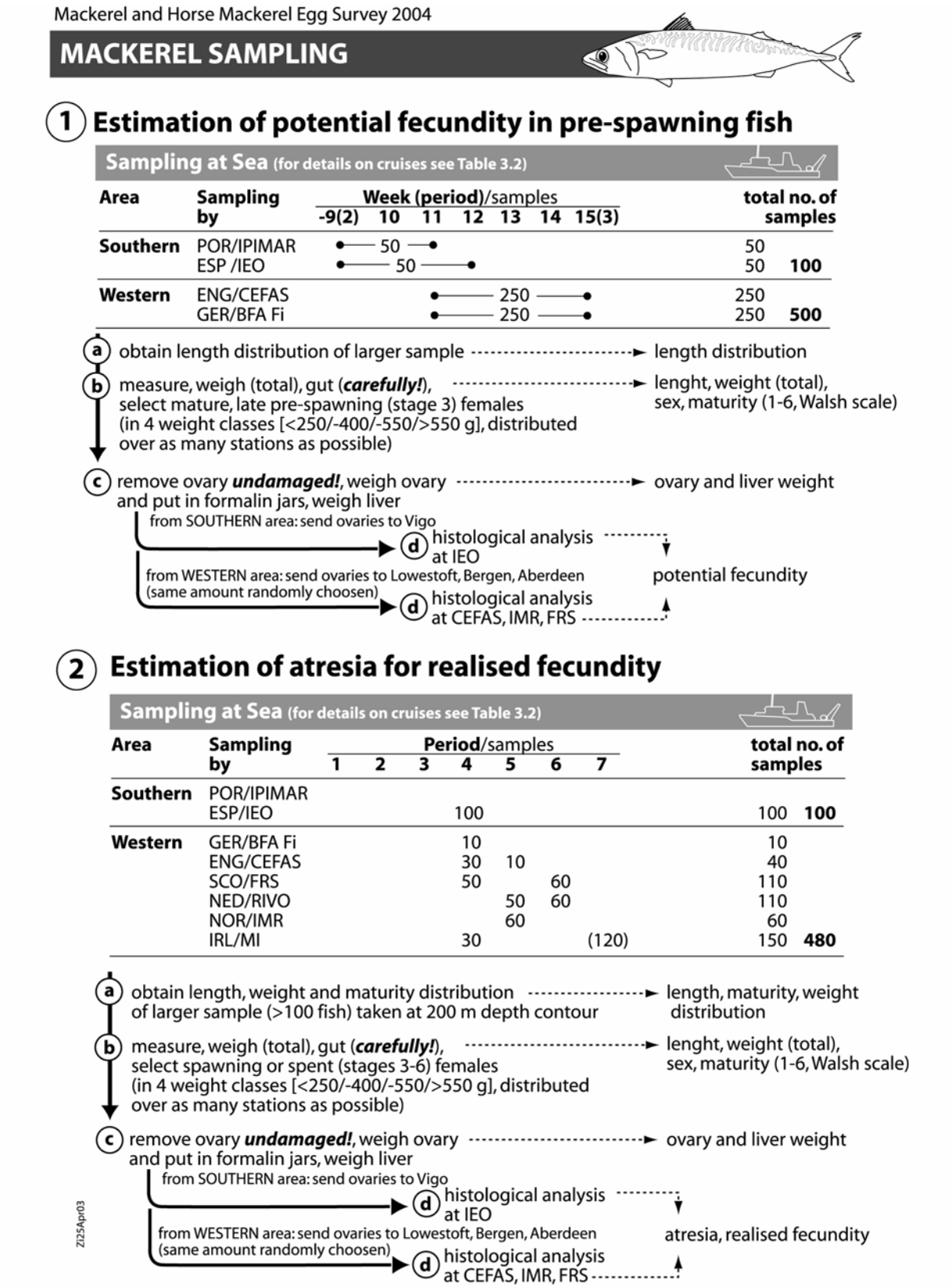
3.4.4 Sampling for mackerel potential fecundity and atresia in the Western and Southern areas

Following WGMEGS decision to use only formaldehyde fixative it will be possible to provide a unified sampling scheme for fecundity and atresia. The temporal coverage and number of ovaries to be collected in the Western and Southern areas for determining potential fecundity and atresia are shown in table 3.4.4.1. Further details of the desired spatial and temporal coverage of fish sampling will be circulated later when the timing of the various surveys has been resolved. Samples must be collected over 10 or more stations selecting 5 fish per weight class as indicated below. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes. 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. In the case of the Western English IBTS survey some samples should be taken from each station, where mature mackerel are caught, to fill the weight classes in table 3.4.4.2 below.

Table 3.4.4.2. Weight classes for sampling females of maturity stages 2-6 (Walsh 1991) for Potential fecundity and atresia

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

Table 3.4.4.1. Adult mackerel sampling programme Flow diagram



Protocol for processing and distribution of ovary sub-samples for either fecundity or atresia analysis

Prior to cruise departure

Procure Eppendorf type tubes and place in suitable racks (See appendix).

Attach a spot label to the Eppendorf lid and add 1.2 ml of 3.6% formaldehyde buffered with 0.1M sodium phosphate (referred to below as 'fixative') to each tube using a dispensor. The label should contain a letter for the collecting vessel / cruise and 3 alpha or numeric characters for the fish to cross reference the fecundity analysis to the fish details

Procure sample bottles for the remaining ovary tissue which 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 capacity jar will be adequate.

Procure capillary pipettes (see appendix)

At sea

Select maturity stages 2-6. Weigh the whole fish and dissect out the ovaries, liver, guts including contents, from the carcass and tabulate the data as below. Divide the samples alternately between England, Norway, Scotland, and Spain. If the ovary is running squeeze out the ovulated eggs prior to processing the ovary. Take a pipette sample (see appendix) from one ovary and place it in a labelled Eppendorf tube. Repeat this twice more to provide 3 replicate samples. Preserve both the sampled and un-sampled ovaries from each fish in a leak proof plastic bottle containing more than twice the combined ovary weight of fixative. Store the Eppendorf tubes and jars containing ovary samples grouped together for each country doing the fecundity analysis.

Processing ovary 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 and pack them in a leak proof bottle. Pack the consignments for each country with a maximum volume of 1000 ml fixative 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 2005 WGMEGS.

Laboratory analysis

Tasks	**Countries**	**Timing for work completion**
Develop image analysis methods with PAS stained Whole mounts by applying protocols developed in RASER to mackerel and horse mackerel [1]	England and Norway	Prior to the October Workshop
Training [1]	England, Scotland and Spain	October Workshop
Prepare and distribute revised fecundity manual	England	December 2005

Determine Auto-diametric calibration (20 fish per country) based on measurements of mean oocyte diameter and oocytes per g ovary. Samples from period 3 must be distributed as soon as possible to complete the method evaluation to agree on a protocol for all the following surveys. A wide range of ovaries within stage 3 and Stage 4 fish should be included in the calibration.	All participating countries	End May 2004
Prepare resin sections from all cassette samples and allocate each sample to fecundity or atresia analysis.	All participating countries	September 2004
Determine fecundity in selected samples by Auto-diametric method.	All participating countries	February 2005 [2]
Determine atresia in selected samples by Stereometric analysis.	All participating countries	February 2005 [2]

[1]This will form the basis of a revised fecundity manual which will be prepared and developed during and following the Workshop.in Lowestoft.

[2] Distribute data for analysis

Table 3.4.4.3 Details required for samples collected to estimate potential fecundity

Ship	Fish Reference number	Position		Fish	Weights			
		Lat	Long	Total Length (mm)	Total (g)	Ovary[1]	Liver[1]	Guts including contents[1]

[1]Endeavour to collect these weights with a resolution of ± 0.1g .

Sample analysis targets for England, Norway, Scotland and Spain participating in estimation of mackerel fecundity and atresia. Each country carrying out the various cruises listed in table 3.4.4.1 is responsible for distributing their sample collection alternately to the countries carrying out the fecundity analysis.

Spawning component	Targets for potential fecundity analysis	Targets for atresia analysis [1]
Southern	100	100
Western	500	480
Total	600	580

[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 3.4.4.1.

Table 3.4.4.4. Details required for samples collected to estimate potential fecundity from fish confirmed as pre-spawning by histology or abscence[1] of hydrated oocytes in distribution or POF in whole mounts.

Ship	Date	Sample reference	Fishing Position (haul)		Fish	Weights				Maximum oocyte diameter	Fecundity Number of oocytes > 0.185 mm
			Lat	Long	Length (mm)	Total (g)	Ovary (g)	Liver (0.1g)	Guts inc. contents (0.1g)		

Indicate the number of proportion of females in prespawning, spawning and spent maturity stages in each sample

Table 3.4.4.5. Details of the mackerel collection to estimate relative atresia collected during the 2001 triennial surveys to be tabulated by period and area

Ship	Date	Sample reference	Position		Maturity stage	Length (mm)	Weights				Histology analysis				
							Total Fish (g)	Ovary (g)	Liver (0.1g)	Guts inc. contents	Presence of spawning markers[1]			Early alpha atresia	
			Lat	Long							Mig nuc	Hyd	POF	Prev[1]	Number

[1] *Present = 1 absent = 0*

3.4.5 Sampling for horse mackerel standing stock fecundity in 2004 for the estimation of indices of realised fecundity in the Western and Southern areas - 2003 and 2004.

Table 3.4.5.1 shows the planned sampling by country in 2003 and 2004 for both southern and western horse mackerel to determine indices of realised fecundity based on lipid content and feeding performance during spawning.

During a period of 3 months only the lipid and dry weight in pre-spawning fish should be estimated. Randomly selected mature females >= 25cm should be individually homogenised to determine their lipid and dry weight content. This will supply information on the changes over time in the lipid and dry weight of pre-spawning fish during the 3 months prior to spawning, when ovaries are developing vitellogenic oocytes.

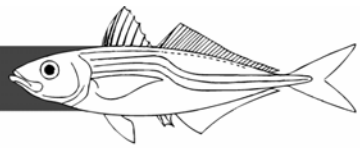
During the spawning season individual mature females >= 25 cm should be collected randomly for the estimation of lipid and water content in combination with both the estimation of the standing stock of vitellogenic oocytes. The classification of stomach fullness using a visual fullness scale and weight of guts including contents will also be carried out.

The above sampling will be used to determine the relationship between the lipid content of mature female fish and the standing stock of vitellogenic oocytes prior to and during the spawning season. Furthermore the monitoring of feeding performance during the spawning season by classifying stomach fullness will provide an estimate of the additional energy gained by feeding. This will be used in addition to information from the Continuous Plankton Recorder to provide a better understanding of Horse mackerel spawning energetics and provide and index of realised fecundity.

Prior to the survey during the 2003 Workshop at Lowestoft the Auto-diametric method will be developed for horse mackerel. This will include an inter-calibration to identify the fecundity in whole mounts relative to the stereometric estimate and to train analysts in the procedures.

The overall sampling programme and procedure for horse mackerel adult parameters is presented in Table 3.4.5.2.

HORSE MACKEREL SAMPLING



1 Estimation of lipid content in pre-spawning fish

Market sampling

Area	Sampling by	Month/samples 10	11	12/03	01/04	02	03	total no. of samples	
Southern	POR	25	25	25				75	
	ESP	25	25	25				75	**150**
Western	NED				50	50	50	150	**150**

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

d analyze fat content per dry weight ----------► fat content, dry weight

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

Sampling at Sea (for details on cruises see Table 3.2)

Area	Sampling by	Period/samples 1	2	3	4	5	6	7	total no. of samples	
Southern	POR/IPIMAR	40	40	20					100	
	ESP/IEO			20	40				60	**160**
Western	GER/BFA Fi			40	20				60	
	ENG/CEFAS			20	20	20			60	
	SCO/FRS				20		30		50	
	NED/RIVO					20	30		50	
	NOR/IMR					20			20	
	IRL/MI				20			(60)	80	**320**

a obtain length distribution of larger sample ----------► 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 3 parallel pipette samples of ovary in pre-filled caps

from SOUTHERN area: processed at the sampling institutes → f histological analysis at IPIMAR, IEO, AZTI

from WESTERN area: send on to IJmuiden, Bergen, Galway (same amount randomly choosen) → f histological analysis at RIVO, IMR, MI

f ----------► vitellogenic oocyte frequency, presence of POFs/atretic oocytes

* *fish can be frozen (carcass and organs together!) between these two steps for further processing in the home labs*

d (thaw), homogenize carcass and organs together

e analyze fat content per dry weight **at sampling lab** to avoid transfers (IPIMAR, IEO, MI, FRS, CEFAS, RIVO, IMR, BFA Fi) ----------► fat content, dry weight

Zi16Apr03

Figure 3.4.5.2. Adult horse mackerel sampling programme Flow diagram

Table 3.4.5.2. Sampling to determine indices of Horse mackerel realised fecundity.

1) Estimation of lipid content and dry weight in pre-spawning fish in 2003 and 2004

Collection of samples

Area	Sampling period	Targets for analysis by country	Total fish
Southern area (VIIIc, IXa)	Oct, Nov, Dec 2003	50 fish per month (75 Spain, 75 Portugal)	150
Western area (VIIbc,VIIjk, VIIIa)	Jan, Feb, Mar 2004	50 fish per month (150 samples Netherlands)	150
Mature female fish >=25 cm should be randomly collected from either surveys or market samples.			300

Tasks

Issues to be resolved by all institutes (Ireland, Netherlands, Norway, Portugal and Spain)	On board or laboratory	Laboratory processes
Locate commercial analysis service or purchase equipment to homogenise fish and to provide data on fat and water content in the homogenate. This requires an analytical standard operation procedure and inter-calibration between countries.	Estimate length (mm) and weight (g) from randomly selected mature females >= 25 cm. Freeze each fish in poly ethylene bag with sample and fish number for later lipid content and dry weight estimation.	Homogenise whole body. Determine lipid content and dry weight per g homogenate. (optional: calorific estimate)

Table 3.4.5.2. Continued.

2) Estimation of lipid content, dry weight, classification of stomach fullness and estimation of the standing stock of vitellogenic oocytes in spawning fish

Collection of samples

Area	Months / Survey periods	Lipid content estimation	Vitellogenic oocytes estimation	Total fish
Southern area (VIIIc, IXa)	Jan - Apr 2004 (survey periods 1-4)	40 per month (80 Spain, 80 Portugal)	40 per month (80 Spain, 80 Portugal)	160
Western area (VIIbc,VIIjk, VIIIa)	Mar - July 2004 (survey periods 3-7)	60 per month Lipid content and dry weight estimation to be carried out by the country collecting the samples	60 per month (100 Ireland, 100 Norway, 100 Netherlands)	300
Each fish to be used for lipid content / dry weight estimation, measurements of vitellogenic oocytes and stomach fullness classification!				460

Tasks

Issues to be resolved by all institutes (Ireland, Netherlands, Norway, Portugal and Spain)	Pre cruise preparations and on board ship procedure	Laboratory processes
Purchase or source analysis service to measure oocyte.. Carry out inter calibration of stereometric fecundity versus Auto-diametric method to identify oocyte size limits for estimation of frequency Determine relationship between Ln mean oocyte number per g ovary and Ln mean oocyte diameter. Develop pictorial scale for stomach fullness (Ireland)	2.5 ml Eppendorf should be filled with 1.2 ml of 3.6% formaldehyde buffered with 0.1M sodium phosphate pH 7.0. Three replicate tubes should be pre-labelled with a printed spot label indexed to each fish record in the standard data recording sheet. Randomly collect mature females >=25 cm. Measure: length (mm), Weigh total fish, carcass, guts including contents, and ovary separately (g). Classify stomach fullness: 1=empty; 2=partly filled; 3=full, 4=super full Remove an ovary sub-samples using a capillary pipette as shown in the appendix and transfer this sample to one of the Eppendorf tubes. Repeat this process three times to provide 3 replicate tubes each containing a sub-sample. Retain and freeze all body parts in one poly ethylene bag identified by the tube label for later lipid content estimation.	Homogenise whole body. Determine lipid content and dry weight per g homogenate. Measure frequency of standing stock of vitellogenic oocytes. Identify presence of POF and atretic oocytes in each sample.

3.4.6 Methodology for taking samples from mackerel and horse mackerel ovaries

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

Method

The capillary pipette will remove an ovary sample of standard weight CV 3% from a stage 3 to 5 ovary but not stage 6 either when the ovary is still in the fish or after dissection. 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 cut out a small piece 0.5cm^3 of ovary and place it in one 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 2 more replicate samples as above
- After use wash the capillary and piston.

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

Figure 3.4.6.1 Method to use a capillary pipette to remove an ovary sample

Push the plunger to the bottom of the glass tube and then push the tube into a hole previously made in the ovary tunica. Pull up the plunger until the sample reaches the first blue line on the glass tube as below. This will provide a sample of 0.106g with a CV3 %. Check there are no voids in the tissue sucked out of the ovary before expelling it into one of the sample tubes containing 3.6% formaldehyde.

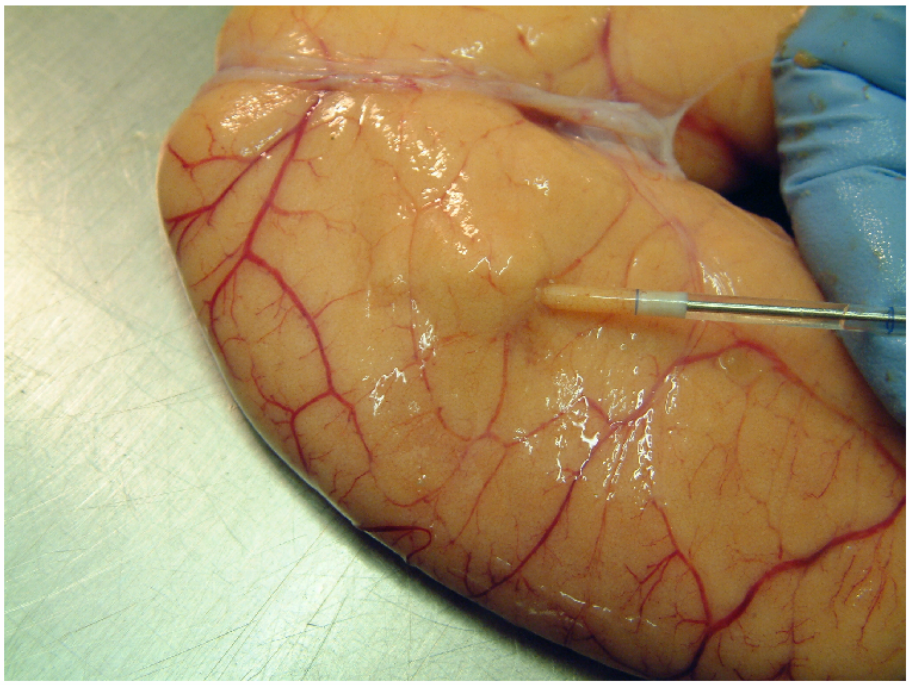


Figure 3.4.6.2 Picture of a rack holding Eppendorf like tubes for 10 fish with 3 replicates identified by spot labels on the lids. During storage a lid fits on top of the rack to keep the tubes in order during transport.

3.5 Data analysis

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

$$\text{Volume filtered } (m^3) = \frac{\text{Flowm-revs} \times \text{Aperture}}{\text{Flowm-calib}} \times \text{Efficiency Factor}$$

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

$$\text{Eggs}/m^2 = \frac{\text{Eggs counted} \times \text{Factor}}{\text{Volume Filtered } (m^3)} \times \text{Depth Sampled}$$

Where:

Flowm-revs. = Number of revolutions of the Flow meter during tow

Aperture = The area of the mouth opening of the sampler in m^2

Flowm-calib. = The number of Flow meter revolutions per metre towed, obtained from the flume or sea calibration in free Flow

Eggs counted = Number of eggs in sub-sample

Factor = Raising factor from the sub-sample to the whole sample

Depth Sampled = The maximum depth of the sampler during the tow in metres

Efficiency Factor = The sampler efficiency from flume or towing tank calibration

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

For stage I **mackerel** eggs:

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

For stage I **horse mackerel** eggs:

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

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

$$\text{Area } (m^2) = (\cos(\text{latitude}) \times 30 \times 1853.2) \times (30 \times 1853.2)$$

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

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

The method was modified for use in the analysis of the 1995 survey data. It is fully described in section 5.3.3 of the report of those surveys (ICES, 1996b). The same methods will be used for the analysis of the 2004 survey data. It is recommended that the Flowmeters and sampling devices deployed in the survey should be calibrated in terms of the volume of water filtered. There are two aspects to calibration: The first requirement is to know and understand the relationship between Flowmeter revolutions and distance travelled through the water. The second is to relate Flowmeter revolutions, whilst mounted *in-situ* in the aperture of a plankton sampler, to volume filtered by the sampler. The only way in which the second aspect can be accurately determined is to calibrate the Flowmeter and sampler under controlled conditions in a circulating water channel or in a large towing tank. These facilities provide independent measures of water or towing speed and also enable water velocity to be measured extremely accurately at numerous positions across the sampler aperture (EU AIR CT94 1911). Such facilities are extremely expensive and alternative methods to calibrate Flowmeters in-situ have been employed by various participants. This usually involves calibration at sea using a reference Flowmeter mounted on the outside of the sampler and two tows in opposite directions to overcome the effects of tides or currents on ship and sampler speed through the water. Such calibrations will provide a crude estimate of volume filtered (under non-clogged net conditions) but it must be remembered that there are differences in water velocity across the aperture of any sampler and that this water velocity profile may change as clogging of the net progresses. However, it is recommended that participants conduct calibrations of their Flowmeters *in-situ* over a range of towing speeds at least at the beginning and end of each survey.

3.6 Co-ordination, Communication, Deadline, Reporting

The co-ordinator of the 2004 western egg survey will be Dave Reid, Marine Laboratory, P.O. Box 101, Victoria Rd., Aberdeen AB9 8DB, Scotland, UK.

The co-ordinator of the 2004 southern egg survey will be Joaquim Pissarra, IPIMAR, Avenida Brasila, 1449-006 Lisboa, Portugal.

Participants who will be surveying during the same time period, should contact each other prior to their cruises to co-ordinate strategies and areas of overlap if any. Co-ordinators will obtain and provide details of vessels communication systems for use in maintaining regular contact during surveys. Contact with cruise leaders from the previous survey is also recommended to give prior indication of any distributional abnormalities.

Data input forms for the survey results and charts showing the proposed trawling positions will be despatched to all participants by the area-coordinators after the meeting of the after the MHMSAWG in September 2003.

The co-ordinator of the western egg survey data base will be Dave Reid.

The co-ordinator of the southern egg survey data base will be Concha Franco, IEO, Avda. Del Brasil, 31, Madrid, Spain.

The two co-ordinators of the egg survey data bases (D. Reid and C. Franco) will be responsible for loading data onto the database, checking their validity and estimating stage I densities. The data base will be available to all participants in the survey.

01 September 2004 is the deadline for sending egg survey results of both mackerel and horse mackerel to the egg survey data base co-ordinators. Data on condition indices and from the autumn preceding the Egg survey should be delivered to the WG from all countries.

The deadline for the analysis of all the samples and data relating to the adult parameters, collected during the 2004 surveys, is in early March 2005.

The next meeting of the ICES Working Group on Mackerel and Horse Mackerel Egg Surveys is proposed to be held on 4 - 8 April 2005, in Bergen.

4 EXAMINE CURRENT AND POTENTIAL FUTURE VARIANCE CALCULATION PROCEDURES (REFERRING TO TOR: "D")

4.1 Geostatistical estimators

A major objective of the EU funded project GBMAF (project QRLT-PL1999-01253) was to develop a geostatistical estimator for the calculation of TAEP and its associated uncertainty for the western mackerel. Previous work by Bez (2002) produced an estimate of egg production and associated uncertainty for Period 2 of the 1989 using transitive geostatistics. However, this application necessitated the assumption that the data were synoptic and temporal variability was not considered. The GBMAF study revealed that other geostatistical techniques, such as Ordinary Kriging and cokriging (see for example Journel & Huijbregts, 1978), provided adequate estimates of TAEP. However, accurate calculation of the associated confidence intervals could not be achieved using the kriging variance, due to the fact that the kriging variance is not dependent on the data values and so should not be used as a measure of uncertainty (Deutsch & Journel, 1998). Experimentation with a relatively new technique, conditional simulation (e.g., Goovaerts, 1997), showed considerable potential for providing good TAEP estimations with robust and straightforward measurements of estimation uncertainty.

In brief, conditional simulation provides a means of generating a number of equally probable surfaces of a variable, based on the conditioning data and the corresponding spatio-temporal autocorrelation structure (Deutsch and Journel, 1998). The most commonly used algorithm is sequential Gaussian simulation (SGS), which requires that the data are normally distributed so that conditional cumulative density functions, from which the realisations are drawn, can be easily derived using simple kriging or co-kriging. The data can be transformed using a normal scores procedure, and the grid node values of a simulation are subsequently back-transformed by referring to the original histogram. Summing over the grid nodes of each simulated surface provides a distribution of possible abundance estimates, from which global confidence intervals can be easily obtained. Furthermore, if the simulated values are conditioned on bathymetric data, making use of the knowledge that the mackerel tend to spawn in the region of the shelf break, we can obtain improved accuracy and precision.

The Stage I egg production data from all the triennial surveys undertaken between 1977 and 2001 were used in the study. Bathymetric data were obtained from NOAA and used to create a depth-related covariate, which was positively correlated with the data with coefficients of 0.27 to 0.46. Since it was necessary to account for temporal as well as spatial uncertainty, the problem was treated as three-dimensional with time as the third dimension. The simulations were done on a grid with a resolution of 15 x 15 nautical miles, by 7 days. This enabled egg production surfaces to be produced for each week of the spawning period, and also allowed egg production curves to be plotted by week rather than by period.

The TAEP estimates obtained using the traditional method (ICES, 2002) and sequential Gaussian simulation are compiled in Table 4.1.1. The 95% confidence intervals have also been calculated by simply adding and subtracting 1.96 times the standard deviation obtained using the SGS method. The final column in Table 1 lists the percentage differences between the estimates made by the Traditional method and the SGS method ((SGS – Trad/Trad)*100).

Table 4.1.1. TAEP estimates obtained using the traditional method and SGS. Confidence intervals are also provided corresponding to the SGS estimations.

	Traditional	SGS				Difference
Survey	TAEP	L. 95%ile	TAEP	U. 95%ile	CV	
1977	1.98×10^{15}	1.22×10^{15}	1.64×10^{15}	2.06×10^{15}	13.0 %	-17.2 %
1980	1.84×10^{15}	1.73×10^{15}	2.14×10^{15}	2.55×10^{15}	9.8 %	16.3 %
1983	1.53×10^{15}	1.23×10^{15}	1.45×10^{15}	1.67×10^{15}	7.8 %	-5.2 %
1986	1.24×10^{15}	1.63×10^{15}	2.09×10^{15}	2.55×10^{15}	11.2 %	68.5 %
1989	1.52×10^{15}	1.43×10^{15}	1.72×10^{15}	2.01×10^{15}	8.7 %	13.2 %
1992	1.94×10^{15}	1.26×10^{15}	1.78×10^{15}	2.30×10^{15}	14.8 %	-8.2 %
1995	1.49×10^{15}	1.91×10^{15}	2.26×10^{15}	2.61×10^{15}	8.0 %	51.7 %
1998	1.37×10^{15}	1.21×10^{15}	1.44×10^{15}	1.67×10^{15}	8.3 %	5.1 %
2001	1.21×10^{15}	1.01×10^{15}	1.18×10^{15}	1.35×10^{15}	7.5 %	-2.5 %

The results in Table 1 indicate that while the majority of estimates are consistent, there are considerable differences between the estimators for 1986 and 1995. The 1986 dataset is characterised by a delayed start to the survey campaign, with no egg densities available prior to mid-May. The traditional method has dealt with this in a far less generous way than the geostatistical method. It could be asserted that the geostatistical method has used more of the information

available and is able to reconstruct the missing data much better than the traditional method. However, it is not clear why there should be such a large disagreement for 1995.

The values of CV range between 14.8% for 1992 and 7.5% for 2001. The CV for 1977 is understandably high, due to the low number of samples taken. The CV for 1992 has an exceptionally high CV, although in this case the main reason is that no data were available until week 10 of the spawning period. The low CV of 7.5% for 2001 suggests that the estimate is extremely precise. However, it should be noted that at present the simulation algorithm assumes that all the modelling parameters and the sample measurements are correct. Therefore they do not take into consideration many potential sources of error. An imminent alteration to the current simulation algorithm will be the introduction of a Monte Carlo procedure which will aim to account for the additional uncertainty due to the variogram model parameters and measurement processes. The joint probability distributions for these parameters are currently being compiled using a Bayesian analysis. Current indications are that the resulting increase in CV will be in the order of 4%. In comparison with the CV of 16% calculated for the traditional method (ICES, 2002), the simulation algorithm should still be able to offer greater precision even with this additional variance. This will be partly due to the use of the bathymetric information.

4.2 References

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4.3 Traditional estimators

The estimation of variances for the total annual egg production (TAEP) and the SSB are performed following the procedures developed by Fryer (ICES 1996). In brief, variance estimation of annual egg production using the Traditional Method can be summarised as follows. For each ICES standard rectangle the variance is calculated as the mean egg production squared divided by the number of times the rectangle was sampled in the period. The variance for the AEP is then the sum of the variances for the rectangles multiplied by the number of days of the survey. However, the method only uses the positive part of the data (i.e. it excludes zero values). It also relies on the assumption that the egg counts are distributed with a constant spatio-temporal coefficient of variation, and does not consider the component of variance due to the uncertainty in the shape of the egg production curve (Augustin *et al*., 1998). Furthermore, the spatio-temporal autocorrelation in the data is not considered.

Beare *et al*. (2002) presented a comparison of three different methods (Traditional, GAM and Geostatistical) for estimating TAEP and its uncertainty. One hundred simulated datasets were prepared using a GAM surface based on the 1995 egg production data with error added from negative binomial distributions at the sampling locations of the 2001 survey. The existing method of variance calculation for the Traditional Estimator was considered inappropriate for the exercise and variances were instead estimated using bootstrap re-sampling. The results of the study revealed that while the Traditional method performed well in terms of estimating the 'true' TAEP, the average CV of the bootstrapped Traditional Estimator TAEPs was more than twice that obtained using the GAM and Geostatistical methods.

The traditional methodology is currently implemented on a Fortran platform and the development of an implementation using an object orientated programming language (i.e. S+, R) is seen as desirable. Aspects of the application still require clarification and a detailed presentation of the methodology to all participants was originally planned for this meeting. Problems related to variance estimation for egg surveys are not unique to the Triennial surveys and the Study Group on the Estimation of Spawning Stock Biomass of Sardine and Anchovy (SGSBSA) has developed methodology which aims at resolving problems specific to egg surveys. Therefore, a joint workshop involving members of WGMEGS and the SGSBSA was proposed by the WGMHSA (ICES 2003) to examine the statistical problems referred to above and assess the possibility of developing a simpler and more inclusive tool for estimating variance of the TAEP. Implications for survey design should also be considered in the Workshop. The WG recommends that the workshop take place before the results from the 2004 survey are analysed.

References

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5 REVIEW OF PROCEDURES FOR EGG SAMPLE SORTING, SPECIES ID AND STAGING (REFERRING TO TOR: "E")

5.1 Problems identified from plankton sample exchange

Three plankton samples were selected from the English (CEFAS) survey, Cirolana 4/01 (Apr-May 2001) which contained large numbers of mackerel and horse mackerel eggs in all stages of development. The samples were relabelled (to prevent participants gaining access to the original results) and passed around each institute in turn. Standard institute protocols were employed, to sort all fish eggs from the samples (or sub-samples). The total numbers of fish eggs were recorded from each sample and mackerel and horse mackerel eggs were identified and counted. A minimum of 100 eggs of each species were taken at random and allocated to development stages. All the eggs sorted and staged were then returned to the samples before they were passed on to the next institute. The results were sent to CEFAS, in a standard spreadsheet format, where the data were collated and summarised.

The results of this plankton sample exchange exercise were presented at this meeting and are clearly disappointing (Milligan and Shaw, WD 2003). However, the results are confused by the fact that the exercise examined three aspects of plankton analysis at the same time. The sorting of eggs from the samples, the identification of those eggs to species and the subsequent staging of the eggs, are discrete components of the analysis but are impossible to separate in these results. Other factors, such as the deterioration in egg quality (making them difficult to stage) and loss of eggs (as the samples were passed from institute to institute) also adversely affected the results. In addition, at least one institute included non-fish eggs in their total egg count, which made comparison of this parameter difficult to interpret. Despite the failings of the 2002 plankton sample exchange it has at least provided a clear path for the running of the egg sorting, identification and staging workshop which will be held at CEFAS, Lowestoft in October 2003 (Section 7.3).

5.2 New procedures for plankton sample sorting

The normal method for separating fish eggs from the rest of the plankton, using microscopes or magnifying lenses, is tedious and time consuming (section 3.3.8 and Milligan and Shaw WD 2003). Recently, Dr G. Eltink of RIVO, Netherlands has developed a simple and faster method for the separation of fish eggs from plankton samples. The technique is described below.

During the tri-ennial egg surveys, plankton samples are transferred from the cod-end of the plankton net and fixed with 4% buffered formaldehyde following the methods described in section ??. The plankton samples are then left in the 4% formaldehyde solution long enough for the fish eggs to become opaque (preferably 1-2 days).

Once the fish eggs are fixed, the formaldehyde is removed by pouring the plankton sample into a 270µm mesh sieve. The sample is then washed from the sieve into a 2 litre, glass beaker with seawater. A normal, garden, pressurised spray can then be used to spray seawater into the plankton sample. This fine, pressurised spray of seawater produces many tiny air bubbles within the sample. The sample is left to stand for five minutes during which time the air bubbles become trapped in the parts of the plankton that have projections (legs, antennae etc). The aerated plankton will rise to the surface and all smooth structures, including the fish eggs, sink to the bottom. It takes more time for the eggs to go to the bottom than for the plankton to go to the surface. It is therefore critical that the sample is not shaken or disturbed as the eggs separate from the plankton. After five minutes the floating plankton and surface liquid can be carefully decanted or siphoned from the top of the sample, leaving the fish eggs on the bottom of the beaker. The glass beaker containing the fish eggs should then be placed over a black tray, at an angle of approximately 45°. The fish eggs will fall/roll to the lower edge of the beaker, which enables the eggs to be easily removed with a pipette for transfer into a vial ready for identification and staging. The aeration process is repeated until no more eggs are found at the bottom of the beaker (a maximum of 4-5 times).

This method was used by RIVO for sorting the Dutch 2001 tri-ennial egg samples and has since been adopted by AZTI as their standard sorting procedure. Until the 'spray technique' has been validated and approved by all WGMEGS participants it is recommended that the entire sample is still resorted using each participants' standard sorting protocols (Milligan and Shaw WD, 2003).

It is recommended that each participating institute trial the effectiveness of the 'spray technique' for removing fish eggs from plankton samples before the egg sorting, identification and staging workshop to be held at CEFAS, Lowestoft. Participants at the CEFAS workshop will fully evaluate the effectiveness of the 'spray technique' and will recommend a standard plankton sorting procedure for use on future WGMEGS survey samples.

5.2.1 Planning for sorting, ID and staging Workshop, Lowestoft, October 2003

It is recommended that each institute has at least one scientist/technician at the egg workshop. It is essential that this representative is the same person who will analyse the majority of their institute's plankton samples from the 2004 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.** A new egg sorting protocol (spray technique) is given in section 5.2 and it is recommended that each participant trials this procedure before the egg workshop. This procedure will be discussed, and possibly updated, prior to being validated 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.

- **Identification of eggs.** Each institute has been asked to try to obtain artificially fertilised fish eggs of mackerel, horse mackerel and other species, which are regularly encountered in tri-ennial egg survey samples. It is hoped that the eggs, from all development stages, can be collected during the 2003 spawning season. These eggs of known species will be used for training and subsequent testing of participants' egg identification skills at the CEFAS workshop.

- **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 2000 egg workshop (ICES, 2001). 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 an 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.

6 DEFICIENCIES AND RECOMMENDATIONS

6.1 General Aspects On Egg Surveys

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

The estimates of SSB from the egg surveys have provided a robust tuning index used in the assessment and updated on a triennial basis. Over the long history of the egg surveys, dating back to 1977, a considerable volume of research work has been generated as a result of close scrutiny of all the parameters used in the calculation of SSB. This research has highlighted a number of problem areas and areas of uncertainty of which the Working Group is aware. These problems potentially affect the precision of the estimate of total egg production and SSB.

The problem areas highlighted by the research include uncertainty in the calculation of some adult parameters, principally in supporting evidence for fecundity changes in mackerel, and whether horse mackerel is a determinate spawner. Both these questions are being addressed by the WG and research is in place to resolve these problems. There is also the potential for problems in the estimation of egg production due to difficulties in egg identification to species and then staging. These issues, particularly staging, were addressed by a workshop held at Lowestoft in 2000. The outcome produced an improvement in agreement between egg readers and a consistency and standardisation of approach. Sorting of the plankton sample and identification to species were highlighted as issues for investigation by the sample exchange programme carried out in 2002. The WG has proposed a new mechanical sorting process, which will be validated for the next workshop at Lowestoft in October 2003. The workshop will also set out to standardise egg species identification for the key species. It is recommended that such workshops be held routinely prior to each triennial survey.

The problems are the subject of continuous investigation at a number of institutes involving valuable research and co-operation over many years. Much has already been accomplished in this respect but it is important that the research in all these areas continues to be encouraged and enhanced in order to further improve the quality of the advice on the management of these stocks.

6.2 ICES 2002 ERRATUM

Clarification of dates used for the calculation of the periods for the 2001 survey.

The 2002 WGMEGS report (ICES 2002) presented a number of different dates for the definition of the periods used for analysis of the egg production data and for producing the annual egg production curve. The confusion arose from a number of different data exploration exercises. The actual dates used for the periods in the final analysis are given in table 6.1. below.

Table 6.1 Period dates for the 2001 egg survey.

Period	Start Date	End Date
1	10th January	9th February
2	10th February	10th March
3	11th March	7th April
4	8th April	12th May
5	13th May	9th June
6	10th June	30th June
7	1st July	31st July

6.3 ICES 2002 Recommendations

1. The WG again strongly recommends that a mackerel egg survey be carried out on a triennial basis in the North Sea. This egg survey should be carried out in the year after the regular surveys on the western stock components. In the most recent years only Norway and The Netherlands have carried out the survey, covering the spawning area three

times throughout the spawning season. However, this coverage is considered as minimal and it would be better to cover the area at least four times to include the onset and end of the spawning. For this reason the WG would like to encourage at least one other nation to participate in the survey.

2. The WG recommends that the next meeting of the group should take place in Bergen from 4th to 8th April 2005. In arranging the meeting it is important that the meeting does not coincide with meeting of the Herring assessment Working Group.

3. The Working recommends that standardisation workshops for fecundity determination, egg identification and staging should be carried out on a routine basis, ideally timed to occur every 3 years immediately prior to the survey.

4. The WG recommends that research on the use of genetic markers for egg identification be continued as a matter of great importance. This work is included in the programme of the MarineEggs project, and is also subject of work in other institutes. Quantitative morphometric measurements acquired by manual or automated image analysis based approaches be developed to improve species and stage identification.

5. The WG recommends that all participating institutes carry out a search into historical data held by their institutes which could help clarify the evolution in key biological factors. The main areas of interest are:

    - Total lipid composition of horse mackerel particularly for periods prior to and during the spawning season. Institutions are encouraged to approach producer/processor organisations who may also hold such data.

    - Data on length and weight of mackerel and horse mackerel, again particularly for the periods prior to the spawning season. However, ANY such data, for any period or location would be desirable. These data should be sent to Dave Reid at FRS for mackerel and Guus Eltink at RIVO for horse mackerel. Data should include fields for location, date, sex and age where available.

    - Institutes are also encouraged to examine relationships between biological parameters and the CPR database.

    Analyses based on these collated data should be presented at the WGMEGS meeting in 2005

6. The working group continues to recommend extending the sampling area as much as necessary in order to delimit the spawning area whenever possible, even when this results in a reduction in the total number of stations.

7. The working group recommends that the calculation of variance for the egg surveys and fecundity estimates be the subject of a specific workshop to be held in collaboration with appropriate statisticians. This should include a clarification of the use of the traditional variance estimator and it's standardisation, as well as more modern techniques e.g. Geostatistics. Implications for survey design should also be considered. Ideally, this workshop should be held in collaboration with SGSBSA which face similar problems.

8. The working group recommends that research into determinacy in horse mackerel be carried out at the Matre facility in Norway. The working group recognises that this work will be carried out without external financial support and greatly appreciates the opportunity presented. Further, it is recognized that further detailed work on this subject carried out at Matre would need to be supported by a collaborative project, ideally under EU auspices.

9. The working group recommends that all participants currently using the new mechanical sampling techniques (the "spray" method) carry out validation trials with "spiked" plankton samples prior to the Lowestoft workshop in 20th – 26th October 2003. Results should be reported to that workshop and to WGMEGS in Bergen April 2005. All participants should also carry out trials of the method prior to the workshop.

10. The working group recommends that all participants carry out a review of their sampling gears (GULF III or Bongo) in relation to the published best practice (see section 5) and report on any deviations from these to the 2005 WGMEGS meeting in Bergen.

11. The working group recommends that all participants review the performance of their Flowmeters and regularly check their calibration in situ. Participants should also investigate the use of non-intrusive Flowmeters for use in the standard samplers. Any reports on progress in this area should be presented to WGMEGS 2005

12. The working group recommends that all labs collecting horse mackerel samples develop or source lipid analysis capability.

13. The WG recommends that institutes involved in fecundity and atresia work develop the appropriate image analysis technology required to carry out the Auto-diametric and stereometric approaches. Institutes are advised to consider that this approach allows the discontinuation of the use of Gilson preservative.

14. The WG STRONGLY recommends that institutes participating in the Lowestoft workshop send an analyst to the meeting who will be actively involved in the collection and analysis of data on the 2004 surveys.

15. The WG recommends that all participants consider including the work carried out to date under the INDICES project as part of their National programmes under the EU Data Directive.

16. The WG recommends that a revised fecundity analysis manual be prepared following the Lowestoft workshop (October 2003) and circulated prior to the 2004 surveys.

7 WORKING DOCUMENTS AND PRESENTATIONS

7.1 Invited Speakers

Reproduction, atresia and fecundity of horse mackerel (*Trachurus trachurus*) in the Saronikos Gulf (Greece).

Invited participant: Constantina Karlou-Riga, Min. of Agriculture, Fisheries Laboratory, Pireus, Greece. Email: fishres@otonet.gr

Abstract

The samples of *T. trachurus* were taken monthly using commercial trawler and purse seine from October 1989 until May 1991. The seasonal developmental changes of the gonads were studied using macroscopic and histological criteria. The mean GSI and HSI were also studied. In each sectioned ovary the following characters were recorded: a) Oocytes that have not begun vitellogenesis, b) Oocytes in early vitellogenic stages, c) Advanced yolked oocytes, d) Migratory-nucleus (MN) stage oocytes, e) Hydrated (H) oocytes and f) Postovulatory follicles (pofs). Pofs were aged following the criteria used for *T. symmetricus.* The sequential alpha, beta, gamma and delta atresia stages were recorded when identified. The ovaries were classified to four atretic states: a) atretic state 0, where no α stage atresia is observed, b) atretic state 1, where <50% of yolked oocyes are in α stage atresia, c) atretic state 2, where ≥50% of of yolked oocyes are in α stage atresia, d) atretic state 3, where no remaining yolked oocyes, but beta or later stages atresia are present. The females classified to atretic states 2 and 3 were considered as postspawning, while the females with either MN stage or H oocytes or yolked oocytes with pofs were considered as spawning.

Females in atretic state 1 showed evidence of past (pofs) or imminent (MN stage or H oocytes) spawning, while those in atretic state 2 had no such evidence. Two successive reproductive periods were identified, the first from October 1989 to July 1990 (onset of spawning observed in December) and the second from December to May 1991 (onset of spawning observed in January). The peak GSI during the first reproductive period occurred in February, while during the second in March-April, whereas the GSI has taken very low values. During the first spawning period atretic state 1 was the most common condition. Females in atretic states 2 and 3 appeared gradually in increased numbers. High atretic state 2 near the end was replaced by atretic state 3 at the end. By the middle of the second period, high levels of atretic states 2 and 3 were noted, and subsequently females in atretic state 0 were increased resulting in an unexpected pattern, thus showing for *T. trachurus* a probable flexibility in adjusting its spawning time to different conditions.

During the first spawning period, the fractions of spawning and postspawning females were estimated as a function of time elapsed since the onset of spawning. The approximate spawning season for the individual female (94 days) was calculated by subtracting the 50% spawning point (67 days) from the 50% postspawning point (161 days). The time where all active females were spawning, while the postspawning rate was accelerated showed the peak of spawning (end of March-beginning of April). The spawning frequency estimation was based on the incidence of pofs and MN stage and/or H oocytes. The mean of percentage females showing imminent spawning in 12 h (late MN stage and/or H oocytes), past spawning 12 h before (presence of new pofs) and 36 h before was calculated as equal to 17.1%. Thus the average female spawned every 5.8 days, while the potential number of batches was estimated as equal to (94/5.8) 16. Using the gravimetric method, the number of MN stage or H oocytes was counted. The batch fecundity estimated as a function of ovary weight, and forcing the regression line through the origin was found to be 205 oocytes/gram female weight. Thus the potential annual fecundity was calculated as equal to 3280 oocytes /gram female weight (205X16).

The evidence for a determinate spawner is: 1) the presence of a gap in oocyte size distribution between the oocytes matured for the season and the reservoir of the immature oocytes taken at the beginning of the season and 2) the product of batch fecundity times the number of batches throughout the season has to be equal to the standing stock of yolked oocytes at the beginning of the season or differs for an amount equal to the oocytes lost by atresia. For *T. trachurus* a continuous oocyte size distribution was shown, while only at the spawning time the mode of hydrated oocytes is separated from the rest. The relative total fecundity from females taken at the beginning of the season was found much less than the potential annual relative fecundity showing that *T. trachurus* is most likely to be considered as an indeterminate spawner.

Table 1. Description of the reproductive stages of horse mackerel female by macroscopical and microscopical criteria

Female maturity stage	Ovary	
	External appearance	Histological appearance
Virgin	Rounded translucent ovaries; less than a quarter of length of body cavity; no oocytes are visible	Well-spaced ovigerous folds orientated towards the center of the ovary; oogonia and primary oocytes at both the chromatin nucleolus and perinucleolus stage; oocyte size 10-60 μ
Developing virgin or resting	Rounded orange -pink ovaries; a quarter to a third of body cavity; no oocytes are visible	Few spaces between ovigerous folds; few oogonia, the majority of primary oocytes at the perinucleolus stage; oocyte size 20-150 μ
Early developing	Orange ovaries; a third to a half of body cavity; oocytes are visible	Oocytes with cytoplasmic vacuoles (lipid droplets); yolk granules first appear in the cytoplasmic periphery while subsequently spread internally; elongated sprindlelike cells constitute the follicle layer; oocyte size 150-400 μ
Later developing	Orange-yellow ovaries; two thirds to whole of body cavity; oocytes are visible	Yolk granules becoming larger (yolk spherules); oil droplets spread throughout the cytoplasm, while at the end of the stage they coalesce and accumulate around the zona radiata i nucleus; granulose cells become cuboidal; oocyte size 400-600 μ
Ripe/ running	Yellow ovaries fill the whole of body cavity; hyaline oocytes visible beneath the thin oocyte membrane may run from vent on slight pressure	Yolk spherules coalesce to globules or plates; oil droplets follow the nucleus migration to the animal pole where the nucleus disperses its content into the cytoplasm; oocyte size 600 -800 μ. By the nucleus dispersion rapid uptake of fluid (hydration) is taken place; zona radiata loosing its striation becomes very thin; oocyte size 700-1200 μ
Partly spent	Flaccid yellow with patches of red ovaries; smaller than those of the previous stage	Present post ovulatory follicles (pof); oocytes in any developing stage including that of ripe; possible oocytes in alpha or subsequent stage atresia
Spent	Small flaccid red ovaries covering a third of body cavity	Possible pof; yolk oocytes where 50% or more are in alpha stage atresia or no yolk oocytes but atretic follicles (beta or later stage atresia) and primary oocytes

The use of parasites as biological tags for stock-identification of Western Horse Mackerel

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Abstract

The multidisciplinary HOMSIR project on stock identification of horse mackerel *Trachurus trachurus* has produced some useful results, particularly from the use of parasites as biological tags. During the horse mackerel spawning period in 2000 and 2001, 50 fish were examined each year for parasites in samples taken at 10 Atlantic stations, from southern Norway to the coast of Morocco. The most important results from the parasite infection data in these samples are as follows.

Samples representing the putative "Western" and "North Sea" stocks were characterized by contrasting patterns of infection with anisakid nematode larvae, which are long-lived parasites that are cumulative with age. Fish in samples from the western area had massive infections of *Anisakis* spp., usually of several hundred specimens, and small infections, usually in single figures, of *Hysterothylacium aduncum*, whereas the reverse was true of fish in the North Sea sample. Anomalous individual fish in the 2000 North Sea sample were identified as migrants from the western area by their characteristic patterns of infection, which differed markedly from all other fish in the sample. In addition, the three different species of *Anisakis* identified from these samples by enzyme electrophoresis show marked differences in their geographical distributions. These two sets of data could be used in combination to discriminate, and estimate the extent of mixing, between stocks of horse mackerel.

The monogenean *Heteraxinoides atlanticus* was found infecting the gills of fish from two samples taken off the west coast of Portugal, from one taken off southern Norway, one from the southern Bay of Biscay, and one from the extreme western Mediterranean. This parasite has previously only been reported from *Trachurus picturatus* caught further south and offshore in the Atlantic and so appears to be a good indicator of migration from this region. It is likely to have a life span of only a few months and so could be used as an indicator of migrations that had occurred within this sort of time scale.

A multivariate cluster analysis of all the most common parasites showed the Atlantic samples falling into three distinct groups corresponding to the currently recognized North Sea, Western and Southern stocks. However, the Western and Southern stocks were less clearly differentiated from one another than either was from the North Sea stock, suggesting a considerable degree of mixing between the two.

7.2 Working Group Members

Genetic identification of fish by species-specific DNA markers for use in stock biomass assessments and detection of commercial fraud (MARINEGGS)

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Abstract

MARINEGGS is a European project with the main objective of improving a fisheries independent method of fish spawning stock biomass estimation by developing PCR based methods for the accurate identification of the early stages of the eggs of 13 commercially important marine species: *Trachurus trachurus, T. mediterraneus, T. picturatus, Macrorhamphosus scolopax, Scomber scombrus, S. japonicus, Gadus morhua, Melanogrammus aeglefinus, Merlangius merlangus, Merluccius merluccius, Merluccius senegalensis, Lepidorhombus whiffiagonis* and *L. boscii*. The species of each group show overlapped distributions and spawning period and their eggs can be confounded in plankton surveys.

Horse Mackerel maturity stages review (1992-2002) in the ICES Division IXa (Portugal and Gulf of Cadiz)

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Abstract

Significative differences have been observed between data on maturity stages identification from macroscopic and microscopic analysis of horse mackerel ovaries. Microscopic data were used to check and control the quality of the macroscopic observations.

Comparison of microscopic maturity stages distribution pattern shows a large dominance of stage 3 during the spawning season and a dominance of stage 5 in the non-spawning season.

Maturity stages distribution during the spawning season, with high proportions of stages 3 and 5, suggests that after a pre-spawning stage 3 females pass through a quick stage 4 to a stage 5 and after that they return again to a stage 3 and starts new batch cycles. This mechanism suggests that there is no microscopic difference between pre-spawning stage 3 ovaries and the subsequent stages 3 observed during the spawning season.

The observed continuous distribution of the oocyte's diameters suggests that horse mackerel is not a determinate spawner.

The evolution of the maturity stages distribution during the spawning season from the period 1992-95 to the recent period 1998-2002 suggests a significative displacement in the horse mackerel spawning season.

Horse Mackerel (*Trachurus trachurus*) evaluation by Daily Egg Production Methods (DEPM) in ICES Division IXa (Portugal and Gulf of Cadiz)

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Abstract

Spatial distribution and abundance estimates of horse mackerel eggs off the Portuguese coast and Gulf of Cadiz during January/February 2002 were obtained during a cruise of the R/V "Noruega" in order to apply Daily Egg Production Methods (DEPM) to evaluate the horse mackerel spawning stock biomass in the area. Two different methods of calculation of Daily Egg Production were used and the estimates obtained with the two ranged between $1.43x10^{11}$ and $4.06x10^{11}$ eggs produced per day. These results are compared and discussed.

Mackerel and Horse Mackerel Egg Production at the Periphery and outside the Western Survey Area: Results from the 2002 *Atlantean* Survey L. Dransfeld[1]; O. Dwane[1]; J. Molloy[1]; C. Kelly[1]; D. Reid[2]

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Abstract

One year after the ICES triennial mackerel and horse mackerel egg survey, a further egg survey was carried out to assess whether significant spawning occurs outside the ICES standard area. 173 ICES rectangles were sampled on the Porcupine, Rockall and Hatton Banks, the Rockall Trough and the Faeroes waters using standard methodology for the collection of mackerel and horse mackerel eggs. Data were analysed to obtain distribution of stage 1 mackerel and horse mackerel eggs and daily egg production in 41 control rectangles inside the standard area and 132 rectangles outside the standard area. In 2002 daily egg production of mackerel was elevated inside the standard area, with rates decreasing off the shelf edge. Some spawning activity took place south and east of the Rockall Bank and south east of the Faeroes Bank extending to west of the Scottish Shelf edge. Low levels of horse mackerel egg production were found west of the Rockall bank and south of the Faeroes Bank. The combined daily egg production per ICES rectangles outside the standard area in 2002 for mackerel and horse mackerel was less than 1% of egg production measured inside the standard area in period 5 and 6, 2001. This indicated that spawning of both species outside the standard area was insignificant. The northern peripheries of the standard area should however be further explored for possible spawning activities and surveys should extend sampling to higher latitudes during the ICES survey program.

Scombrid eggs identification using genetic markers

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Abstract

Atlantic mackerel (*Scomber scombrus*) is one of the most abundant fish species and also one of the largest fishery resources in European waters in North Atlantic. Chub mackerel (*Scomber japonicus*) is distributed in eastern Atlantic from the Canaries and the Azores, commonly north to the Bay of Biscay. Eggs and larva of these two species are morphologically similar. Both species have common spawning area, which make difficult their identification. Then, a genetic method to identify them should be very helpful to assess the spawning stock size of these species.

Species-specific primers were designed for species with eggs of mackerel type. As the specificity was not complete a Multiplex PCR was performed with the two sets of primers in individuals of the two species. On the other hand, primers to amplify different fragments from both species in order to obtain markers for SSCP were designed and this technique and MSP-PCR technique was applied to the two species.

With all these methods eggs and larvae of *S. scombrus* and *S. japonicus* were identified.

Horse Mackerel (*Trachurus trachurus*, L. 1758) have a Determinate or Indeterminate fecundity?

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Abstract

The reproductive strategy of horse mackerel was studied by comparing the variation of the total number of oocytes during the spawning period at three different periods (beginning, middle and ending). It was observed an increase of the total number of oocytes during the spawning season as well as the mean number of total oocytes by units of area (cm^2). The mean radius (μm) of oocytes along the spawning season showed a decrease. According to these results horse mackerel is probably an indeterminate spawner.

Estimation of the total annual egg production and associated uncertainty of Atlantic mackerel (*Scombrus scombrus*) using conditional simulation

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Abstract

This Working Document assesses the use of conditional simulation to improve on the accuracy and precision of the estimation of the total annual egg production (TAEP) of Atlantic mackerel over the traditional methodology. While standard geostatistical methods, such as collocated cokriging, can provide unbiased estimates, calculation of the global estimation uncertainty is less straightforward. Conditional simulation offers a simple method for obtaining confidence intervals by generating a number of equiprobable realisations of the variable surface, based on the available data and the spatiotemporal correlation structure. Sequential Gaussian simulation (SGS) is used to calculate the TAEP and associated uncertainty for each of the 9 surveyed years (1977 to 2001) to date. The results are compared to those obtained using the traditional method. The CVs varied between 14.8% and 7.5%, although it was acknowledged that the current methodology did not account for a number of additional sources of uncertainty. However, the results demonstrated the potential of conditional simulation as a valuable tool for the estimation of TAEP and its associated uncertainty.

Spawning frequency and batch fecundity of horse mackerel, *Trachurus trachurus* (L.), in the Saronikos Gulf (Greece)

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Abstract

Horse mackerel *Trachurus trachurus* (L.) is a multiple spawning fish with most probably an indeterminate fecundity. Histological sections of gonads were used to identify the hydrated oocytes, the migratory-nucleus stage oocytes n and the new and old post-ovulatory follicles. Spawning frequency determination of the based on the mean percentage estimation of the females that occurred in different states such as migratory-nucleus stages oocytes and post-ovulatory follicles during two successive reproductive periods, was found to averages once every 5.8 and 4.8days, respectively. High spawning frequency was observed at the peak of spawning. Relative batch fecundity of 205 oocytes/fish weights was estimated by the hydrated and migratory-nucleus method. Since potential annual spawnings were found to be equal to 16, potential annual fecundity could be 3280 oocytes/g fish weight.

Biological aspects of Horse Mackerel (*Trachurus trachurus* L. 1758) in the Bay of Biscay in 1987 and 1988

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Abstract

Weight/length relationships, monthly evolution of condition factor, gonosomatic index, maturity stages evolution, sex ratio and mean length at first maturity of Horse Mackerel in the Bay of Biscay are presented. The results have been compared by sexes and areas.

Biological aspects of Mackerel (*Scomber scombrus* L. 1758) in the Bay of Biscay from the Basque Country catches in the period 1987-1993

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Abstract

Fish total and gutted weight and length relationships, monthly evolution of condition factor, sex ratio, maturity stages and gonosomatic index monthly evolution, mean length and age at first maturity, otoliths weight and length relationships, growth equation and stomach repletion monthly evolution of mackerel (*Scomber scombrus*, L.1758) in the Bay of Biscay are presented.

7125 mackerel from commercial landings are studied. They are caught mainly in the southeastern Bay of Biscay (ICES Divisions VIII a,b,c), in the period 1987-1993. The length range of specimina is between 10.7 and 50.3 cm of total length. The spawning period extends from February and June, with a peak in March-April. 50% of males reach their first maturity at 27.0 cm of total length and females do at 29.1 cm, and both sexes mature at about 1.7 years old. Sex ratio observed below 40 cm length range is close to 1:1, but above this length females are found predominant. The total length (mm) – total weight (g) relationship is described by the multiplicative function: $W = 0.00000207263 * L^{3.212}$; r = 0.980.

In the period 1987-1993, 4686 exemplars are aged by means the otolith (sagitta) reading. Age Length Keys for quarters and year are established. Fish aged 0-15+ years old are present in the samples. The parameters of the Von Bertalanffy growth equation for both sexes combined and all the years together considered are: $L_\infty = 45.88$ cm, $k = 0.196$ years^{-1} and $t_0 = -3.026$.

Horse Mackerel (*Trachurus trachurus*, L. 1758) feeding in the southern Bay of Biscay: seasonal and length variations

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Abstract

Results of a comparative plankton sample sorting, egg identification and staging exercise, 2002

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Abstract

The results of an EU funded mackerel and horse mackerel egg staging workshop (ICES, 2001) showed good agreement between WGMEGS participants in the allocation of mackerel and horse mackerel eggs to the various development stages. A recommendation of this workshop was for a sample exchange to be conducted following the 2001 tri-ennial surveys. The aims were to help maintain standards in egg staging and also to address the potential problems of sorting and identification of fish eggs.

Three plankton samples were selected from the English (CEFAS) survey, Cirolana 4/01 (Apr-May 2001) which contained large numbers of mackerel and horse mackerel eggs in all stages of development. The samples were passed around each institute in turn. Standard institute protocols were employed, to sort all fish eggs from the samples (or sub-samples). The total numbers of fish eggs were recorded from each sample and mackerel and horse mackerel eggs were identified and counted. A minimum of 100 eggs of each species were taken at random and allocated to development stages. All the eggs sorted and staged were then returned to the samples before they were passed on to the next institute. The results were sent to CEFAS, in a standard spreadsheet format, where the data were collated and summarised.

The results showed significant differences between the participants which are extremely difficult to interpret. The main difficulty is in separating the various components of plankton analysis (the removal of eggs from the rest of the plankton, the identification of those eggs and the staging of identified eggs). On reflection, the exercise was not well designed however it still raises some concerns for the plankton results from the tri-ennial surveys. These concerns will be addressed by an egg staging workshop to be held at CEFAS, Lowestoft during October 2003.

Diel patterns in spawning by Mackerel and Horse Mackerel along the continental shelf west of the British Isles estimated using direct observations of multiple-stage egg data

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Abstract

The aim of this study is to describe the times of day when spawning by Atlantic mackerel and horse mackerel along the continental shelf west of the British Isles are most likely to occur. This was done by using multiple egg stage data from the International Tri-Annual Egg Surveys. Times of day at which spawning events occur were back-calculated from the raw data. Egg development rate equations were employed to estimate how the duration between fertilization and hatching varies with respect to sea temperature. In order to minimize the uncertainty in the back calculated times of day,

different statistical re-sampling techniques were used. The results show that horse mackerel is most likely to spawn during early morning, while mackerel are more likely to spawn at night. It should be noted that the diel-spawning signal for mackerel is much weaker that that observed for horse mackerel. Nevertheless, diel signals for both species are weak and it is clear that spawning events can occur at any time of the day for both mackerel and horse mackerel. The re-sampling method used to calculate spawning times from observational multiple egg stage data, and the possible reasons for the differences in diel spawning activity between the two species are discussed.

Horse mackerel egg staging for Daily Egg Production Method

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Abstract

With the aim to apply Daily Egg Production Method (DEPM) to horse mackerel the embryonic development of horse mackerel were classified in 11 stages. Two experiments of incubation of fertilized horse mackerel eggs were carried out during two research cruises: the first at RV "Noruega" in March 1989 and the other at RV "Mestre Costeiro"'s cruise in January 2002. Egg stages descriptions and their duration was clarified using those artificially fertilised eggs, incubated at no controlled temperature (≈18º C). The model that better fitted data is presented as the polynomial equation $y = -0.003x^2+0.286x+0.218$

Advances in methods to estimate realised fecundity and consideration of an indirect approach based on condition indices

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Abstract

This working document gives some information on what is required to improve the precision of realised fecundity estimates and the aims of EU funded research to meet these requirements. In the first part we extend the application of the Auto-diametric method to a more diverse range of ovaries containing hydrated or asynchronous oocyte populations to determine oocyte number and frequency. The final section considers indirect methods of assessing annual realised fecundity in mackerel using condition indices of the gut (including contents), liver, carcass and ovary. Examination of the gut condition indicated that mackerel feed little during the peak spawning period when multiple spawning markers were found in the ovary. It was estimated that in 2001 the Western mackerel spawning component invested 16% of their body weight in reproduction albeit on a limited number of samples available for the analysis (n= 30 fish).

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