FSS Survey Series No. 2007/04

Mackerel Egg survey, June 26th – July 16th, 2007

by

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Keywords: Mackerel, Horse mackerel, eggs, surveys, plankton.

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Abstract

Every three years the International Council for the Exploration of the Sea (ICES) coordinates a series of mackerel and horse mackerel egg surveys covering the eastern Atlantic from Gibraltar to the north coast of Scotland between January and July. The aim of this survey programme is to assess the northeastern Atlantic mackerel and horse mackerel stock. The Marine Institute participates in this programme and covers stations in the Celtic Sea and West of Ireland. Plankton samples were collected at 88 stations, and the eggs they contained were preserved in 4% buffered formaldehyde. Preliminary analysis shows that egg numbers were concentrated close to the shelf edge, around the 200m contour line. Seven fishing hauls were made to collect mackerel and horse mackerel samples for fecundity analysis. Samples were collected to ensure maximum temporal and geographical spread.

1 Introduction

Every three years the International Council for the Exploration of the Sea (ICES) coordinates a series of mackerel, *Scomber scombrus,* and horse mackerel, *Trachurus trachurus*, egg surveys covering the eastern Atlantic from Gibraltar to the north coast of Scotland between January and July. The aim of this survey programme is to estimate the spawning stock biomass of the northeastern Atlantic mackerel and horse mackerel stock. The Marine Institute participates in this programme and in this survey covered stations in the Celtic Sea and west of Ireland.

This was the last of nine surveys covering the Celtic Sea, west of Ireland and west of Scotland. Five further surveys targeted the Bay of Biscay and the Cantabrian Sea. This reflects the spawning area of both mackerel and horse mackerel. Results from onboard sample analysis of egg numbers are presented, and full laboratory analysis of the histological samples will be carried out in the coming months. The data will be submitted to the Working Group on Mackerel and Horse Mackerel Egg Surveys, WGMEGS, in March 2008. Preliminary data will be used by the Working Group on Mackerel, Horse Mackerel, Sardine and Anchovies, WGMHMSA, in September 2007.

2 Materials and Methods

2.1 Scientific Personnel

Name	Service area/Affiliation	Role
Brendan O'Hea	MI - FSS	Scientist-in-charge
Eugene Mullins	MI - FSS	Scientist
Shane Shannon	MI - FSS	Scientist
Orla Hannify	MI - FSS	Scientist
Dermot Fee	MI - FSS	Scientist
Stephen Comerford	GMIT	Student
Aisling Smith		Student

2.2 Survey Plan

2.2.1 Area of operation

The survey was carried out in the Celtic Sea and West of Ireland, from 47° 45N to 55° 45N, and from 6° 15W to 16° 15W. This covered ICES areas Via, VIb, VIIb, VIIc, VIIg, VIIh, VIIj, VIIk, VIIIa and VIIId, (Figure 1). Survey sites were at 0.5 degrees spacing, both latitudinally and longitudinally. The survey was adaptive, and while theoretical eastern and western limits were set, in practice the presence or absence of eggs dictated moving to the next transect. The protocol called for two consecutive zero samples before making a transect change, however due to the large sampling area, for this survey, it was modified to change transects after two low samples or one zero figure. Survey protocols called for the survey area to be sampled on alternate transects. The intervening transect could be sampled on the return leg, if sufficient time remained.

2.2.2 Specific operations

Plankton Hauls

At each station the GULF VII plankton sampler was towed at four knots on a V-shaped profile. The GULF was deployed over the stern, using a winch with 11mm co-axial cable in an armoured sheath. The water column was sampled to within five metres of the bottom, or a maximum depth of 200m. Each sample lasted 40 minutes. If a thermocline of greater than 2.5°C difference in temperature over a depth of 10m was encountered the tow was halted 10m below the thermocline, and the sampler was recovered. Attached to the sampler was a real-time CTD and flowmeter system, (Pronet), which collected temperature and salinity data, and measured the volume of water filtered during

the tow. Once back aboard the cod-end was removed, a second cod-end was attached and the plankton net was washed down. The cod-ends were then brought to the lab, and the plankton sample was washed out. The sample was preserved in 4% buffered formalin. It was examined under a microscope after an hour and any eggs and fish larvae were removed. A second examination took place after 36 hours. A count was kept of mackerel and horse mackerel stage 1 eggs, mackerel and horse mackerel eggs of later stages, and other fish eggs. Note was also taken of the volume of water sampled by the GULF during each haul, as well as the salinity at 20m, and the water temperature at 5m, 20m, 50m, 100m, and deepest temperature. We were also asked to collect samples of Greater Pipefish, *Entelurus aequorous,* from the plankton hauls.

Fishing Hauls

As part of the survey samples of mature mackerel and horse mackerel were collected at various latitudes. Fishing sites were selected close to the 200m contour line (Figure 2). Hauls were made using a herring pelagic net. Sampling targets were 100 mackerel gonads of stage 3 or greater, and 50 horse mackerel samples, over four weight categories. Four 25µl replicate samples were collected from one gonad of each fish, and stored in pre-weighed tubes containing 1.2ml of 3.6% buffered formalin. The sampling protocols for both species are attached in the appendix.

Acoustic sampling

The Simrad ER-60 split-beam transducer was run throughout the survey. This provided depth information for the plankton hauls.

2.3 Equipment and system details and specifications

GULF VII plankton sampler

11mm armoured co-axial cable

Pro-net CTD and flow sensor

Pro-monitor CTD and flow sensor

Pelagic Herring net

Simrad ER-60

2.4 Protocols used

The protocols for the gonad sampling of the mackerel and horse mackerel are listed in the 2006 WGMEGS report and in the appendix. The gonads of both species were staged using the Walsh scale.

Results

Plankton Hauls

A total of 88 hauls were carried out, over eight transects, (Figure 1, Table 1). Due to the large volume of material in the samples the eggs were separated from the rest of the plankton using the "spray technique method". In this method the sample is placed in a conical container with a tap at the bottom. Pressurised water from ordinary garden sprayers is forced through the sample. This produces large amounts of bubbles which attach to hairs etc. of planktonic organisms and causes them to float. Eggs, being smooth and round, sink to the bottom of the cone and can be decanted off. This technique is repeated a number of times to ensure the removal of all the eggs.

Mackerel stage 1 eggs were recorded at 73% of stations and horse mackerel stage 1 eggs were recorded at 88% of stations, (Figures 3 & 4). The largest numbers of stage 1 Mac eggs were found on transects 2 and 3, at 445 and 302 respectively. Similarly the largest concentrations of HOM eggs were also found on these transects, at 586 and 490 respectively. Most of the larger hauls of stage 1 mackerel eggs were found to the east of the 200m contour line. In the case of HOM the larger egg hauls were made closer to, or on, the 200m line.

Fish Hauls

A total of 7 hauls were carried out, (Figure 2). Horse mackerel were caught in low numbers in all hauls. Mackerel were recorded in six of the hauls and was the dominant species in four of them. Other species caught were Boarfish, *Capros aper*, Hake, *Merluccius merluccius*, Blue whiting, *Micromesistius poutassou* , Greater Pipefish, *Entelurus aequorous* , Herring, *Clupea harrengus*, John Dory, *Zeus faber*, and Monkfish, *Lophius piscatorius.*

Samples were collected for all four of the mackerel weight categories, but only the two smallest horse mackerel classes. In total 70 mackerel and 39 horse mackerel samples were collected. These will be worked up in the laboratory over the coming months. As well as the samples required for maturity, flesh samples were collected from the mackerel for genetic analysis by IMR in Norway, and flesh samples were collected from the mackerel and horse mackerel for stable isotope analysis by scientists in the Marine Institute. A separate sample of 50 mackerel was collected for scientists at IMR, to look at parasites. Samples of Greater Pipefish, *Entelurus aequorous,* were also collected for colleagues in IMARES, in Holland.

3 Discussion and Conclusions

The survey was quite successful. Due to the large survey area it was unlikely that we would cover the proposed sampling area. We lost approximately 1.5 days due to bad weather, and a further day was lost when we encountered problems with one of the high pressure hydraulic pipes which controlled the winch. On this survey most of the identification and staging took place on the survey, rather than bringing the eggs back to the lab. as happened earlier in the year. All egg data from both surveys carried out by the Marine Institute this year were passed on to the data coordinator in early August. The early fecundity work is nearly complete, and the atresia work will be finished for the WGMEGS working group in April.

During the survey an attempt was made to artificially fertilise mackerel eggs for a research project onshore. Ripe males and females were collected from Haul 6. The eggs were stripped from the fish and a quantity of milt was added to them. They were left to fertilise for an hour. However due to the low numbers of eggs stripped initially the numbers of eggs that fertilised successfully were low.

Acknowledgements

Much appreciation is expressed to the skipper, Ciaran Flanagan, and crew of the *Celtic Explorer.* Their many skills kept the survey functioning. Thanks are also expressed to the scientists and students who worked on the survey.

References\Bibliography

ICES, 2006. Report of the working group on mackerel and horse mackerel egg surveys. ICES CM 2006/LRC:09

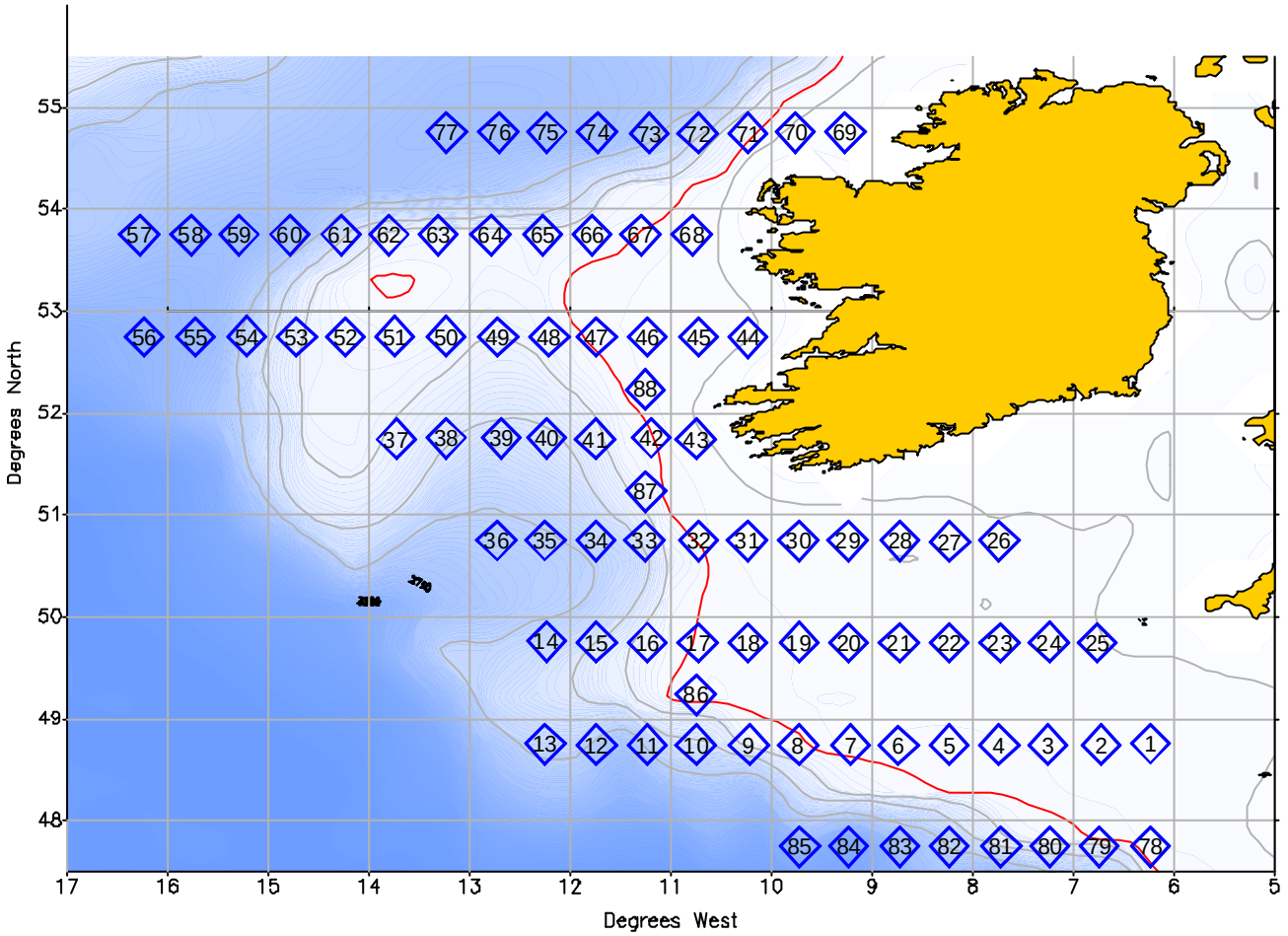

Figure 1 Survey plankton stations.

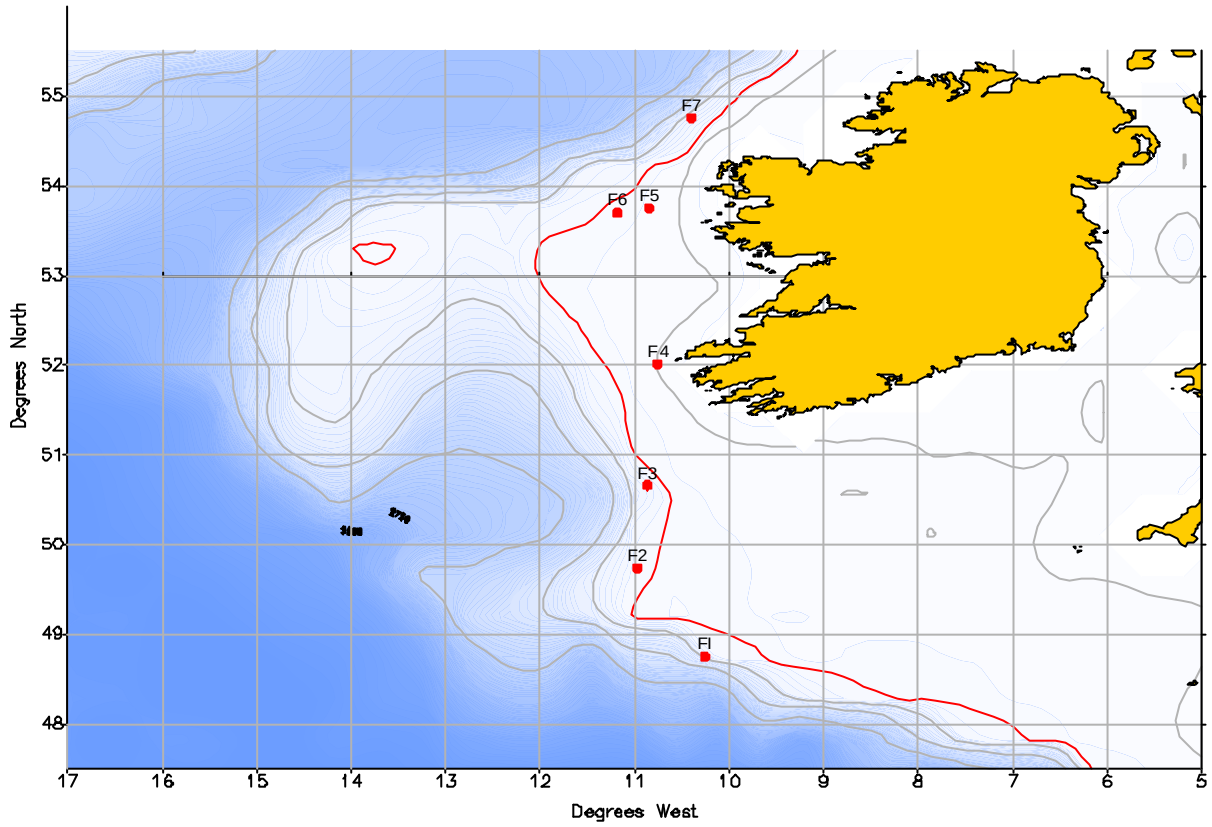

Figure 2 Fishing stations.

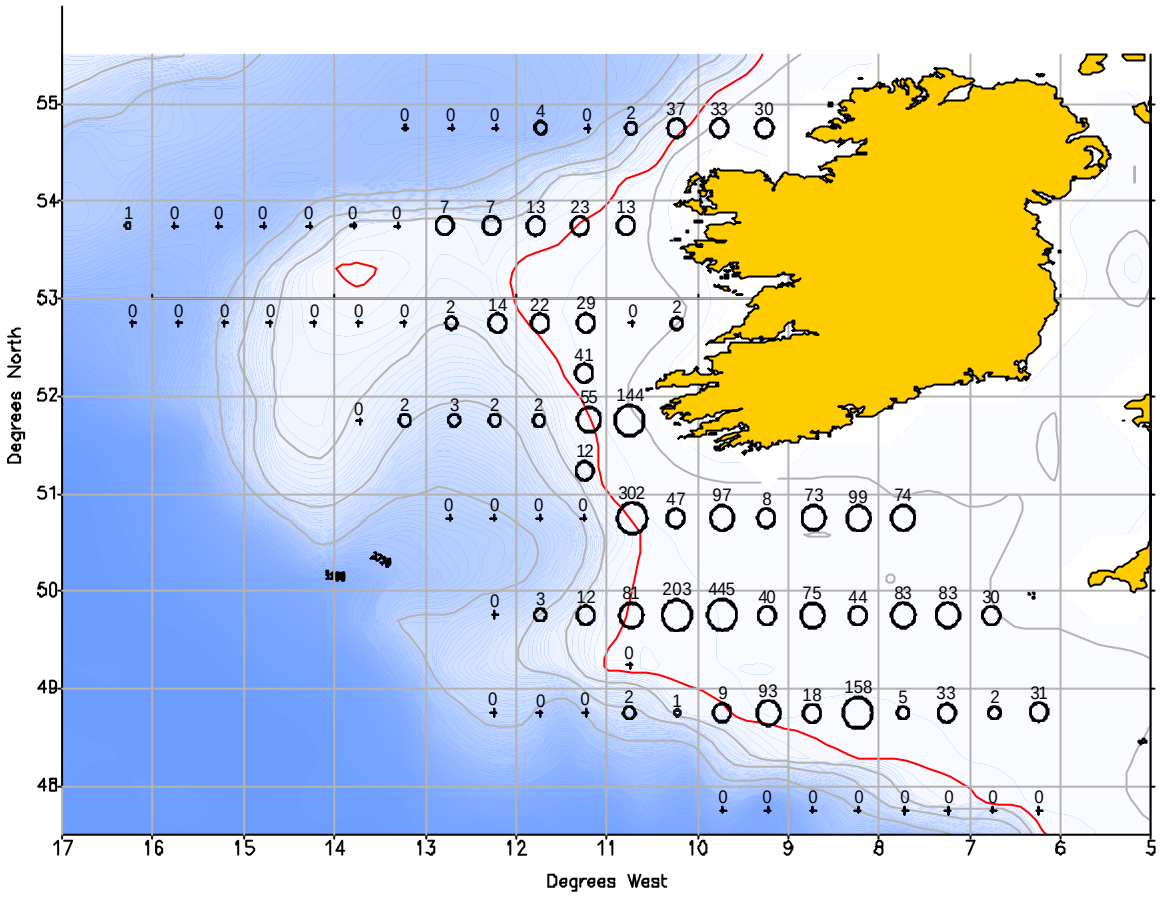

Figure 3 Numbers of Stage 1 mackerel eggs.

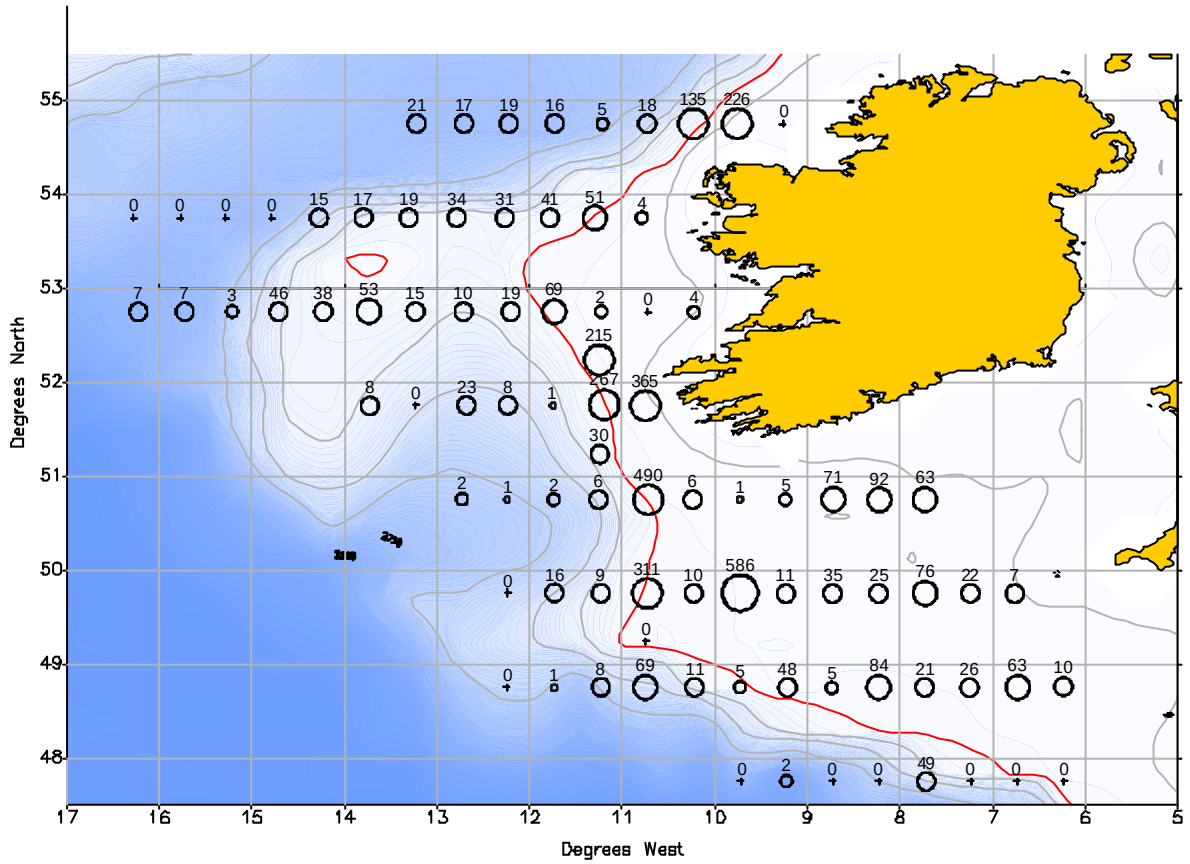

Figure 4 Numbers of Stage 1 horse mackerel eggs.

Table 1 Plankton stations and associated Stage 1 egg numbers.

Haul no	Date	Time Start	Lat deg	Lat Min	Long Deg	Long Min	Sample Depth Metres	Mac 1a	Hom 1a
1	28/06/07	15:23	48	45.58	6	14.16	125	31	10
2	28/06/07	18:20	48	45.06	6	43.63	130	2	63
3	28/06/07	21:10	48	44.9	7	15.06	135	33	26
4	29/06/07	00:00	48	45.09	7	44.12	145	5	21
5	29/06/07	03:31	48	45.06	8	13.93	142	158	84
6	29/06/07	07:00	48	44.7	8	44.14	160	18	5
7	29/06/07	09:47	48	44.99	9	12.92	154	93	48
8	29/06/07	12:30	48	45.1	9	43.7	173	9	5
9	29/06/07	15:12	48	45.1	10	13.23	150	1	11
10	29/06/07	21:30	48	45.06	10	45.04	201	2	69
11	30/06/07	00:25	48	45.17	11	13.78	201	0	8
12	30/06/07	04:06	48	44.86	11	44.01	201	0	1
13	30/06/07	07:45	48	45.22	12	14.59	200.3	0	0
14	30/06/07	23:17	49	45.61	12	14.06	200	0	0
15	01/07/07	02:22	49	45.35	11	43.99	199.2	3	16
16	01/07/07	05:30	49	45.15	11	13.85	203.5	12	9
17	01/07/07	11:08	49	45.08	10	43.74	146.6	81	311
18	01/07/07	14:13	49	45.1	10	13.66	132	203	10
19	01/07/07	17:02	49	45.34	9	43.59	55.9	445	586
20	01/07/07	19:31	49	44.88	9	14.03	59.1	40	11
21	01/07/07	22:08	49	44.91	8	43.78	59.4	75	35
22	02/07/07	00:51	49	45	8	13.93	73.9	44	25
23	02/07/07	03:37	49	45.13	7	43.79	64.2	83	76
24	02/07/07	06:09	49	45.18	7	14.4	61.3	83	22
25	02/07/07	08:37	49	44.94	6	45.56	50.7	30	7
26	02/07/07	18:10	50	45.01	7	43.93	52	74	63
27	02/07/07	20:55	50	44.77	8	13.39	55.9	99	92
28	02/07/07	23:15	50	45.12	8	43.19	51.2	73	71
29	03/07/07	01:30	50	45	9	14.45	53.9	8	5
30	03/07/07	03:43	50	45.04	9	43.67	51.7	97	1
31	03/07/07	06:11	50	44.94	10	14.38	60.2	47	6
32	03/07/07	08:26	50	44.99	10	43.07	157.5	302	490
33	03/07/07	13:25	50	45.03	11	15.38	202.5	0	6
34	03/07/07	16:14	50	44.99	11	44.32	202.9	0	2
35	03/07/07	19:15	50	45.19	12	14.55	202.6	0	1
36	03/07/07	22:16	50	45.18	12	43.76	203	0	2
37	04/07/07	08:43	51	44.81	13	43.55	200.4	0	8
38	04/07/07	11:53	51	45.27	13	13.48	200.3	2	0
39	04/07/07	15:46	51	45.17	12	40.93	203.7	3	23
40	04/07/07	18:32	51	45.25	12	14.02	200.7	2	8
41	04/07/07	21:32	51	45.01	11	44.84	200	2	1
42	05/07/07	00:49	51	45.57	11	11.63	185	55	267
43	05/07/07	03:26	51	44.99	10	44.96	130.6	144	365
44	06/07/07	11:22	52	44.75	10	13.61	94.9	2	4
45	06/07/07	13:52	52	45.03	10	43.11	117.4	0	0

46	06/07/07	16:22	52	45.1	11	13.82	121.5	29	2
47	06/07/07	18:52	52	45.03	11	44.02	162.9	22	69
48	06/07/07	21:10	52	45.03	12	12.24	200	14	19
49	06/07/07	23:35	52	45.03	12	42.81	200	2	10
50	07/07/07	01:59	52	45.18	13	13.98	200	0	15
51	07/07/07	04:20	52	45.25	13	44.19	200.4	0	53
52	07/07/07	06:35	52	45.04	14	13.68	200.8	0	38
53	07/07/07	08:53	52	45.02	14	42.68	201	0	46
54	07/07/07	11:15	52	45.21	15	12.71	195	0	3
55	07/07/07	13:23	52	45.07	15	43.39	200	0	7
56	07/07/07	15:40	52	45	16	13.52	200	0	7
57	07/07/ 07	22:36	53	44.8	16	16.58	201.1	1	0
58	08/07/07	00:50	53	45.01	15	45.93	200.4	0	0
59	08/07/07	02:57	53	45	15	16.97	200	0	0
60	08/07/07	05:07	53	45.03	14	47.04	199.6	0	0
61	08/07/07	07:21	53	44.97	14	16.46	200.4	0	15
62	08/07/07	09:35	53	45.14	13	47.62	200.8	0	17
63	08/07/07	11:47	53	45.03	13	18.34	200.6	0	19
64	08/07/07	14:02	53	45.14	12	47.22	200.1	7	34
65	08/07/07	16:15	53	45.06	12	16.13	200.3	7	31
66	08/07/07	18:25	53	44.95	11	46.92	200	13	41
67	08/07/07	20:33	53	44.95	11	17.77	200	23	51
68	08/07/07	22:57	53	45.09	10	47.24	141	13	4
69	09/07/07	15:58	54	45.16	9	15.71	82.8	30	0
70	10/07/07	18:33	54	45.19	9	45.53	91.6	33	226
71	10/07/07	20:35	54	45.08	10	13.92	113	37	135
72	11/07/07	00:34	54	45.09	10	43.81	200.9	2	18
73	11/07/07	02:41	54	45.04	11	12.79	200.7	0	5
74	11/07/07	04:49	54	45.34	11	43.69	199.9	4	16
75	11/07/07	07:03	54	45.21	12	13.52	200.8	0	19
76	11/07/07	09:08	54	45.24	12	42.48	200.7	0	17
77	11/07/07	11:20	54	45.21	13	13.26	199.1	0	21
78	13/07/07	14:19	47	44.96	6	13.79	50.3	0	0
79	13/07/07	16:51	47	45.01	6	44.26	161.4	0	0
80	13/07/07	19:32	47	44.9	7	14.01	167.5	0	0
81	13/07/07	21:58	47	44.93	7	42.95	200.5	0	49
82	14/07/07	00:32	47	45.1	8	13.7	200.2	0	0
83	14/07/07	03:02	47	45	8	43.44	200.7	0	0
84	14/07/07	05:37	47	45.1	9	13.65	200.6	0	2
85	14/07/07	08:11	47	45.08	9	42.94	200.7	0	0
86	14/07/07	19:20	49	14.57	10	44.81	150.5	0	0
87	15/07/07	08:35	51	13.97	11	14.58	190.5	12	30
88	15/07/07	15:35	52	14.18	11	14.89		41	215

Appendices

Survey Narrative

Date	Events
Tuesday, June 26th:	Mobilisation took place in Galway. Problems were encountered with the mackerel net so both herring nets were brought instead. Both the ProNet and ProMon systems were connected and checked.
Wednesday, June 27th:	Left port at 02.00. Steamed all day. Held scientific meeting to run through the various sampling protocols and assign watches. Discussed work programme with skipper and bosun at evening meeting.
Thursday, June 28th:	Arrived at first sampling station at 14.55. After discussions with the skipper it was decided to start the sampling at 48° 45N, 06° 15W, outside the English Channel separation zone. Ran some tests on the CTD winch. First station started at 15:23. Proceeded westwards. Three stations carried out
Friday, June 29th:	Continued westwards. Carried out fishing haul 1 at 48° 45.14N, 10° 15.47W. It produced a bag of Boarfish, with a small number of mackerel. Went back on transect but lost the weight off the GULF. Once this was replaced we encountered problems with the software. This was eventually rectified and we restarted sampling. Seven stations were carried out for the day.
Saturday, June 30th:	We reached the end of transect 1 at 08:30 and turned north. The weather disimproved during transit, and we were forced to delay sampling for the rest of the day. We started transect 2 at 23:17. Four stations had been carried out.
Sunday, July 1st:	Continued along transect 2. Carried out fishing haul 2 at 49°44.25 N, 10° 58.35 W. The haul contained a small bag of mixed fish, including seven mackerel and one horse mackerel. Due to time constraints it was decided not to fish again, and we turned back onto the transect. Seven stations were carried out
Monday, July 2nd:	Finished transect 2 at 08:54, and turned northwest to transect 3. Arrived on station at 18:10. Carried out seven stations.
Tuesday, July 3rd:	Continued on transect 3. Stopped to carry out fishing haul 3 at 50° 39.79N, 10° 51.98W, in 180m of water. After 40 minutes the haul provided a box of mackerel and a small number of horse mackerel. Of these twelve mackerel and four horse mackerel were sampled. Returned to transect and carried out eight stations for the day. Finished transect 3 at 22:55 and turned northwest.
Wednesday, July 4th:	Arrived at transect 4 at 08:43. Five stations were carried out.
Thursday, July 5th:	Two more stations were carried out to finish transect 4. At 04:00 the ship turned north. At 08:30 fish were seen on the sounder and fishing haul 4 was carried out at 52° 00.97N, 10° 45.37W. It produced three boxes of mackerel, some boarfish and blue whiting, but only six horse mackerel. Of these only two were sampled. At this stage the ship headed to the Shannon estuary for shel-

	ter.
Friday, July 6th:	Arrived on transect 5 at 11:22. Six stations were carried out
Saturday, July 7th:	Continued along transect 5 until 16:20 when we turned north. Arrived at transect 6 at 22:35. Eight stations were carried out.
Sunday, July 8th:	Continued east on transect 6. Eleven stations were carried out, and transect 6 was completed at 23:30.
Monday, July 9th:	Carried out two fishing hauls. Haul 5 at 53° 44.88N, 10° 53.03W produced a small bag of herring as well as smaller numbers of mackerel, horse mackerel and blue whiting. A second haul was made at 53° 43.79N, 11° 09.97W. This produced a mixed bag of mackerel, horse mackerel, boarfish, blue whiting and blackfish. The horse mackerel were sampled for the programme. The mackerel were examined to determine if any were spawning males and females. Two females were selected and their eggs were stripped. Milt from four males was added to the eggs, and they were allowed to fertilise. After an hour the eggs were examined but only a few hundred had fertilised. The ship turned north to the next transect. Arrived at transect 7 at 16:00. Carried out one station. At this stage it was decided to head to Killybegs to repair a leak in the hydraulic system controlling the GULF.
Tuesday, July 10th:	Hydraulic pipe was replaced and left Killybegs at 13.30. Arrived back on transect at 18:33. Fishing haul 7 was carried out at 54° 45.41N, 10° 24.10W. Four boxes of mackerel and 1.5 boxes of horse mackerel were caught, as well as smaller numbers of blue whiting and blackfish. Two stations were carried out.
Wednesday, July 11th:	Continued along transect 7. After a conversation with the survey coordinator we were asked to finish transect 7 at 54° 45N, 13° 15W. We were asked to then turn south to a new transect off the French coast at a latitude of 47° 15N. We were to carry out as many stations as our remaining survey time would allow. This was due to the large numbers of horse mackerel eggs found on our first transect. We were hoping to find the southern boundary of these eggs.
Thursday, July 12th:	Still heading south. Carried out a GULF flowmeter calibration west of Mizen Head at 51° 21N, 10° 12W.
Friday, July 13th:	Started transect 8 at 14:19. Four stations were carried out for the day.
Saturday, July 14th:	Continued along transect 8. Finished the transect at 08:52. Steamed north towards Galway. Carried out GULF station at 49° 15N, 10° 45W. Five stations carried out.
Sunday, July 15th:	Carried out the final two GULF stations at 51° 15N, 11° 15W, and 52° 15N, 11° 15W. The survey is completed and we headed for Galway.
Monday, July 16th:	Arrived in port at 07.00.

Horse mackerel sampling procedure at sea

Before the cruise:

Fill the labelled 2,5 ml eppendorf tubes with 1,2 ml of 3,6% buffered (sodium phosphate) formaldehyde (see excel-file : Buffered formaldehyde) and measure the weight (± 0,0001 g).

During the cruise:

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

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

Select females in maturity stages 3-6 (see table 3.1.2 WGMEGS 2006) from the subsample for fecundity analysis. Be sure to divide the females equally into the 4 weight categories: < 150g, 151-250g, 251-350g and >351g.

Measure or take:

- Total length
- Total weight
- Maturity
- Otoliths for age reading
- Weight of gut, ovary and liver
- Stomach fullness (1: empty, 2: filled, 3: full and 4: bursting)

Ovary sampling:

- From the ovary take 4 * 25µl samples with a pipette and immediately put each sample in individual coded eppendorf tubes.
- Make sure that all the ovary samples are covered with formaldehyde.
- Freeze and label the gutted fish separately in plastic bags for lipid measurements. Be sure to use the same code for the eppendorf tubes and frozen fish for each individual

After the cruise :

Measure the weight of the eppendorf tubes containing the sample.

Send all the frozen fish to IMARES, see address in table 1.

Send the eppendorf samples for analysis to the different institutes referred to in table 1.

Colour code	Country	Institute and address	Responsible person
Blue	Norway	IMR, Nordnesgaten 50,PB 1870, 5817 Bergen-Nordnes, Norway	Merete Fonn
Pink	Ireland	MI, Rinville, Oranmore, Co. Galway, Ireland	Brendan O'Hea
Green	Netherlands	IMARES, Haringkade 1, 1976 cp Ymuiden, Netherlands	Cindy van Damme
White	Spain	IEO, Cabo Estay-Canido, 36280 - VIGO (Pontevedra) Spain	Jose Ramon Perez

Fecundity whole mount analysis procedure for horse mackerel

Transfer the unstained sample to a tray and try to separate the oocytes.

Under the microscope check for spawning markers, if there are hydrated oocytes, atretic hydrated oocytes or ≥ 5 POFs, the sample should not be analysed for fecundity.

Note the present or absence of atretic oocytes.

Count all the oocytes >175μm in the sample.

Distribute the sample randomly in the tray and measure the diameter for size distribution for all oocytes >175μm in 1/3 of the sample.

Formula to calculate the total fecundity:

Number of oocytes / weight of the pipette sample * ovary weight

Formula to calculate the potential fecundity:

Total fecundity / total fish weight

Mackerel sampling procedure at sea

Before the cruise:

Fill the labelled 2,5 ml eppendorf tubes with 1,2 ml of 3,6% buffered (sodium phosphate) formaldehyde (see excel-file : Buffered formaldehyde) and measure the weight (± 0,0001 g).

During the cruise:

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

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

Select females in maturity stages 3-6 (see table 3.1.2 WGMEGS 2006) from the subsample of 100 for DNA, fecundity and atresia analysis. Be sure to divide the females equally into the 4 weight categories: < 250g, 251-400g, 401-550g and >551g.

Measure or take:

- Total length
- Total weight
- Maturity
- Otoliths for age reading
- Weight of gut, ovary and liver

DNA sampling:

- Cut a tissue sample, (roughly 10x5x5 mm), of the muscle from the thick muscle behind the head. Put each tissue sample in a 1.5 ml eppendorf tube in absolute alcohol.

Ovary sampling:

- From the ovary take 4 * 25µl samples with a pipette and immediately put each sample in individual coded eppendorf tubes.
- Make sure that all the ovary samples are covered with formaldehyde.

Atresia sampling:

- Place the other ovary in a bottle filled with 3.6% buffered, (sodium phosphate) formaldehyde.
- Make sure that all the ovary sample is covered with formaldehyde

After the cruise:

Measure the weight of the eppendorf tubes containing the sample.

From the fixed half ovary cut two 5mm thick slices and put them in a labelled cassette. If the ovary is very large you may have to use two cassettes. Separate the cassettes into 4 colour coded bottles filled with 70% ethanol.

Send the cassettes and eppendorf samples for analysis to the different institutes referred to in table 1.

Table 1

Colour code	Country	Institute and address	Responsible person
Blue	Norway	IMR, Nordnesgaten 50,PB 1870, 5817 Bergen-Nordnes, Norway	Merete Fonn
Red	Ireland	MI, Rinville, Oranmore, Co.Galway, Ireland	Brendan O'Hea
Yellow	Scotland	FRS, Marine Laboratory, Victoria Road, Torry, Aberdeen AB9 8DB, Scotland.	Findlay Burns
White Even numbers	Spain	IEO, Cabo Estay-Canido, 36280 - VIGO (Pontevedra) Spain	Jose Ramon Perez
White Uneven numbers	Spain	AZTI, Foundation Herrera Kaia, Portualde z/g 20110, Pasaia, Basque Country, Spain	Maria Santos

Parasites sampling:

Select new fish from the catch to freeze individually in plastic bags. The total number of fish should be 50 for each cruise, see table 2.

Table 2

Area	Sampled by	Period / number of fish				
		1	2	3	4	5
Southern	POR / IPIMAR	50				
	ESP / IEO	50	50			
	ESP / AZTI			50		
Western	GER / BFA Fi	50				
	IRL / MI					50
	SCO / FRS		50			
	NED / IMARES			50		
	NOR / IMR				50	