

# **Mackerel Egg Survey, July 8<sup>th</sup> - 28<sup>th</sup> 2010**

**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 102 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. Ten 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, west of Ireland and west of Scotland.

This was the last of twelve surveys covering the Celtic Sea, west of Ireland, west of Scotland and Faeroese waters. 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 2011. Preliminary data will be used by the Working Group on Widely Distributed stocks, WGWIDE, in August - September 2010.

## 2 Materials and Methods

### 2.1 Scientific Personnel

Name	Service area/Affiliation	Role
Brendan O'Hea	MI - FSS	Scientist-in-charge
Dermot Fee	MI - FSS	Scientist
Marcin Blaszkowski	MI - FSS	Scientist
Tobi Rapp	MI - FSS	Scientist
Kyle Sweeney	MI - FSS	Scientist
Edward Farrell	Student	Scientist
Dave Wall	IWDG	Cetaceans

### 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° 15N to 56° 15N, and from 3° 45W to 15° 15W. This covered ICES areas Via, VIIb, VIIc, VIIg, VIIh, VIIj, VIIk, VIIIa and VIII d, (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. The GULF was deployed at a rate of roughly 10m per minute. If a thermocline of greater than 2.5°C difference in temperature over a depth of 10m was encountered the tow was halted 20m 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 on board 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.

### ***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 mackerel pelagic net. Sampling targets were 100 mackerel gonads of stage 3 to stage 6, and 40 horse mackerel samples from stage 3 to stage 5, over four weight categories. Three 25µl replicate samples were collected from one gonad of each fish, and stored in tubes containing 3.6% buffered formalin. For mackerel the second ovary was stored, undamaged, also in 3.6% buffered formalin. The sampling protocols for both species are attached in the appendix.

A sample of 10 mackerel stomachs were also collected from each fishing tow for Faeroese scientists. They wish to check if adult mackerel are major predators on Blue whiting eggs and larvae.

## **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 Mackerel net

## **2.4 Protocols used**

The protocols for the plankton are listed in the 2009 WGMEGS report. The gonad sampling of the mackerel and horse mackerel are listed in the 2009 WGMEGS report, and in the appendix of this report.

### 3 Results

#### **Plankton Hauls**

A total of 102 hauls were carried out, over ten 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 an ordinary garden sprayer 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. Once the bulk of the eggs have been extracted by spraying, the sample is examined under the microscope to remove any remaining eggs.

Mackerel stage 1 eggs were recorded at 41% of stations and horse mackerel stage 1 eggs were recorded at 42% of stations, (Figures 3 & 4). The largest numbers of stage 1 Mackerel eggs were found in the Celtic Sea on transects 3, 4 and 5. The highest count for mackerel was 109 at station 51. All other stations recorded less than 50 stage 1 eggs. For horse mackerel the greatest concentrations of stage 1 eggs were found on the Porcupine Bank, on transects 6 and 7. The highest count was 551, recorded at station 27.

A thermocline was recorded at all stations inside the 200m contour on the first six transects.

#### **Fish Hauls**

A total of 10 hauls were carried out, (Figure 2). Horse mackerel were caught in large numbers in 50% of the hauls, but were not caught at two stations. Mackerel were caught in big numbers on the two most northerly transects, but were in low numbers further south. Other species caught were Boarfish, *Capros aper*, Hake, *Merluccius merluccius*, and Blue whiting, *Micromesistius poutassou*. Boarfish were caught in great numbers, ca. 1tonne hauls, on transects 1 and 2.

Samples were collected for three of the four mackerel weight categories, predominately in the 251 – 400g class. 86% of the samples were in this size range. For Horse mackerel samples were caught in the three largest size classes. 68% of the fish were in the 251 – 350g category. In total 80 mackerel and 40 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, the stomachs were collected from the ten mackerel in each haul to check for the presence of Blue whiting eggs or larvae. A sample of 100 mackerel fin clips was collected for a researcher in the Marine Institute.

## **4 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 half day was lost when we had to exchange one of the crew.

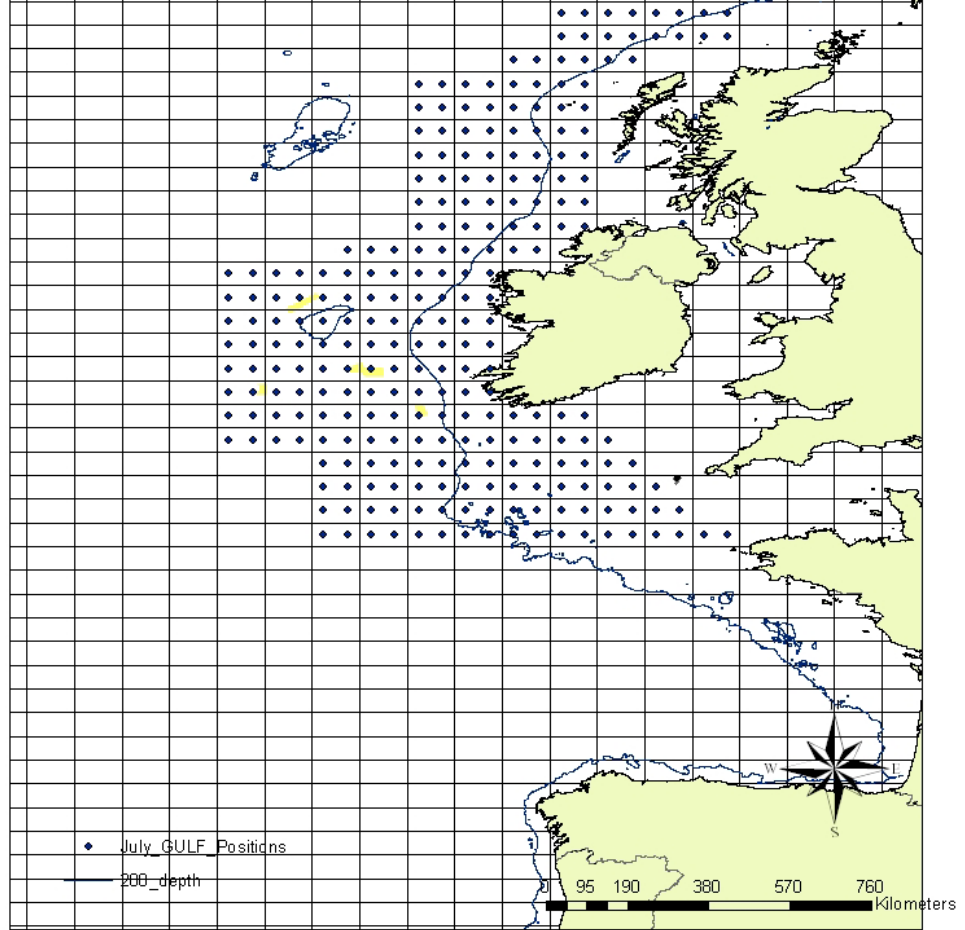
While the potential survey area was large the eggs were less dispersed than earlier in the year. On most transects it was possible to turn north within two or three stations after passing the 200m contour line. Mackerel eggs were found in small amounts at most stations in the Celtic Sea. The greatest numbers of Horse mackerel were found on the Porcupine Bank.

On this survey most of the identification and staging took place on the survey, rather than bringing the eggs back to the laboratory 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.

## **Acknowledgements**

Much appreciation is expressed to the skipper, Dennis Rowan, and crew of the RV *Celtic Explorer*. Their many skills kept the survey functioning. Special thanks are expressed to Gordon Furey for managing to overcome the numerous technical problems. Thanks are also expressed to the scientists and students who worked on the survey.

Refer



Mackerel

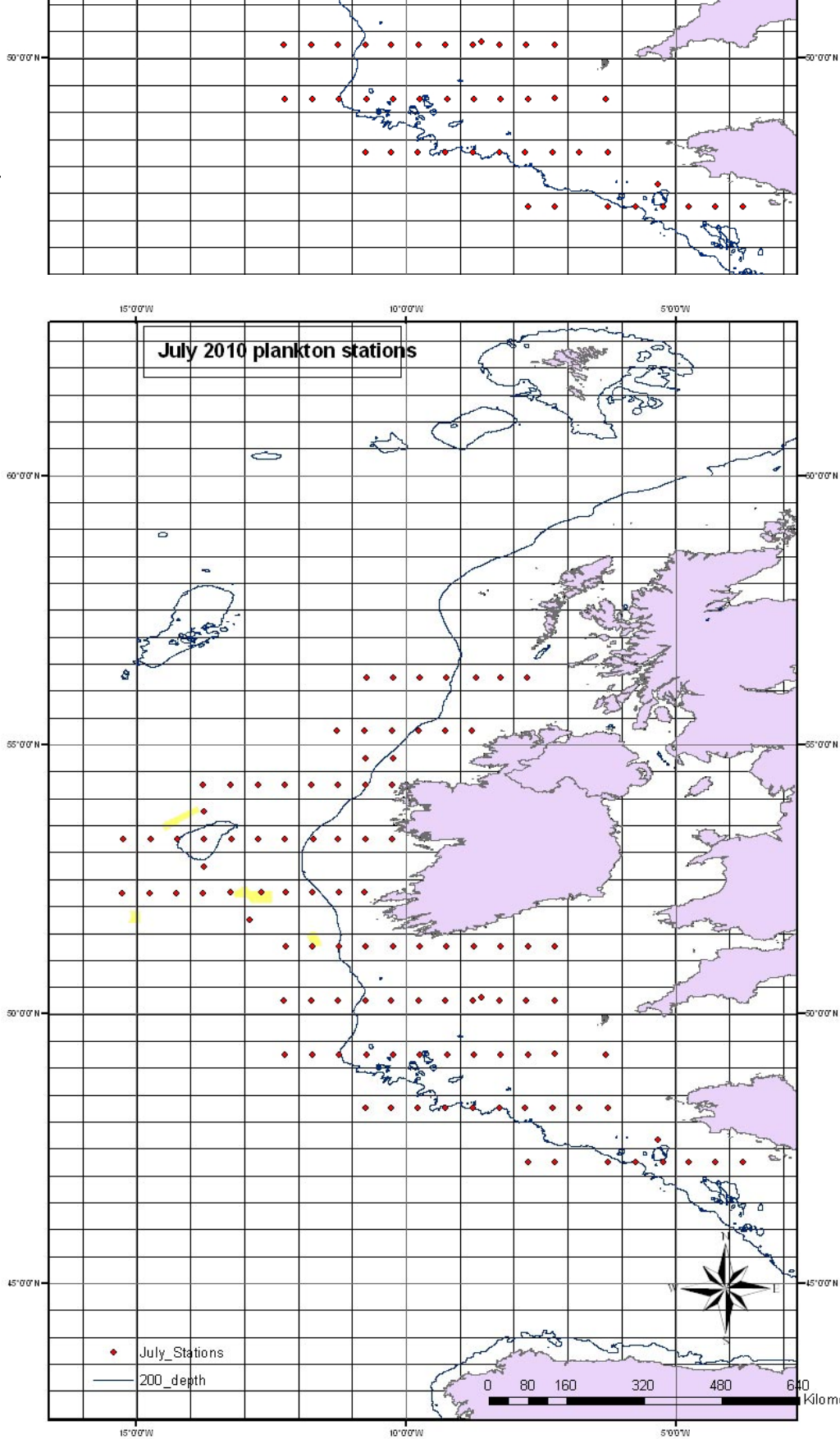


Figure 1 Survey plankton stations.

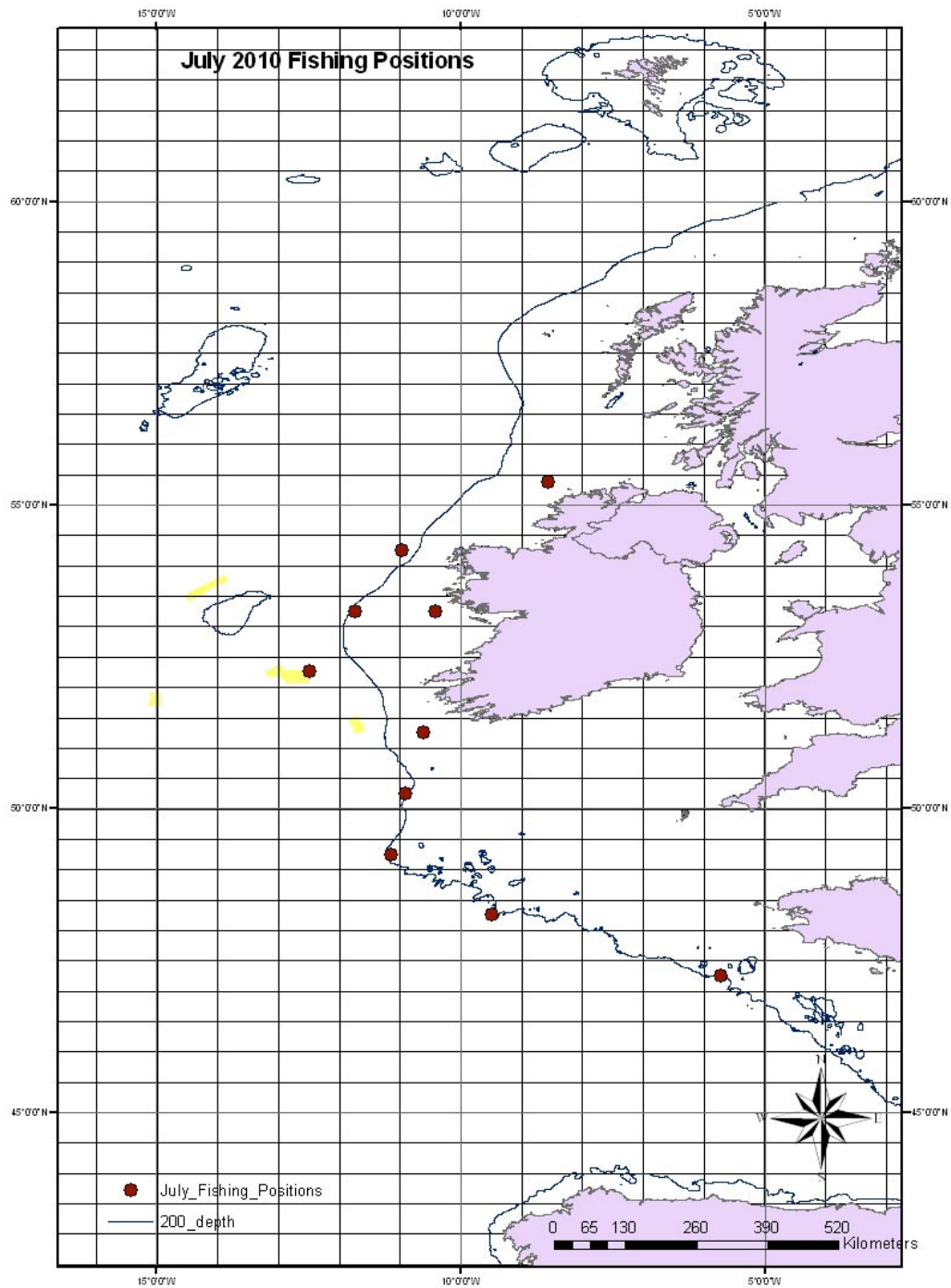


Figure 2 Fishing stations.

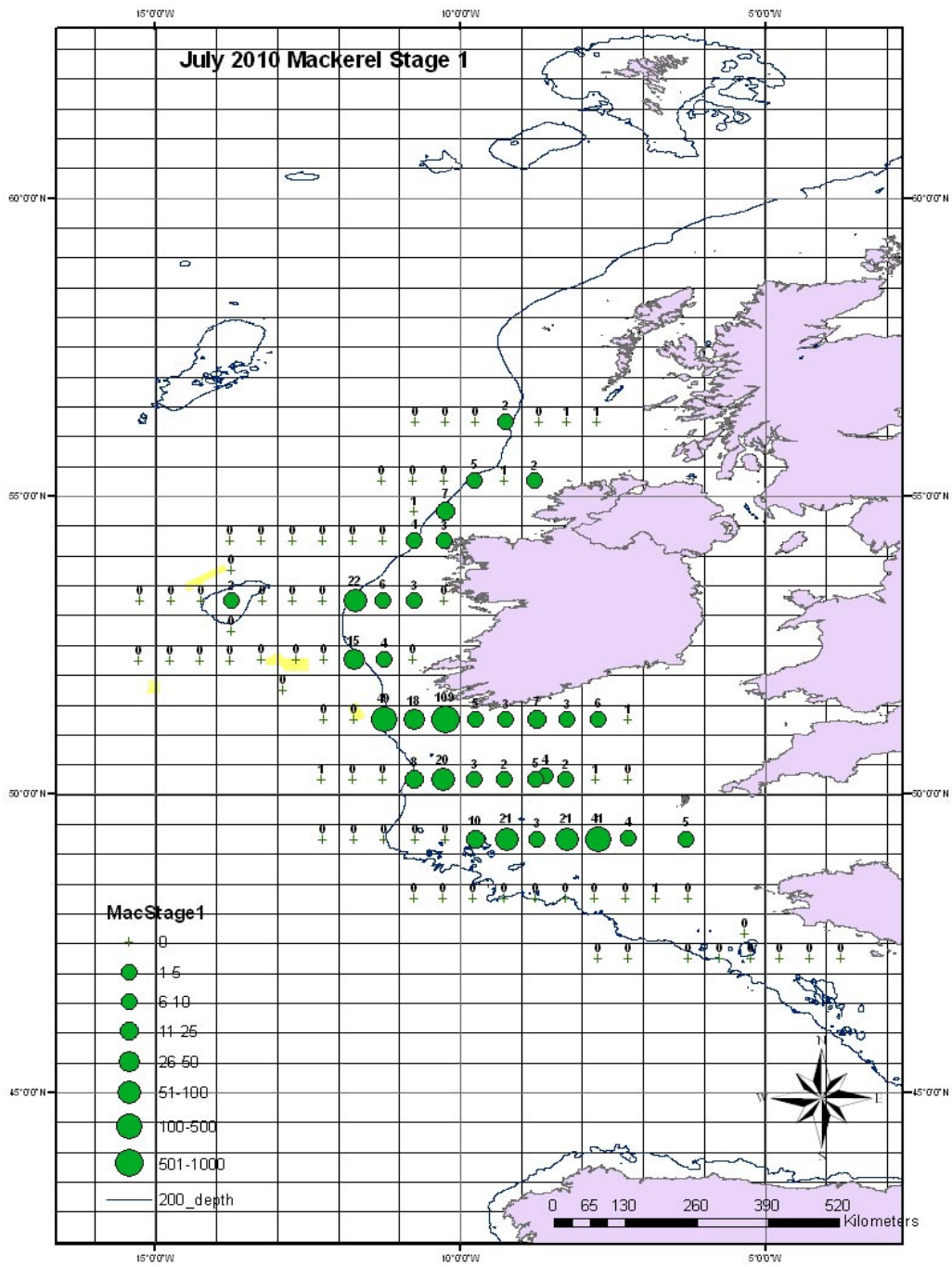


Figure 3 Numbers of Stage 1 mackerel eggs.

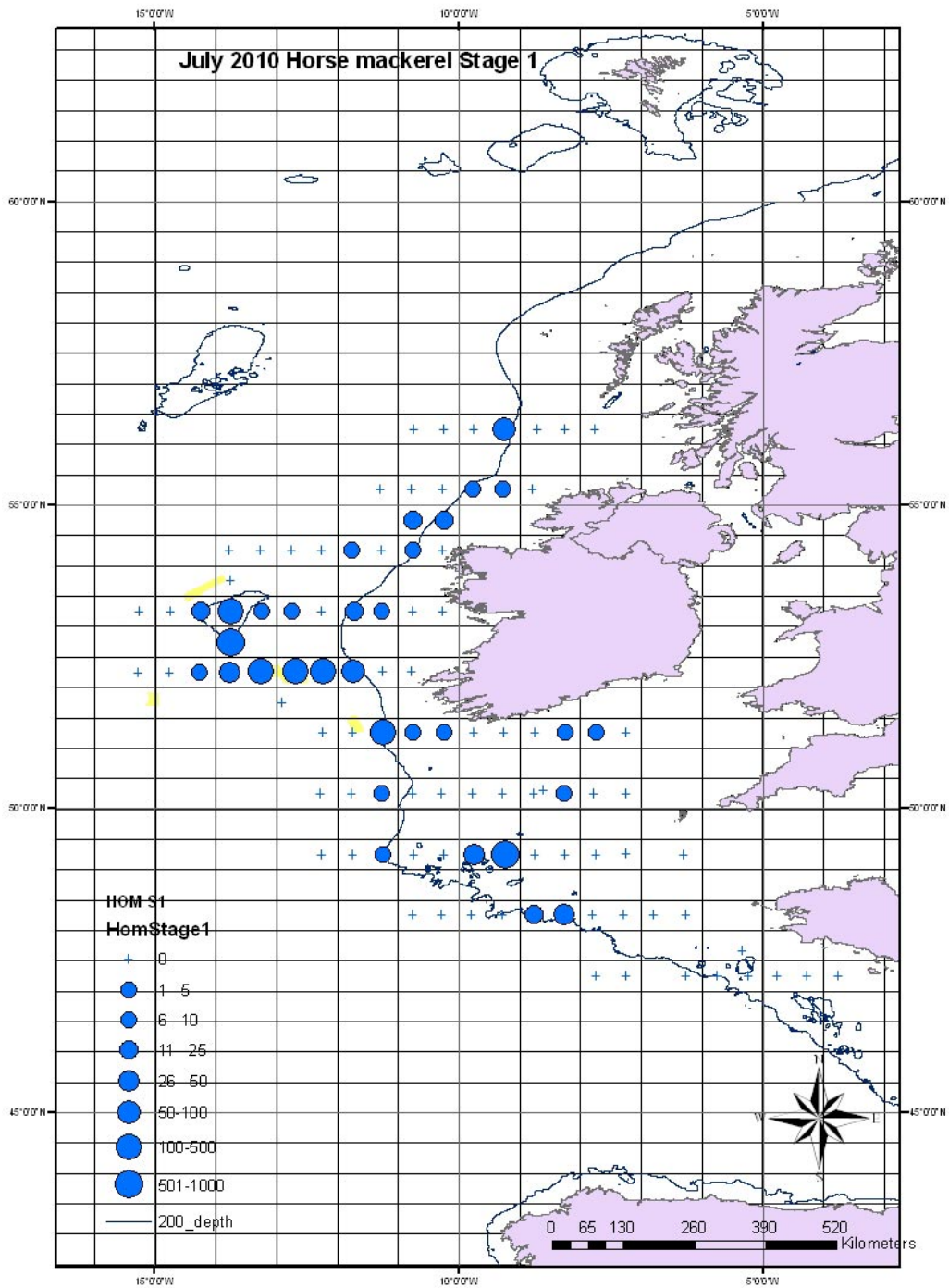


Figure 4 Numbers of Stage 1 horse mackerel eggs.

Table 1 Plankton stations and associated Stage 1 egg numbers.

Haul no	Date	Time Start	Latitude	Longitude	Sample Depth Metres	Mac 1a	HOM 1a
1	09/07/2010	14:35	50.3082	-8.5993	110.0	4	
2	10/07/2010	13:32	47.6622	-5.3190	50.5		
3	10/07/2010	21:01	47.2508	-3.7378	45.1		
4	10/07/2010	00:14	47.2503	-4.2502	50.1		
5	11/07/2010	02:53	47.2500	-4.7478	60.4		
6	11/07/2010	05:23	47.2497	-5.2323	56.5		
7	11/07/2010	07:54	47.2500	-5.7360	65.6		
8	11/07/2010	11:37	47.2500	-6.2413	200.2		
9	11/07/2010						
10	11/07/2010	18:33	47.2480	-7.2387	200.1		
11	11/07/2010	20:53	47.2498	-7.7393	200.1		
12	12/07/2010	14:10	48.2505	-10.7553	200.2		
13	12/07/2010	16:33	48.2513	-10.2715	81.7		
14	12/07/2010	18:42	48.2517	-9.7778	198.4		
15	12/07/2010	22:57	48.2502	-9.2758	193.6		1
16	13/07/2010	01:38	48.2502	-8.7500	170.2		23
17	13/07/2010	03:58	48.2508	-8.2542	195.5		33
18	13/07/2010	06:10	48.2508	-7.7798	174.4		
19	13/07/2010	08:26	48.2488	-7.2845	160.5		
20	13/07/2010	10:53	48.2503	-6.7785	64.3	1	
21	13/07/2010	13:28	48.2497	-6.2578	61.1		
22	15/07/2010	11:33	49.2480	-6.2877	61.6	5	
23	16/07/2010	08:08	49.2518	-7.2365	60.4	4	
24	16/07/2010	12:04	49.2492	-7.7393	60.3	41	
25	16/07/2010	15:00	49.2495	-8.2447	66.0	21	
26	16/07/2010	18:05	49.2500	-8.7382	60.9	3	
27	16/07/2010	21:45	49.2505	-9.2337	60.5	21	551
28	17/07/2010	00:53	49.2500	-9.7468	70.0	10	29
29	17/07/2010	03:29	49.2498	-10.2415	61.2		
30	17/07/2010	06:12	49.2502	-10.7372	61.0		
31	17/07/2010	10:48	49.2495	-11.2333	62.3		4
32	17/07/2010	13:26	49.2498	-11.7440	188.8		
33	17/07/2010	16:16	49.2505	-12.2445	201.0		
34	17/07/2010	23:47	50.2493	-12.2753	196.5	1	
35	18/07/2010	02:12	50.2495	-11.7545	200.0		
36	18/07/2010	04:44	50.2502	-11.2620	166.3		7
37	18/07/2010	08:16	50.2497	-10.7555	67.1	8	1
38	18/07/2010	10:15	50.2498	-10.2688	66.4	20	
39	18/07/2010	12:25	50.2497	-9.7563	70.4	3	
40	18/07/2010	14:37	50.2493	-9.2613	70.2	2	
41	18/07/2010	16:52	50.2502	-8.7595	62.2	5	
42	18/07/2010	19:02	50.2500	-8.2600	61.7	2	3
43	18/07/2010	21:06	50.2500	-7.7665	62.5	1	
44	18/07/2010	23:14	50.2498	-7.2445	63.1		
45	19/07/2010	05:40	51.2498	-7.2357	62.7	1	

46	19/07/2010	08:49	51.2487	-7.7257	63.3	6	5
47	19/07/2010	11:47	51.2497	-8.2340	61.9	3	3
48	19/07/2010	14:21	51.2498	-8.7427	60.4	7	1
49	19/07/2010	16:44	51.2503	-9.2398	62.8	3	
50	19/07/2010	19:15	51.2500	-9.7402	61.8	5	
51	19/07/2010	21:52	51.2512	-10.2347	62.3	109	5
52	20/07/2010	01:24	51.2502	-10.7423	70.6	18	5
53	20/07/2010	03:59	51.2508	-11.2405	191.2	49	67
54	20/07/2010	06:48	51.2501	-11.7373	178.0		
55	20/07/2010	09:38	51.2492	-12.2275	202.1		
56	20/07/2010	15:37	51.7475	-12.8925	200.6		
57	20/07/2010	19:48	52.2513	-13.2612	176.6		93
58	20/07/2010	22:07	52.2501	-12.6788	200.2		74
59	21/07/2010	02:32	52.2510	-12.2265	193.4		88
60	21/07/2010	05:30	52.2503	-11.7452	178.0	15	44
61	21/07/2010	07:59	52.2503	-11.2518	66.3	4	
62	21/07/2010	10:16	52.2502	-10.7650	63.1		
63	21/07/2010	22:27	53.2492	-10.2498	85.1		
64	22/07/2010	02:05	53.2492	-10.7437	80.3	3	
65	22/07/2010	06:43	53.2497	-11.2568	130.2	6	5
66	22/07/2010	09:10	53.2488	-11.7147	170.6	22	18
67	22/07/2010	13:40	53.2503	-12.2427	201.1		
68	22/07/2010	16:01	53.2497	-12.7483	198.7		6
69	22/07/2010	18:23	53.2500	-13.2440	187.4		7
70	22/07/2010	20:41	53.2497	-13.7402	155.1	2	95
71	22/07/2010	22:56	53.2495	-14.2352	196.1		19
72	23/07/2010	01:19	53.2498	-14.7433	201.1		2
73	23/07/2010	04:08	53.2495	-15.2410	200.2		
74	23/07/2010	12:48	52.2500	-15.2580	200.2		
75	23/07/2010	15:11	52.2498	-14.7500	200.4		
76	23/07/2010	17:32	52.2498	-14.2613	173.4		3
77	23/07/2010	19:51	52.2498	-13.7605	200.5		26
78	23/07/2010	23:48	52.7407	-13.7498	200.3		400
79	24/07/2010	06:38	53.7590	-13.7498	185.6		1
80	24/07/2010	10:12	54.2495	-13.7605	179.0		1
81	24/07/2010	12:23	54.2502	-13.2502	200.2		
82	24/07/2010	14:34	54.2502	-12.7502	200.2		
83	24/07/2010	16:44	54.2505	-12.2518	200.8		
84	24/07/2010	18:55	54.2505	-11.7578	200.9		3
85	24/07/2010	21:09	54.2520	-11.2568	200.1		
86	25/07/2010	00:24	54.2500	-10.7562	181.1	4	5
87	25/07/2010	02:35	54.2495	-10.2568	79.4	3	1
88	25/07/2010	10:59	55.2508	-11.2747	189.6		
89	25/07/2010	13:15	55.2502	-10.7620	200.2		2
90	25/07/2010	15:26	55.2497	-10.2503	198.2		1
91	25/07/2010	17:27	55.2498	-9.7633	115.5	5	3
92	25/07/2010	19:27	55.2498	-9.2688	95.9	1	10
93	25/07/2010	21:24	55.2505	-8.7748	85.1	2	
94	26/07/2010	06:18	56.2502	-7.7430	40.7	1	
95	26/07/2010	08:37	56.2500	-8.2433	158.6	1	

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96	26/07/2010	11:05	56.2505	-8.7047	120.6		
97	26/07/2010	13:27	56.2502	-9.2397	200.9	2	38
98	26/07/2010	17:39	56.2498	-9.7382	200.8		1
99	26/07/2010	20:28	56.2490	-10.2320	201.5		1
100	26/07/2010	23:13	56.2498	-10.7330	192.1		
101	27/07/2010	09:30	54.7488	-10.2440	110.2	7	18
102	27/07/2010	12:05	54.7505	-10.7432	201.3	1	24

## Appendices

### Survey Narrative

Date	Events
<b>Thursday, July 8<sup>th</sup>:</b>	Mobilisation took place in Galway. We sailed at 13.30 and headed towards France, with our first transect being 47.25N.
<b>Friday, July 9<sup>th</sup>:</b>	Still on passage. We deployed the Pronet to test the equipment but found problems with the data being transmitted by the flowmeters. The GULF was recovered and repairs were carried out. A second deployment was carried out in the afternoon. The flowmeters returned good data this time but problems were found with the depth data. The GULF was recovered to the surface but the equipment was indicating it was still at 40m depth. This haul was regarded as a valid haul and the eggs were collected from the sample.
<b>Saturday, July 10<sup>th</sup>:</b>	Still on passage. A further test tow was carried out to ensure that everything was working correctly. All was fine. We reached 47.25N 03.75W at 20:40 and commenced the survey. We started by carrying out a CTD cast using the ship's Seabird 911. This was to confirm the temperature data collected on the journey south was in the correct range. The first plankton sample was started at 21:00. Problems were encountered on tow 4 when all real time data was lost during the ascent. The haul was repeated and ran successfully.
<b>Sunday, July 11<sup>th</sup>:</b>	Continued along transect 1. Fishing haul 1 was carried out at 09:36 in 210m of water. The tow lasted 58 minutes and produced approximately one tonne of blue whiting, horse mackerel and a small number of mackerel. The mackerel and horse mackerel were sampled. The plankton tows continued afterwards but very few eggs were found until haul 8. On hauls 9 and 10 the same problem was encountered with the real time data on the ascent. It was eventually tracked down to a software issue. Station 11 was carried out at and produced no eggs. We broke off transect 1 at 22:15 and steamed to transect 2.
<b>Monday, July 12<sup>th</sup>:</b>	Transect 2 started at 14:10 after a passage of 130 miles. All the equipment is working properly. Four plankton hauls were carried out for the day. Fishing tow 2 was carried out at 20:44 for 75 minutes. It produced approximately 2 tonnes of Boarfish with some Horse mackerel.
<b>Tuesday, July 13<sup>th</sup>:</b>	Continued on transect 2. We encountered a few stations near the 200m contour line that contained large numbers of what appear to be Boarfish eggs. We finished transect 2 at 13.40 and steamed to Cornwall to change a member of the crew.
<b>Wednesday, July 14<sup>th</sup>:</b>	Headed out from Cornwall to start transect 3. Arrived on station at 20:00. Conditions too bad to shoot the GULF, strong winds and 6m swell. Conditions should improve by mid-morning tomorrow.
<b>Thursday, July 15<sup>th</sup>:</b>	Weather still poor. One station carried out at 11:30 but conditions disimproved again.

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<b>Friday, July 16<sup>th</sup>:</b>	Started sampling at 08:00. Forecast looking good for the next few days. Continued along transect 3.
<b>Saturday, July 17<sup>th</sup>:</b>	Continued along transect 3. We carried out fishing haul 3 at 09:30. We fished for 1 hour and caught two baskets of mixed fish, one basket of which was boarfish. Finished transect 3 at 17:00 and steamed to transect 4. Arrived at transect 4 at 23:45.
<b>Sunday, July 18<sup>th</sup>:</b>	Fished tow 4 at 07:00 for 30 minutes. We caught roughly four baskets of mixed fish, including a sunfish. Eleven of the horse mackerel and two of the mackerel proved to be suitable for fecundity sampling. With the wind behind us we made good time between stations and finished transect 4 at 23:30. We turned north to transect 5.
<b>Monday, July 19<sup>th</sup>:</b>	Arrived on station on transect 5 at 05:40. Continued stations to the west.
<b>Tuesday, July 20<sup>th</sup>:</b>	We fished haul 5 at 00:15. We towed through the pelagic layer approximately 25m below the surface. Three boxes of mackerel and horse mackerel were caught, as well as a Blue shark. None of the horse mackerel were mature, but eight of the mackerel were suitable for sampling. We finished transect 5 at 10:20 and steamed northwest to transect 6. On the way we carried out a plankton haul at 51.44N 12.50W. We arrived on station at 19:50 and turned east. Fished haul 6 at 23:45 for 45 minutes. It produced six baskets of mackerel and horse mackerel. The bulk of the mackerel were small one year olds. A number of horse mackerel were suitable for fecundity sampling.
<b>Wednesday, July 21<sup>st</sup>:</b>	Continued along transect 6. Finished transect 6 at 10:30 and steamed northeast to transect 7. Bad weather delayed the transit and we were forced to slow down. We arrived on transect at 22:30 and started sampling. A fishing tow, haul 7, was made at 23:45 but produced no fish.
<b>Thursday, July 22<sup>nd</sup>:</b>	Continued on transect. Due to difficulties retrieving the GULF in heavy seas operations were suspended for three hours. Sampling resumed at 06:30. Fishing tow 8, lasting one hour, took place at 10:30. Large marks of fish were seen throughout the haul but they proved impossible to catch. The haul consisted of a half basket of fish, mainly mackerel. However many of these fish were suitable for fecundity sampling.
<b>Friday, July 23<sup>rd</sup>:</b>	Finished transect 7 at 05:00. We steamed south to 52.15N to pick up four stations on the western edge of transect 6, the western edge of the Porcupine Bank. These stations were finished at 20:30. We then steamed north to transect 8 at 54.15N. We picked up two further stations on this transit across the top of the Bank.
<b>Saturday, July 24<sup>th</sup>:</b>	We arrived at transect 8 at 10:00 and turned east. We carried out fishing tow 9 at 23:00 for 45 minutes. Large marks of fish were seen. The haul consisted of six baskets of mackerel and two baskets of horse mackerel. Many of the mackerel were spent but were suitable for sampling.
<b>Sunday, July 25<sup>th</sup>:</b>	Transect 8 was finished at 03:00 so we steamed northwards. We arrived at transect 9 at 11:00 and started sampling. This transect was short and was finished by 22:00. We turned north towards transect 10. As we steamed north fishing haul 10 was carried out at 23:15 for one hour and produced one basket of mixed fish, about 50% of which were mackerel. These were checked but only one was sampled.

<b>Monday, July 26<sup>th</sup>:</b>	We arrived at transect 10 at 06:00 and started sampling. During the afternoon we carried out a series of flowmeter calibration tows. We finished transect 10 at 23:45 and turned for Galway.
<b>Tuesday, July 27<sup>th</sup>:</b>	As we steamed towards Galway we carried out two plankton tows at 54.45N, west of Killybegs, starting at 09:30. We finished these stations at 12:45 and headed for home. The scientific equipment was disassembled and packed away. The fishing labs were washed down.
<b>Wednesday, July 28<sup>th</sup>:</b>	Arrived in Galway docks at 07:00. Demobbed ship and all scientists disembarked by 12.00.

## 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

### 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 3-5 (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. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes.

### Measure or take:

- Total length
- Total weight
- Maturity
- Otoliths for age reading
- Weight of gut, ovary and liver. (if it is not possible to take these weights at sea, take the pipette and atresia samples and fix the remainder of the ovary. Subsequently weigh the ovary in the lab. Gut and liver should also be frozen and weighed in the lab. The fixed and frozen weights should be corrected to fresh weights.)

### Ovary sampling:

- From the ovary take 3 \* 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.

After the cruise :

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	Selene Hoey
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  $\geq 5$  POFs, the sample should not be analysed for fecundity.

Distribute the sample randomly in the tray and measure the oocyte diameters for the whole sample.

Count all oocytes  $>185\mu\text{m}$  in the sample

Formula to calculate Potential Fecundity:

Pot. fec. = Number of oocytes / weight of the pipette sample \* ovary weight

Formula to calculate Relative Potential Fecundity:

Rel. Pot. Fec. = Potential 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

### 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 from the subsample of 100 ( if there are less than 100 fish take them all) for fecundity and atresia analysis. Be sure to divide the females equally into the 4 weight categories: < 250g, 251-400g, 401-550g and >551g. If the size range of fish is restricted in the catch the remaining sample quota should be taken from the more abundant classes to fill the weight classes.

### Measure or take:

- Total length
- Total weight
- Maturity
- Otoliths
- Weight of gut, ovary and liver. (If it is not possible to take these weights at sea, take the pipette and atresia samples and fix the remainder of the ovary. Subsequently weigh the ovary in the lab. Gut and liver should also be frozen and weighed in the lab. The fixed and frozen weights should be corrected to fresh weights.)

### Ovary sampling:

- From the ovary take 3 \* 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:

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**

<b>Colour code</b>	<b>Country</b>	<b>Institute and address</b>	<b>Responsible person</b>
Blue	Norway	IMR, Nordnesgaten 50,PB 1870, 5817 Bergen-Nordnes, Norway	Merete Fonn
Red	Ireland	MI, Rinville, Oranmore, Co.Galway, Ireland	Selene Hoey
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