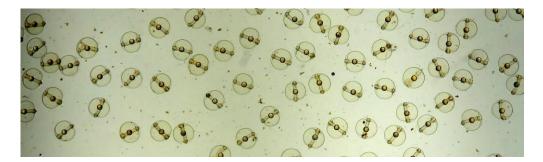
Atlantic mackerel and horse mackerel egg survey 2013: Dutch participation

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Report number C100/14



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Summary

In 2013 the international Atlantic mackerel and horse mackerel egg survey was performed. This year the entire spawning area was sampled by 10 institutes from 9 different countries: Faeröer Islands, Germany, Iceland, Ireland, Norway, Portugal, Scotland, Spain and The Netherlands. Sampling started in February along the Portuguese coast and continued until July west of Scotland. The Dutch institute IMARES participated in the survey in May and June on board 'RV Tridens'. IMARES covered the area of the Northern Bay of Biscay and Southern Celtic Sea once each month.

In both months a total of 234 ichthyoplankton samples were taken with a Gulf VII plankton torpedo with a Seabird CTD mounted on top. Also adult fish samples for the estimation of fecundity and atresia were taken using a pelagic trawl.

Due to technical problems and bad weather only part of the survey area was covered in the first sampling period in May. In June the assigned sampling area was successfully sampled. However, numbers of both mackerel and horse mackerel eggs found in the samples were lower compared to 2010, despite the fact that the adult fish caught still showed signs of upcoming spawning or recent spawning markers. Mackerel eggs were found in low numbers, but at more different stations in May compared to June. Numbers of horse mackerel eggs were higher in June compared to May, as expected. Highest numbers of both mackerel and horse mackerel eggs were found around the 200 m depth contour.

Temperature at the surface and 20 m depth were higher in June compared to May. Salinity at 20 m depth was the same in both periods.

1. Introduction

Every three years an international Atlantic survey is carried out by different European institutes to monitor the spatial and seasonal distribution of Atlantic mackerel and horse mackerel. During this survey mackerel and horse mackerel eggs are sampled using a plankton torpedo or bongo nets. The survey covers the whole spawning area and season. It starts along the Portuguese coast in February and continues until July when the waters west of Scotland are sampled.

The mackerel and horse mackerel egg survey is coordinated by the ICES working group for mackerel and horse mackerel egg surveys (WGMEGS).

England and France started the egg survey in the western area in 1977. The Netherlands participates since 1983. Nowadays participating countries and sampling area have expanded. In 2013 the following countries participated in this survey: Faeroes Islands, Germany, Iceland, Ireland, Norway, Portugal, Scotland, Spain and The Netherlands.

The method used to estimate mackerel spawning stock biomass is the so-called Annual Egg Production Method (AEPM). The theory behind this method is simple: estimate the total number of eggs produced during the entire spawning season. Dividing the total egg production by the numbers of eggs produced by a single female gives an estimate of the female spawning stock biomass. The ratio between female and male mackerel gives an estimate of the total spawning stock biomass. This method is simple but requires an accurate estimate of the total fecundity (total number of eggs produced by a single female in one spawning period) of a female. Total fecundity can only be estimated for determinate spawners, spawners which develop all oocytes prior to spawning. But horse mackerel and very probably mackerel are indeterminate spawners (the females keep recruiting new oocytes after spawning has started). Hence part of the oocytes are already spawned while others are still recruited and it is therefore impossible to estimate total fecundity.

In 2013 we also attempted to carry out the Daily Egg Production Method (DEPM). This method requires an accurate estimate of the daily egg production at the peak spawning period and batch fecundity estimates in order to estimate the numbers of eggs which a single female produces per day. But for the DEPM also an estimate of the daily spawning fraction is needed. Hence this method requires a more intensive sampling of the adult fish. The DEPM can be used for both determinate and indeterminate spawners.

2. Aim of the project

The purpose of this project is to monitor the spatial distribution and seasonal patterns in the appearance of mackerel and horse mackerel eggs in the eastern Atlantic. IMARES, on board the 'RV Tridens' sampled the part of the Bay of Biscay and Celtic Sea in both period 4 (May) and period 5 (June). IMARES uses a Gulf VII plankton sampler to sample fish eggs. Additionally, pelagic trawl hauls were carried out to collect adult mackerel and horse mackerel to estimate fecundity. These data will be combined to provide a fisheries-independent estimate of the spawning stock biomass of the western mackerel stock and egg production of horse mackerel.

This report contains the results of Dutch participation in the international mackerel and horse mackerel egg survey 2013.

3. Methods

3.1 Gears for sampling of plankton and adult fish

Fish eggs are sampled with a "High Speed Plankton Sampler Gulf VII" (Fig. 3.1) (referred to as 'torpedo' in the remainder of the report) with a plankton net with 280 μ m mesh size. A small skrips-depressor of 35 kg is attached to the torpedo. The volume of water filtered during each haul is measured using an internal Valeport electronic flowmeter. An external Valeport flowmeter is also mounted on the frame, to check for blowing of the net due to large amounts of phyto- and microzooplankton in the water that can clog the net.

On top of the torpedo a Seabird 911plus CTD with a Benthos PSI 916 altimeter is mounted to monitor in live view the depth of the torpedo in the water column and the bottom depth under the torpedo. The CTD also measures temperature and salinity.



Adult fish samples were taken using the pelagic 5600 trawl.

Figure 3.1. Gulf VII plankton torpedo.

3.2 Fishing method

This survey is carried out on board 'RV Tridens'. The speed during fishing with the plankton torpedo is 5 knots through the water. At each station a 'double oblique' haul is performed (Fig. 3.2). The Gulf VII sampler is lowered to 5 m above the sea floor or, at deeper stations, to 200 m depth. To ensure enough water is filtered during the haul, haul duration should at least be 10 minutes. At stations with shallow depth a double 'double oblique' is performed without the torpedo breaking the surface of the water. In this way each 10 meters of the water column is sampled 1 minute going down and going up. In case of a thermocline stronger than 2.5°C over 10 meters the sampler is lowered to 20 meters below the thermocline. Eggs cannot float through a thermocline, hence it is not necessary to sample below one. In each period a calibration haul should be carried out to calibrate the flowmeters. During the calibration the torpedo without the codend is lowered to 20m depth. The torpedo is hauled at constant depth for 30 minutes at a speed of 5 knots through the water.

During this haul the flowmeter revolutions, water track and bottom track are registered. This is repeated in the exact opposite direction in order to rule out any influence of water and tidal currents on the calibration.

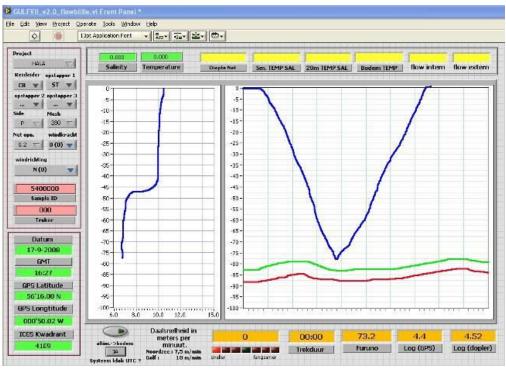


Figure 3.2. Illustration of an oblique haul in the Labview program.

3.3 Sampling grid

IMARES is asked by WGMEGS to sample the Celtic Sea and Northern part of the Bay of Biscay in May (period 4 of the international egg survey; Annex A) and June (period 5; Annex A). Due to results of the cruise in period 3 we were asked just prior to the start of the cruise in period 4 to also sample the transect at 51.45°N. Hence Annex A is different from the proposed sampling grid in the original survey program.

3.4 Sample processing on board

3.4.1 Plankton samples

As soon as the torpedo is on board the vessel, the sample (Fig. 4.2) is brought to the hydrographic lab. The fresh sample is immediately fixed in 4% buffered formaldehyde. After 12 to 24 hours of fixation, the fish eggs are separated from the other plankton using the 'spray method': the sample is sprayed until few eggs remain in the last spray. Then the whole plankton sample is sorted to check for remaining eggs. Eggs that have only been fixed in the 4% formaldehyde solution for 12 hours are photographed and put on 96% ethanol for genetic analyses.

Eggs are photographed and identified to species using image analysis (Fig. 4.3). All eggs are counted, measured, identified to species and staged. For mackerel and horse mackerel eggs, per sample, at least one hundred eggs are measured and the development stage is determined. The remaining mackerel and horse mackerel eggs are counted. If the sample contains a lot of eggs these are all sorted from the

sample, and then subsampled using a 'Folsom'-splitter ensuring at least 100 mackerel and horse mackerel eggs are staged.

For quality assurance sorting of the samples is checked. During period 4 all samples were sorted for fish larvae after spraying, during which was checked if eggs remained in the sample. During period 5 4 samples per 'sprayer', with different total amounts of plankton, were checked to see if eggs are properly sorted. If > 5% of the total number of larvae and eggs remain in the samples, all samples of this person were checked and numbers adjusted.

3.4.2 Adult fish samples

In principal all the fish were put on the conveyor belt and all mackerel and horse mackerel were collected from the catch. If the catch was large a random sample of 4 baskets of mackerel and horse mackerel is selected. Total weight of mackerel and horse mackerel is measured. One hundred mackerel and horse mackerel are taken randomly from the catch. If less than 100 were caught all individuals have been measured. Of each individual length, weight, sex, maturity and otoliths were taken.

From the 100 mackerel, females in development stage 3 to 6 were collected. In period 4 only 46 female mackerel needed to be sampled for fecundity and atresia in our sampling, divided over all the trawl hauls. In period 5 51 female mackerel needed to be sampled.

In period 4 no horse mackerel females needed to be sampled for fecundity because the DEPM method was used for horse mackerel and the expected spawning peak is in period 5. In period 5 at each transect a fishing haul needed to be performed. From each haul 30 horse mackerel females in development stage 3 to 6 needed to be collected and also 30 females with hydrated eqgs needed to be sampled.

Of each female, length, weight, maturity, age and ovary weight has been collected. Of the ovary one whole lobe was put in 3.6% formaldehyde for atresia sampling. From the other lobe 2 25 μ l and 2 100 μ l pipette samples were collected and put in 3.6% formaldehyde for mackerel. For horse mackerel only 2 100 μ l pipette samples were collected. Also a teaspoon full (2-3 g) of oocytes has been collected for histological conformation of the maturity stage.

Of one mackerel 10 25 μ l pipette samples were taken for a ring test between analyzing institutes.

3.4.5 Fertilized eggs

While sorting out the catch, running mackerel, horse mackerel and hake were separated. The gonads from the running males and females were extracted as soon as possible. Using alcohol and seawater rinsed scalpels the gonads were cut open and put in a sieve in clean sea water in order to fertilize hydrated eggs. After one hour the gonad remains were removed and the fertilized eggs are transferred to a clean sieve and put in the experimental tank with running seawater.

At the start of development fertilized eggs were sampled every few hours to ensure development stage 1B eggs are sampled. From development stage 2 sampling can be reduced but all stages should be collected up till hatching.

All eggs sampled were photographed on board and put into 96% ethanol and 4% formaldehyde solution.

3.4.6 24 hour-sampling

Both in period 4 and period 5 one station was planned to be sampled continuously for 24 hours. At the same position a plankton haul was carried out, followed by a pelagic trawl haul, followed by another plankton haul and continued for 24 hours. The reason for this is to exactly establish the timing of spawning of mackerel and horse mackerel. The plankton samples were treated as the regular samples. For the adult fish samples each haul 25 adult mackerel and horse mackerel were sampled if available, which were treated as in the regular trawl hauls.

3.4.7 GCxGC-MS analyses

From each pelagic trawl haul 10 mackerel have been selected and frozen for GCxGC-MS analysed in the lab. This allows for diet analyses of mackerel to identify and compare feeding and uptake of chemicals in the past months and past days.

3.4.8 Sampling of mackerel larvae

In period 4 a PhD student from Hamburg University participated in the egg survey on board the Tridens. During his PhD he investigates condition of fish larvae, especially mackerel, in relation to food availability. He sorted out the fish larvae from the regular samples after fixation in 4% formaldehyde. We took 5 extra samples with the torpedo solely for the collection of fish larvae, because for DNA/RNA analyses it is not possible to use formaldehyde fixed larvae. At these 5 extra stations also a vertical haul with a microzooplankton net have been carried out.

3.5 Sample processing in the lab upon return from the survey

3.5.1 Plankton samples

Remaining samples from the 2nd period need to be sorted, analysed and checked for sorting.

3.5.2 Adult fish samples

Upon return in the laboratory, screening and fecundity samples will be send out immediately to the analysing institutes. The IMARES screening samples will first be checked with histology for spawning markers. If no spawning markers are visible the samples will be analysed for fecundity. If spawning markers do occur, this sample will be analysed for atresia.

After fixation of at least 14 days in 3.6% formaldehyde the ovary lobes for atresia estimation are ready to be cut. From each lobe one or two whole sections (depending on the size of the ovary) of 0.5 cm thickness will be put in individual cassettes and sorted in 70% alcohol. The atresia samples will then be send to the various analysing institutes.

3.6 Calculation of the number of eggs

The total number of eggs in the water is calculated using the below formulas:

The volume filtered is obtained from the formula:

Volume filtered =
$$\frac{\text{area of mouth opening } (m^2)^* \text{efficieny factor}^* \text{flowmeter revolutions}}{\text{flowmeter calibration constant}}$$

The numbers per square metre at each station can be calculated as:

$$n/m^{2} = \frac{eggs \ per \ sample \ (n) * sampler \ depth \ (m)}{volume \ filtered \ (m^{3})}$$

4. Results

Date and time

From (harbour)	Date	Time (UTC)	To (harbour)	Date	Time (UTC)
Stellendam	06-05-2013	10:00	Brest	09-05-2013	08:30
Brest	09-05-2013	16:30	Cork	18-05-2013	13:30
Cork	20-05-2013	4:15	Cork	22-05-2013	14:30
Cork	03-06-2013	14:00	Brest	14-06-2013	15:00
Brest	16-06-2013	09:30	Scheveningen	20-06-2013	07:00

Crew Cindy van Damme (cruise leader) Kees Bakker Ineke Pennock (week 19-21) Hanz Wiegerinck (week 23-25)

Volunteers

Fionne Kiggen (week 19-21) Anne Martens (week 19-21), freelance journalist Sjors Treffers (week 19-21) Bastian van Benthem (week 23-25) Florian Lang (week 23-25)

Guests

Patricia Browne (Irish observer) Maik Tiedemann (University of Hamburg, week 19-21) Jacobus van der Zwan (week 23-24), observer from the pelagic trawler fleet

Deviations from sampling plan

When we arrived in the sampling area in week 19 we were faced with bad weather conditions (south)west of Ireland which forced us to go south in our survey area instead of carrying out the proposed alternating transect sampling grid starting north. In the first week, we also lost a considerable amount of time due to technical problems with the winches which forced us to go in to the harbour of Brest to collect spare parts. Next to the time lost because of bad weather, in period 4 we lost 2 full days due to fishing trials and winch repairs.

Bad weather circumstances in the second week also caused serious delay. Of the 129 planned plankton stations and 4 planned fishing hauls (Fig. 3.3.1) we sampled 94 plankton stations and carried out 2 valid fishing hauls (Fig. 4.1.1). We could not sample the planned 24 hour-sampling station. We managed to cover the southern-most transects as planned, but the remainder of the area we only performed plankton sampling around the 200m depth contour, the area were we expected most of the spawning to occur.

In period 5 (June) we sampled 140 plankton stations and carried out 13 valid fishing hauls (Fig. 4.1.2). We also sampled the 24 hour-sampling stations successfully. On the interlacing transects we left out 4 plankton stations at the end of transects because the surrounding stations did not contain fish eggs.

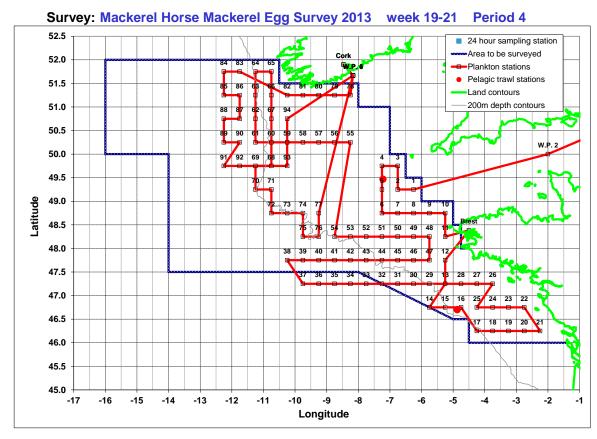


Figure 4.1.1. Sampled station grid in weeks 19-21 (Period 4).

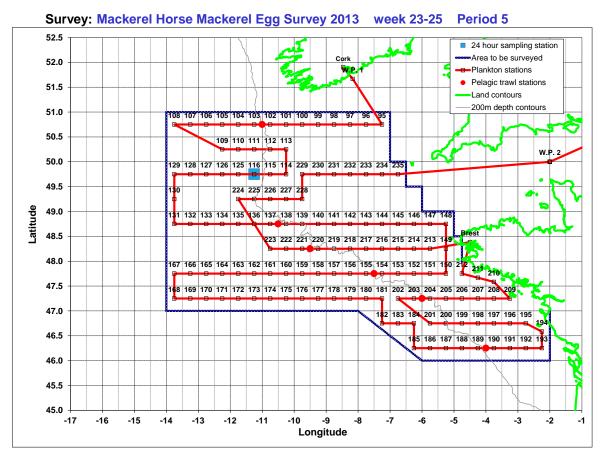


Figure 4.1.2. Sampled station grid in weeks 23-25 (Period 5).

Damage to sampling materials

No damage to the sampling gear occurred during this survey. Even though we experienced bad weather circumstances no damage to the sampling gear or the portside plankton winch occurred despite other things on board sustaining damage.

Survey

May, week 19-21 (Period 4)

Left Stellendam harbour on Monday 6 May 2013 at 10:00 (UTC). Due to the drought the water level in the Slijkgat was low, however we managed to move through it with only 50 cm water underneath the stern. The alternative would have been to go through the inner waters to the harbour of Rotterdam which would have meant getting to the North Sea at 20:00. Compliments to the captain and crew of the Tridens for taking the Slijkgat option!

Steaming through the English Channel to the first planned station at 49.15N 6.15W. The winches for plankton fishing were equipped with new cables, hence new connections for the plankton torpedo had to be made. On Tuesday afternoon we performed a successful trial haul with the plankton torpedo. Before the survey the control and software of the winches for trawl fishing had been revised, but no time was left to carry out trials before the survey and tweak the settings of the fish winches. Therefore a mechanic from Bakker-Sliedrecht joined the survey in order to get the correct settings for fishing. A first trial with the pelagic trawl was done on Tuesday afternoon. Setting of the trawl went well, but the hauling of the trawl could only be done at very slow speed. Increasing the speed caused the winches to

run into overload. It took considerable time to get the trawl back on board. Tuesday evening some more, unsuccessful, trials were carried out to solve the winch control problems.

Due to the time lost with the winch trials, we arrived on 7th May at 23:22 (UTC) on the first station. There were technical problems with the communication between the plankton lab to the bridge and/or deck. This has occurred and reported many times before on other plankton surveys as well. This problem would be solved with a stationary VHF station in the plankton lab, but so far this solution has not been implemented.

We were able to sample two stations before we had to stop due to the bad weather. We could restart again on 8th May at 6:01 (UTC). After two plankton stations another trial was carried out with the pelagic trawl. In order not to waist too much time we decided to keep the catch of this haul and use it for collecting adult samples. Again the trawl could only be hauled at the lowest winch speed. But we had a 2 ton catch of mostly small juvenile mackerels, and also some adult mackerels and horse mackerels were caught, enough to collect samples for fecundity estimates. We also caught a female porbeagle. We resumed plankton sampling at 14:16 (UTC). We continued sampling until 9th May 3:46 (UTC). At that time we were in front of Brest harbour and had received orders from Rijkswaterstaat to go into harbour to collect a new software filter that was transported from the Netherlands. We docked in Brest at 8:30 (UTC). The new software filter did not prove to be the solution to the problems. While in harbour we sorted the plankton samples and entered the data from the pelagic trawl haul. We left Brest harbour at 16:30 (UTC).

On Friday we did another trawl haul at 6:58 (UTC). Again there were problems with hauling the net back on board. The catch contained lots of blue whiting, horse mackerel and also some large mackerel, just enough females to collect all the fecundity samples we needed for this period. We also caught some 'running' hake and tried to fertilize eggs, but this was not successful. The eggs were not running freely so it might have been just too early for fertilization, but we also needed to wait a few hours before the fertilized eggs could be moved from the buckets of salt water to the salt water tanks with free running sea water and air. The pump for the salt water tanks needed to be repaired before it could be used. Both of these reasons might have caused the failure of the fertilization experiment.

Even though we did not need other adult samples still another pelagic trawl trial was carried Saturday morning. This time the net could not be set, because the winches went in overload before the doors were even in the water. Another trawl trial was carried out on Sunday early morning. In order to have a chance of catching some spawning mackerel and horse mackerel the next trial was carried out at night. The shooting of the net started at 01:00 (UTC). However, this time the winch of the trawl sensor broke down. It took an hour to repair this. At 4:45 (UTC) the net was hauled. The catch contained > 95% boarfish and a few mackerel and horse mackerel that were badly damaged by the boarfish. This was the last trial with the pelagic trawl and on Monday evening after station 47 (12:04 UTC) we steamed back to Brest to drop off the winch mechanic. Because of this steam we lost another 5.5 hours of sampling time.

We resumed plankton sampling Monday evening at 21:17 (UTC). Tuesday at 11:00 (UTC) we decided, after a plankton haul were both ground and water speed varied from 2 to 5 knots, that we needed to cease plankton sampling and wait for better weather. We lost the sample at this station because the pump providing salt water to the plankton lab stopped working. We could not get the sample out of the codend without salt water and it took over half an hour to fix the problem. Hence the temperature of the sample was too high causing distortions to the fish eggs and other plankton and which made it unsuitable for further use. As we were in the shelf edge area (the area where we expected highest mackerel and horse mackerel egg abundances) we waited at the station to resume the survey once the weather improved. Because we were stationary it was still possible to sort out and identify eggs, so that work continued.

On Wednesday 06:00 (UTC) we evaluated the situation as the sea state was still too bad to sample and would not improve in near future. We decided to move north, to the only area where weather conditions allowed plankton sampling: southwest of Ireland. We resumed sampling Wednesday at 17:17 (UTC) at 50.15N 8.15W. We continued sampling to the west until we reached the 200m depth contour and the shelf edge. From then on we followed the 200m depth contour north. When we arrived at the northern most transect the weather had slightly improved in the south, so we decided to move south again along the 200m depth contour. This week we sampled the last station at Friday evening at 21:01 (UTC) at 48.45N 9.15E. We arrived in Cork on Saturday 18 May at 13:30 (UTC). The first plan was that two mechanics would fly into Cork for the winch repair during the last three days of this survey period. However, by the time we arrived in Cork, the mechanics seemed to know what caused the problem with the winch control and it was decided to repair this later, during the survey break.

On Monday 20 May we left Cork at 4:15 (UTC) and we had another full 2.5 days for plankton sampling. We started with a calibration of the flowmeters. As we suspected the internal flowmeter showed too few revolutions. The external flowmeter worked correctly and with the expected revolutions per m. The internal flowmeter should give a 20% higher value than the external one due to the design of the torpedo, pulling the water in the nosecone and thus increasing the flow in the torpedo. However, during calibration the revolutions of the internal flow meter were only half of the external flowmeter revolutions. After consulting the international survey coordinator we decided to start the plankton sampling at the beginning of transect 51.15N and move west. When reaching the shelf edge we moved south again in order to ensure a good sampling of the shelf edge area. The weather was finally favourable and we could sample plankton without any problems. On Tuesday evening and Wednesday the pump feeding salt water to the plankton lab gave many problems and salt water flow frequently stopped. This pump needed to be repaired for the next survey in June.

The last plankton station at 50.45N 10.15W was sampled on Wednesday 22 May at 5:12 (UTC). From that position we steamed back to Cork for the inter-survey break. During the steaming we continued sorting and identifying fish eggs. We arrived in Cork 22 May at 14:30 (UTC).

During the May survey we also performed 4 extra Gulf hauls (instead of 5 hauls planned) for the collection of mackerel larvae for the PhD-student from Hamburg University. During the survey we had to move the microzooplankton sampling from the CTD winch to the big crane on deck, because the hydraulics of the CTD-winch broke down.

The PhD student also collected the larvae from all the plankton stations and at the same time checked for remaining fish eggs. Almost all samples were sorted correctly during the first sorting, few eggs remained in the samples. Only at two stations a lot of sardine eggs which float easily were left in the sample. The remaining eggs were also counted and identified.

A freelance journalist joined the survey during the May survey and prepared a written manuscript for the VPRO-magazine and website wetenschap24.nl, as well as pictures for the Resource magazine, a radio interview and a movie about the mackerel and horse mackerel egg survey.



Figure 4.2 The codend with the plankton sample.

June, week 23-25 (Period 5)

We returned to Tridens in Cork Monday 3rd June around lunchtime. We left Cork Harbour for the second leg of the survey at 14:00 (UTC) which was earlier than planned as the captain did not want to sail out of Cork in the middle of the night. The weather was good and we sampled our first station at 20:18 (UTC). During the inter-survey break the winch for the pelagic trawls was repaired and tested and the pump for the salt water was replaced. Hence we experienced no technical problems. Due to the good weather we made good progress. We did a first pelagic trawl haul on Tuesday morning. The catch consisted of lots of juvenile blue whiting, horse mackerel, hake and some mackerel. On Tuesday we also started sorting and identification of the fish eggs.

We continued plankton sampling until we arrived at the position of the planned 24 hour station on Wednesday 5th of June at 16:00 (UTC). At this station we remained for 24 hours to collect plankton and pelagic trawls hauls one after the other. We managed to take 7 pelagic trawl hauls and 19 plankton hauls. All pelagic trawls contained horse mackerel. The first haul did not contain mackerel, the second only one and from the third haul onwards numbers of mackerel started to increase. We saw running horse mackerel in the middle of the night and running mackerel in the afternoon. Spawning of mackerel and horse mackerel occurs in the same area but at different times of the day. Kees managed to do the plankton hauls during the 24 hours, while in the fish lab we had 6 hours on 3 hours off shifts with the three fish samplers. Our volunteers ran 6 hours on 6 hours off shifts to do the administration for the hauls and helped with storing the fecundity samples. It was a long but fruitful 24 hour sampling.

After this we continued along the transect west to collect plankton samples. Friday morning it was discussed with the captain to go in to Brest harbour for the break on Sunday, since we planned to be at the end of the transect close to Brest on Saturday evening. We agreed to this plan and it was told to the crew. However, an hour later it appeared not to be possible to get in contact with the agent so we needed to continue the survey and go in to Brest the second weekend.

During Friday afternoon we lost some time because we supposedly hit a buoy and we had to turn around to check if there was any damage to the buoy. No damage, not even a scratch could be seen on the buoy. On Friday evening we had another successful pelagic trawl haul. The catch consisted of blue whiting, horse mackerel, hake, mackerel and some mesopelagic fish. Plankton sampling continued without any problems on Saturday and Sunday morning. The next pelagic trawl haul was carried out on Sunday morning. This was a short haul because boarfish went into the net so it was decided to haul after 15 minutes fishing. The catch consisted of 95% boarfish and some damaged horse mackerel and mackerel. Only the complete fish were collected and sampled.

The weather remained good and we continued plankton sampling until the next pelagic trawl haul on Wednesday morning. This catch consisted of loads of small mackerel (approx. 10 cm) and some blue whiting. The mackerel seemed to be big for this year's juveniles but too small for last year's youngs. The tiny otoliths confirmed that these are young of the year mackerel and consulting of colleagues and literature shows that at this size mackerel are aged 3 to 4 months. Hence, we have had early spawning in this area this year and loads of them survived the early larval stages of life. It was quite exciting to get this catch.

On Wednesday 12th June between 18:00 and 19:00 (UTC) the radio interview, that was produced by the freelance journalist in period 4, was aired. We managed to listen to it using the internet in the planktonlab.

On Thursday morning we discovered that the fridge where our fecundity samples were stored, was turned off for defrosting. This was the second time this cruise that the fridge has been turned off without telling IMARES crew. Luckily we discovered it in time so our samples could be moved to the other fridge. On Thursday evening we performed the last pelagic haul before the weekend break. There was mackerel visible on the acoustics while we were setting the net so it was decided to turn around while fishing. It took a long time before we were back on the spot where the mackerel was spotted. And after one hour of seeing fish going into the net I thought the catch was big enough and asked for the net to be hauled. However, the reply from the captain was that we weren't at the spot yet and continued fishing. After another 20 minutes the net was hauled and the result was a huge catch and even the rope for pulling in the codend broke because of the weight. Over 90% of the catch was boarfish. We only took out some mackerel and horse mackerel, which was 6 baskets and a quarter of the horse mackerel which was 5 baskets. As I have repeatedly told the crew we only need 100 mackerel and 100 horse mackerel of the regular tows, this catch was for most part a complete waste of fish.

On Friday at 10:10 (UTC) we sampled the last plankton station for the break. In the afternoon we carried out a calibration of the flowmeters. We arrived on Friday at 15:00 in Brest.

Sunday 16th June we left Brest again at 9:30 (UTC). The first plankton station was sampled at 13:11 (UTC). The weather was not so favourable with a low pressure area just west of us, but due to changing wind directions the waves were not too bad so we could continue plankton sampling. Due to the bad weather though we lost some time. On Monday morning we performed the last pelagic trawl haul of this survey. There were some problems with the Marelec, the counter of the winch cable on starboard side did not work. However, we were able to set the net with the trawl sonar. The catch consisted of boarfish and horse mackerel, but the horse mackerel were still alive and not damaged so we could take samples for fecundity.

On Monday the weather improved slowly but because of the low pressure area still west of us and the surrounding stations of the stations at the west end of the interlacing transects did not contain fish eggs it was decided to drop the last stations of these transects. On Tuesday the weather improved further and we had no problems conducting the plankton sampling. We sampled the last plankton station on Tuesday afternoon at 14:22 (UTC).

While on board, all samples were sorted for fish eggs and all eggs were photographed. Only 15 plankton samples remained to be identified back in the lab in IJmuiden additionally, all fecundity samples still have to be analysed.

We arrived in Scheveningen on Thursday 20th June at 07:00 (UTC).

When we needed to put our sampling equipment and personal luggage on board before the survey this was almost impossible and dangerous because the vessel was in the dock in Stellendam. When we arrived in Scheveningen we docked at the Rijkswaterstaat quay. At this quay the distance between the vessel and the main land is about 1 m and it was not possible to hand our survey samples and materials to people ashore. When asking the boatswain they were not willing to help us lift our stuff with the crane to the main land. We had to ask the first mate and when he ordered the crew to help us a crane was lowered onto the deck for us to load our samples and materials onto. When we had filled the crate it was coffee time and we had to wait another half hour for the crew to return. Then again we had to ask the first mate if they could lift our materials from the deck before we were helped by the crew.



Figure 4.3. Horse mackerel, mackerel and other fish eggs and zooplankton in a sample.

Sample-id's

Plankton hauls 2013.5400171 - 2013.5400405 Fishing hauls 2013.5400121 - 2013.5400135

Samples and data

During the survey in May (Period 4) a total of 94 (including 1 invalid haul) plankton stations with CTD measurements, 2 valid fishing hauls and 3 calibration tows were performed only part of the proposed sampling area. At each plankton station a double oblique haul was performed and minimum sampling time was 10 minutes.

In this period we also performed 4 extra Gulf trawls to collect mackerel larvae for RNA/DNA analysis and performed 4 vertical microzooplankton hauls to collect microzooplankton for feeding studies of mackerel larvae. In this period the freelance journalist on board prepared a manuscript for the VPRO-gids and

website wetenschap24.nl, as well as pictures for the Resource magazine, a radio interview and a movie about the mackerel and horse mackerel egg survey.

During the survey in June we performed 13 fishing hauls, all were valid. We sampled 140 plankton stations with CTD measurements and performed 2 calibration hauls. All plankton hauls were valid, except for one station were the net was clogged due to the amounts of phytoplankton in the water.

In both May and June we collected 14 plankton samples for genetic analyses. Unfortunately, we were not able to fertilize eggs, so have no validated samples for genetic analysis. Samples numbers for genetic analysis: 5400218, 5400219, 5400225 (maybe too long in formaldehyde?), 5400226, 5400227, 5400253, 5400254, 5400373 (maybe too long in formaldehyde?), 5400390, 5400396, 5400397, 5400398, 5400399, 5400400.

4.1 Remarks for the next surveys

Technical issues

During this survey we had many things on Tridens that had direct influence on the work that could be carried out and so, on the data collected, being:

- The breakdown of the winch for fishing
- The breakdown of the CTD-winch
- The breakdown of the salt water pumps for the plankton lab
- The breakdown of the salt water tanks for experiments

Other serious technical problems influencing the work were the 'bow thruster' that again did not work, problems with the communication over the VHF which is needed to safely carry out plankton sampling, and breakdowns of the internet connection during the June cruise. As all surveys by IMARES are internationally coordinated, the scientists need to be able to stay in contact with the other vessels and survey coordinator and to send data and updates on the progress of the survey. Thus we need 24-hour and fast internet connection.

Next to that, smaller things happened like the cupboard doors in the fish lab falling out of the cupboard (luckily no-one got hurt), broken chairs in the down-stairs dry lab, constant loads of water on the fishing deck and/or in the wet lab, the taps in the cabins are leaking or are difficult to open and close (when you take a shower you run the risk of getting burnt by the hot water).

It is a no use trying to clean the fish lab as (dirty) water runs constantly from the fish deck into the fish lab because the plumbing doesn't work properly. Part of the problem arose because the captain asked to put more ballast in front of the vessel for stability reasons. The hosepipes in the lab are however at the back. As a result, the fish lab was constantly a stinking swimming pool.

In summary:

- The VHF communication problem needs to be solved. If a stationary VHF with an antenna outside is installed in the plankton lab than the communication problems will be solved.
- Chairs in the dry lab need to be replaced by new chairs as the current chairs are broken.
- Fish winch control needs to be repaired.

As a result from all problems described above, the quality of the samples collected is questionable. There are technical measures that can be taken to improve the quality of the plankton sampling and working circumstances in the lab. Other measures than already named above:

- It is necessary to install a big airing cupboard over the sink and formaldehyde container rack. When sorting out the plankton samples you still smell formaldehyde in the lab, even though the air extraction is on. Above the table where the flow is the strongest the smell of formaldehyde is strong, indicating that the fume hood is not strong enough. Smelling formaldehyde fumes means that the concentration of formaldehyde is above legal levels.
- The gimbal in the table should be removed and replaced by a light box in the table on which we can sort our samples. The current gimbal in the table where the plankton samples are sorted is dangerous as fingers can easily get stuck. Also when the movement of the vessel is strong the gimbal is swinging too much to be able to sort the samples.
- Currently the torpedo is set behind the vessel. This means that the first 5 m of the water column is not sampled correctly because of the disturbance of the water by the thruster. If a crane and winch would be installed to lower the torpedo from the side this problem would be solved. During the proposed refit a lab with a door on the side could be installed from which the torpedo and the CTD/rosette samples could be deployed. In this case IMARES personnel can also rinse the net themselves and this would not require a crew member anymore. As a result IMARES has control of the rinsing and collection of the plankton samples which means that catch handling will happen more consistently as less people are involved.

If not solved already, all issues mentioned above should be solved as soon as possible, as it limits or even prevents the conduction of a scientific survey.

5. Conclusions

5.1 Mackerel eggs

Numbers of mackerel eggs found in the samples in both periods, May and June, were low compared to the previous survey in 2010 (Fig. 5.1 - 5.4). However when all results of the international surveys were combined, it showed that mackerel spawning started already in February-March in the southern part of the Bay of Biscay. In fact peak spawning if western mackerel occurred already in the first sampling period (ICES, 2014). Highest numbers were found around the 200m depth contour.

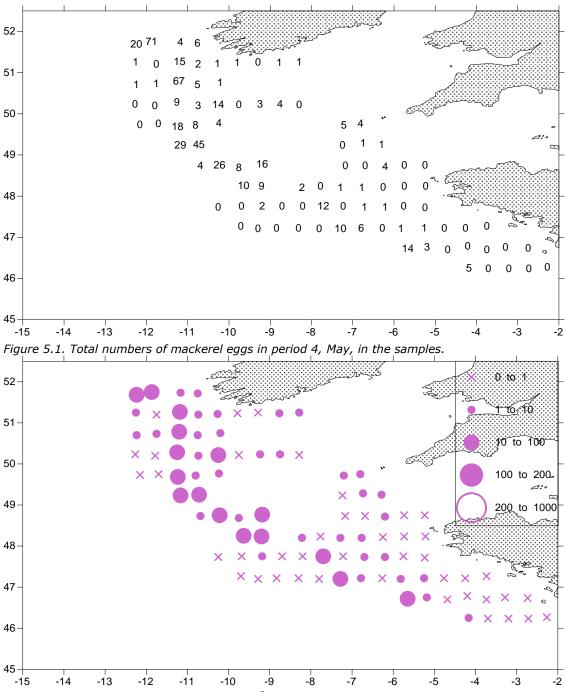
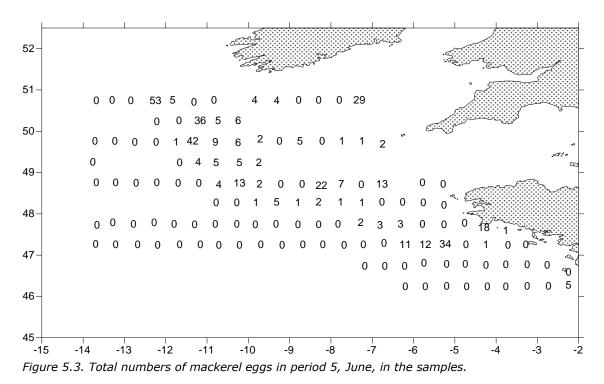


Figure 5.2. Numbers of mackerel eggs per m^2 in period 4, May, in the samples.



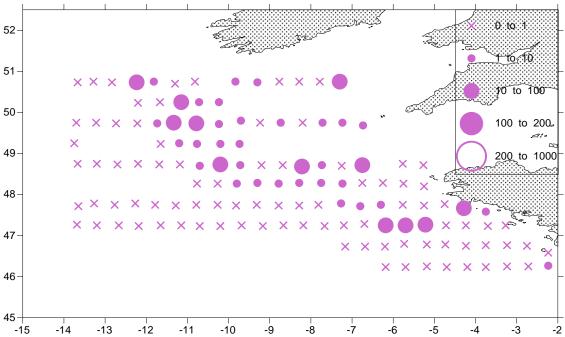


Figure 5.4. Numbers of mackerel eggs per m^2 in period 5, June, in the samples.

5.2 Horse mackerel eggs

The numbers of horse mackerel eggs found in the samples in both periods, May and June, were slightly lower compared to the survey in 2010 (Fig. 5.5 - 5.8). As expected the numbers of horse mackerel eggs found in June were higher compared to May. Highest numbers were found on the deep side of the 200m depth contour.

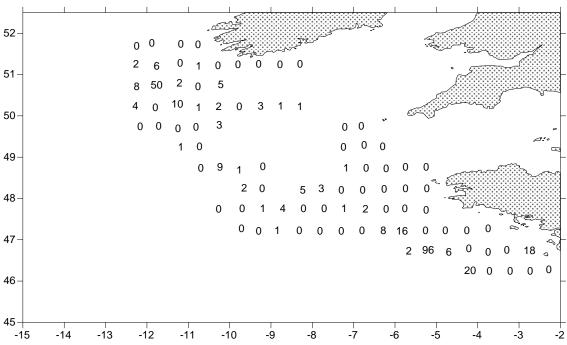


Figure 5.5. Total numbers of horse mackerel eggs in period 4, May, in the samples.

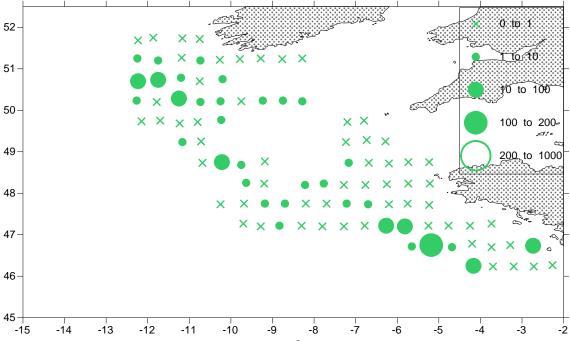
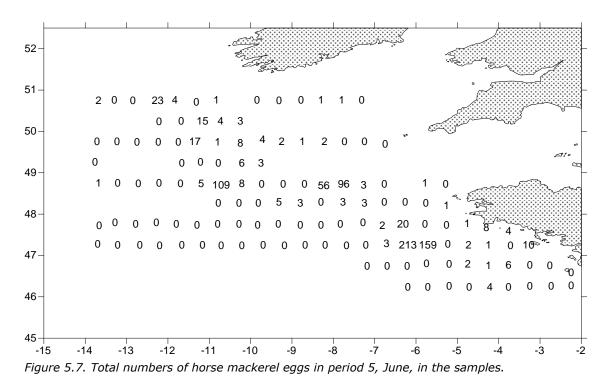


Figure 5.6. Numbers of horse mackerel eggs per m^2 in period 4, May, in the samples.



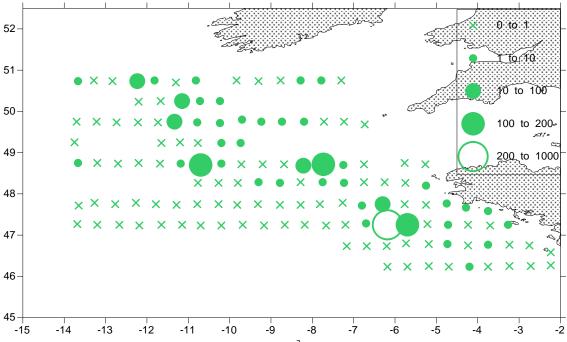


Figure 5.8. Numbers of horse mackerel eggs per m^2 in period 5, June, in the samples.

5.3 Adult fish samples

In total we performed 15 pelagic trawl hauls, 2 in period 4 and 13 in period 5. Of these hauls, 13 contained mackerel and 14 contained horse mackerel. We managed to collected 44 mackerel ovary samples in period 4 and 34 in period 5. Of horse mackerel we only planned to collect fecundity samples in period 5. We managed to collect 171 horse mackerel ovary samples.

At the 24-hour sample we collected 31 mackerel and 109 horse mackerel fecundity samples. Overall we manage to collect all requested fecundity samples.

We were able to collect one sample of mackerel in both periods for GCxGC-MS analyses.

5.4 Fertilized eggs

We caught running hake in period 4. We tried to fertilize eggs but this wasn't successful. In period 5 we only caught running mackerel and horse mackerel at the 24-hour station. However, we were too occupied with the collection of the fecundity samples and were not able to fertilize mackerel or horse mackerel eggs.

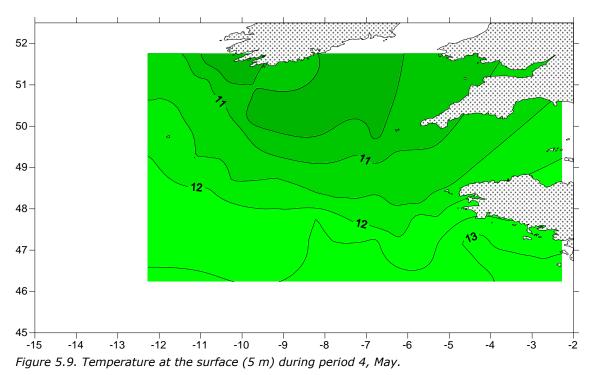
5.5 Samples for genetic analyses

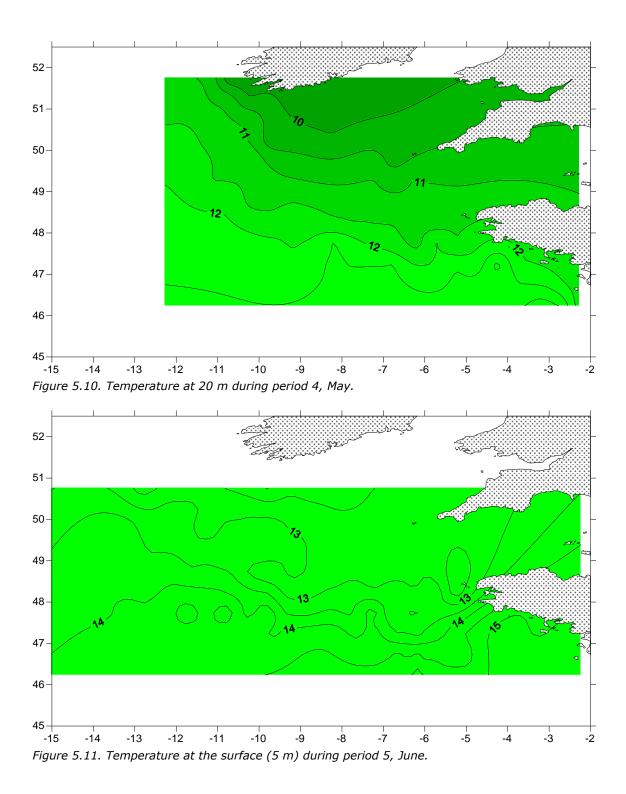
In both periods we collected 14 plankton samples for genetic analyses. These samples have been fixed on formaldehyde for at least 12 hours but shorter than 24 hours and were then transferred to 96% ethanol after they had been photographed for visual identification. Samples numbers for genetic analysis: 5400218, 5400219, 5400225 (over 12 hours in formaldehyde), 5400226, 5400227, 5400253, 5400254, 5400373 (over 12 hours in formaldehyde), 5400390, 5400396, 5400397, 5400398, 5400399, 5400400.

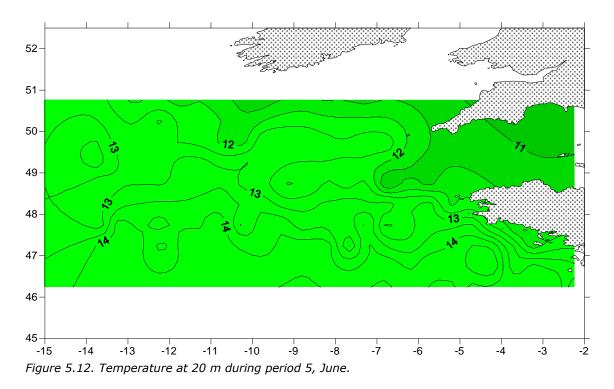
Since we were not able to fertilize eggs, we have no validated samples for genetic analysis.

5.5 Hydrographical data

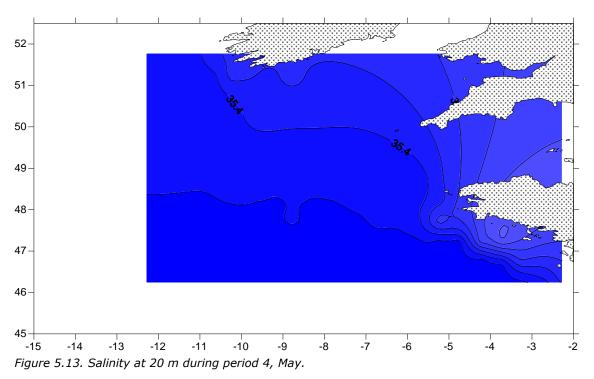
Temperatures in May at both the surface (5 m) and 20 m depth were lower compared to June (Fig. 5.9 – 5.12). In May no thermoclines were seen at the plankton stations, while in June a few stations on the continental plateau showed a thermocline just below 20 m depth.

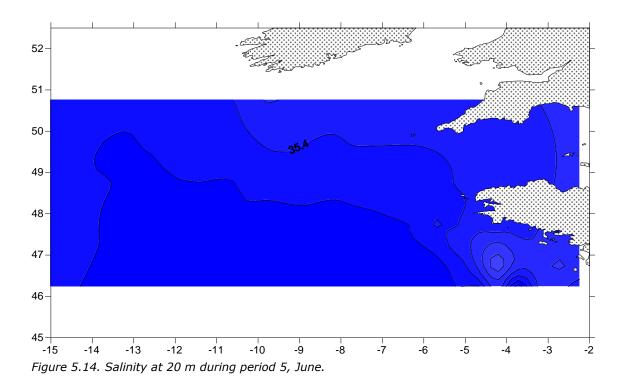






Salinity pattern at 20 m depth was comparable in both May and June (Fig. 5.13 – 5.14).





5.6 Mackerel larvae

During the survey in period 4 we visited four stations on which we performed 4 extra Gulf hauls for the collection of mackerel larvae and a vertical haul with a microzooplankton net and the CTD for the sampling of microzooplankton for feeding studies of mackerel larvae. The PhD student also collected the mackerel and other fish larvae from all the plankton stations. All fish larvae will be analysed at the University in Hamburg.

5.7 Publications

The freelance journalist, Anne Martens, on board during the May survey, prepared a written manuscript for the VPRO-gids and website wetenschap24.nl, as well as pictures for the Resource magazine. All were published in June. She also a produced a radio interview which was featured on Wednesday 12 June. She is still working on a movie about the mackerel and horse mackerel egg survey.

6. Acknowledgements

We would also like to thank all the volunteers, Fionne Kiggen, Sjors Treffers, Anne Martens, Bastiaan van Benthem and Florian Lang for their enthusiasm and much appreciated support and help during the survey. Also our Irish observer Patricia Browne and the PhD-student Maik Tiedeman are thanked for their support and help. Without the help and support of these people we would not have been able to take as many samples and work up as many samples as we did. They also creates a happy atmosphere with their never wavering enthusiasm, even during the bad weather, in the fish and plankton labs.

Special thanks is due to Anne Martens for her preparation of the manuscripts on the egg survey for the VPRO-gids, wetenschap24-website, Resource and Hoe?zo! radio.



Figure 6.1. The scientific crew in period 4 (from left to right: Patricia, Fionne, Kees, Cindy, Ineke, Sjors, Maik and Anne).



Figure 6.2: The scientific crew in period 5 (from left to right: Patricia, Kees, Florian, Bastiaan, Hanz and Cindy).

7. Quality Assurance

7.1 Check on the sorting of the plankton samples

For quality assurance the sorting of eggs from the plankton samples is checked. In period 4 all plankton samples were checked for remaining eggs while collecting the fish larvae. Only few eggs (<5%) remained in the samples. In period 5 4 samples of each 'sprayer' were sprayed again by another 'sprayer'. In most samples no eggs remained and in some few eggs (<5%) remained.

7.2 International calibration of egg identification and fecundity and atresia analyses

Before the survey international workshops, Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM), are held to calibrate egg species identification and egg staging and one workshop to calibrate fecundity and atresia estimation. 4 IMARES specialists participated in this workshop. Results are described in the WKFATHOM (ICES, 2012) report.

Every participating institute is requested to collect ring test samples for fecundity and atresia analyses. These are analysed after the surveys and discussed and reported by WGMEGS in the 2014 report.

7.3 ISO qualification

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

References

- ICES (2012). Report of the Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM). ICES CM 2012/SSGESST:17, 209pp.
- ICES (2014). Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS). ICES CM 2014/SSGESST:14, 110 pp.

Justification

Report number:	C100/14
Project number:	4301211070

The scientific quality of this report has been peer reviewed by a colleague scientist and the head of the department of IMARES.

Approved: ing. I.J. de Boois Project leader surveys

Signature:

Date:

1) deboois July 2014

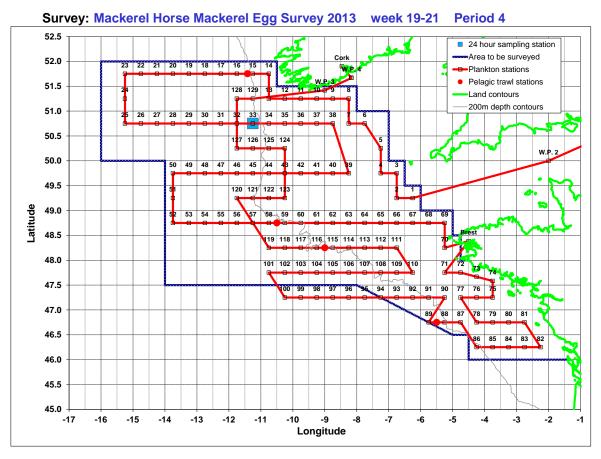
Approved:

Drs. J.H.M. Schobben Head of department Fish

Signature:

Date:

Th July 2014



Appendix A. Proposed sampling grid

Figure 1. Planned sampling grid in weeks 19-21 (Period 4).

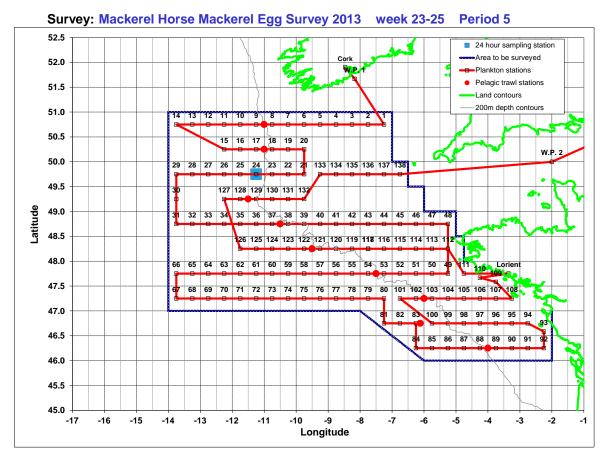


Figure 2. Planned sampling grid in weeks 23-25 (Period 5).