Horse mackerel fecundity in relation to lipid content

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Abstract

During the 2004 mackerel and horse mackerel triennial egg survey fecundity of horse mackerel (*Trachurus trachurus* L.) was estimated using a new gravimetric and image analysis system. The fecundity estimates and mean oocyte diameters showed great variation between and within different institutes. Horse mackerel fecundity increased during the spawning season and showed no decline at the end of the spawning season. This indicates that this species is likely to be an indeterminate spawner.
The lipid content was estimated from whole horse mackerel collected during the three months prior and during the spawning season. Lipid content rapidly decreased during the period of ovary development prior to the onset of spawning, but remained rather constant during spawning. A relationship between fecundity and lipid content could not be found.
A time series of lipid content estimates collected annually or triennially might provide an index for changes in fecundity over time. Sampling needs to be carried out prior to the onset of spawning, when fat content is at its highest.
Annual stomach content estimates collected in successive years might provide an index for food availability and feeding success. The stomach analysis carried out in 2004 indicated that horse mackerel was feeding only at a low level during the spawning period.

Keywords: horse mackerel, Trachurus trachurus, fecundity, gravimetric analysis, lipid content, feeding, indeterminate spawning

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Introduction

Horse mackerel (*Trachurus trachurus*) is considered to be an indeterminate spawner (Gordo et al. in prep; ICES, 2003a, 2005; Karlou-Riga and Economidis, 1997). Fish adopting such a spawning strategy augment their potential fecundity (the standing stock of vitellogenic oocytes in the ovary prior to spawning) from the previtellogenic oocyte population (*de novo* vitellogenesis) (Greer Walker et al. 1994). In this situation potential fecundity is likely to be a

substantial under estimate of total fecundity (the number of eggs spawned from the ovary over the spawning season).

Only the total annual egg production is used as spawning stock biomass index when carrying out the assessment of western horse mackerel, because of the absence of a reliable fecundity estimate (ICES, 2003a, 2003b, 2005). This method implies that fecundity is constant over time. However, the assessment could be vulnerable to changes in fecundity as has been shown for other species such as mackerel (*Scomber scombrus*) (ICES, 2002). For a more reliable horse mackerel assessment an index of fecundity over time is needed.

Total body energy content prior to the start of spawning and uptake of energy from feeding during the spawning season are likely to be the parameters that regulate total fecundity and it is likely that in conjunction they would determine the total annual fecundity of a female. It is suggested that these parameters may be used as an index for interannual variation in horse mackerel fecundity.

Lucio and Martin (1989) showed that body condition factor of horse mackerel does not appear to change during the spawning season. This is due to the replacement of fat by water. Total amount of lipid content might, therefore, give a better indication of fecundity.

Prior to the 2004 survey horse mackerel fecundity was estimated by a histometric method. Whole ovaries were preserved on formaldehyde and two transverse slices were cut and embedded in resin. The embedded samples were cut in 5 µm thick sections and glued to glass slides. From these slides oocytes were counted using a Weibel M168 multipurpose grid (Weibel and Gomez 1962). Fecundity was estimated using the method of Emerson et al (1990). Prior to this work an improved fecundity methodology, based on image analysis, had been described by Thorsen and Kjesbu (2001). Trials on plaice and mackerel (ICES, 2003a, 2005) were promising and it was decided to adopt this procedure for estimation of the standing stock of fecundity in horse mackerel.

We will discuss a new method, using a solid displacement pipette, to remove fecundity samples for gravimetric fecundity analysis (Hunter et al. 1989). A staining procedure based on Periodic acid Schiffs or toluidine blue was used to increase the definition of partially yolked oocytes for image analysis. The method was then applied to determine the standing stock of fecundity in horse mackerel collected in the Eastern Atlantic from the Cantabrian coast to west of Ireland over the spawning season whilst the stock's population egg production was assessed concurrently in icthyoplankton surveys (ICES, 2005). This data is discussed to question the assumption that horse mackerel has an indeterminate spawning strategy. Observations on total body mass lipid content and feeding intensity were assessed in relation to the spawning season to assess the value of these parameters as indices of total individual egg production.

Method

Sample collection

During the 2004 international triennial mackerel and horse mackerel egg survey samples were collected from freshly caught horse mackerel following the method described in the WGMEGS reports (ICES, 2003a, 2005). For each female horse mackerel sampled, different biological parameters were measured: total length, weights of the whole body, liver, stomach and ovary. Ovaries were extracted and samples of known volume were sucked up with a solid displacement pipette (Drummond Scientific Company Wiretrol II) inserted into the ovary through a cut in the tunica wall. Triplicate pipette samples were taken from each fish and preserved separately in 2 ml of 3.6% buffered formaline.

Stomachs were removed from the fish and the stomach fullness was estimated. Fullness was divided into four categories: empty (1), partially full (2), full (3) and stuffed (4).
After this procedure the whole fish, including intestines and the remains of the ovaries, were frozen separately for lipid content analysis in the laboratory.

Fecundity analysis
The calibration between the nominal pipette volume and sample weight removed is shown in Table 1. An additional collection of fecundity samples was made in 2005 to test the accuracy of the pipette under field conditions. Triplicate 25 µl samples were taken from mackerel ovaries and preserved as above but the tube and fixative was weighed prior to departure. The sample weight was determined by difference after correcting for vapour loss from the tube over the interval between sampling and weighing. Vapour loss was estimated in an identical set of tubes containing fixative where no sample was added.
The analysis of the samples was performed by four institutes. Samples south of 48°N were analysed by institute IEO in Spain. Each of the triplicate samples collected north of 48°N was sent to a different institute, MRI (Ireland), IMR (Norway) or RIVO (Netherlands), to be analysed. In addition some images of oocytes and samples were sent around for direct comparison of oocyte diameter measurements and fecundity estimates.
Vitellogenic oocytes are opaque and difficult to analyse by image analysis. To enhance the contrast, oocytes are therefore coloured using PAS-staining (Periodic acid followed by Schiff's reagent) or toluidine blue. The PAS staining is more intense, when the oocytes are more advanced in maturation. Whereas in toluidine blue all oocyte stages are coloured equally.
Fecundity is determined by counting the number of vitellogenic oocytes in the ovary sample. Distinguishing between previtellogenic and vitellogenic oocytes is possible by measuring oocyte diameter. For this study the oocyte diameter threshold for vitellogenic oocytes was set at 185 µm (ICES, 2005). Fecundity can then be calculated using the formula:

$$F = N/s * W$$

where F is fecundity, N is the total number of vitellogenic oocytes in the subsample, s is the subsample weight and W is total weight of the ovaries or total fish weight.
Only horse mackerel which showed no sign of spawning were used for the analysis. However in the field it can be difficult to assign the maturation stage. In the stained ovary samples hyaline and post-ovulatory follicles (POF's) can be distinguished. Whenever hyaline oocytes or POF's were encountered in the samples, these were rejected for fecundity estimation.
No distinction is made between potential and residual fecundity, which corresponds to respectively the fecundity prior to spawning and during spawning, because it is not possible to distinguish between fish that have not yet spawned from those that have already spawned (the batch interval period is longer than duration of the post-ovulatory follicle stage). Therefore only the term fecundity is used within this document.

Lipid measurement
In the laboratory the whole fish (the carcass including head, skin, bones, intestines and ovaries) was shredded. The remains were homogenised and duplicate samples were analysed for lipid and water content according to Bligh and Dyer (1959) or its adaptation by Smedes (1999).

Results

Fecundity

Fecundity samples were collected during the egg survey covering the whole western area and spawning period (Figure 1). Sampling for fecundity was done from March till July 2004 and triplicate samples of 282 fish were collected.
Due to circumstances some ovaries were sampled using a larger 100 µl pipette instead of the recommended 25 µl. These large samples highly increased the analysis time per sample and it was decided to try and subsample these by weight. Total weight of the ovary samples fixed in formaldehyde was different from the supposed fresh weight (Figure 2). Differences range from –20 to 50% of the fresh weight. No statistical differences were found between the institutes. Because of the difference from the supposed weight and the variance in sample weight it was decided to also weigh some samples taken with the 25 µl pipette. These results also show the sample weight to differ from the supposed fresh weight (Figure 2), though the differences and variance are smaller compared to the larger sample sizes. The performance of the pipette to remove a constant sample weight was in 2005 also determined directly from field observation. From the weight gained after adding sample (Figure 3) over 40 days when the samples were collected. The sample tubes on average lost 2.4 mg (se 0.0006) of vapour over the 40 day interval from filling to when the tube plus sample were reweighed to determine sample weight. After applying this correction the majority (89%) of samples were +/– 3 mg from the expected weight of 26 mg but in 11% of samples the pipette was under filled.
Some images exchanged to compare oocyte diameter measurement irrespective of differences in the staining method. The results showed no statistical difference in oocyte diameter measurements between the institutes (Table 2). However, there was a difference in the number of oocytes that were measured automatically by the image analyses system. Also the total number of oocytes measured differed.
A further test with 21 samples being analysed by both Ireland and the Netherlands again showed the differences in oocyte diameter and fecundity estimate to be insignificant (Table 3).
Comparison of oocyte diameters of the triplicate subsamples showed a significant difference (Table 4). Irish mean oocyte diameter is 7% higher compared to the Dutch measurements. Fecundity also showed significant difference between the three institutes (Table 4). Irish mean fecundity estimate was lowest, while Dutch and Norwegian estimates were 9 respectively 12% higher.
Over the spawning season, fecundity (number of oocytes per gram female fish) changed, both above and below 48°N (Figure 4). In the northern area fecundity was 672 at the start of the sampling season, but fell to a minimum of 215 at day 112. From then on there was a gradual increase to 1152 oocytes per gram female at the end of the spawning season. No latitudinal patterns or significant differences could be found in the area north of 48°N.
In the area south of 48°N there was an increase in fecundity towards the end of the spawning season (Figure 4). Mean fecundity is 176 at the start of the spawning season and increased to 725.
There is no significant difference in fecundity between the areas above and below 48°N (Table 5), but variance is considerable. Especially in horse mackerel with low mean oocyte diameter variance in fecundity is high (Figure 5). There is a significant difference in mean oocyte diameter (Table 5) between the two areas.

Lipid content

Sampling for lipid content was done prior to and during spawning. Sample collection started in October 2003 in the most southern part of the western horsemackerel distribution area and ended in July 2004 in the northern part (Figure 1). Over the whole period a total of 509 fish were sampled for lipid analysis.
Figure 6 shows the relationship between lipid content and dry weight in horse mackerel. The slope of the relationship is significantly lower ($P<0.001$) and the variance in the data is higher in the area below 48°N compared to the area above.
Lipid content decreased rapidly during the development of the ovaries prior to the spawning period and remained at a constant level during spawning (Figure 7). Although an increase in fecundity could be seen lipid content remained constant at a low level (Figure 8). This pattern in lipid content could be seen in areas, north and south of 48°N.

Stomach content
During the spawning season 270 female horse mackerel were analysed for stomach fullness. Sampling was done during night and day time but no significant diurnal differences in stomach fullness were found. During sampling no signs of regurgitation were found. 87% of the sampled fish had empty stomachs (Table 6). Only 11 fish (4.1%) had a full or stuffed stomach.

Discussion

Fecundity
The trial with the mackerel ovary samples shows the assumption of the fresh sample weight being 26 µg not entirely correct. Taking pipette samples of fish in early maturation or recovering from spawning was difficult because the oocytes are tightly packed in the ovary tissue. This may result in air being trapped in the pipette sample or connective tissue being included in the sample. This could explain the differences found between the supposed and observed sample weight. It was concluded that to reduce the variance around an assumption of a constant sample weight the tubes should be weighed before and after the sampling was carried out at sea.
Although the trials with exchanged images and samples showed promising results, significant differences in oocytes diameter measurement and fecundity estimates were found between the three analysing institutes. Part of this can be explained by methodological errors. As discussed before, pipette sampling can be difficult in early matured or recovering fish. Also, not all stained oocytes are recognised by the analysis program and some oocytes need to be measured by hand. Differences in these oocytes measurements result in higher or lower fecundity estimates.
Fecundity increases towards the end of the spawning season. This may be an indication for *de novo* vitellogenesis and indeterminate spawning of horse mackerel. This increase was also seen during the 2001 egg survey (ICES 2002).

Fecundity and energy content
Lipid content showed a sharp decline from the start of sampling indicating that the peak possibly occurred prior to sampling. Lucio and Martin (1989) showed a sharp increase in lipid content from October to November, with the peak occurring in November, for horse mackerel in the Bay of Biscay. If lipid content is to be used as an index for fecundity in stock assessment the sampling for lipid content should be carried out annually or triennially during the period of highest lipid content. Additional sampling should be carried out over the course of a year to understand the relation between lipid content and seasonal changes.

The results of this study show no evidence of feeding during spawning. Earlier studies on horse mackerel diet in the Atlantic, North Sea and Adriatic show that many stomachs are empty, especially during the spawning season (Cabral and Murta, 2002; Dahl and Kirkegaard, 1986; 1987; Jardas et al. 2004; Sahrhage, 1970). These studies also show a clear diurnal feeding pattern. No such pattern was found in this study, probably due to the low number of stomachs containing prey remains.

Conclusion

The results of the study show that there is still research needed to find a reliable method for estimation of the standing stock of vitellogenic oocytes in horse mackerel. One important issue to solve is the standardisation of methods when different institutes are involved.

1. Sampling of the ovaries with the pipettes should be standardised.
2. The tubes for preserving ovary subsamples should be weighed before and after sampling at sea in order to get reliable subsample weights.
3. Differences in the PAS and toluidine blue staining should be analysed.
4. Additional sampling of lipid content and feeding should be carried out over the course of a year prior to the mackerel and horse mackerel egg survey in 2007.
5. Together with the lipid sampling, ovary development and fecundity analyses should be carried prior to and during the 2007 survey in order to understand the relationship between energy content and fecundity.

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Figure 1. Sampling stations in the western area. Crosses are stations which were sampled during the spawning season. Dots are stations which were sampled for lipid content prior to the onset of spawning.

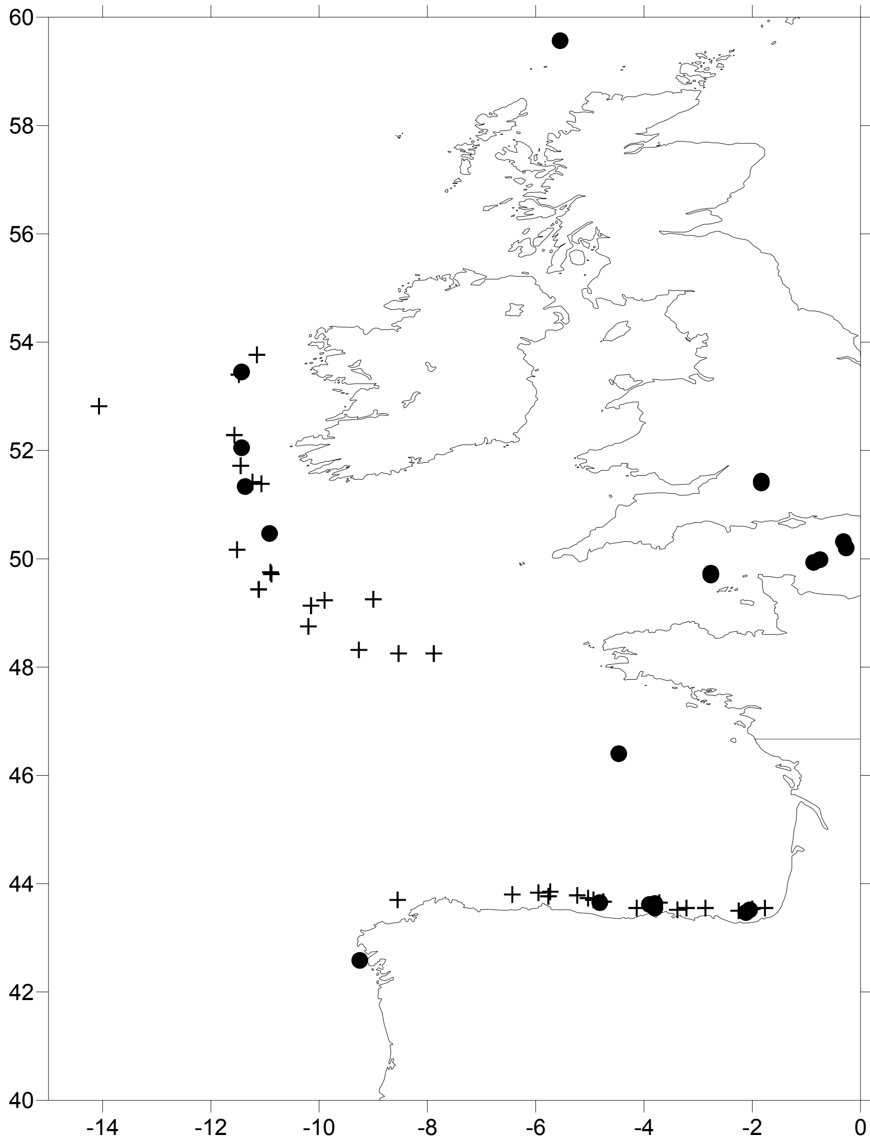

Figure 2. Percentage difference between fresh ovary sample weight and sample weight after preservation in formaldehyde (NL (25) and NOR (25) are differences for the 25 µl samples weighted by Netherlands and Norway and SP (100) and NL (100) are differences for the 100 µl pipette samples weighted by Spain and Netherlands).

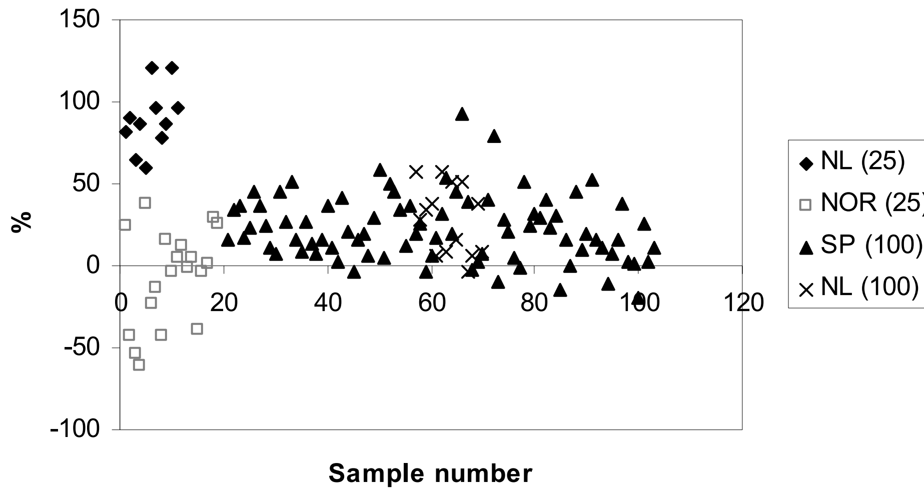

Figure 3. Frequency of sub sample weights (n=60) taken with a 25 µl pipette from 30 mackerel ovaries during 2005.

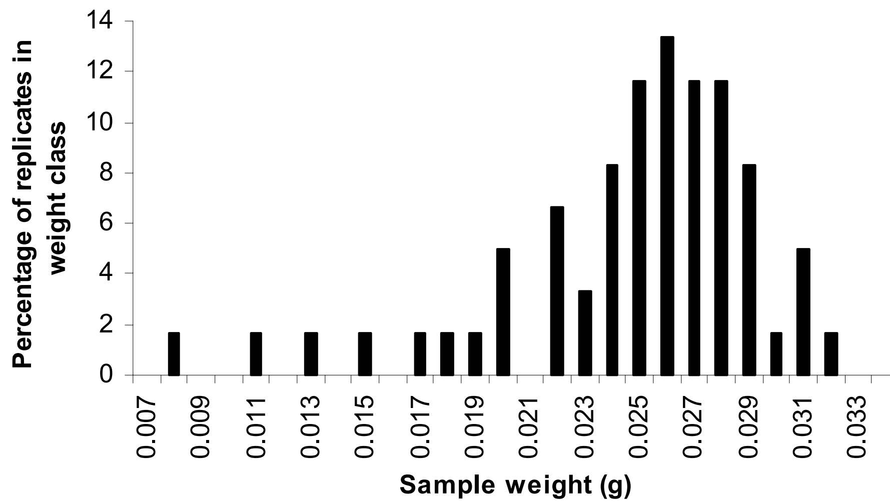

Figure 4. Fecundity over the spawning season for the areas above and below 48°N.

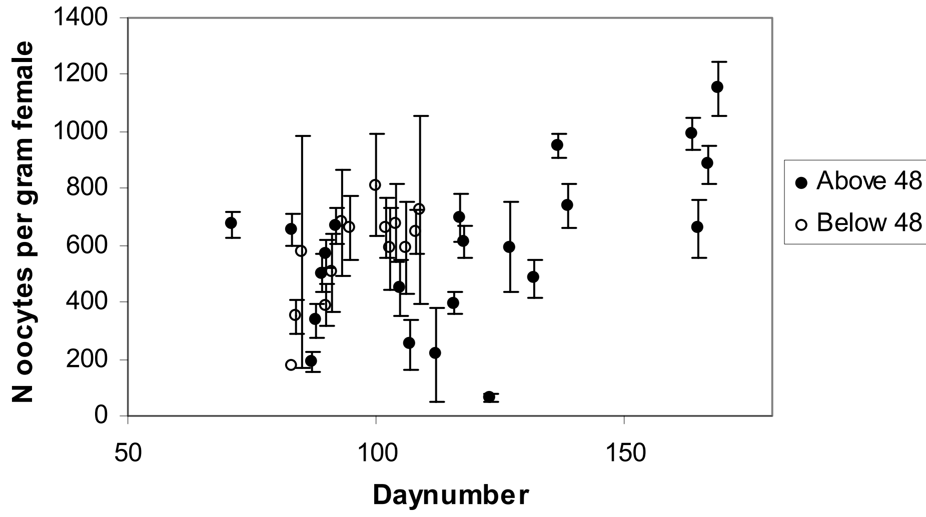

Figure 5. Relationship between mean oocyte diameter and fecundity in horse mackerel.

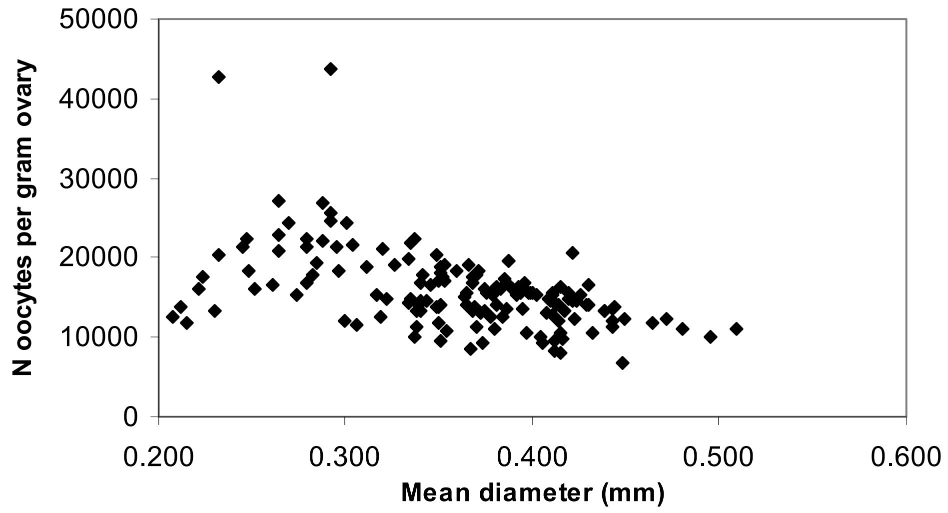

Figure 6. Horsemackerel lipid content in the western area above and below 48°N.

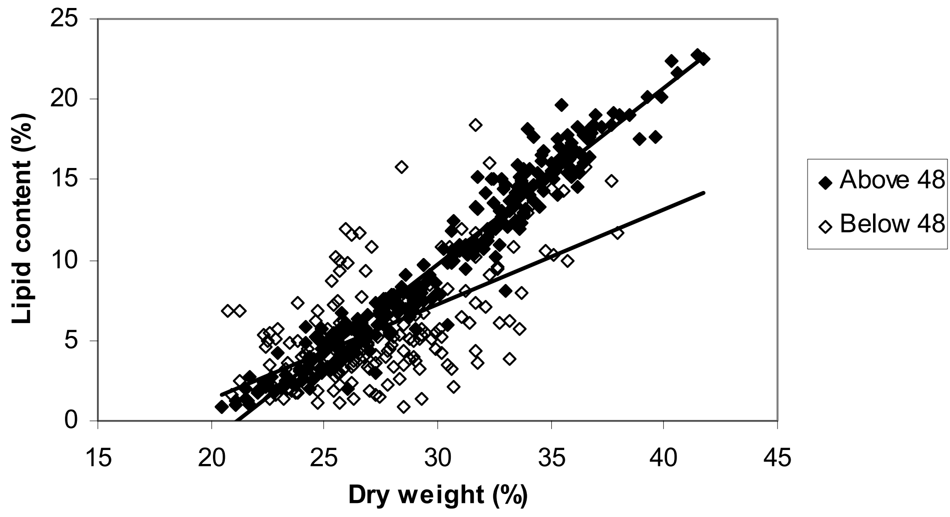

Figure 7. Changes in lipid content of horse mackerel prior to and during the spawning season.

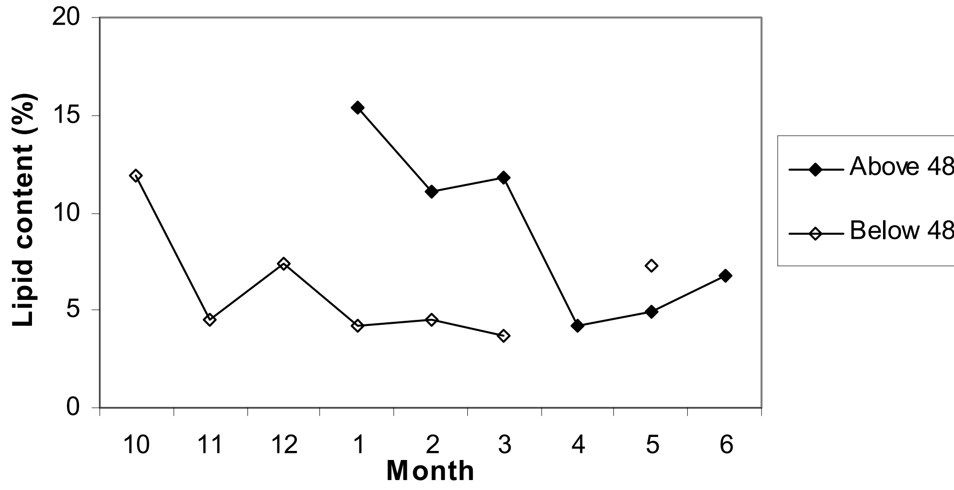

Figure 8. Relation between lipid content and fecundity of horse mackerel during the spawning season.

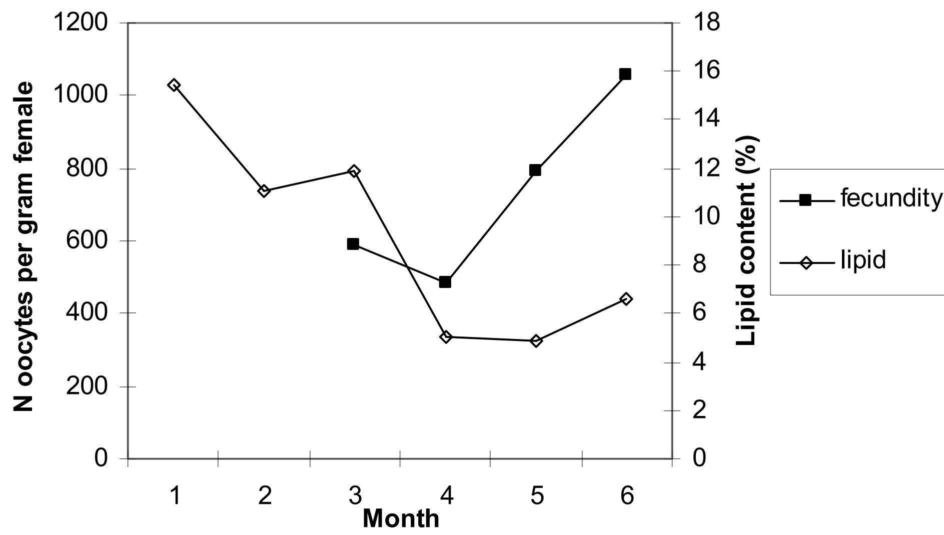

Table 1. Average sample weight removed in 10 replicates taken from a fresh sole ovary using a solid displacement pipette with a nominal volume of 25, 50 and100 µl.

Nominal volume (ml)	Sample weight (g)	CV %
0.025	0.02599	1.84
0.050	0.05221	0.88
0.100	0.10529	3.26

Table 2. Comparison of oocyte diameter measurements between institutes, based on images.

	RIVO (Netherlands)	MRI (Ireland)
Mean oocyte diameter (mm)	0.378	0.383
STDEV oocyte diameter	0.135	0.131
Total number of oocytes measured	185	140
Number of oocytes measured automatic	116	96
Number of oocytes measured by hand	69	44
Comparison (P)	0.780	0.780

Table 3. Comparison between institutes of oocyte diameter measurements and fecundity estimates. (21 samples were analysed by both institutes).

	RIVO (Netherlands)	MRI (Ireland)
Mean oocyte diameter (mm)	0.318	0.335
STDEV oocyte diameter	0.070	0.079
Comparison diameter (P)	0.471	0.471
Mean fecundity	52150	42238
STDEV fecundity	25132	27771
Comparison fecundity (P)	0.232	0.232

Table 4. Comparison between institutes of horse mackerel fecundity estimates based on triplicate subsample analyses.

	RIVO (Netherlands)	MRI (Ireland)	IMR (Norway)
Mean oocyte diameter (mm)	0.356	0.380	-
STDEV oocyte diameter	0.066	0.077	-
Comparison diameter (P)	0.002	0.002	-
Mean fecundity	140054	128540	143359
STDEV fecundity	9859	9748	23412
Number of sample triplicates	163	163	46
Comparison fecundity (P)	0.001	0.001	0.001

Table 5. Comparison between north and south of 48°N of horse mackerel oocyte diameter and fecundity (N oocytes per gram female).

	North of 48ºN	South of 48ºN
Mean oocyte diameter (mm)	0.369	0.307
STDEV oocyte diameter	0.073	0.045
Comparison diameter (P)	0.001	0.001
Mean fecundity	634	607
STDEV fecundity	388	305
Comparison fecundity (P)	0.550	0.550

Table 6. Stomach fullness of spawning horse mackerel

Stomach fullness	Stomachs	%
Empty	235	87.0
Partially full	24	8.9
Full	10	3.7
Stuffed	1	0.4
Total	270	100