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Fecundity in relation to lipid content in North Sea herring

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Foreword

I took part in a project for Wageningen IMARES in Ijmuiden. I wrote this thesis as a graduation study for Inholland College were I study Animal care.
This study gives me the opportunity to experience the work that biologists are engaged in. At IMARES there are also working opportunities for Polytechnic (HBO) students.
In this research I worked in laboratory, performed data management and collected samples in the field. In the laboratory I have worked for 8 weeks, on a desk for 5 weeks and out on the sea for 3 weeks to collect fish samples and fertilized fish eggs. My graduation lasted for 16 weeks all together.

For this project I would like to thank Cindy van Damme for all the help and patience with my project. She gave me the opportunity to follow this apprenticeship at IIMARES

Summary

For this project there are two spawning types examined. These two spawning types are the autumn and winter spawning herring.
In the summer they feed on zooplankton that can grow because of the amount of phytoplankton, which lives from sunlight.
The fecundity of herring depends on when they spawn. The least fecund are the spring spawners, more fecund are the winter spawners, still more are the autumn spawners and the most fecund are the summer spawners . *(Hickling, 1940)*
Some are tempting to relay these differences on the plankton cycle. *(Hickling, 1940)*

The Formulation of a problem:
Is there a relationship between de fecundity and the amount of lipid in the North Sea herring and is it possible that the lipid content can be used as an index for the fecundity.

With as objective:
Determine the relationship between fecundity and lipid content in North Sea herring and study on the basis of atretic oocytes if 'down regulation' occurs.
This objective is going to solve with a few sub objectives:

- Describe the spawning grounds of the herring in the North sea
- Note the similarity between the fecundity in the past and the founded results with this project.
- Compare two lipid content measuring methods:
    - Bligh and Dyer method (Old method),
    - Distell fish fat meter (New method)
- Analyse what the variation of the lipid content could be as a result of the fecundity in the herring.
- Analyse the ovaries for atretic oocytes,
- Analyse if down regulation is taking place.

The definition of fecundity of herring is the number of eggs that will be spawned.
In the past there have been done some researches about fecundity.
The total fecundity most researchers found at fish under the 27 cm were between the 20000 and 30000 oocytes per ovary and above 27 cm between 30000 and 40000 in Winter spawners. (*Hickling, 1940; Bridger, 1961; Zijlstra, 1973)*
For autumn spawners the number of oocytes is between 40000 and 45000 for herring under 27 cm and between 50000 and 85000 for herring above 27 cm. *(Zijlstra, 1973; Burd, 1974; Almatar, 1984)*

There must been found some biological parameters on the herring samples.
Biological parameters of herring collected are:

- spawning type,
- age,
- length,
- weight,
- ovary weight,
- lipid content,
- fecundity.

It is possible to determine the lipid content by two different methods. These are: the Bligh and Dyer method and the Distell fish fat meter method. The Distell fish fat

meter is electronic and the Bligh and Dyer method is chemical. The Bligh and Dyer method costs a lot more time to use compared to the Distell fish fat meter.

The fecundity can be determined by the binocular microscope, which is connected with the computer by a camera. On the computer is installed the program: ImageJ.
A module in ImageJ can count and measure the diameters of the oocytes in the ovary samples. These two variables could give a relation between oocytes diameter and fecundity. The fecundity can then be determined from this relationship.
Also it's important to look at the same time at atretic oocytes. Atretic oocytes are a sign of 'down regulation'.

There are three variables to give conclusions to:

- literature study,
- lipid content,
- fecundity.

The conclusions from my literature study are that there are two different species of Herring swimming in the North Sea. These are the Autumn and Winter spawners. They eat in the central North Sea where they don't spawn. Here they eat zooplankton. In the past there were no studies done about Fecundity against lipid content. There are also no Fecundity studies done about 'down regulation'.

The lipid content conclusions are that there is not a good relationship between the Distell fish fat meter and the Bligh and Dyer method. The fecundity in relation to the lipid content from the Fat meter is:

- Autumn spawners: A high Fecundity shows a high lipid content.
- Winter spawners: A low Fecundity shows a high lipid content.

For the first time atretic oocytes have been seen in ovary samples of North Sea herring. There is also 'down regulation' noticed in North Sea herring.

Summary Dutch
(Samenvatting Nederlands)

Voor dit onderzoek zijn twee paaitypes onderzocht uit de Noordzee: de herfst en de winter paaiende haring. In de zomer leven deze twee soorten van zoöplankton, welke op zijn beurt weer leeft van Fytoplankton. Fytoplankton voedt zich doormiddel van fotosynthese en maakt hierbij gebruik van zonlicht.

De hoeveelheid fecunditeit hangt af van het paaitype van de haring. De minste fecunditeit wordt gevonden bij de lente paaiende haring, iets meer fecunditeit wordt er gevonden bij de winter paaiende haring. Nog iets meer bij de herfst paaiende haring en het meeste bochten is te vinden bij de zomerpaaiende haring . Enkele onderzoekers zeggen dat dit afhangt van de planktoncyclus. *(Hickling, 1940)*

De probleemstelling:
Welke relatie bestaat er tussen het vetgehalte en de fecunditeit in Noordzee haring en kan vetgehalte dienen als index voor fecunditeit.

Deze probleemstelling heeft als doelstelling:
Bepaal wat de relatie is tussen fecunditeit en vetgehalte in Noordzee haring en onderzoek of er ‘down regulation’ plaats vindt. Atretische oocyten zijn een teken, dat er ‘down regulation’ plaats vind. “Down regulation” wil zeggen dat het aantal oocyten in the kuit verminderd naarmate de vissen verder komen in de geslachtsrijpheid cyclus.
Deze doelstelling zal worden opgelost aan de hand van verschillende sub doelstellingen:

- Beschrijf de paaigebieden van de haring in de Noordzee.
- Schrijf de verschillen op tussen de fecunditeit van vroeger en van dit onderzoeksverslag.
- Vergelijk twee methoden die het vetgehalte bepalen:
    - de “Bligh and Dyer” methode, (oude methode)
    - de Distell visvet meter. (nieuwe methode)
- Analyseer wat de variatie van het vetgehalte doet met de fecunditeit.
- Analyseer de kuiten op atretische oocyten.
- Analyseer of er ‘down regulation’ plaats vindt.

De definitie van fecunditeit bij haringen is het mogelijke aantal eieren dat de haring kan gaan paaien.
In het verleden zijn er verschillende onderzoeken gedaan naar fecunditeit. De totale fecunditeit die de meeste onderzoekers vonden bij winter paaiende haring was bij vissen die minder lang waren dan 27 cm een aantal van tussen de 20000 en 30000 oocyten en boven de 27 cm was dit tussen de 30000 en 40000 oocyten. (*Hickling, 1940; Bridger, 1961; Zijlstra, 1973)*
Bij herfst paaiende haring ligt dit aantal hoger, hierbij ligt het aantal bij vissen kleiner dan 27 cm tussen de 40000 en 45000 en bij vissen groter dan 27 cm ligt dit aantal tussen de 50000 en 85000 oocyten. *(Zijlstra, 1973; Burd, 1974; Almatar, 1984)*

Om alle resultaten te verkrijgen zullen er een aantal methoden verricht moeten worden. Er zullen een aantal biologische parameters gemeten worden aan de haringmonsters.
Deze parameters zijn:

- paaitype,
- leeftijd,
- lengte,
- gewicht,
- kuitgewicht,
- vetgehalte,
- fecunditeit.

Het vetgehalte kan op twee manieren bepaald worden. Deze twee manieren zijn de 'Bligh and Dyer' methode en de methode met de Distell vis vetmeter. De Distell vis vetmeter is een elektrische methode en de "Bligh and Dyer" methode een chemische. De "Bligh and Dyer" methode kost hierdoor meer tijd dan de vetmeter.

The fecunditeit kan bepaald worden met een binoculair die aangesloten staat door middel van een camera met de computer, waarop het programma ImageJ draait. ImageJ kan de oocyten tellen en de diameters bepalen. Deze twee variabelen kunnen een index weergeven. Als deze er is kan de fecunditeit bepaald worden met de diameter doormiddel van een formule.
Het is ook belangrijk dat er tegelijkertijd wordt gekeken naar atretische oocyten. Atretische cellen kunnen een oorzaak zijn van "down regulation".

Er zijn drie verschillende variabelen waar conclusies aan gegeven kunnen worden:

- literatuurstudie,
- vetgehalte,
- fecunditeit.

De conclusies vanuit de literatuur zijn:
Er zijn twee verschillende soorten haring in de Noordzee. Deze zijn de winter en de herfst paaiende haring. Deze voeden zich in de centrale Noordzee waar deze niet paaien. Hier eten ze zoöplankton.
In het verleden zijn er geen studies verricht naar 'down regulation'.

De conclusies voor het vetgehalte zijn:
Er is geen goede relatie tussen de vetmeter en de gehalten die uit de "Bligh and Dyer" methode zij gekomen. De fecunditeit in relatie tussen het vetgehalte bij de twee verschillende paaitypen is geworden:

- Herfst paaiende haring: een hoge fecunditeit geeft een hoog vetgehalte weer.
- Winter paaiende haring: een lage fecunditeit geeft een hoog vetgehalte weer.

Voor de eerste maal ooit is er in Noordzee haring atretische cellen gevonden wanneer de fecunditeit bepaald wordt. Tevens is er 'downregulation' waargenomen bij de Noordzee haring.

Table of contents

1. Introduction

1.1 Background

1.1.1 North Sea Herring

Herring fecundity has been studied for many years. The data goes back in time till 1891. There are different spawning types living in the North Sea. In this thesis is examined the autumn spawning and the winter spawning herring. The autumn spawning herring spawns at the North of the North Sea near the coast of Scotland and Shetland. The winter spawning herring spawns 'down' in the North Sea, in the English Channel and the Southern North Sea. The winter spawning herring is also named 'Downs herring'. (*Dicky-Collas, 2005)*
At the time the herring don't spawn they all migrate to the centre of the North Sea. Here they feed on Zooplankton that can grow because of the amount of phytoplankton in the North Sea. These phytoplankton feeds by photosynthesis and has a large amount of sunlight needed for photosynthesis. This is why the Herring only swims to the centre of the North Sea in the summer months. (*Corten, 1996)*
Here they find enough food supply to get enough lipid reserves for migrating to the spawning grounds and produce and mature the oocytes.
Little is known about the factors controlling the number of eggs in a herring. There are two things mentioned by former researchers:

1) environmental factors like food supply (Lipid content),
2) a genetic fixation.

(Zijlstra, 1973)

1.1.2. Fecundity and lipid content

The definition of fecundity is de possible reproduction capacity of an organism or population. For fish this means the number of eggs that possible will be spawned. The lipid content could be an index for the fecundity. This research project reflects the possibility of a relation between the two variables.

Fecundity can be divided in two different fecundities: potential fecundity and realized fecundity. The meaning of potential fecundity is the amount of fecundity that the herring maximum can spawn. And the realized fecundity is the fecundity that the Herring has spawned. It is possible that atresia is a factor can change potential fecundity into realized fecundity. Atresia is the process that oocytes during the maturation cycle are being resorbed by the fish, thus these oocytes never contributes to the reproduction of the herring. *(Kurita, 2003)*
Less Oocytes in the ovary through the maturity cycle is also called 'down regulation' Figure 1 shows in a drawing what this maturity cycle looks like. (*Oskarsson, 2002)*

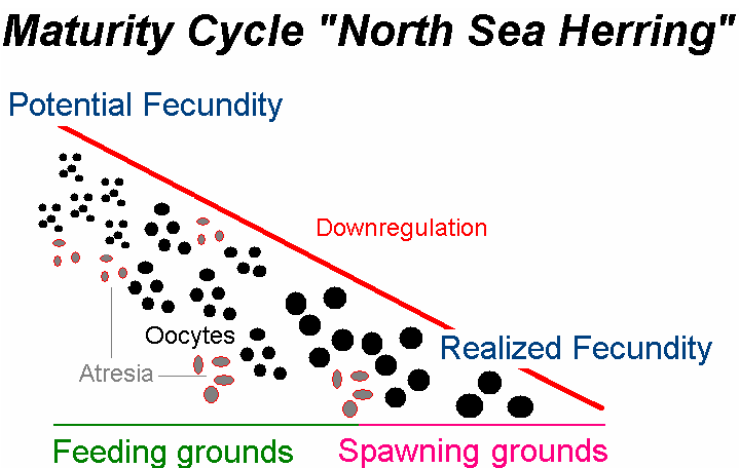


Figure 1: The Maturity cycle in North Sea herring.

Figure 1 shows that when the herring goes further into the maturity cycle, the amount of oocytes is reduced. There are two causes: Atresia is a factor that can reduce the oocytes by resorption in the herring. Also larger Oocytes further in the maturity cycle makes the space in the ovary less. This process is named ‘down regulation’. (*Oskarsson, 2002)*

1.2 The reason for this research

The numbers of herring in the North Sea has decreased for the last decades with rock bottom in the late 80’s. The last 2 decades the herring stock is increasing again. IMARES gives on advice on the state of the spawning biomass of the herring. Ministers set the quota regulations based on the state of the spawning biomass. For example: how much herring is swimming now in the North Sea or how much breed is growing to adult fish and what is the number of eggs they spawn or could spawn. The last example is were this research is going about. How many eggs can a female herring spawn? An other question is what is the reason that the certain herring spawn less then other spawning types. If IMARES knows the amount of eggs the Herring could spawn, then is it possible to give a solution to the other questions. The Dutch “new” herring is caught at the end of May. At this time of the year the herring has a lipid content of 16 percent or more. But in the year 2006 the herring reached the high lipid content later then the end of May. *(Damme, 2006)* The North Sea herring was 2 weeks late. It could be that because of the low lipid contents the amount of fecundity is less. This could also result in the recruitment of new herring being low also. And this will be a problem for the future.

1.3 Formulation of a problem

At the start of the research there is a problem to solve. The short formulation of the problem that will be solved:

Is there a relationship between de fecundity and the amount of lipid in the North Sea Herring and is it possible that the lipid content can be used as an index for the fecundity.

H0: The further into the maturity cycle, less lipid and less oocytes.

1.4 Literature

1.4.1 Fecundity measured by other researchers throughout the years.

Fulton (1891) has taken a research of fecundity of winter spawning Herring. Fulton found a fecundity of 32470 eggs in fish larger then 27 centimetres. The same research was repeated by Farron in 1938. Farron (1938) found an average fecundity of 30043 eggs at winter spawning Herring in the North Sea. These two arrays do not have a significant difference.

Mitchell (1913) found a fecundity of the Herring at three times greater than they found by Fulton. The herring that Mitchell observed were summer spawned Herring. Farron has found that, in Herring with the same length, spring spawners fecundity is about half of the fecundity of autumn spawners, though the eggs are 25% larger. The Fecundity of Herring seems to depend on when they spawn. The least Fecund are the spring spawners, more fecund are the winter spawners, still more are the autumn spawners and the most fecund are the summer spawners. Some researchers are tempting to relay these differences on the plankton cycle *(Hickling, 1940).*

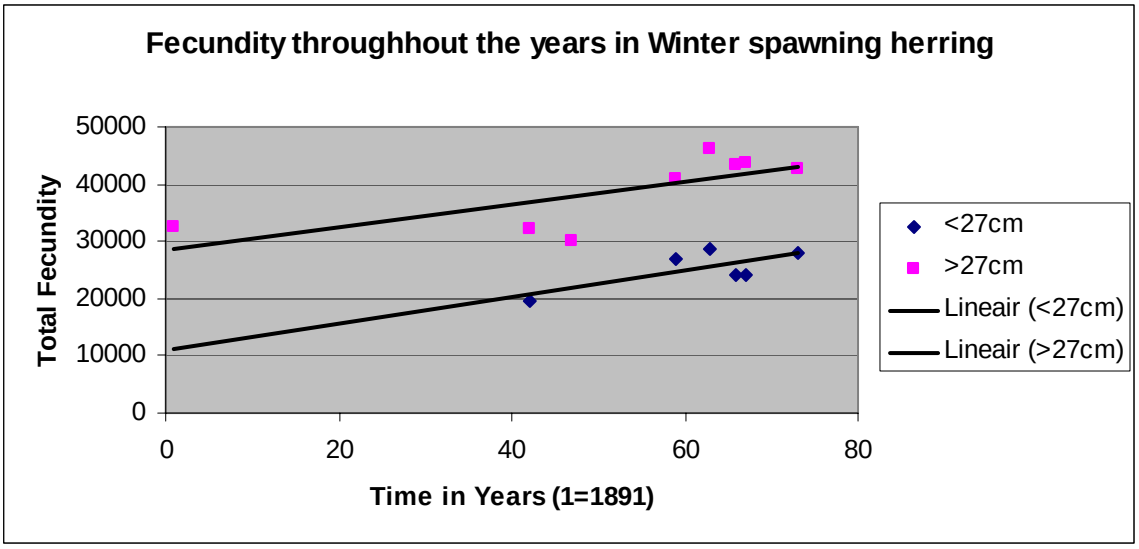
In 1933, *(Hickling, 1940)* Hickling estimated fecundity in Downs Herring (winter spawning) larger then 27 cm of 32200 eggs.
By Simpson (1950) there has been found an estimate of 41000 eggs. (*Bridger, 1961)*
Zijlstra spread his research from 1954 till 1957, he has found an estimate of 46200. *(Zijlstra,1973)*
When Baxter (1957) estimated the fecundity, the amount of eggs he found was 43400. Bridger (1958) discovered a fecundity of 43600 (*Bridger, 1961)* and Zijlstra had a fecundity of 42714. *(Zijlstra,1973)*

Between the lengths of 22- 26 cm they found different estimates. Hickling found an estimate of 19660. The estimate that Simpson found was 26860 eggs. (*Bridger, J.P. 1961)* Zijlstra has got an estimate of 28750. *(Zijlstra,1973)* Baxter's results were 24300 eggs, (*Bridger, J.P. 1961)* Bridger had 24280 and Zijlstra 28000 *(Zeilstra,1973).* Al these estimates were found in winter spawning Herring. In table 1 and figure 1 the mean fecundity of two length groups is tabulated. There seems to be an increase in fecundity over the years.
(*Bridger,1961)*

Table 1: Fecundity in winter spawning Herring for two different length groups. The number of observations is shown between the brackets.

Length / Year	22-26cm	27-29 cm
1891 (Fulton)	-	32470
1933 (Hickling)	19660 (108)	32200 (25)
1938 (Farron)	-	30043
1950 (Simpson)	26860 (57)	41000 (62)
1954 -1957 (Zijlstra)	28750 (92)	46200 (60)
1957 (Baxter)	24300 (55)	43400 (39)
1958 (Bridger)	24280 (60)	43600 (25)
1964 -1966 (Zijlstra)	28000 (80)	42714 (90)

(*Hickling, 1940; Bridger, 1961; Zijlstra, 1973)*



Graph. 1: this graph shows the differences between fecundities throughout the years. 1 is in this graph equal to 1891: the first time there has been found fecundities in literature.

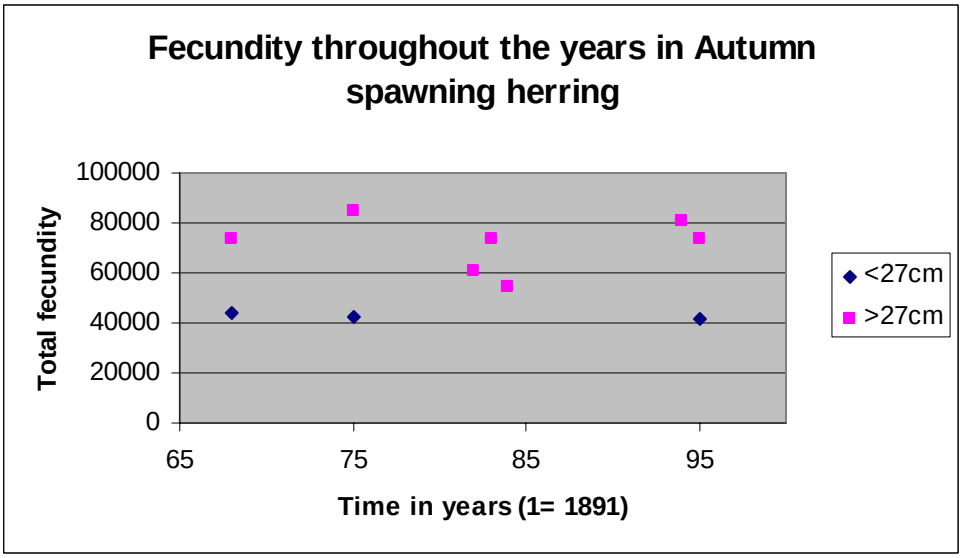
Autumn Spawners spawn at the North of the North Sea. Table 2 shows the different fecundities throughout the years founded by Baxter (1957), Zijlstra (1964-1966) and Almatar (1971-1973, 1983, 1984).

Table 2: Fecundity in autumn spawning Herring at two different length groups. The number of observations is shown between the brackets.

length	22-26 cm	27-29 cm
Year		
1957 Baxter	44250	73300
1964-1966 (Zijlstra)	42339 (106)	84833 (90)
1971 (Almatar)	-	60960
1972 (Almatar)	-	73300
1973 (Almatar)	-	54400
1983 (Almatar)	-	80933
1984 (Almatar)	41250	73500

(Zijlstra, 1973; Burd, 1974; Almatar, 1984)

The reason why the length is used is: fecundity is general related to their weight and their length. *(Hickling, 1940; Bridger, 1961; Zijlstra, 1973).* Weight is more dependent on the condition of the fish and length is less dependent on the condition.



Graph 2: this graph shows the differences between fecundities throughout the years. 1 is in this graph equal to 1891: the first time there has been found fecundities in literature.

1.4.2 Different lipid estimate methods

There are four different methods to estimate the content of lipid in fish. The methods are three non-destructive methods: NIR (Near infrared reflectance), NMR (Nuclear magnetic resonance) and the Distell fish fat meter.

The Bligh and Dyer method is a more destructive way to estimate lipid in fillet or the whole fish.
NIR estimates the lipid content in the whole fish or fillet. NIR spectroscopy is used to estimate the chemical composition of fish. Although this method can't estimate yet the lipid content of herring or herring filet.
NMR can be used for an alternative of NIR but due the size of the magnets it's not portable. When it's not portable the method is useless for surveys.
The Distell fish fat meter, developed in the 1990's operates in the microwave region and measures the dielectric properties and the water content in the sample (*Nielsen et al, 2005).* The method is based on the fact that there is a balance between the water and lipid content in the fish. The electric strip on the Distell fish fat meter measures the water content. Through a calibration value, the water content can be converted into the lipid content. By calibration on the special relationship for herring is it possible to determine the lipid content in herring. However Nielsen's (2005) opinion was that the results of the Distell fish fat meter are slightly inferior to the NIR (*Nielsen et al, 2005).* But we could not use this method.

With the Bligh and Dyer method it's important to homogenize the fish with a blender. The Bligh and Dyer method costs more time then the other methods. The Bligh and Dyer method will be compared with the Distell fish fat meter in this project.

1.5 Objectives

The Objective of this research is:

Determine the relationship between fecundity and lipid content in North Sea herring and study on the basis of atretic oocytes if ‘down regulation’ occurs.

The sub objectives are:

- Collect information about the herring from the North Sea through a literature study. The following points should be described in this report to complete the project.
    - describe the spawning grounds of the herring,
    - enumerate the natural food of the herring,
    - note down the difference or the similarity between the fecundity and the lipid content throughout the years.
    - compare the Distell fat meter with the Bligh and Dyer method.
- Collect data on the herring that would be of significance for the further going of the study:
    - lipid content,
    - age,
    - fecundity,
    - weight,
    - gutted weight,
    - ovary weight,
    - stomach content,
    - spawning type.

- Analyse the data and explain what kind of relation these parameters have with the fecundity in relationship with the lipid content.
- Analyse what the variation at the lipid content could be as a result for the fecundity of the herring.
- Analyse the oocytes on vitellogenetic oocytes and atretic oocytes.
- Analyse if down regulation is taken place.
- Calculate the relation between the lipid content and the fecundity at the herring samples.
- Substantiate the founded data from the calculation of the relation between the lipid content and the fecundity of the herring samples.
- Evaluate the founded results.
- Judge all results critically.

2. Material en Methods

2.1 Biological parameters measured on the Herring.

2.1.1 Material

1. scalpel,
2. cutting board with length measure instrument (picture 1),
3. balance,
4. formalin,
5. jars,
6. herring,
7. vessels: Tridens and Scotia,
8. pencil,
9. fill in templates,
10. computer,
11. excel,
12. Distell fish fat meter.

2.1.2 Method

This will be put into effect in several steps:

1) The herring is caught at different places in the North Sea. See figure 2.

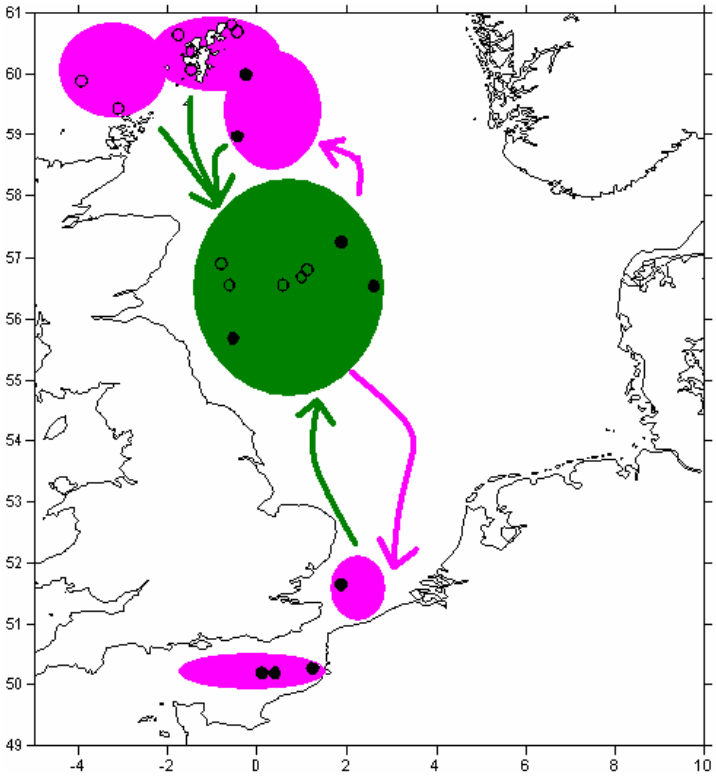


Figure 2: Spawning and feeding grounds herring in the North Sea. The pink spots in the north are the spawning grounds for the autumn spawning herring. The pink spots in the south are the spawning grounds for the winter spawning herring. Each year they all come back to the green feeding grounds in the central North Sea. The open dots represent the surveys with the vessels: Scotia or Tridens (7) and the closed dots are from the market samples. The time of collecting data is from June till December.

2) Fifty females will be separated from the males. This will be done by cutting the herring with a scalpel (1) from the anus till the head. This is shown on picture 1. Female herring has an ovary and male herring has milt. The further the female is in her spawn development, the larger is the ovary. The stage of ovary development is subdivide into 4 stages:

   Stage 2 Immature: This is the stadium before the female is sexually mature. After the fish is started to be sexually mature, she will never go back to stadium 2.

   Stage 4 Mature: This stage starts on the point when the Herring starts to be sexually mature and ends on the point when it's the time of spawning.

   Stage 6 Spawning: It's time to spawn.

   Stage 8 Rest: The spawning time is finished and the herring is in rest. She goes back to stage 4.

3) The weight and length (from nose till tail) will be noted on the fill in template (9).
4) Age and spawning type of herring is read from otoliths. Otoliths are hearing bones in the back of the head. From rings on the bones (just like trees) the age can be read. The otoliths will be examined by others.

Picture 1: A Herring on a cutting board with length measure instrument.

5) **New lipid content estimating method. (Distell fish fat meter (12))**
   The lipid content of the fish will be estimated with the electronic Distell fish fat meter. The Distell fish fat meter can be used for many species. By calibrating the Distell fish fat meter on "herring"; it can estimate the lipid content.
   The method is based on the fact that water and lipid are in balance in the fish. Every species of fish haves a different relation between the lipid and water content. This is why the Distell fish fat meter must be calibrated to herring.
   The electronic strip measured the water content and with a calibration value it can estimate to lipid content. This is also the reason why access water should be wiped off, otherwise it is possible that there comes many water between the Distell fish fat meter and the fish and it estimates a wrong lipid value when the fat meter is placed on the flank of the fish. This must be done on both flanks after which the fat meter estimates the average content (picture 2).
   Place the Distell fish fat meter on the filet of the fish. The centre of the Distell fish fat meter should be at the height of the dorsal fin.


Picture 2: The Distell fish fat meter.

Old lipid estimating method. (Bligh and Dyer)
After measuring the lipid content from the herring with the Distell fish fat meter five of the 50 herring will be kept for the Bligh and Dyer method. This will be done for control of the estimates of the Distell fish fat meter.

6) After estimating the lipid content the intestines will be thrown away, but the ovary must be kept. From the ovary the oocytes will be counted for the fecundity. Also the stomach contents will be observed. Stomach contents is divided into four different categories:
    1. Empty
    2. Filled
    3. Full
    4. Overfull
7) The fish is weighed without the intestines (gutted weight). Also the ovary will be weighted. (ovary weight)

8) The ovary is kept in a jar (5) with formalin (4) for estimating the fecundity.

9) Fill in all data in a template and use the excel spreadsheet (11) to work out the data.

2.2 Bligh and Dyer Lipid definition

2.2.1. Material

The material or appliance that should be used for the Bligh and Dyer method is:

1. 20 ml methanol each sample,
2. 20 ml chloroform each sample,
3. demy – water,
4. fish samples,
5. spin dryer tubes with a top, volume 80 ml,
6. flat bottom cups,
7. blender,
8. centrifuge,
9. Ultra Turrax ,
10. hot plate,
11. balance (0,01 gram),
12. analytic balance (0,001 gram),
13. measure cups 50 ml or 100 ml,
14. Bligh and Dyer template,
15. pipette,
16. 20 ml measure cup,
17. blower.

2.2.2. Method

Each fish will be examined twice. Once the filet and once the whole fish (with the filet).

1) Preparation

- Fill in the name of the fish on the Bligh and Dyer template (14). The amount of meat that is needed for the Bligh and Dyer method is depended on the fish species. For each fish the amount is determined. This amount indicates the maximum of Lipid that solves in the Chloroform. Also the amount of demy-water needed is estimated.
- Take the filets from the herring with a knife. It is important that the skin is also removed. After every new fish clean the cutting board and knife so that there is no lipid transfer between the herring.
- In the bags were the herring were kept in the freezer is water containing fat left. This must also go into the blender with the herring.
- Homogenize the filet and the fish with the filet (4), in a blender (7).
- After each fish clean the blender cup.
- Each fish has to go into a 50 ml measure cup. Be sure that each measure cup is labelled. (picture 3)

Picture 3: Homogenized fish. In picture 3a is shown a herring. Picture 3b shows the herring with on top of the picture the filet and on the bottom the other parts of the fish. On 3c is seen the fish inside the blender and 3d shows the homogenize fish inside the 50 ml measure cups (13).

- From every measure cup a small amount of fish is used for the Bligh and Dyer method. Weigh the small amount of sample in a spin dryer tube on an analytic balance (0,001 gram) (12). See picture 4a. Duplicate only the first sample for the reliability of the method.

2) Extractions

- First add the amount of demy- water (3) that is calculated by the Bligh and Dyer template, into the spin dryer tube (5).
- Then dose 20 ml of methanol (1) into the spin dryer tube.
- Place the tube with the methanol and demy-water on the balance. Set the balance (11) on zero. Add 10 ml chloroform (2) into the tube with methanol and water and weigh the tube. This amount represents the weight of only the chloroform. Write this number down on the Bligh and Dyer template.
- Homogenize the mixture in 30 seconds with the Ultra Turrax (9). The volume ratio is tabled in table 3 at the letter A.
- Add once more 10 ml of chloroform into the mixture. Weigh only the chloroform on the balance. Homogenize the fluid in 30 seconds with the ultra Turrax. (Table 3: B)
- After 30 seconds add 10 ml of demy-water to the mixture. Homogenize once more for 30 seconds (Table 3: C). Be certain that the top is on the tube when the mixing is finished, otherwise the chloroform will vaporize. These handlings are shown in picture 4b.

Table 3: The volume ratio of the mixtures at the extractions.

	Chloroform	methanol	demy- water
A	1	2	Calculated by the B and D template
B	2	2	,,
C	2	2	,, +10 ml

(Dao, Q.T. and M.M. de Wit; 2005)

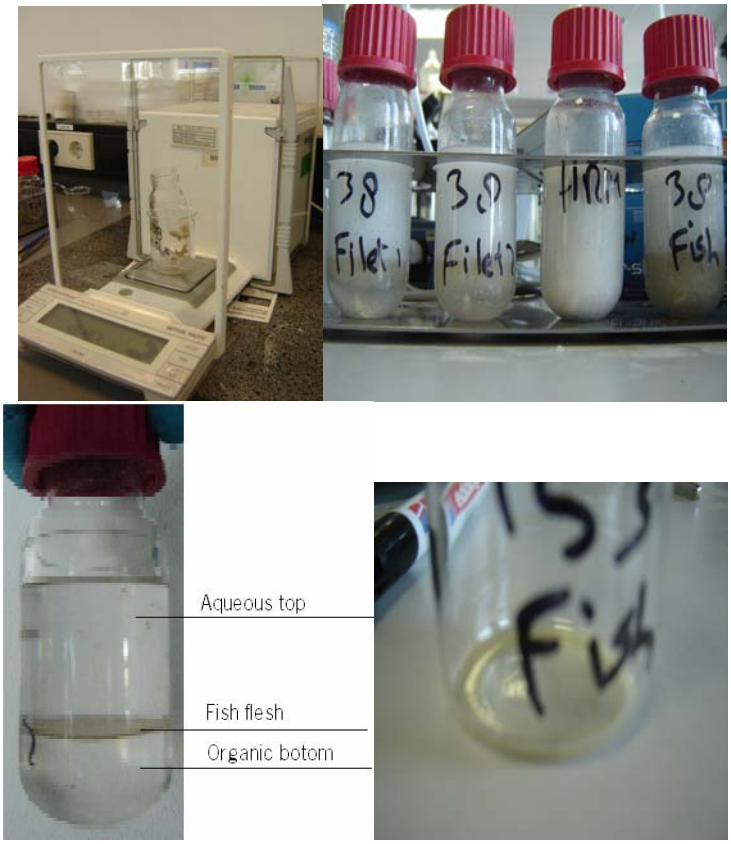
3) Centrifuge

- Centrifuge the tubes at 3750 RPM for 10 minutes. The tubes across each other must have the same weight, if they do not the centrifuge (8) will be out of balance and won't work.
- After centrifuge, the tube shows a two layer system, an aqueous top and an organic bottom. The aqueous top contains Methanol and demy-water and the organic bottom contains lipid and chloroform. See picture 4c.

4) Lipid estimating

- Place the measure cup on the balance. Set the balance on zero. Pipette (15) the aqueous top and try to create a small opening into the organic bottom. Pipette 10 ml from the organic bottom and transport this into a 20 ml measure cup (16). Then weigh the measure cup with the 10 ml organic bottom and write the weight of the organic bottom down.
- Place the measure cup on the hot plate (10). Be certain that the blower (17) blows, otherwise the mixture won't remain mixed.
- If the chloroform is vaporized only the lipid will remain. This is shown as a brown-yellow film on picture 4d. Weigh the lipid on the analytic balance, because of the small amount it has to be weighted with 3 decimals.

(Dao, Q.T. and M.M. de Wit; 2005)

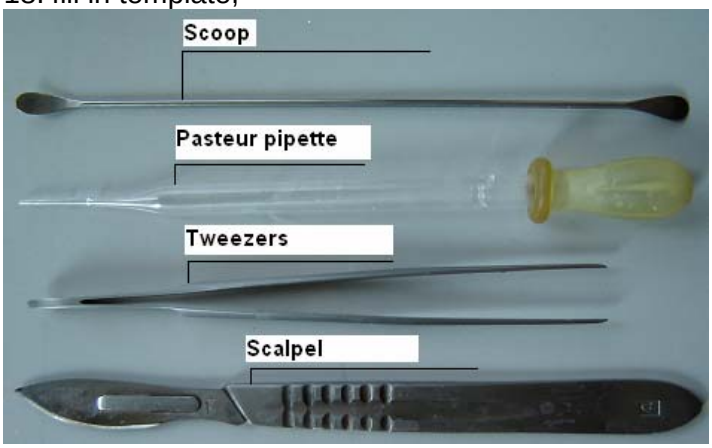


Picture 4: The Bligh and Dyer Method. On 4a is shown the spin dryer tube on the analytic balance with the sample of homogenizes herring. 4b shows the spin dryer tube filled with 20ml chloroform, 20 ml methanol, 17, 2 ml demy water and 1, 3 grams of homogenized herring. After spinning in the centrifuge the fluids and the sample looks like picture 3c. In 3d is the lipid shown that has left after vaporizing the chloroform from the organic bottom in a 20 ml measure cup.

2.3 Estimate the Fecundity

2.3.1 Material

1. ovary jars,
2. Pasteur pipette (picture 5),
3. balance (0,01 gram),
4. analytic balance (0,001 gram),
5. acid cabinet,
6. demy-water,
7. Formalin 3,6%,
8. closable cup (picture 6),
9. not closable cup,
10. bowls with a gauze bottom (picture 6),
11. computer with image J,
12. binocular with a camera,
13. scoop (picture 5),
14. blotting paper,
15. scalpel (picture 5),
16. special designed dish (picture 6),
17. tweezers (picture 5),
18. fill in template,



Picture 5: A scoop, Pasteur pipette, tweezers and scalpel.

2.3.2. Method

1) Preparation

- The samples are numbered from 1 till 350, herring 1 is caught on 06-06-2007 and 350 on date 24-11-2007. The oocytes grow larger when the herring is nearer to the spawning time.
- Because of the lack of time it's important to make a partition of the samples. Every sample number which contains a five will be researched after 100. For example: sample 105-115-125-135 etcetera. Before 100 the oocytes are too young and small so it's not reliable to lay them under the binocular. Only with slices these oocytes can become part of the research. From the oocytes is cutted a cross section, from the inside the oocytes are checked for atresia and maturity stage.
- There are 25 samples. Every sample will be duplicated, thus the samples for the start of the research contains 50 samples.

2) Remove the oocytes from the ovary.
   - Get a jar (1) with an ovary on formalin (7). Work in the acid cabinet (5). In advance weigh the small bowl with the gauze bottom (10) on the analytic balance (4) and set the balance on zero.
   - Take the ovary with tweezers (17) out of the jar. Dry the ovary for 2 seconds each side on a piece of blotting paper (14) and put the ovary on a balance (3). Weigh the ovary and write the ovary weight down on the fill in template (18).
   - The ovary consists of two parts. Make an incision with a scalpel (15) on one of the two parts.
   - Scrape with a scoop (13) approximately 0,100 grams from the ovary and transport the oocytes into a small bowl with a gauze bottom. Weigh the oocytes on the analytic balance. Write the amount of the weight down. Repeat the former handling for the duplicate.
   - Place the bowls into a cup (9) filled with demy-water. The Formalin will solve in the demy-water (6).

Picture 6: A bowl with a gauze bottom, a closable cup which contains the bowl with the gauze bottom and the cover for the closable cup. From the bowl with the gauze bottom there is made a special designed dish to count the oocytes.

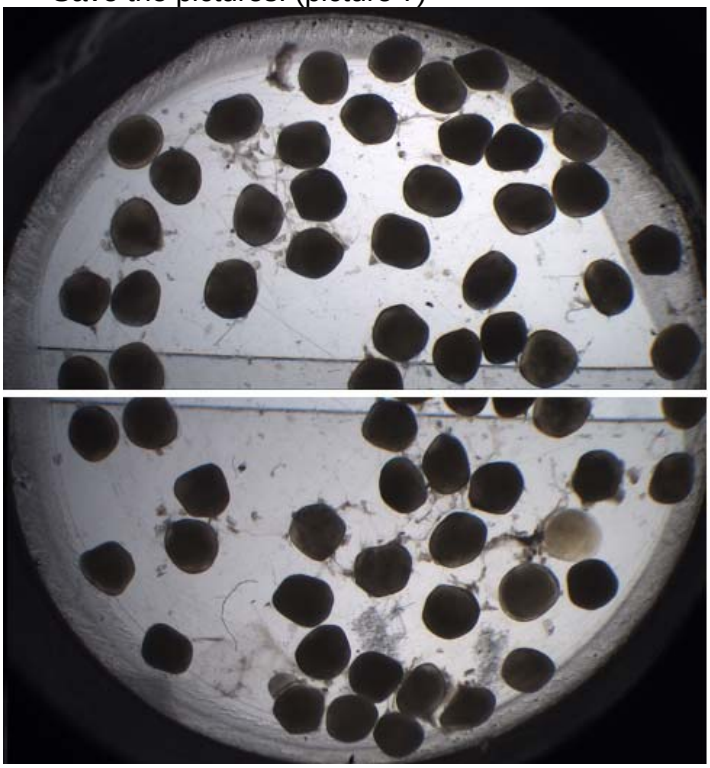
3) Prepare the oocytes for the binocular (12).

   - Make the oocytes loose from each other with a narrow pipette, called Pasteur pipette (2). Use the pipette for several times till the oocytes are not attached anymore. It's very difficult to count the oocytes if they are attached.
   - Use demy-water to rinse the pipette if the oocytes are loose to make sure that there are no oocytes left in the pipette. Also rinse the sides of the cup for lost oocytes.
   - Place the holder of the bowls in a closable cup (8). It's important that the oocytes don't dry up or get loose by spill.

4) Observe the oocytes under the binocular.

   - Transfer the oocytes with the pipette into a special designed dish (16). This dish is designed to make it possible to take only 2 pictures with de camera on the binocular. This special designed dish is shown on picture 6.
   - Start the computer and play the program: ImageJ (11).
   - Start the camera on the binocular with ImageJ to get a live view.
   - First calibrate the program to the right linear measure.

- Make a picture of the top side of the sample. And after that the bottom side. Save the pictures. (picture 7)

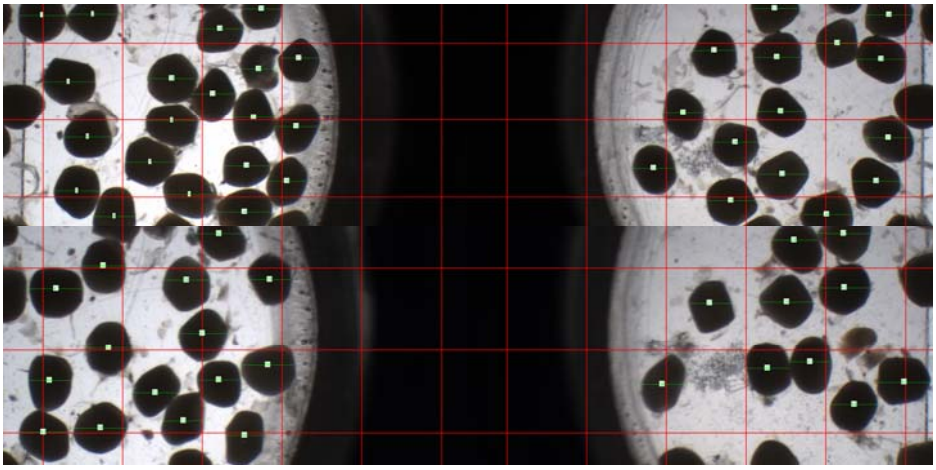


Picture 7: Pictures of the oocytes made by the binocular. Picture 7a represents the top side of the special dish and picture 7b represents the bottom side. The centre the line is seen which separates the two sides.

5) Counting the Oocytes.

   - Count the oocytes automatically counted by pushing number “1” button on the keyboard. After counting save the counting as xls.
   - The oocytes which are not automatically will have to be counted manually by pushing the “R” button. On the screen appears a circle. Go with the circle to not numbered oocytes and push the “M” button for counting. Repeat this handling till all oocytes are numbered. Save the numbered oocytes.
   - Push the “F” button on Atretic oocytes to mark them with a red dot. Repeat this handling also for oocytes from the bottom pictures which are lying on the centre line and counted automatically. After counting all oocytes, note the number of artetic oocytes and all red dots.

6) Estimate the diameter.
   - Push the “0” button to make a picture of 1/3 of the sample. Repeat this handling for all pictures of the sample. Save the automatically measured oocytes. The pictures are seen on picture 8.
   - The not automatically measured oocytes have to be counted manual. Measure with a line the diameter. Always draw this line the same direction. Save the measured oocytes.
   - Repeat all handlings on sample 2.

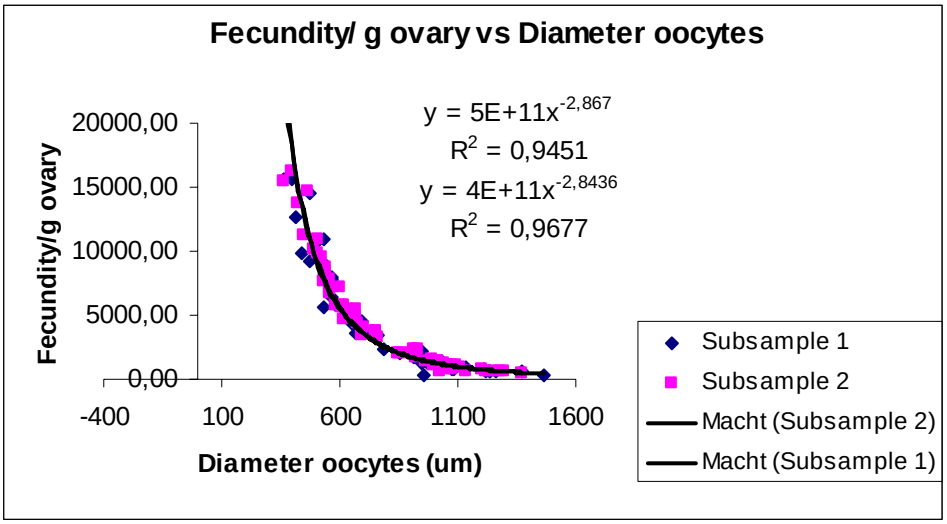


Picture 8: Measuring the diameters of the oocytes.

7) Estimate Fecundity with the diameter
   - o After estimating enough fecundity and diameters make in the excel spreadsheet a graph.
   - o On the x-as the diameters and on the y-as the Fecundity.
   - o Use a power line to calculate a possible relation between the two variables. If their is shown a high chi-quadrant (reliability) the diameters can be used for the estimate of the fecundity with a formula. For example a chi-quadrant of 0,90 represent a percentage of 90 percent reliable.
   - o If the chi quadrant is high it is possible to estimate the fecundity a lot faster then the former manner. The formula that is calculated could be used to calculate with the estimated diameter the fecundity. The advantage is that it is possible to estimate more fecundities.
   - o Measure many diameters for the reliability of the fecundity. Make 4 pictures of the whole sample and measure from all samples together more then 100 oocytes. If there are less then 100 oocytes measured, measure the not measured oocytes manual. Take care that not only the large or small oocytes are counted, because there have to be a good average between the oocyte diameters to calculate a reliable fecundity (picture 8).

3. Results

3.1 Fecundity



Graph 1 Fecundity/g ovary related to the diameter of the oocytes

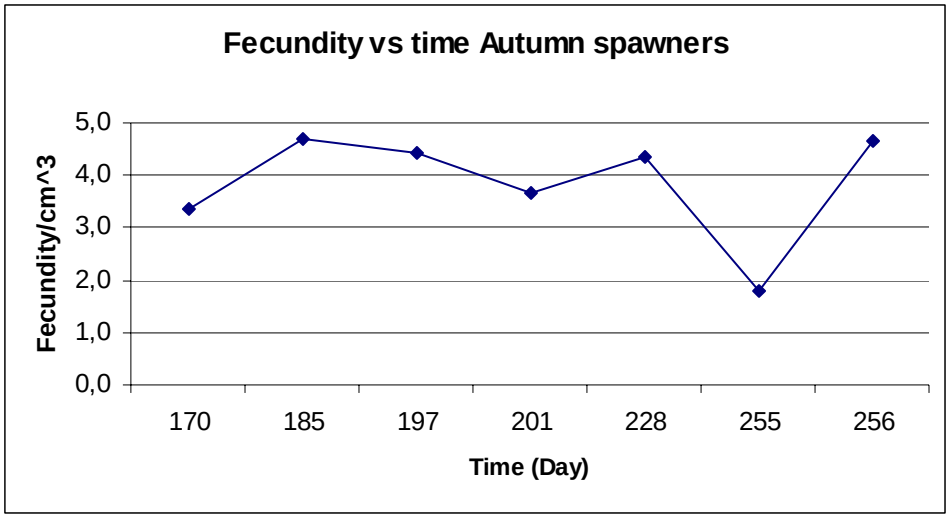
Graph 1 shows the relation between each sub sample and the diameter of the oocytes. This graph gives a good view that the diameters of the oocytes are in relation with the fecundity/g ovary. It is also shown that when the diameters grow, the amount of fecundity is decreasing. The oocytes growing during the maturity cycle.

The relation of sub sample 1 has a reliability (Chi- quadrant) of 94,5 percent. This relation is to calculate with the formula:

Fecundity/g ovary = 5*10^11*(Diameter oocytes^-2,867)

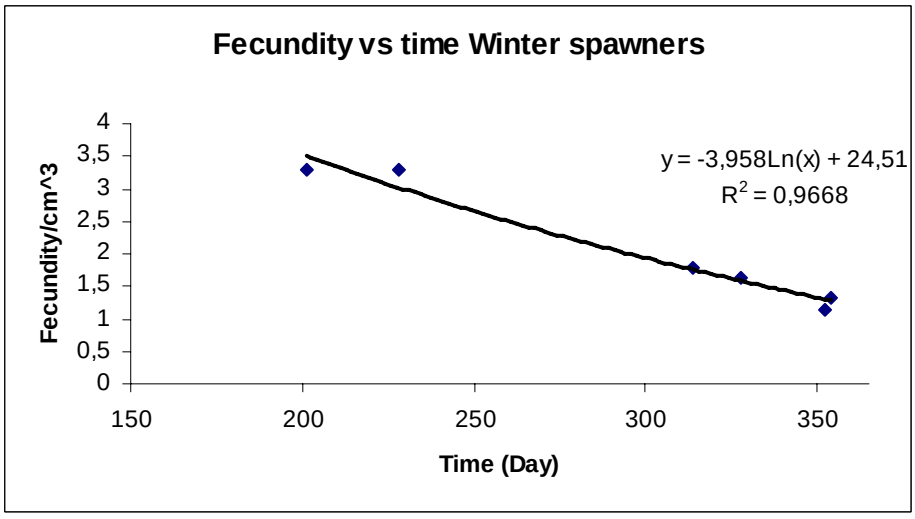
The relation of sub sample 2 has a reliability of 96,8 percent. This relation is to calculate with the formula:

Fecundity/g ovary = 4*10^11*(Diameter oocytes^-2,8435)



Graph 2 Fecundity for a period of time in Autumn spawning herring. (Day 1 represents the first of January and day 365 recommend 31 December)

Graph 2 describes the Fecundity per cubical centimetre for a period of time. I use this parameter, because of the length is not variable to condition. The first spot is the eight of June and the last spot is the thirteenth of September. From day 170 till 228 the herring are feeding but from 228 they go further to the spawning ground they get a decreasing fecundity. But this graph show on day 256 an increasing of fecundity.



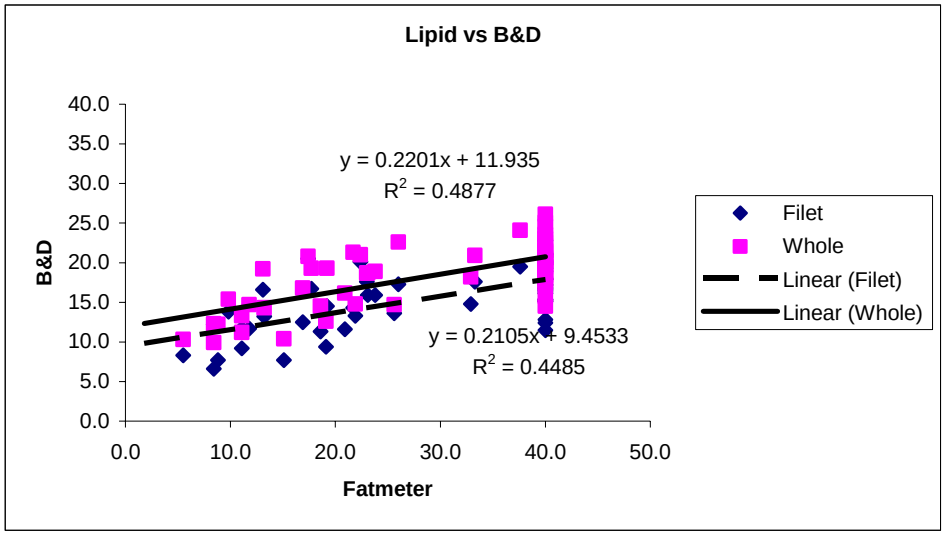
Graph 3 Fecundity for a period of time in Winter spawning herring. (Day 1 represents the first of January and day 365 recommend 31 December)

Graph 2 describes the Fecundity per cubical centimetre for a period of time. The first spot is the eight of June and the last spot is the twentieth of December. This line shows a good down regulation through time. On day 200 the herring are still in the feeding ground, but when they go further into the maturity cycle the fecundity/cm^3 are decreasing.

The formula what represents a reliability of 96,7 percent is:

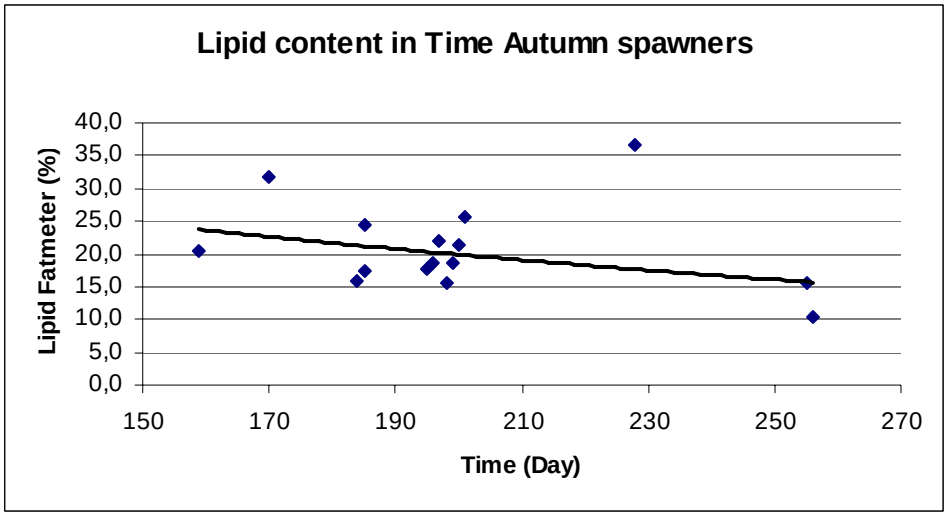
Fecundity/cm^3= -3,958*Log(Time)+ 24,51

3.2 Lipid content



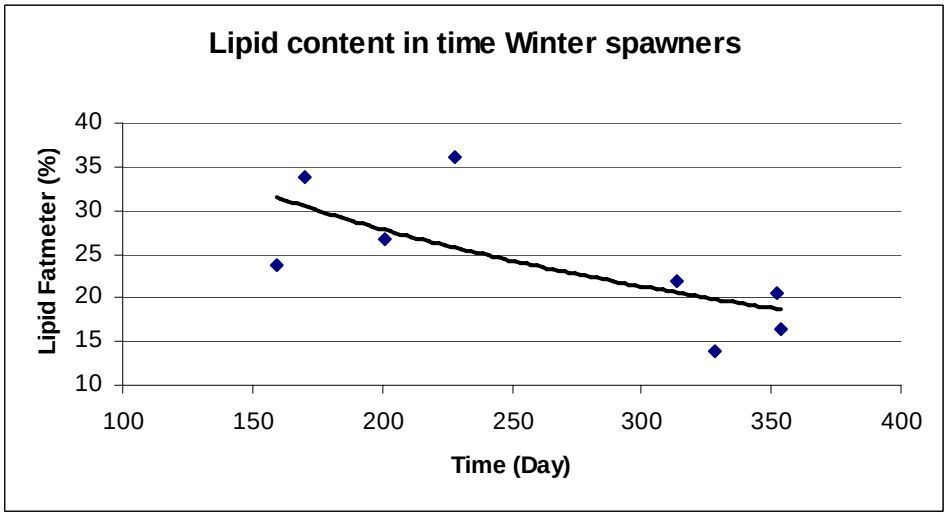
Graph 4 the Bligh and Dyer Lipid determination method (old Method) compared to the electronic Distell fish fat meter (new method).

Graph 4 shows a relationship between the Bligh and Dyer method and the newer Fat meter method. In the end of the lines the amount of percent lipid stops direct at 40 percent. This is because the Distell fish fat meter doesn't go over 40 percent. This graph shows that there is a reliability of 44 or 48 percent.



Graph 5 Lipid content for a period of time in Autumn spawning herring.

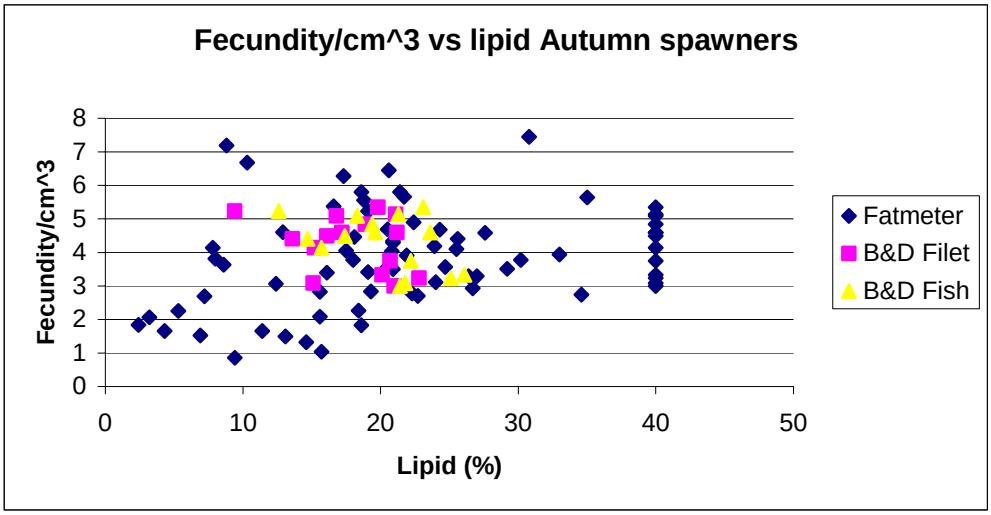
Graph 5 shows the lipid content for autumn spawners for a period of time. This graph shows that the lipid content is decreasing for a period of time, but the graph has a point that is different from the rest. This point represents day 230 and is the point that represents the herring which are taken lipid content from at IMARES in the lab. The other points are measured at the vessels. On IMARES the lipid content is estimated at another temperature then on the Vessels. The Distell fish fat meter could give a higher measurement at lower temperature since it uses conductivity to measure water content.



Graph 6 Lipid content for a period of time in Winter spawning herring.

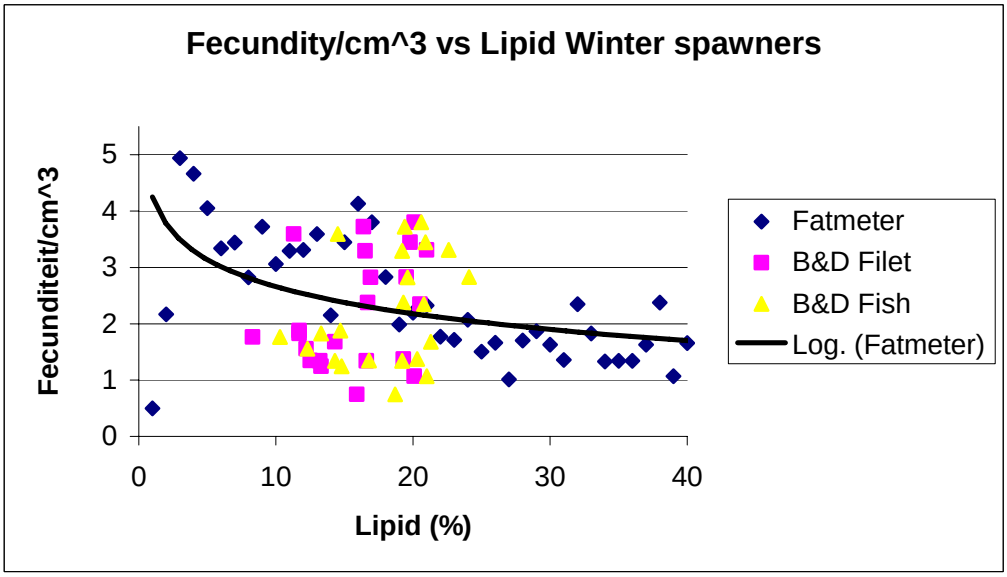
Graph 6 shows the lipid content in Winter spawners for a period of time. In this Graph point 230 days is like the autumn spawners also very high compared to the other points on the graph.

3.3 Relation between fecundity and lipid content



Graph 7 Fecundity per cubic centimetre herring versus lipid content in Autumn spawning herring.

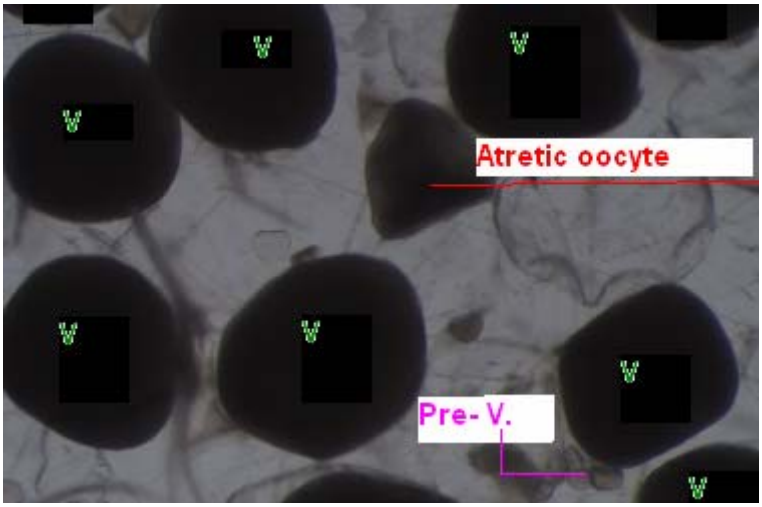
Graph 7 shows the Fecundity/cm^3 autumn spawners in relation with the lipid content. This has been done in 2 different methods: the Bligh and Dyer method en the Distell fish fat meter. The lipid of the Bligh and Dyer method is measured from the filet and the whole herring. The Distell fish fat meter shows that when the lipid content is high the fecundity/cm^3 is high also. The fecundity is measured by cubic centimetre, because the length is not a variable that is depending on condition. The other variable weight is depending on condition. It is therefore likely that length correction is the best variable to determine influence of condition on fecundity.



Graph 8 Fecundity per cubic centimetre herring versus lipid content in Winter spawning herring.

Graph 8 shows the Fecundity/cm^3 Winter spawners in relation with the lipid content. This has been done in 2 different methods: the Bligh and Dyer method en the Distell fish fat meter. The lipid of the Bligh and Dyer method is measured from the filet and the whole herring. This graph shows that with the measurements of the Distell fish fat meter the fecundity/ cm^3 are decreasing when there is more lipid inside the herrin

3.4 Atresia



Picture 9: Picture of Atretic oocyte in the middle of Vitellogenic oocytes (V) and Pre-Vitellogenic oocytes (Pre-V) made by a binocular (40x)

Picture 9 shows that there are atretic cells in the ovaries of North Sea herring. In picture 9 is seen that the atretic oocyte is smaller then the vitellogenic oocytes. It is also not as round as the other oocytes. Further in the picture there are shown pre-vitellogenic oocytes. These are oocytes which have not become vitellogenic yet.

4. Discussion

Researchers throughout the years:
In the past there has been a lot of research performed on fecundity. This was also done in herring. For many years researchers thought that the potential fecundity of herring the same was as realized fecundity. So they took a sample from a “x” number of herring and they thought that this was de realized fecundity. In the past the fecundity was related to weight and length, that’s today the same but it is also related to the time of the year and the maturity cycle. And so it is difficult to compare my results with the fecundities In the past, because there is not an article in the past that is telling about the month or place where the fish are caught.

Sample from Aberdeen (autumn or winter spawning herring):
There are herring in our data which were not yet identified to spawning type. The reason why is because there was a lack of time. These herring swam in the area were they spawn.
In Graph 2 in chapter results there is a ‘down regulation’ noticed, but on the end there is shown a higher point. These point shows the herring which is caught near Aberdeen in September. These herring were swimming in the spawning area of the autumn spawning herring. But the fecundity is very high in these herring and also the oocytes are very small for the time of the year in autumn spawning herring. But they swim this far in the spawning ground of the Autumn spawners we considered that they have as spawning type: autumn spawner. But it is possible that this herring swam on the far North side of the feeding ground so they could be winter spawning herring. If this point goes to graph 3 which shows the down regulation of the Winter spawners this point is fitting well into the graph to show still down regulation.

Lipid content Distell fish fat meter:
Herring Numbers 1-200 has been taken with the Distell fish fat meter on IMARES in the fish cutting room. In the fish cutting room the temperature is colder then on the vessels, so it is possible that the Distell fish fat meter has given other estimates then the numbers that the Distell fish fat meter has given on the vessels. In Graph 4 and 5 in chapter results on day 230 (this are the numbers that were measured on IMARES) shows in both graphs, autumn and winter spawning herring, high numbers. Both these points on the graphs are far from the line were the other points are. So it is likely that the Distell fish fat meter estimates higher lipid contents when the temperature is lower.
The Distell fish fat meter could not show lipid contents higher then 40%, this is shown in graph 4. Also all of these samples were measured at IMARES as well.

Lipid content versus the Fecundity/cm^3
In the two graphs (Graph 7 and 8 in the chapter results) which are trying to found out a relation between the fecundity/cm^3 and the lipid content there are strange things going on. The first is that the lipid content is high when the fecundity is high also. This is going on at the autumn spawning herring, but at the winter spawning herring it is very much the opposite way. The fecundity is high when the lipid content is low. My H0 was: The further into the maturity cycle, less lipid and less oocytes. But this is only true for the autumn spawning herring. For winter spawning herring this is not true and the H0 will be dismissed. When the herring swims further to the spawning ground they could not feed that much anymore, so it was unlikely to say that the lipid content increased. Also because of that they have to produce and mature oocytes. Only the Distell fish fat meter shows this results. The old method, the Bligh and Dyer

method, shows no relation at all. So it can be that the Distell fish fat meter is not giving the correct data. It is possible that the Bligh and Dyer method gives the right value.
Also the Distell fish fat meter has estimated the herring lipid over the whole maturation period and the Bligh and Dyer method only a sub sample from the first 200 samples. The Bligh and Dyer method shows only the time that the herring spend in the feeding ground and not after the feeding ground when the herring swims toward the spawning ground. It is likely that the lipid content estimated with the Bligh and Dyer method is only increasing and not decreasing. The lipid content with the Bligh and Dyer method is only estimated for control of the reliability of the lipid content.

'down regulation'
Graph 1 in the chapter results is showing a relation between the diameter and the fecundity/g ovary. This relation represents less fecundity while the oocytes are growing. 'Down regulation' is that the fecundity is decreasing while diameters are growing and there is less room for many oocytes and the numbers are decreasing through atresia. This graph shows this 'down regulation'. But what is the amount of fecundity in time for each spawning type? Is the amount also decreasing as graph 1? In the paragraph "Sample from Aberdeen" is shown that there is a 'down regulation' noticed before the Aberdeen sample in autumn spawning herring. At winter spawning herring there is even a better 'down regulation' noticed with a reliability of 97 percent. Without the Aberdeen samples graph 1 compared with graph 2 and 3 are comparable.

Atretic oocytes
Looking for Atretic oocytes from the outside to determine the oocytes is a time spending and a not very reliable method. The difference between the different atretic oocytes is high. It is never certain to say how many atretic oocytes their are in a sample. The only way to know for certain is by the use of histology. The disadvantage is that making histological slices costs for one sample a day or more. But with looking at other slices and at other atretic cells is it possible to say that there are atretic cells noticed in the whole mount ovary samples of North Sea herring.

5. Conclusion

Literature study:

- There are two different Herring examined in this thesis which are swimming in the North Sea:
    - Autumn Spawners: Spawn at the North of the North sea (east cost of Scotland)
    - Winter Spawners: Spawn at the south of the North Sea (English channel and Southern North Sea)
- The natural food of the herring is zooplankton.
- There have been no studies done in the past on Fecundity in relation to Lipid content.
- No Fecundity studies have been done about down regulation.

Lipid content:

- There is no certain relationship between the Distell fish fat meter and the Bligh and Dyer method.
- The fecundity in relation to the Fecundity by using only the Distell fish fat meter is:
    - Autumn spawners: A high Fecundity shows a high lipid content.
    - Winter spawners: A low Fecundity shows a high lipid content.

Fecundity:

- There are Atretic oocytes seen for the first time in North Sea herring.
- ‘Down regulation’ has not been identified before in North Sea herring. ‘Down regulation’ is seen in autumn and winter spawning herring, as seen in the relation between the Fecundity/g ovary and the diameters.

6. Recommendations

Lipid content:

- The Distell fish fat meter shows different values compared to the chemical Bligh and Dyer method and is probably depending of temperature. This can be solved to perform a little project on: “what is the value deviation of the Distell fish fat meter at a x-temperature”. If this is done then it’s perhaps possible to calculate the real Lipid content from the Distell fish fatmeter meter. It is possible that there is then a relation between the Distell fish fat meter and the Bligh and Dyer method.
  Also if there is a good calculation between The Distell fish fat meter and temperature and the values are reliable, this method saves then a lot of time in comparison to the Bligh and Dyer method. For example in an 8 hours working day it is possible to measure from 7 fish the lipid content. And for the Distell fish fat meter are this approximately 50 per hour. Each method is done by one person.
- In this research project the samples for the Bligh and Dyer method were taken only from the first 2 months of the research. This is the time where the herring still swims in the feeding ground. It is not possible to make a Bligh and Dyer against Time table to notice down regulation. For the future project it is to recommending that the Bligh and Dyer samples were taken from the whole maturity cycle. And maybe it is better to find a relation between Distell fish fat meter and Bligh and Dyer.

Fecundity:

- Counting fecundity is a very time consuming procedure. In the future I recommend a way to save time. Some ideas to save time are:
  - One picture used for each sample. For this idea there has to be an other binocular or camera. Because to examine a 100 mg sub sample it is needed to get a bigger picture of the sample.
  - Less sub samples, instead of from each fish two sub samples maybe only from each 50 samples only the first 5 two sub samples for the reliability and the rest only one.
  - The automatic counting method doesn’t count all oocytes, because some places in the special designed dish are to dark to count these oocytes as well. It can be a light problem so lighter could be a solution.
  - An other problem of the computer is that it doesn’t count all oocytes automatically when they lay against each other. This can be solved with a larger special designed dish or Petri scale. But then it is not possible to make a whole picture with our camera. So then the first point comes again.

Atretic, POF's and hydrated oocytes.

- Looking for Atresia, POF;s or hydrated oocytes from the outside to determine the oocytes is a time spending and a not very reliable method. Maybe it is a way to count and estimate diameters under the microscope and an other person make slices to know certain that the oocytes are atretic. But making slices costs days. But it is the only manner to know for sure.
- It is also an idea that there could be a project about atretic oocytes. This project must be going about atretic oocytes from the outside and atretic oocytes from the inside with a lot of pictures. (Pictures from atretic oocytes from the outside are very rare). This could be a manual to see atretic oocytes from the outside and makes the project more reliable.

Recommendations for Inholland College

- For the most research project there is needed data software. Some use SPSS, some use SAS, but every computer has excel. I think that this is the most important program to learn the basics. I know now a lot about SPSS, but at IMARES they work with excel or SAS. Thus it is likely that I work with the excel spreadsheet. I think that at school there has to be more attention going to this windows spreadsheet.
- Also the English part of my education is not very high. Holland is depending of trade. IMARES is also an international institute so this research project has to be in English. In the beginning my English vocabulary was not very large. So it was difficult to express myself in this project. Maybe it is a recommendation to give some more classes in English or English as a subject will give a lot of good too many students.

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Appendices

Appendix 1 Biological parameters

Herring stations:

Catch dates:

Species	Sample_ID	Ship	Catch date year	Catch date month	Catch date day
Herring	Cindy1	SCH22-23	2006	6	8
Herring	Cindy2	SCH22-23	2006	6	19
Herring	Cindy3	SCH22-23	2006	7	20
Herring	Cindy4	SCH22-23	2006	8	16
Herring	Cindy5	SCH22-23	2006	9	12
Herring	Cindy6	SCH22-23	2006	11	10
Herring	Cindy7	SCH22-23	2006	11	24
Herring	RAB1	SCH123	2006	12	18
Herring	RAB2	SCH123	2006	12	20
Herring	Scotia1	Scotia	2006	7	13
Herring	Scotia2	Scotia	2006	7	14
Herring	Scotia3	Scotia	2006	7	15
Herring	Scotia4	Scotia	2006	7	16
Herring	Scotia5	Scotia	2006	7	17
Herring	Scotia6	Scotia	2006	7	18
Herring	Scotia7	Scotia	2006	7	19
Herring	Tridens1	Tridens	2006	6	29
Herring	Tridens2	Tridens	2006	7	3
Herring	Tridens3	Tridens	2006	7	4
Herring	Tridens4	Tridens	2006	7	4
Herring	Tridens5	Tridens	2006	7	5
Herring	Aberdeen		2006	9	

Sample dates:

Species	Sample_ID	Ship	Sample date year	Sample date month	Sample date day
Herring	Cindy1	SCH22-23	2006	6	9
Herring	Cindy2	SCH22-23	2006	6	21
Herring	Cindy3	SCH22-23	2006	7	24
Herring	Cindy4	SCH22-23	2006	8	17
Herring	Cindy5	SCH22-23	2006	9	13
Herring	Cindy6	SCH22-23	2006	11	11
Herring	Cindy7	SCH22-23	2006	11	24
Herring	RAB1	SCH123	2006	12	18
Herring	RAB2	SCH123	2006	12	20
Herring	Scotia1	Scotia	2006	7	13
Herring	Scotia2	Scotia	2006	7	14
Herring	Scotia3	Scotia	2006	7	15
Herring	Scotia4	Scotia	2006	7	16
Herring	Scotia5	Scotia	2006	7	17
Herring	Scotia6	Scotia	2006	7	18
Herring	Scotia7	Scotia	2006	7	19
Herring	Tridens1	Tridens	2006	6	29
Herring	Tridens2	Tridens	2006	7	3
Herring	Tridens3	Tridens	2006	7	4
Herring	Tridens4	Tridens	2006	7	4
Herring	Tridens5	Tridens	2006	7	5
Herring	Aberdeen		2006	9	

Catch places:

Species	Sample_ID	Ship	Position (N)	Position (E-W)	E-W	Latitude dec	Longitude dec
Herring	Cindy1	SCH22-23	56.32	2.36	E	56.53	2.60
Herring	Cindy2	SCH22-23	57.15	1.52	E	57.25	1.87
Herring	Cindy3	SCH22-23	60.00	0.16	W	60.00	-0.27
Herring	Cindy4	SCH22-23	58.59	0.27	W	58.98	-0.45
Herring	Cindy5	SCH22-23	55.41	0.33	W	55.68	-0.55
Herring	Cindy6	SCH22-23	51.39	1.52	E	51.65	1.87
Herring	Cindy7	SCH22-23	50.16	1.14	E	50.27	1.23
Herring	RAB1	SCH123	50.11	0.06	E	50.18	0.10
Herring	RAB2	SCH123	50.11	0.23	E	50.18	0.38
Herring	Scotia1	Scotia	59.27	3.07	W	59.45	-3.12
Herring	Scotia2	Scotia	60.42	0.27	W	60.70	-0.45
Herring	Scotia3	Scotia	60.49	0.35	W	60.82	-0.58
Herring	Scotia4	Scotia	60.39	1.47	W	60.65	-1.78
Herring	Scotia5	Scotia	60.23	1.28	W	60.38	-1.47
Herring	Scotia6	Scotia	60.05	1.29	W	60.08	-1.48
Herring	Scotia7	Scotia	59.54	3.57	W	59.90	-3.95
Herring	Tridens1	Tridens	56.54	0.49	W	56.90	-0.82
Herring	Tridens2	Tridens	56.48	1.07	E	56.80	1.12
Herring	Tridens3	Tridens	56.41	0.59	E	56.68	0.98
Herring	Tridens4	Tridens	56.33	0.34	E	56.55	0.57
Herring	Tridens5	Tridens	56.33	0.38	W	56.55	-0.63
Herring	Aberdeen						

Biological parameters

Biological parameters winter spawning herring :

Sample_ID	Number	Length	Spawning type	Ovary weight
Cindy3	*124*	25.5	W	2.3
Cindy3	*135*	28.5	W	24.0
Cindy3	*137*	29.6	W	24.1
Cindy3	*138*	27.2	W	22.2
Cindy3	*142*	27.1	W	2.6
Cindy3	*144*	30.9	W	29.2
Cindy4	*151*	24.5	W	3.8
Cindy4	*153*	26.5	W	4.4
Cindy4	*159*	26.5	W	5.9
Cindy4	*163*	27.5	W	9.4
Cindy4	*170*	29.0	W	14.1
Cindy4	*176*	26.0	W	2.5
Cindy4	*179*	25.0	W	4.3
Cindy4	*184*	27.5	W	12.5
Cindy4	*185*	27.5	W	7.9
Cindy4	*190*	29.0	W	18.0
Cindy4	*193*	29.5	W	10.7
Cindy6	*255*	24.5	W	21.7
Cindy6	*261*	27.0	W	35.5
Cindy6	*265*	25.5	W	37.8
Cindy6	*266*	29.0	W	35.3
Cindy6	*270*	25.5	W	31.2
Cindy6	*275*	26.0	W	38.4
Cindy6	*280*	24.0	W	16.5
Cindy6	*285*	24.5	W	12.1
Cindy6	*287*	23.5	W	12.0
Cindy6	*290*	22.5	W	11.0
Cindy6	*295*	26.5	W	36.4
Cindy6	*299*	26.0	W	21.3
Cindy7	*302*	26.5	W	31.8
Cindy7	*305*	27.0	W	41.0
Cindy7	*310*	26.5	W	33.4
Cindy7	*312*	26.0	W	28.9
Cindy7	*315*	26.0	W	30.0
Cindy7	*320*	25.5	W	26.6
Cindy7	*322*	27.0	W	35.3
Cindy7	*325*	26.5	W	25.2
Cindy7	*330*	25.5	W	15.8
Cindy7	*332*	25.0	W	25.2
Cindy7	*335*	26.5	W	26.0
Cindy7	*340*	25.5	W	20.0
Cindy7	*345*	27.0	W	27.0
Cindy7	*350*	25.0	W	20.0
RAB1	*5*	25.4	W	20.0
RAB1	*10*	29.5	W	61.0
RAB1	*13*	27.8	W	47.0
RAB1	*15*	25.5	W	31.0
RAB1	*19*	26.2	W	21.0
RAB2	*25*	27.8	W	44.0
RAB2	*28*	26.4	W	32.0
RAB2	*35*	27.8	W	54.0
RAB2	*45*	25.8	W	33.0

Biological parameters *winter spawning herring*: Lipid content

Sample_ID	Number	Lipid (fat meter)	Lipid (B&D) filet	Lipid (B&D) whole
Cindy3	*124*	20.8		
Cindy3	*135*	33.2		
Cindy3	*137*	30.3		
Cindy3	*138*	31		
Cindy3	*142*	17.7		
Cindy3	*144*	29.6		
Cindy4	*151*	40	16.9	19.6
Cindy4	*153*	40	16.4	19.4
Cindy4	*159*	35.8		
Cindy4	*163*	40	16.5	19.2
Cindy4	*170*	40	21	22.6
Cindy4	*176*	18.6	11.3	14.5
Cindy4	*179*	35.8		
Cindy4	*184*	40	19.8	20.9
Cindy4	*185*	31.9		
Cindy4	*190*	40	20.1	20.6
Cindy4	*193*	37.6	19.5	24.1
Cindy6	*255*	20.3		
Cindy6	*261*	27.5		
Cindy6	*265*	37.9		
Cindy6	*266*	21.5		
Cindy6	*270*	18.7		
Cindy6	*275*	20.7		
Cindy6	*280*	20		
Cindy6	*285*	26.2		
Cindy6	*287*	16.7		
Cindy6	*290*	16.7		
Cindy6	*295*	11.7		
Cindy6	*299*	18.2		
Cindy7	*302*	9.6		
Cindy7	*305*	17.4	20.5	20.8
Cindy7	*310*	11.1	11.7	13.3
Cindy7	*312*	11.6		
Cindy7	*315*	13.1	16.6	19.2
Cindy7	*320*	13.2	13.2	14.3
Cindy7	*322*	23.3		
Cindy7	*325*	17.7	16.7	19.3
Cindy7	*330*	22.4	20.1	21
Cindy7	*332*	20		
Cindy7	*335*	5.5	8.3	10.3
Cindy7	*340*	8.4	12.2	12.3
Cindy7	*345*	11.8	11.7	14.7
Cindy7	*350*	16.9	12.5	16.8
RAB1	*5*	20.6		
RAB1	*10*	21.7	14.3	21.3
RAB1	*13*	40	19.3	20.3
RAB1	*15*	27.2		
RAB1	*19*	23.1	15.9	18.7
RAB2	*25*	13.9		
RAB2	*28*	21.9	13.3	14.8
RAB2	*35*	22		
RAB2	*45*	13.7		

Biological parameters winter spawning herring : Fecundity

Sample_ID	Number	Mean Total Fecundity	SD Mean	Fecundity/ gram ovary (auto diametric)	FEC/CM^3 fish
Cindy3	*124*	35946.705	190.3877		2.167896509
Cindy3	*135*	114358.3	409.9278		4.940070252
Cindy3	*137*	120950.69	566.6591		4.663727982
Cindy3	*138*	81495.23		3675.92	4.049724493
Cindy3	*142*	66444.754		25954.98	3.338511105
Cindy3	*144*	101566.83	1660.155		3.442520038
Cindy4	*151*	41549.696		11039.95	2.825332727
Cindy4	*153*	69322.226	1889.713		3.72507375
Cindy4	*159*	56953.965	3824.554		3.060457403
Cindy4	*163*	68539.204		7254.51	3.295649172
Cindy4	*170*	80780.974	521.09		3.312188844
Cindy4	*176*	63152.385	1252.078	3824.25	3.593103372
Cindy4	*179*	33574.249		7834.62	2.148751946
Cindy4	*184*	71680.764	553.0103		3.446708404
Cindy4	*185*	85887.674	214.5683		4.12983555
Cindy4	*190*	92689.435		5150.46	3.800460676
Cindy4	*193*	72623.301		6771.70	2.828850115
Cindy6	*255*	29195.901	547.8179		1.985288522
Cindy6	*261*	43213.739		1217.97	2.195485385
Cindy6	*265*	38532.452	1460.108		2.323839363
Cindy6	*266*	43131.835		1223.25	1.768495443
Cindy6	*270*	28399.197		909.94	1.712716646
Cindy6	*275*	36389.159	513.8583		2.070389119
Cindy6	*280*	20838.045		1265.98	1.50738174
Cindy6	*285*	24428.269	611.2595		1.661094903
Cindy6	*287*	13163.21		1094.20	1.014280879
Cindy6	*290*	19373.676		1767.67	1.700844014
Cindy6	*295*	34764.026	945.7703		1.868066997
Cindy6	*299*	28638.81		1343.28	1.629427087
Cindy7	*302*	25296.41		795.48	1.359318634
Cindy7	*305*	46188.868	371.946		2.346637587
Cindy7	*310*	33968.222		1015.80	1.825303959
Cindy7	*312*	23352.391		809.16	1.328652208
Cindy7	*315*	23540.86	411.477		1.339375283
Cindy7	*320*	22207.982		834.89	1.33933298
Cindy7	*322*	32092.253		908.36	1.630455372
Cindy7	*325*	44191.456	1393.662		2.374655886
Cindy7	*330*	17730.702		1122.20	1.069314363
Cindy7	*332*	25867.994		1024.88	1.655551586
Cindy7	*335*	32884.362	1736.629		1.767062053
Cindy7	*340*	25798.668		1288.00	1.55588232
Cindy7	*345*	36990.948	22.75932		1.879334839
Cindy7	*350*	21000.164		1052.64	1.34401051
RAB1	*5*	12734.283	213.3633		0.777093653
RAB1	*10*	43103.153		706.61	1.678970204
RAB1	*13*	29600.799		629.80	1.377745624
RAB1	*15*	17314.048	177.2457		1.04418648
RAB1	*19*	13457.641		640.84	0.74828158
RAB2	*25*	26226.455	659.4536		1.220689494
RAB2	*28*	22918.78		716.21	1.245603208
RAB2	*35*	34789.566	360.1748		1.619252664

RAB2	*45*	20956.769	667.2298	1.220296043

Biological parameters: autumn spawning herring

Sample_ID	Number	Length	Spawning type	Ovary weight
Aberdeen	*55*	27.4	A	46
Aberdeen	*60*	27.8	A	66
Aberdeen	*65*	28.3	A	83
Aberdeen	*70*	25.9	A	58
Aberdeen	*75*	27.0	A	57
Aberdeen	*80*	26.5	A	56
Aberdeen	*85*	29.9	A	70
Aberdeen	*95*	26.7	A	59
Aberdeen	*100*	27.0	A	48
Cindy2	*78*	25.5	A	4.4
Cindy2	*89*	26.0	A	2.3
Cindy2	*96*	26.0	A	6.3
Cindy3	*105*	29.8	A	24.1
Cindy3	*107*	26.9	A	17.1
Cindy3	*110*	27.5	A	18.2
Cindy3	*115*	26.1	A	6.7
Cindy3	*122*	28.9	A	21.0
Cindy3	*125*	28.6	A	18.7
Cindy3	*128*	28.7	A	19.0
Cindy3	*140*	28.5	A	23.9
Cindy3	*145*	31.2	A	42.3
Cindy4	*154*	27.0	A	6.2
Cindy4	*161*	27.0	A	10.1
Cindy4	*162*	28.5	A	35.1
Cindy4	*164*	26.5	A	9.2
Cindy4	*165*	27.5	A	23.1
Cindy4	*167*	29.0	A	34.8
Cindy4	*168*	28.0	A	12.3
Cindy4	*172*	29.5	A	53.6
Cindy4	*173*	30.0	A	53.4
Cindy4	*174*	29.5	A	49.5
Cindy4	*180*	27.5	A	26.6
Cindy4	*186*	27.5	A	12.1
Cindy4	*191*	30.0	A	40.1
Cindy4	*195*	29.0	A	13.6
Cindy4	*196*	29.0	A	51.9
Cindy4	*200*	31.0	A	63.2
Cindy5	*205*	30.0	A	48.9
Cindy5	*210*	31.0	A	50.0
Cindy5	*213*	28.5	A	63.9
Cindy5	*215*	29.0	A	35.1
Cindy5	*220*	27.5	A	29.5
Cindy5	*225*	27.5	A	33.9
Cindy5	*226*	30.0	A	61.9
Cindy5	*230*	30.0	A	34.9
Cindy5	*235*	29.5	A	43.4
Cindy5	*239*	29.0	A	40.3
Cindy5	*240*	30.0	A	21.3
Cindy5	*245*	28.0	A	41.9

Cindy5	*248*	31.5	A	44.7
Cindy5	*250*	31.5	A	40.7
Scotia2	*S05*	30.0	A	23.0
Scotia2	*S10*	30.0	A	23.0
Scotia3	*S15*	31.0	A	26.0
Scotia4	*S20*	29.0	A	12.8
Scotia4	*S25*	28.5	A	14.6
Scotia5	*S30*	30.5	A	31.4
Scotia5	*S35*	29.0	A	16.4
Scotia6	*S40*	28.5	A	10.3
Scotia6	*S45*	28.5	A	19.4
Scotia6	*S50*	26.0	A	9.0
Scotia6	*S55*	31.5	A	27.5
Scotia7	*S100*	32.5	A	63.8
Scotia7	*S60*	32.5	A	41.6
Scotia7	*S65*	31.0	A	40.4
Scotia7	*S70*	30.0	A	19.8
Scotia7	*S75*	31.0	A	30.1
Scotia7	*S80*	33.0	A	64.6
Scotia7	*S85*	32.0	A	40.2
Scotia7	*S90*	31.0	A	48.5
Scotia7	*S95*	30.0	A	31.0
Tridens2	*N05*	28.9	A	13.3
Tridens3	*N10*	28.5	A	10.4
Tridens4	*N15*	27.4	A	8.7
Tridens4	*N20*	27.2	A	8.0

Biological parameters *autumn spawning herring*: Lipid content

Sample_ID	Number	Lipid (fat meter)	Lipid (B&D) filet	Lipid (B&D) whole
Aberdeen	*55*	12.4		
Aberdeen	*60*	7.8		
Aberdeen	*65*	8.8		
Aberdeen	*70*	8		
Aberdeen	*75*	10.3		
Aberdeen	*80*	8.6		
Aberdeen	*85*	18.6		
Aberdeen	*95*	12.9		
Aberdeen	*100*	15.6		
Cindy2	*78*	29.2		
Cindy2	*89*	34.6		
Cindy2	*96*	30.2		
Cindy3	*105*	25.5		
Cindy3	*107*	24.7		
Cindy3	*110*	27		
Cindy3	*115*	22.3		
Cindy3	*122*	24.3		
Cindy3	*125*	26.4		
Cindy3	*128*	33		
Cindy3	*140*	23.9		
Cindy3	*145*	24		
Cindy4	*154*	40	15.1	21.8
Cindy4	*161*	40	21	21.4
Cindy4	*162*	40	15.2	15.7
Cindy4	*164*	40	20.7	22.2
Cindy4	*165*	35		
Cindy4	*167*	25.6	13.6	14.7
Cindy4	*168*	40	20.1	26.1
Cindy4	*172*	40	21.1	21.3
Cindy4	*173*	40	17.2	19.6
Cindy4	*174*	40	19.8	23.1
Cindy4	*180*	40	16.1	17.4
Cindy4	*186*	40	22.8	25.1
Cindy4	*191*	19.1	9.4	12.6
Cindy4	*195*	40	21.2	23.6
Cindy4	*196*	40	16.8	18.3
Cindy4	*200*	40	18.9	19.4
Cindy5	*205*	9.4		
Cindy5	*210*	3.2		
Cindy5	*213*	7.2		
Cindy5	*215*	6.9		
Cindy5	*220*	2.4		
Cindy5	*225*	15.6		
Cindy5	*226*	22.7		
Cindy5	*230*	14.6		
Cindy5	*235*	4.3		
Cindy5	*239*	5.3		
Cindy5	*240*	15.7		
Cindy5	*245*	13.1		
Cindy5	*248*	18.6		
Cindy5	*250*	11.4		

Scotia2	*S05*	20.5
Scotia2	*S10*	16.1
Scotia3	*S15*	20.6
Scotia4	*S20*	19.3
Scotia4	*S25*	21.9
Scotia5	*S30*	18
Scotia5	*S35*	18.8
Scotia6	*S40*	19.1
Scotia6	*S45*	17.5
Scotia6	*S50*	18.4
Scotia6	*S55*	26.7
Scotia7	*S100*	30.8
Scotia7	*S60*	20.3
Scotia7	*S65*	22.4
Scotia7	*S70*	20.9
Scotia7	*S75*	16.6
Scotia7	*S80*	17.3
Scotia7	*S85*	18.1
Scotia7	*S90*	20.9
Scotia7	*S95*	21.7
Tridens2	*N05*	20.9
Tridens3	*N10*	27.6
Tridens4	*N15*	20.8
Tridens4	*N20*	21.4

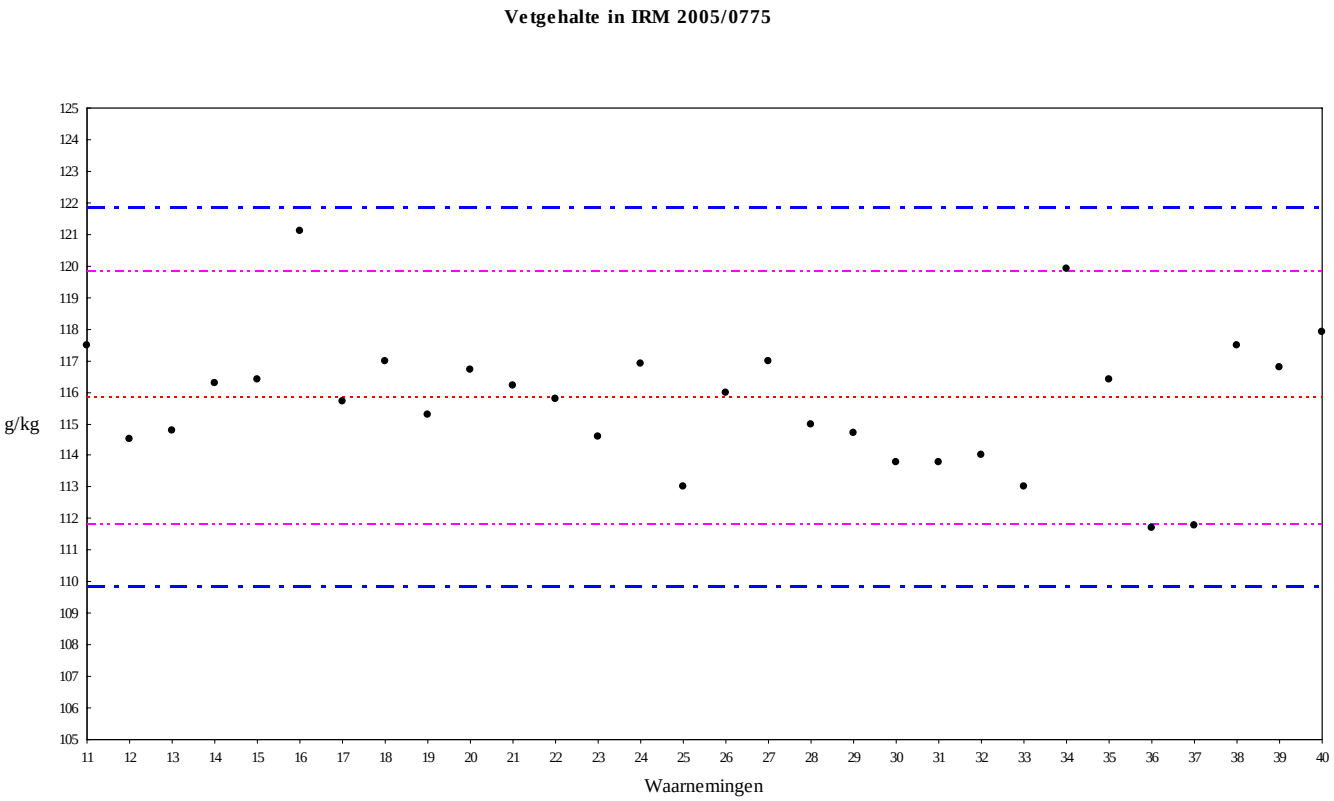
Biological parameters autumn spawning herring: Fecundity

Sample_ID	Number	Mean Total Fecundity	SD Mean	Fecundity/ gram ovary (auto diametric)	FEC/CM^3 fish
Aberdeen	*55*	63020	6505.382		3.063562
Aberdeen	*60*	88832.41717		1345.95	4.134634
Aberdeen	*65*	163095	4108.29		7.195837
Aberdeen	*70*	66232.43924		1141.94	3.812163
Aberdeen	*75*	131385	403.0509		6.67505
Aberdeen	*80*	67646.54656		1207.97	3.63503
Aberdeen	*85*	155050	5444.722		5.800403
Aberdeen	*95*	87615	5423.509		4.603039
Aberdeen	*100*	55598.44588		1158.30	2.824694
Cindy2	*78*	58272.56946	3388.562		3.514339
Cindy2	*89*	48071.43103	3203.573		2.735061
Cindy2	*96*	66352.79158	6242.209		3.775193
Cindy3	*105*	108549.1406	2278.099		4.101829
Cindy3	*107*	69385.73512		4062.40	3.564621
Cindy3	*110*	68463.27689		3772.08	3.291998
Cindy3	*115*	49387.29356	1202.408		2.777754
Cindy3	*122*	113079.4221		5387.30	4.684789
Cindy3	*125*	77072.33463	3439.841		3.294583
Cindy3	*128*	93160.86145		4903.20	3.940831
Cindy3	*140*	96955.25491		4053.31	4.18829
Cindy3	*145*	94419.80814	10351.64		3.108847
Cindy4	*154*	60689.56814		9849.63	3.083349
Cindy4	*161*	59057.26972		5832.80	3.00042
Cindy4	*162*	95759.26178		2727.83	4.136626
Cindy4	*164*	69756.84642		7552.01	3.748428
Cindy4	*165*	117328.5693	59.44488		5.641644
Cindy4	*167*	107324.921		3082.64	4.400546
Cindy4	*168*	73048.36648		5933.05	3.327641
Cindy4	*172*	131784.5112		2458.65	5.13332
Cindy4	*173*	124141.9903		2323.28	4.597851
Cindy4	*174*	137315.4275		2776.33	5.348762
Cindy4	*180*	93372.65317		3516.07	4.489744
Cindy4	*186*	67215.34164		5564.66	3.231992
Cindy4	*191*	141164.4758		3517.35	5.228314
Cindy4	*195*	111956.5875	3808.482		4.590454
Cindy4	*196*	124090.261		2391.38	5.08796
Cindy4	*200*	144334.9445		2284.05	4.844918
Cindy5	*205*	23043.20841	8372.423		0.853452
Cindy5	*210*	61578.14167		1232.55	2.067005
Cindy5	*213*	62380.56781		975.92	2.694727
Cindy5	*215*	37230.66459	3913.289		1.526535
Cindy5	*220*	38274.95479		1297.90	1.840419
Cindy5	*225*	43237.63348	3335.628		2.079045
Cindy5	*226*	72875.30028		1177.88	2.699085
Cindy5	*230*	35658.76966		1022.33	1.320695
Cindy5	*235*	42599.04873	11177.06		1.659334
Cindy5	*239*	54869.43153		1362.54	2.249761
Cindy5	*240*	28000.35594		1313.34	1.03705
Cindy5	*245*	32764.06931	4450.712		1.492532

Cindy5	*248*	56953.43515		1275.55	1.822167
Cindy5	*250*	51667.8931		1270.42	1.653062
Scotia2	*S05*	126615	4065.864		4.689444
Scotia2	*S10*	91792.75069		3990.99	3.399732
Scotia3	*S15*	192140	8456.997		6.449599
Scotia4	*S20*	69175.73114		5404.35	2.83635
Scotia4	*S25*	90593	12078.8		3.913452
Scotia5	*S30*	107075.473		3410.05	3.773901
Scotia5	*S35*	135546	12640.24		5.557669
Scotia6	*S40*	79063.41961		7676.06	3.415396
Scotia6	*S45*	93702	5212.791		4.047756
Scotia6	*S50*	39760.44424		4417.83	2.262201
Scotia6	*S55*	91712.5	2916.815		2.934248
Scotia7	*S100*	255563.1926		4005.69	7.444717
Scotia7	*S60*	119323.0234		2868.34	3.475955
Scotia7	*S65*	145844	5142.081		4.895572
Scotia7	*S70*	94640.01515		4779.80	3.505186
Scotia7	*S75*	160132	4256.783		5.37518
Scotia7	*S80*	225496.0185		3490.65	6.274759
Scotia7	*S85*	146328	3411.083		4.465576
Scotia7	*S90*	127716.3071		2633.33	4.287077
Scotia7	*S95*	152830	23235.53		5.66037
Tridens2	*N05*	104604.5	470.226		4.33368
Tridens3	*N10*	106080	10589.63		4.582463
Tridens4	*N15*	82911	3814.134		4.030514
Tridens4	*N20*	116760	1074.802		5.802129

Appendix 2 Bligh and Dyer

Quality control template:



Haring/Makreel filet in blik IRM 2005/0775	Periode opbouw kaart 6/24/2005 t/m 4/6/2007
Totaal aantal waarnemingen voor opbouw kaart = 40 waarvan 0 waarnemingen geschrapt.	
Vet B&D analyse volgens ISW A004	**Kaart 1**

26-Jan-07	30-Jan-07	31-Jan-07	1-Feb-07	14-Feb-07	14-Feb-07	15-maart-07	20-maart-07	22-maart-07	27-maart-07	28-maart-07	29-maart-07	3-Apr-07	6-Apr-07
EB	EB	QD	EB	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
27	28	29	30	31	32	33	34	35	36	37	38	39	40