



**Methods, directions and basic results  
to the lab work on female Atlantic cod  
(*Gadus morhua*) gonad samples**

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Final report to the course  
"LÍF038M Research project in biology for foreign students"

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Reykjavík, June 2014

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# 1 Brief introduction

The lab work is part of a comprehensive PhD project on Atlantic cod (*Gadus morhua*) reproduction conducted by Fraser Cameron at the Marine Academic Research in Iceland (MARICE) situated at the University of Iceland. The work was done within the course “Research project in biology for foreign students”. The aim of the work is to examine female gonad samples, collected in the Eastfjords of Iceland in spring 2013, in respect of spawning time. Several oocyte parameters, e.g. mean diameter, roundness, etc. were measured and some basic analysis on the data was conducted.

As working hypothesis it is proposed that the variation in oocyte diameters will decrease as the spawning progresses, i.e. as relatively more hydrated oocytes are inside the gonads. Additionally the average oocyte diameter should increase with proceeding spawning time.

This report is not written using the classic sequence of a scientific report. It firstly summarizes the method and gives directions to be used as a manual for a replication at a later date. Secondly it shows the results of the measurements and introduces a brief data analysis.

Additionally to the lab work the author was involved in some days of field work on the sea, supporting Fraser and his colleagues on gathering new data for the PhD project (fish tagging, acoustic and sonar monitoring).

## 2 Material, method and directions

### 2.1 Material and method

All gonad samples were collected between end of March and mid of June 2013 in the Eastfjords of Iceland. After the collection they were fixed in formalin and frozen till March 2014, before the lab work begun. As determined by Fraser Cameron the selection criterion was the Gonadosomatic Index (GSI). From all samples ten samples with the biggest and ten samples with smaller GSI were chosen for the lab work. If a sample was not suitable, e.g. the oocytes were dissolved, the sample with the next smallest / biggest GSI was taken. These samples from the list of big or small GSI could not be investigated: 130417002 and 130502002 (oocytes dissolved), 130509014 and 130425009 (not found). See Table 1 for an overview on the chosen samples.

Following equipment and software was used for the measurement:

- Stereomicroscope Leica MZ9.5, completely equipped

- Software Pixelink Capture
- Software Image-Pro Plus, Version 7.0
- some transparent object holder(s)
- 1 pipette
- A pair of tweezers
- 1 water bottle with small pipe as outlet

## 2.2 Directions

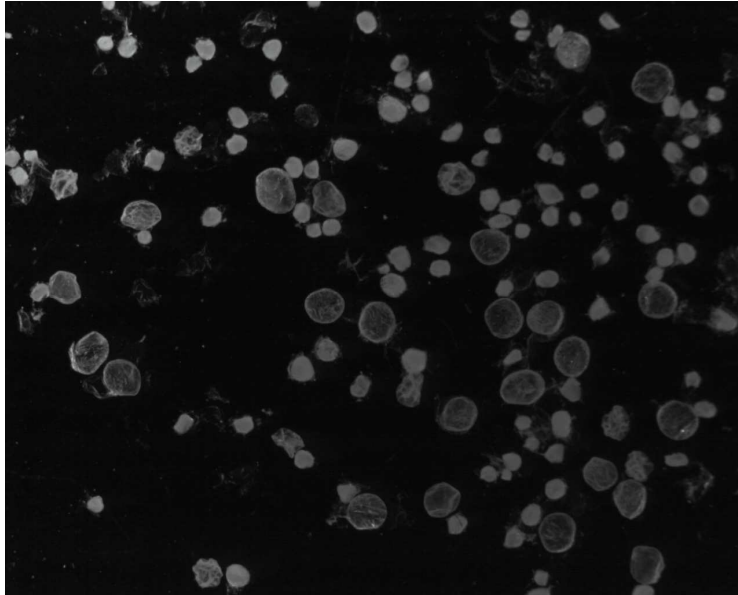
Below are the executed steps listed, which may serve as directions for a rerun of the measurement.

1. Setting of Pixelink Capture:  
For the test use oocyte samples as described under point (3). To get satisfying pictures for the measurement, test the different settings of the Software Pixelink Capture (Exposure, White Balance, Saturation, etc.).  
-> Save the setting for Pixelink Capture (Presets - Save).
2. Creation of a reference calibration for Image-Pro Plus:  
Calibrate the zoom using a length reference (e.g. a calibration scale).  
-> Create a reference calibration for Image-Pro Plus (Measure - Calibration - Spatial Calibration Wizard - Calibrate the active image - name the reference calibration, mm, Create a reference calibration - Draw Reference Line - Position Reference Line over length reference and set the units - Finish)

Don't change the zoom any longer. If you are not sure whether the zoom is still the same, do a test calibration. After the calibration each gonad sample is examined following the same order.

3. -> Separate some oocytes (including as little formalin as possible) from the gonad sample using a pipette and put it onto a transparent object holder. (Due to different composition of the gonad samples varying numbers of oocytes might be collected.)  
-> Wash the formalin away from the oocytes with water inside the object holder using a water bottle with a small pipe as outlet.  
-> Put the object holder onto the microscope with the backlight on.  
-> Remove fibres, tissue, etc. using tweezers.  
-> Arrange most of the oocytes in the middle of the object holder and separate them using tweezers that they don't stick together.

4. -> Apply the saved Preset in PixeLink Capture. If the setting is not ideal for the current sample, adjust it. -> Save the picture (Save As... \*.tif - Snapshot) and rename it with the ID-no. (fish-no.). The result is a snapshot of the oocytes for further analysis with the image analyzer Image-Pro Plus (for an example see Fig. 1).



*Fig. 1: Example of a snapshot made by PixeLink Capture (130421006\_1.tif).*

5. Wash the object holder and the tools thoroughly under running water.
6. If needed, repeat points (3) - (5) for a second, third, etc. picture of this gonad sample. Rename the pictures with ID\_1.tif, ID\_2.tif, etc.
7. Time for the measurements:
  - > Open the picture in Image-Pro Plus.
  - > Set the calibration (Measure - Calibration - Select Spatial...) and save the current document.
  - > Measure - Count/Size.
  - > Adjust the Options as required (Outline, Object#, Label Colour, Fill Holes, Clean All Borders, etc.).
  - > Set the Measurements and Filter Ranges as required (Measure - Select Measurements - Area, Diameter (max), Diameter (mean), Diameter (min), etc.).
  - > Save the Settings for later use (File - Save Settings).
  - > Automatically Bright Objects, Apply Filter Ranges.
  - > Count: In case of unconsidered, stuck-together or unwanted items use the Edit commands (Split Objects, Draw/Merge Objects, Toggle Objects On/Off, etc.).
  - > Save the outlines (File - Save Outlines - ID\_x.scl) for later traceability (for



an example see Fig. 2).

-> Save the data (File - Data to File - ID\_x.csv).

(You could also add the new data to an already existing file by using Append Data to File or Data to Clipboard and Paste, but don't loose the overview within this big file!)

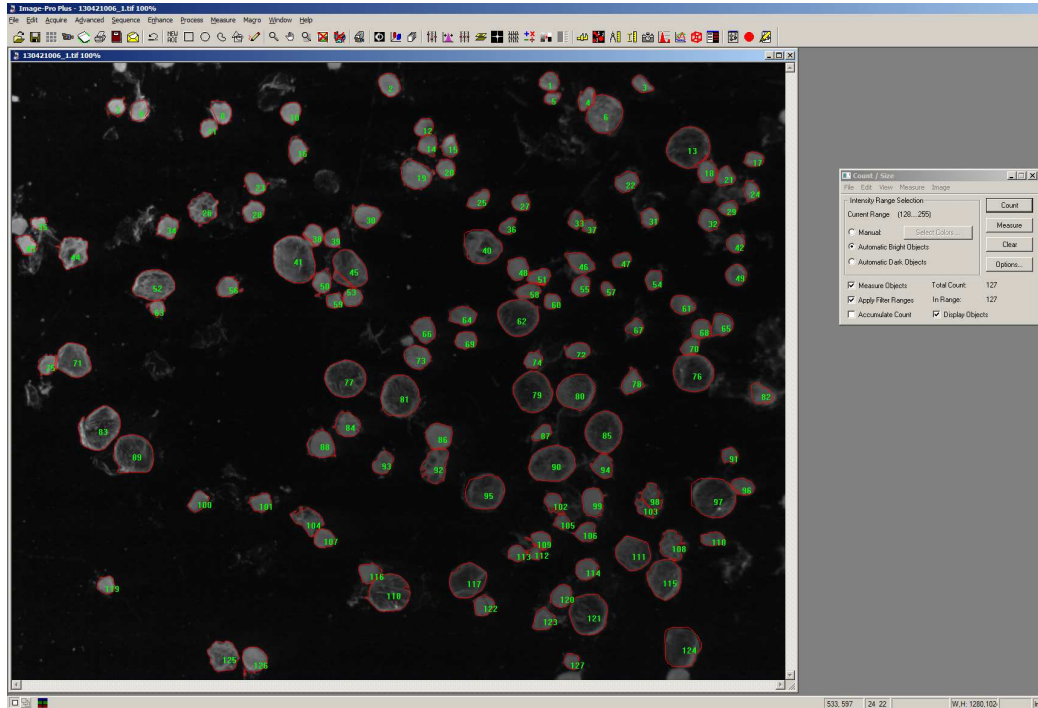


Fig. 2: Example of a picture incl. outlines in Image-Pro Plus (130421006\_1.tif + 130421006\_1.scl).

Per investigated picture you should have the following files: Picture (ID\_x.tif), Outlines (ID\_x.scl), Data (ID\_x.csv)

After the data gathering you may manipulate the data-files either using R, a text editor or a spreadsheet program (e.g. Excel). But be aware about the weakness of spreadsheet programs as interpreting and changing data internally!

Results of the measurements were 20 pictures, 20 outline-files and 20 csv-files. Additionally one picture per gonad sample with different oocyte samples was taken for further use at a later date. The csv-files were not ideal formatted for direct use in R, so they had to be prepared within a text editor (Notepad) before reading it into R. In R additional information on individual fish (ID, Length, Weight, GSI, HSI) as well as the number of counted oocytes per sample were added and a new csv-file for each sample was created.

Below is an example of the R-commands for the data manipulation for later use:

```

# reading the individual sample
Data <- read.csv("130518007_1.csv")

# adding columns
Data$ID <- "130518007_1"
Data$Length <- 60
Data$Weight <- 2.108
Data$GSI <- 2.3
Data$HSI <- 19.2
Data$Egg_count <- 44

# creating data for each sample
Data130518007_1 <- Data

# writing csv for each sample
write.csv(Data, "130518007_1_allData.csv", row.names=FALSE)

```

At least all csv-files were merged to one comprehensive csv-file with the oocyte measurements including individual fish data for further analysis.

### 3 Basic results & analysis

An overview of all 20 investigated gonad samples incl. measurements and calculations is shown in Table 1. As described before GSI was the selection criterion of the gonad samples, so the table is sorted by GSI and the two groups of GSI are displayed. The average oocyte diameter of all samples is 0,783 mm, the maximum diameter is 1,530 mm and the minimum diameter is 0,375 mm. The standard deviation of the mean diameter varies from 0,033 to 0,291.

Table 1: Investigated samples incl. measurements and calculations sorted by GSI, 2 groups: GSI big (orange), GSI small (yellow).

ID	Length [cm]	Weight [kg]	Liver [kg]	Gonad [kg]	GSI	HSI	D_mean [mm]	D_max [mm]	D_min [mm]	D_SD	Count
130425007	70	3,995	0,073	0,929	23,3	1,8	1,136	1,380	0,630	0,116	38
130503019	64	2,06	0,382	0,426	20,7	18,5	0,746	1,356	0,468	0,201	75
130421006	62	3,268	0,526	0,648	19,8	16,1	0,740	1,412	0,404	0,254	127
130412002	73	4,45	0,7	0,85	19,1	15,7	0,640	1,253	0,456	0,156	84
130421015	70	4,504	0,136	0,844	18,7	3,0	0,624	1,280	0,399	0,133	94
130413010	65	3,694	0,506	0,676	18,3	13,7	0,597	1,293	0,375	0,151	228
130502013	70	4,09	0,756	0,728	17,8	18,5	0,796	1,436	0,481	0,291	67
130430014	70	2,074	0,25	0,362	17,5	12,1	0,655	1,530	0,436	0,198	77
130430013	59	5,35	0,828	0,922	17,2	15,5	0,697	1,351	0,432	0,221	75
130417010	74	4,18	0,528	0,718	17,2	12,6	0,646	1,064	0,377	0,134	48
<b>Mean GSI big</b>	<b>67,7</b>	<b>3,767</b>	<b>0,469</b>	<b>0,710</b>	<b>19,0</b>	<b>12,8</b>	<b>0,728</b>	<b>1,335</b>	<b>0,446</b>	<b>0,186</b>	<b>91,3</b>
130517001	75	4,205	0,818	0,162	3,9	19,5	0,864	1,256	0,581	0,176	76
130505009	73	3,194	0,536	0,12	3,8	16,8	0,695	0,935	0,377	0,113	45
130427002	61	2,84	0,532	0,1	3,5	18,7	0,836	0,981	0,666	0,058	37
130503013	66	3,346	0,564	0,108	3,2	16,9	0,675	0,917	0,502	0,091	55
130508009	66	3,242	0,642	0,102	3,1	19,8	0,924	1,243	0,445	0,156	63
130503003	65	2,754	0,52	0,082	3,0	18,9	0,820	1,075	0,551	0,094	42
130506010	73	3,708	0,594	0,086	2,3	16,0	0,793	1,285	0,559	0,167	47
130518007	60	2,108	0,404	0,048	2,3	19,2	0,819	1,018	0,449	0,116	44
130509005	64	3,424	0,428	0,074	2,2	12,5	0,748	0,927	0,596	0,087	20
130419005	66	2,614	0,09	0,054	2,1	3,4	1,214	1,304	1,124	0,033	34
<b>Mean GSI small</b>	<b>66,9</b>	<b>3,144</b>	<b>0,513</b>	<b>0,094</b>	<b>2,9</b>	<b>16,2</b>	<b>0,839</b>	<b>1,094</b>	<b>0,585</b>	<b>0,109</b>	<b>46,3</b>
<b>Mean all samples</b>	<b>67,3</b>	<b>3,455</b>	<b>0,491</b>	<b>0,402</b>	<b>10,9</b>	<b>14,5</b>	<b>0,783</b>	<b>1,215</b>	<b>0,515</b>	<b>0,147</b>	<b>68,8</b>

*D\_mean ... average oocyte diameter*  
*D\_max ... max. oocyte diameter*  
*D\_min ... min. oocyte diameter*  
*D\_SD ... standard deviation*  
 (all above derived from Parameter "Diameter\_mean")  
*Count ... number of measured oocytes*

When looking at all samples there is no significant change from the standard deviation of the mean oocyte diameter by time ( $p > 0,05$ ) (see R-output and Fig. 3).

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-5.678314e+00	4.441185e+01	-0.1278558	0.8996807
Date_num	4.465308e-05	3.404134e-04	0.1311731	0.8970933

Additional two size groups were introduced (fish length till or over 65 cm). To have a clearer view on the varying relations it was plotted with different graphs (Fig. 3, Fig. 4).

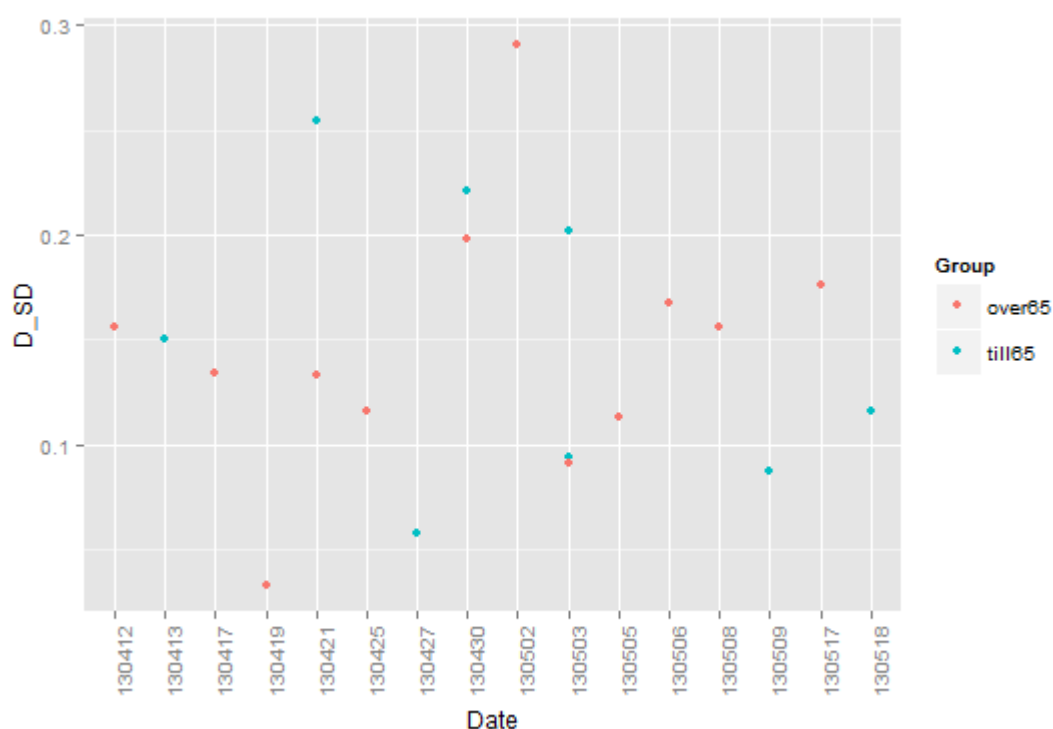


Fig. 3: Standard Deviation of the mean oocyte diameter ( $D_{SD}$ ) by time (Date),  
 2 size groups: fish length till 65cm (blue), over 65 cm (red).

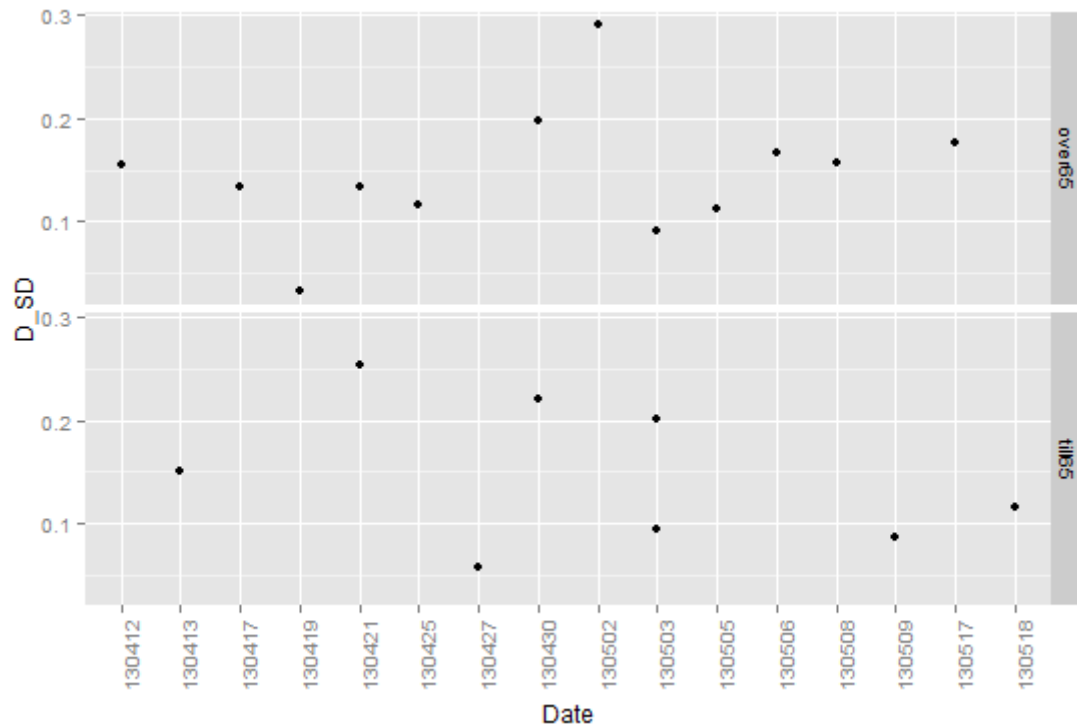


Fig. 4: Standard Deviation of the mean oocyte diameter ( $D_{SD}$ ) by time (Date), divided into 2 size groups: fish length till 65cm, over 65 cm.

A linear regression for all samples shows no significant change of the mean oocyte diameter by time ( $p > 0,05$ ) (see R-output and Fig. 5).

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-2.748412e+01	1.107634e+02	-0.2481335	0.8068398
Date_num	2.166676e-04	8.489930e-04	0.2552054	0.8014577

The same size groups as before are used for the discrimination and to have a clearer view on the varying relations different graphs were plotted too (Fig. 5, Fig. 6).

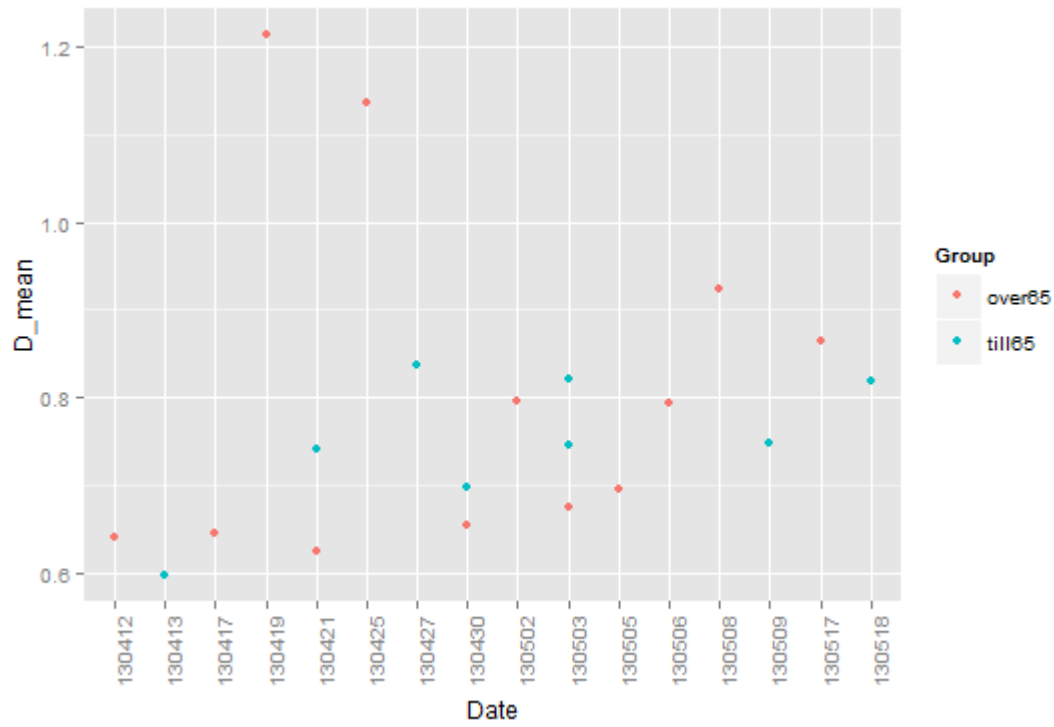


Fig. 5: Mean oocyte diameter ( $D_{mean}$ ) by time (Date), 2 size groups: fish length till 65cm (blue), over 65 cm (red).

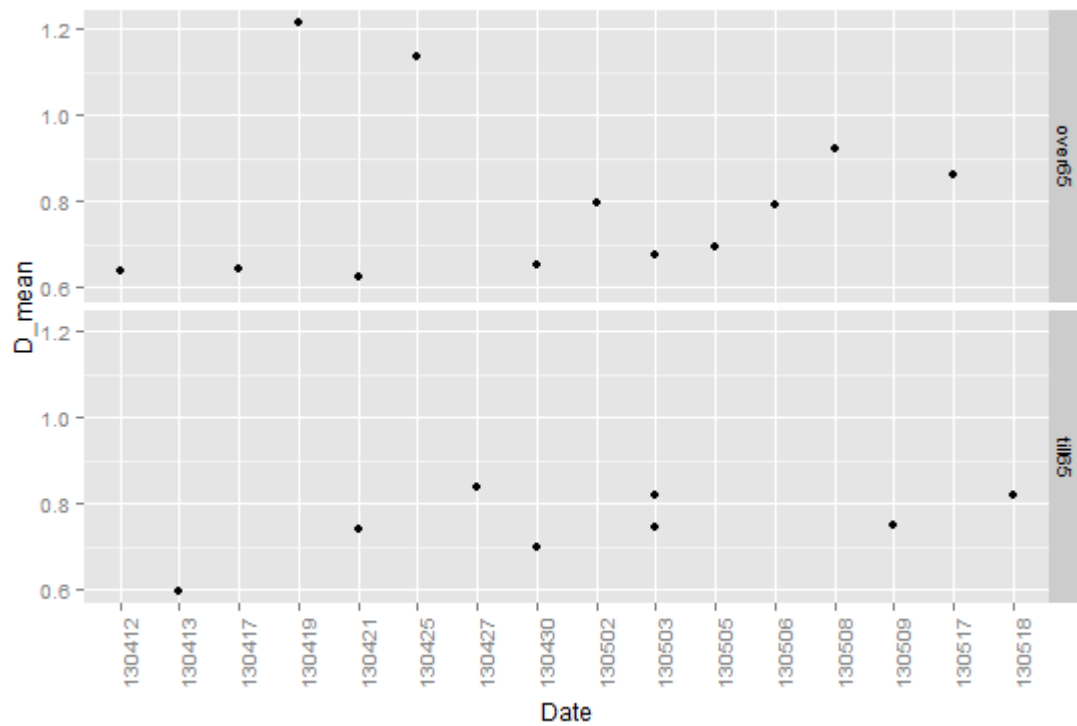


Fig. 6: Mean oocyte diameter ( $D_{mean}$ ) by time (Date), divided into 2 size groups: fish length till 65cm, over 65 cm.

## 4 Personal conclusion to the lab work

The focus of the lab work was on testing and exploring the equipment and software, gathering data from a small sample size, and giving directions as well as sharing the experience with this report. As mentioned in the introduction, therefore the report is not written as a classic scientific report. I am aware about the missing references within the report. Some scientific papers about GSI, HSI, annual egg production, etc. were studied, but they don't match with the current work, so they are not considered. Due to time conflict further literature research was not possible.

From the snapshots it is obvious that there are two different types of oocytes. The bigger and more transparent ones are already hydrated oocytes which were close to being released during spawning, while the smaller and darker ones are not yet hydrated. The hydrated oocytes should be 1,2 mm and larger in diameter (information by Gudrun Marteinsdottir), but they may be smaller due to shrinking in the formalin.

The GSI was determined as selection criterion of the gonad samples and this leads to difficult analysis of the data. To test the variation in oocyte diameters or average oocyte diameter in relation to spawning time it probably would have been better to take random samples or to use the fish length as selection criterion and create different size groups.

It has to be kept in mind that the sample size is quite small to gain statistically significant results. There was not more time left to work in more detail on the statistical analysis. The data is stored at the lab and ready for further analysis, for example additional examination on the variation between and within the size groups. The GSI as the selection criterion of the gonad samples leads to two groups of data, and this has to be considered when reading the results. It is crucial to distinguish between the two GSI groups when examining the data more detailed and to consider these differences.

Last but not least I would like to thank my supervisor Gudrun for the possibility to participate on this research project, where I could gain lots of new skills in practical lab and field work as well as I got to know some real experts in the field of fish and marine biology and ecology. Finally I would like to thank my colleagues Fraser, Niall, Will, Jed, Freydís, Tim, Halldór, the Icelandic fishermen from the field work and the other guys in the lab for their support and kindness over the time.

It was a great time at MARICE and at the Háskóli Íslands!

Takk fyrir!