Estimation on seasonal and bathymetric difference in condition of Icelandic cod (*Gadus morhua*) using common metrics

Guðrún Kristín Ragnarsdóttir

Líf– og umhverfisvísindadeild
Háskóli Íslands
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12 eininga ritgerð sem er hluti af
Baccalaureus Scientiarum gráðu í líffræði

Leiðbeinandi/endur
Prof. Guðrún Marteinsdóttir
Bruce McAdam

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Hér með lýsi ég því yfir að ritgerð þessi er byggð á mínum eigin athugunum, er samin af mér og að hún hefur hvorki að hluta til né í heild verið lögð fram áður til hærri prófgráðu.

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Guðrún Kristín Ragnarsdóttir

Ágrip

Aðal okruforði í Atlantshafs þorski (*Gadus morhua*) er varðveittur í lifrinni á formi lípíða. Orkuforðinn sveiflast í samræmi við gnægð fæðu og endurspeglar árstíðabundið ástand fisksins á hverjum tíma. Metlar, eins og lifrarstuðullinn (HSI) og líkams ástands stuðullinn Fulton´s K (K) sem og mælingar á magni lípíða í lifur þorsksins, eru notaðir til að meta ástand hans. Niðurstöður fengust um að ástand væri breytilegt milli árstíða og dýpis. Mismunandi metlar gáfu hins vegar mismunandi niðurstöður. HSI og orkuinnihald lifrarinnar sýndu svipað mynstur en annað mynstur fékkst með K. Af þessum ástæðum er ályktað svo að HSI og orkuinnihald lifrar séu jafngildar mælingar á skammtíma ástandi en K sé ekki hægt að nota í sama tilgangi.

Lykilorð: þorskur, orkuforði, ástand, metlar, árstíðir, dýpi, mynstur

Abstract

In Atlantic cod (*Gadus morhua*) the main energy reserves are stored in form of lipids in the liver. Energy reserves fluctuate in response to abundance of food and reflect the annual condition of the fish each time. Metrics, such as the hepatosomatic index (HSI), the body condition index Fulton´s K (K) and measurements of the lipid content of the cods liver, are used to assess the condition. Condition was found to wary between seasons and depths. Different patterns emerged from different condition indexes. HSI and energy content of the liver showed similar patterns but no such pattern was observed for K. Therefore it is concluded that HSI and energy content are equivalent measures of cod short term condition while K cannot be used in the same purpose.

Key words: Atlantic cod, energy, condition, metrics, seasons, depths, patterns

Acknowledgments

I would like to thank my instructors, Prof. Guðrún Marteinsdóttir and Bruce McAdam for assistance and directing me into right paths while creating this project. I also would like to thank Hlynur Bárðarson for his endless patient and help with statistics and Birna Reynisdóttir for the collaboration with the liver analysis. Last but not least I would like to thank my family for her support.

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1 Introduction

In fishes, energy in form of lipids is stored in tissues and used when needed during all major life history events such as growth, gonad development, spawning behavior and migration. This energy fluctuates in response to the abundance of food (Lambert & Dutil 1997a). In gadoid species, like the Atlantic cod (*Gadus morhua*), the protein reserves are stored in the trunk muscles, while lipid reserves is primarily stored in the liver (Lambert & Dutil 1997a). The energy reserves of the liver are mobilized prior to mobilization of muscle energy content (Auðunsson 1999).

Food availability and the prey type is a key factor for good condition. The Atlantic cod (*Gadus morhua*) is a generalist carnivorous species for which physical characteristics of the prey affect the condition (Dos Santos et al. 1993). Prawn, for example, is considered to be a lean prey while herring and capelin are rich in lipids (Dos Santos et al. 1993). Dos Santos et al. (1993) found that liver size and liver fat content increased with increased levels of fat in the diet.

Very little is known about the seasonal cycle of condition of Icelandic cod (Pardoe et al. 2008). However Auðunsson (1999) reported that for the Atlantic cod an increase in lipids or liver size has been observed during late summer or autumn until the end of the year. In the autumn, when the cod start preparing for the spawning in the following spring an increase in lipids and size of liver is detected. Similarly, when the spawning season starts and as the spawning progresses, the liver lipids decrease and reach a minimum at the end of the spawning season. This annual feeding and spawning cycle therefore results in a large variation in liver fat content. This variability can also wary from one year to another due to environmental changes and food availability (Auðunsson 1999).

Pardoe and Marteinsdóttir (2009) report that for cod North of Iceland, capelin biomass better explained variability in the condition factor whereas temperature explained more variability in growth rates.

Metrics are often used to assess fish condition. The hepatosomatic index (HSI) describes the liver mass relative to body mass for individual fish. Fulton´s K describes the body shape or the proportion of weight in relation to the length of the fish. If K is high then the weight of the fish is relatively great for its length and the fish contains a greater proportion of muscle compared to fish with lower K. These two metrics show different results (Pardoe & Marteinsdóttir 2009) as they are not based on the same factors and therefore it is not wise to use them separately as they might respond to environmental variation at a different rate (Pardoe & Marteinsdóttir 2009).

In this study energetic condition of Icelandic cod was observed. The condition was then observed between seasons and between bathymetrical variations similar as reported in other papers (Mello & Rose 2005, Lambert & Dutil 1997a, Pardoe & Marteinsdóttir 2009).

The Icelandic cod stock has been divided into two main groups depending on their behavioral and genetic difference. These are fishes that follow deep- or shallow water migrations. The

shallow water fish stays mostly above the 200 meters depth and appears to follow the seasonal trend in temperature. The deep water fish however migrates to deeper and cooler water below 200 meters and engages vertical movement. During spawning migrations, the depth range of the two types overlaps (Pálsson & Thorsteinsson 2003). Pardoe studies (2008, 2009) show bathymetric and seasonal trends in condition of Icelandic cod.

The aim with this study was to use the weight of the liver and the lipid content to estimate the condition of Icelandic cod. That information will then be used to look into if there is any seasonal difference in condition of the cod and if the condition is different between seasons.

The hypothesis :
H_1 : Energetic condition shows patterns of variation with bathymetry (of catch location).
H_2 : Energetic condition show a seasonal pattern of variation.

2 Materials and methods

2.1. Sample collection

The dataset used in this report is made by combining two sets of data. One contains cod sampled by the MARICE group at the University of Iceland in 11 and 24 of March and 3 April in 2009, a total of 148 samples. The other dataset is a bigger subset of a dataset from the Marine institute of Iceland (Hafró) which was collected for a project called "Grandskoðum þann gula" and has a total of 873 samples. Those data were collected in the year 2007, 2008 and 2009 throughout the year and at different locations around Iceland. Data from all years and periods were combined for all tests.

The analysis of the liver sample form MARICE dataset was made in collaboration with Birna Reynisdóttir. We took the liver samples to a lab at Matis ohf. and processed them there as described later.

In total the dataset contains 1021 cod sampled which each have several measurements but not all used for this study. These included length, total weight, liver weight, gutted weight, age, sex, maturity stage and water and fat content of the liver. Also included were dates and locations of catch for all the Hafró data but not an exact location for the smaller dataset.

In some cases some of the information is missing and some samples needed therefore to be excluded from the statistical analysis.

2.2. Dividing the data

In order to assess the hypothesis about bathymetric and seasonal variation the data were divided into fish caught at deep water stations (n= 372; blue tags in Fig.1) and fish caught at shallow stations (n= 603; red tags in Fig 1) i.e. following Pardoe, H., Thordarson, G. and Marteinsdóttir, G. (2008).

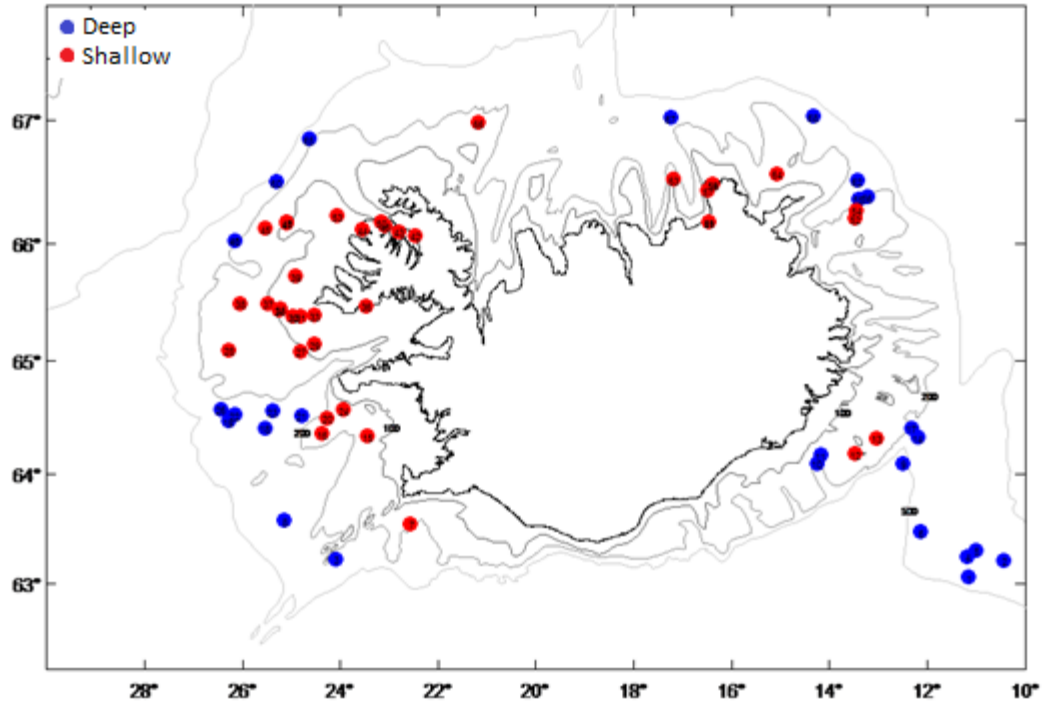

Figure 1: *Sampling locations. Blue represent deep sampling locations at more than 200 meters depth and red represent shallow sampling locations at less than 200 meters depth.*

Then the data were further divided by seasons.

Table 1 : *Division into seasons and number of samples sampled form deep and shallow water.*

SEASON	MONTH	DEEP	SHALLOW
1. Late winter	Jan, Feb, Mar	143	201
2. Spring – early summer	Apr, May, Jun	0	94
3. Late summer – fall	Jul, Aug, Sep	91	30
4. Early winter	Oct, Nov, Dec	148	278

2.3. Estimation of water content in liver

The liver sample analysis was made at Matís ohf. in October 2011. The method is based on drying a sample and the loss is calculated as water content of the sample. Note that other things than water can evaporate when heated more than 100°c such as short lipid chains and all the chemicals we can smell. All the water usually does not vapor at 100°c because part of it is bond to proteins. With increasing temperature some of this water evaporates (ISO 6496 1999).

A full list of consumables and equipment can be seen in Appendix 1.

Execution

The liver samples were retrieved from freezer at c.a. -23°c and taken to the lab. At the lab a subsample of the liver sample was taken as it was thawing and crushed manually in a plastic cap with a mortar and made uniform.

Porcelain bowls were marked with the samples´ numbers and then weighted with approximately tablespoon of sand and a glass stick with the accuracy of 0.0001 g. Then a approximately of two grams of the liver sample were added to the bowl, weighted and then blended to the sand using a glass stick.

The sample was then dried in the heating cabinet at 103.2 °C for at least 4 hours. In the end the sample in the porcelain bowl was cooled down for 30 minutes in the desiccator and then weighed for the last time.

All numbers (or calculations) were recorded in a book with the samples´ number, date and the weights.

Calculation

$$\% \text{ water} = \frac{(S2-S3)}{(S2-S1)} \times 100 \qquad \text{Equation (1)}$$

Where :

S1 is the weight of the bowl
S2 is the weight of the bowl and the sample
S3 is the weight of the bowl and the sample after drying

2.4. Fat estimation with the soxhlet method

Fat from dried liver sample was processed with petroleum ether and then washed when the ether had been removed.

Execution

The sample has to be prepared correctly to ensure that the right outcome of the fat content is reached.

When the sample had been weighed after drying it was removed from the porcelain bowl with a plastic spoon, cotton and petroleum ether and placed in a filtering thimble. It is carefully guarded so that nothing is left behind in the bowl, on the glass stick or on the plastic spoon. The filtering thimble is placed in pre weighed aluminum cup and weighed agene. Then the sample is run through the Soxhlet extractor machine and lipids extracted. After extraction the non soluble portion remains are cooled in desiccators and then weighed for further calculations (AOCS 1997).

Calculation

$$\% \text{ Fat} = \frac{(F2-F1)}{g \text{ (sample)}} \times 100 \qquad \text{Equation (2)}$$

Where :

F1 is the weight of aluminum cup with boiling stones
F2 is the weight of aluminum cup with fat and boiling stones
g is the sample weight

2.5. Condition indexes

Fulton´s condition factor and the hepatosomatic index are frequently used as indicators of the condition of cod (Lambert & Dutil 1997b, Pardoe et al. 2008, Dutil et al. 2006). These indices have been shown to be good indicators of the general condition in term of protein and lipid reserves of cod (Lambert & Dutil 1997b).

The hepatosomatic index (HSI) describes the liver mass relative to body mass for individual fish (equation (4)). The Fulton´s K (K) is used to evaluate the weight–length relationship in fish and converts it into a single statistic that gives a simple indicator of the "well being" of a fish (equation (3)). The general assumption is that a heavier weight for a given length corresponds to a better condition (Lambert & Dutil 1997b). However the Fulton´s K is dependent on length of the fish, it assumes that fish of different size but with the same condition factor, have the same shape. Therefore Fulton´s K is not a good metric for fish of different size (Eyjólfsson et al. 2001).

Calories were calculated for each sample as amount of energy stored in the liver (equation (5)). For comparison the energy was then calculated as calories per gram of body mass (equation (6))

Fulton´s condition factor $K = (GW/L^3) \times 100$	Equation (3)
Hepatosomatic index $HSI = (LW /GW) \times 100$	Equation (4)
Calories (kcal) = LW x (percent fat / 100) x 9	Equation (5)
Energy per body mass = calories / GW	Equation (6)

Where GW is gutted weight in grams and L is fish length in centimeters and LW is the weight of the liver in grams. Note that the Fulton´s condition factor K does not depend at all on the liver weight and is therefore not suitable for measuring short term condition of cod.

Gutted weight is more accurate, as stomach contents and gonad maturation can vary significantly between seasons and regions.

3. Data analysis

Fulton´s condition factor (K), the hepatosomatic index (HSI) and the liver lipid percentage were calculated for each individual fish.

Statistical analysis were performed with the statistical package R (R Development Core Team 2009), with a critical level of significance at $\alpha = 0{,}05$. For some of the statistical analysis the data were log-transformed for better normal distribution. ANOVA was used to find out if measurement varied significantly.

The data were separated at the 200 meters depth contour and classified as either shallow (<200 m) or deep (>200 m) as Pardoe et al. did (2008). This division is based on studies of two groups of Icelandic cod; one group almost exclusively inhabits shallow water above 200 meters and the other group which spends most of his time in deeper cooler waters (Pardoe 2008). During spawning migrations, the depth range of the two groups overlaps (Fig. 1).

4. Results

A total of 873 samples were analyzed by the Marine Research Institute of Iceland and a total of 148 samples were analyzed by Birna Reynisdóttir and me. Some samples had to be discarded because several of reasons, for example when crucial information like liver weight or total weight were missing. Some samples were discarded because of some mistakes in the lab work that resulted in a total water and lipid content more than 100%. In addition one liver sample was thrown away because it was very small, the plastic bag was open and the sample had dried up and could not be processed.

The total number of samples used for the statistical analysis was 975.

4.1. Fat and Water

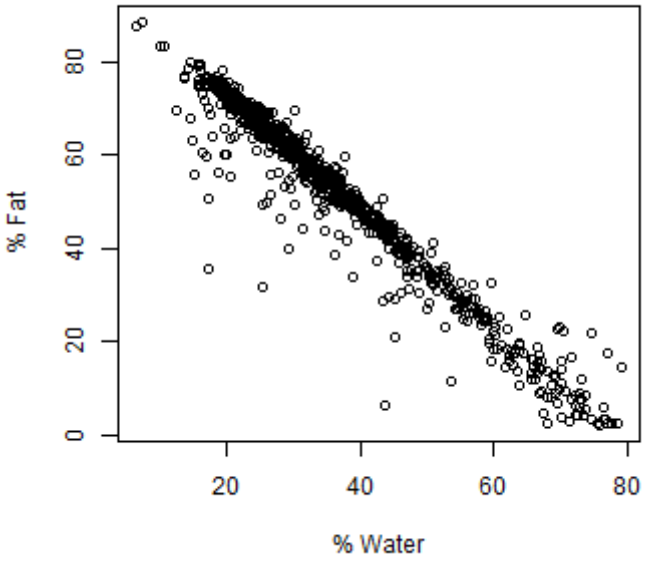

Figure 2: *The ratio of water plotted agenst fat ratio for each individual.*

There is a strong negative correlation (r^2 = -0.97) between the amount of fat (lipids) and water in the liver as expected. As the lipid content of the liver increases the water content decreases. Therefore lipids are largely responsible for the variation in liver composition and thereby energy content.

4.2. HSI and lipids

Specific lipid content of the liver varied between 2% and 88.78% on a wet-weight basis. The HSI index varied from 0,61% up to 18,21%.

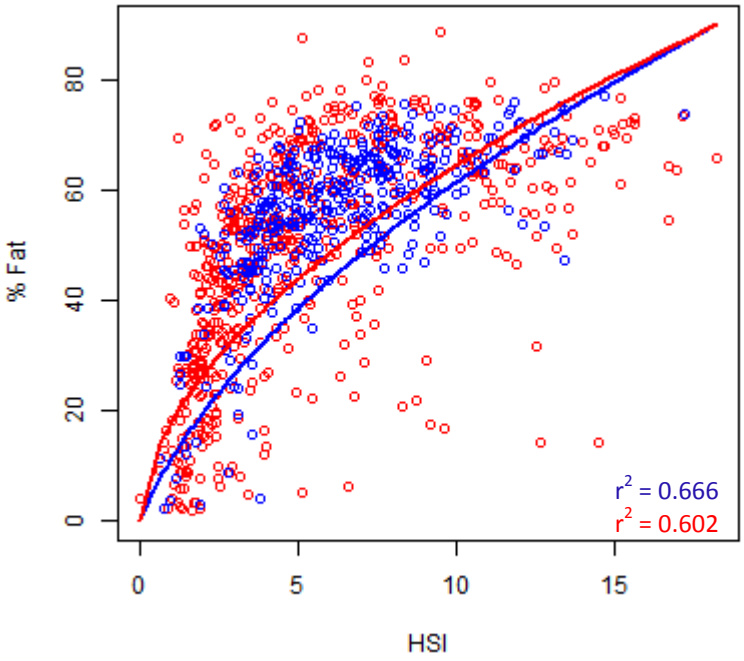

Figure 3: *The relationship between the percentage of lipids in the liver and HSI according to depth. Red represents fishes caught in shallow water (>200m) and blue represent fishes caught in deep water (<200m). Each ring represents individual fish.*

Lower hepatosomatic index (HSI) is followed by decrease in liver fat (lipids) content with a massive drop as the HIS goes under c.a. 7% (Fig. 3). This shows that when the liver is relatively small it contains lesser amount of lipids than relatively bigger liver who contains high amount of lipids.

Same trend in the lipid – HSI relationship were observed between samples from both depth ranges. The correlation for deep water fish is slightly stronger (r^2 = 0.666) than for shallow water fish (r^2 = 0.602).

4.3. Energy

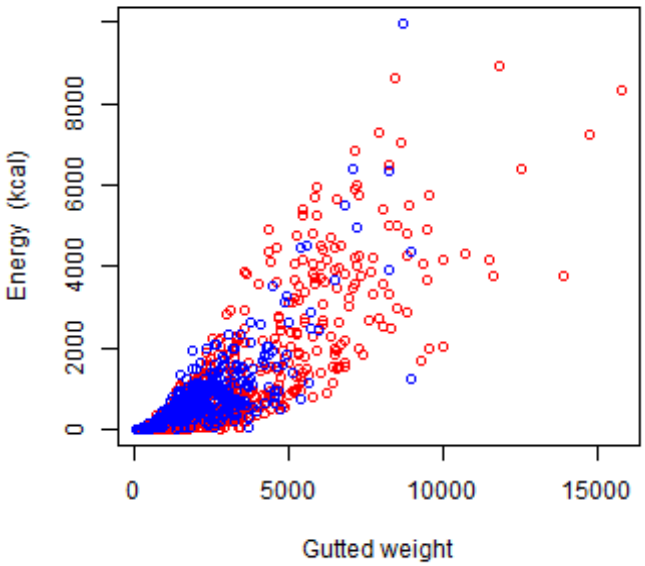

Figure 4: *The relationship between energy and gutted weight for deep water fish in blue and shallow water fish in red.*

Most of the cods are lighter than 5 kilos and store energy less than 2000 kcal. Few cods from the dataset are heavier than 10 kilos and store high amount of energy.

Positive correlation was observed between calories in liver and gutted weight (r^2 = 0.765, Fig.4).

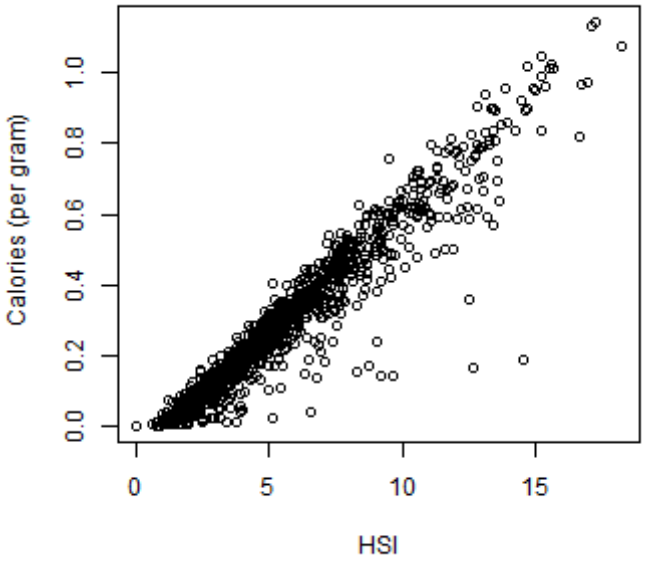

Figure 5: *The relationship between energy per gram of body weight and HSI.*

When calories had been calculated as the amount of calories per gram of gutted body weight (equation 6), the correlation between energy and HSI was strong (r^2 = 0.954, Fig. 5). There is no correlation between HSI and K (r^2 = 0.094).

4.4. Difference between depths

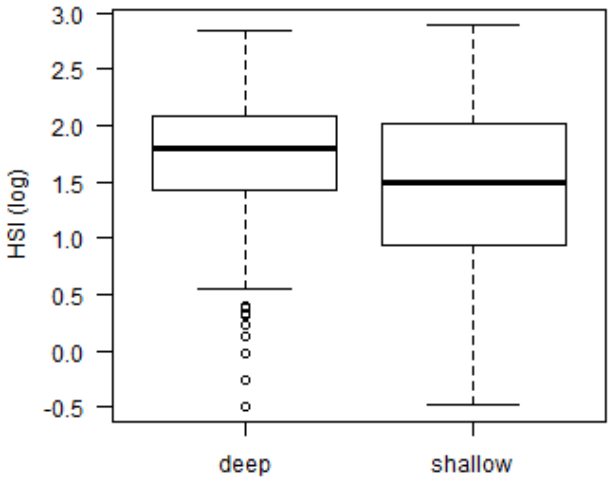

Figure 6 : *Difference in HSI between depths*

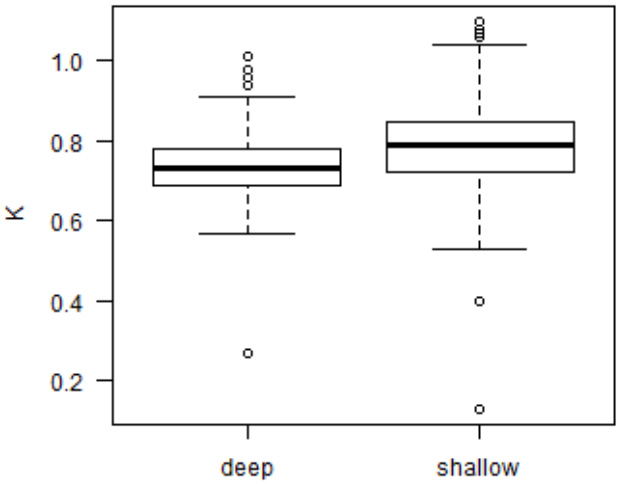

Figure 7 : *Difference in K between depths*

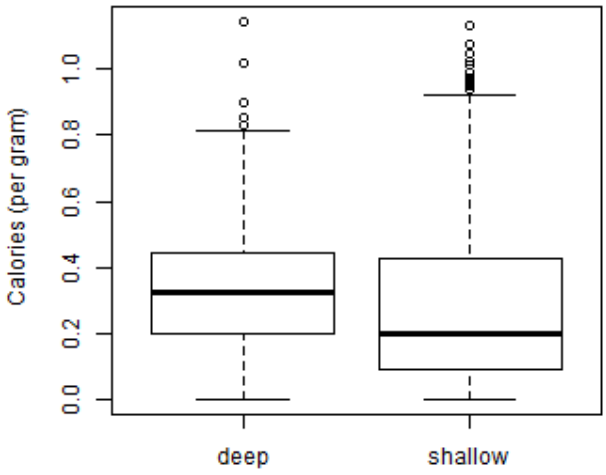

Figure 8 : *Difference in energy between depths*

There is not a significant difference in HSI between depths (ANOVA, p = 0.243). However there is a signifigant difference (ANOVA, p > 0,001) in K betveen deep (>200 m) and shallow (<200 m). There is a significant difference in energy betveen depths (ANOVA, p > 0,001).

4.5. Seasonal differences in HSI, K and energy

Seasonal variability in HSI was observed from the data.

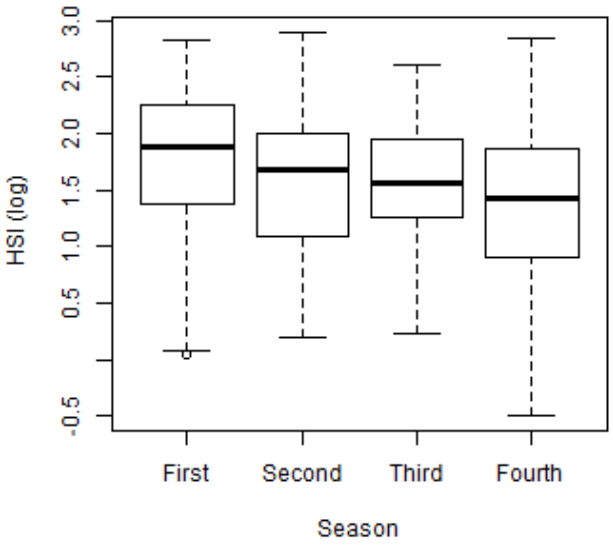

Figure 9: *Seasonal difference in HSI. First season is January-March, second season is April-June, third season is July-September and fourth season is October-December.*

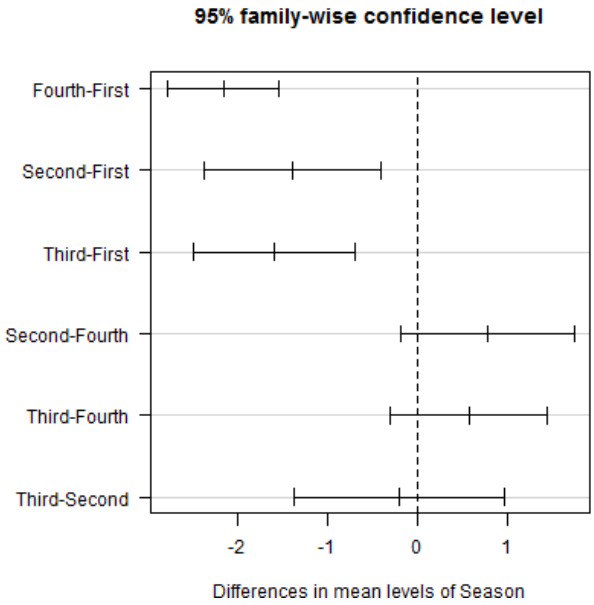

Figure 10: *Tukey's honestly significant difference (HSD) multiple comparison test to determine which season comparisons were significant. If a line crosses the zero vertical dotted line, there is not a significant difference between those seasons.*

There is a significant difference in HSI between seasons (ANOVA, P<<0.001) but this difference is not between all seasons (tukeyHSD, Fig. 10). There is a significant difference between fourth- and first season, second- and first season and third- and first season. Other seasons do not differ significantly.

Seasonal variability in K was observed from the data.

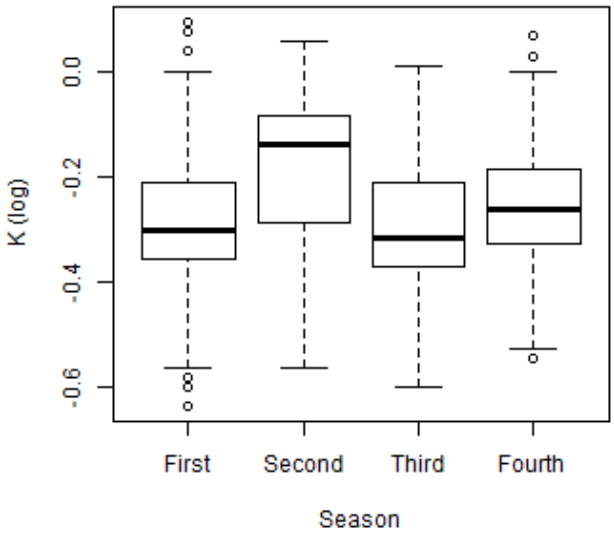

Figure 11: *Seasonal difference in K. First season is January-March, second season is April-June, third season is July-September and fourth season is October-December.*

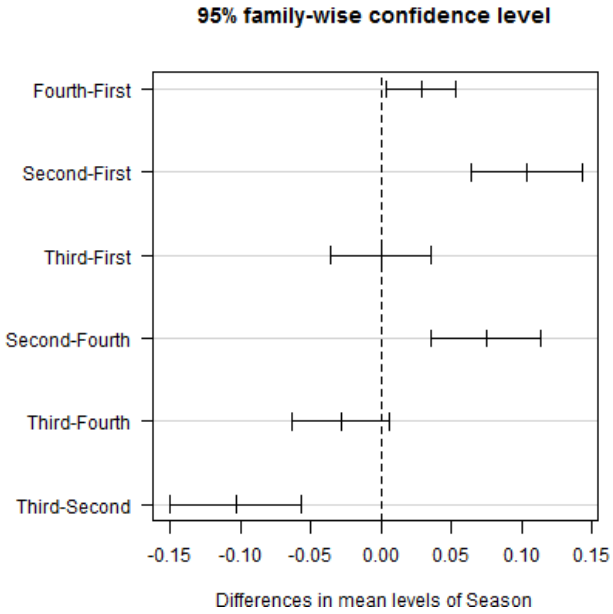

Figure 12: *Tukey's honestly significant difference (HSD) multiple comparison test to determine which season comparisons were significant. If a line crosses the zero vertical dotted line there is not a significant difference between those seasons.*

There is a significant difference in K between seasons (ANOVA, p<<0,001). Difference in K is not observed between all seasons (TukeyHSD Fig. 12). There is a significant difference between fourth- and first season, second- and first season, second- and fourth season and third- and second season. Third- and first season and third- and fourth season do not differ significantly.

Seasonal viability in energy was observed from the data.

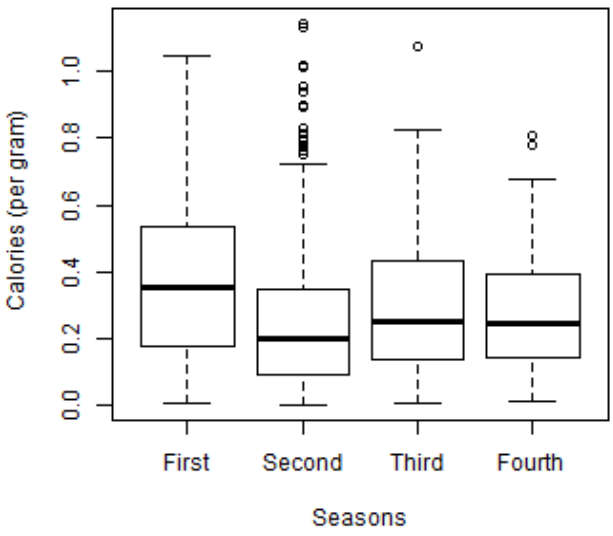

Figure 13: *Seasonal difference in energy. First season is January-March, second season is April-June, third season is July-September and fourth season is October-December.*

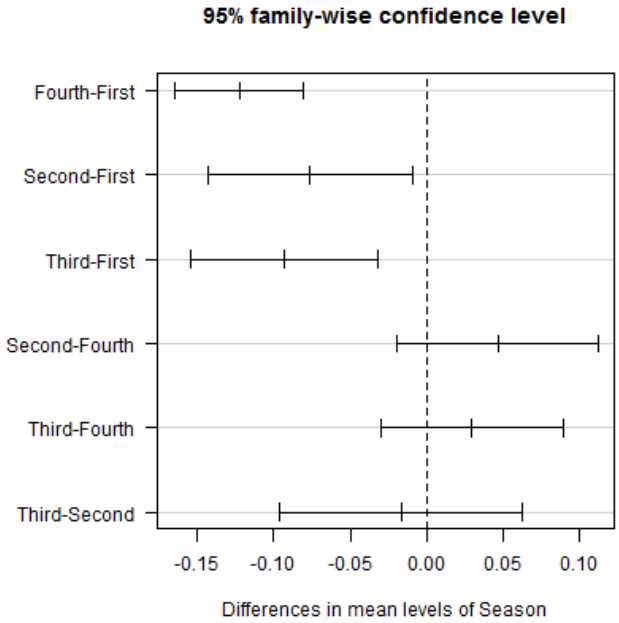

Figure 14: *Tukey's honestly significant difference (HSD) multiple comparison test to determine which season comparisons were significant. If a line crosses the zero vertical dotted line, there is not a significant difference between those seasons.*

There is a significant difference in energy per gram of body weight between seasons (ANOVA, p<<0,001). This viability in energy is not observed between all seasons (TukeyHSD Fig. 14). There is a significant difference between fourth- and first season, second- and first season and third- and first season. Other seasons do not differ significantly.

There is a similar pattern for HSI and energy through seasons (Fig. 9 and 13) but this pattern is not observed for K (Fig. 11). Both HSI and energy are highest in the first season and then reduce.

5. Discussion

This study shows same results as previous studies have shown that simple condition indices such as the condition factor (K) and the HSI can clearly be used as indicators of the energy reserves of the cod. Moreover the determination of water content of liver provides accurate measurements for estimating the energy content of the liver (Lambert and Dutil 1997a). These condition indexes can therefore be used to monitor seasonal changes in energy reserves of the cod. They can as well be used to monitor stocks and variations in environmental conditions, as the energy available for the different processes influencing cod productivity can be measured (Lambert and Dutil 1997a). However as observed those indices tell different stories. HSI and energy content show similar seasonal trend (Fig. 9 and 13) which was not the same trend there was observed by K (Fig 11). This might be a reason to rather use HSI and energy content than K as condition index for monitoring seasonal changes in energy reserves of cod.

5.1. Fat and water

In this research the fat and water composition of the liver were observed but neither the livers protein content nor trace elements which can vary dramatically. It has been reported that trace element generally decrease as fat content of the liver increases. Though, the Icelandic cod stock is very little polluted (Auðunsson 1999). Lambert and Dutil (1997a) found a strong inverse relationship between energy and water content in the liver, as the proportion of lipids in the liver increased the energy content increased as well and thereby the energy reserves of the cod. So when the liver contains a minimum amount of lipids it contains little energy reserves that could be mobilized. From such high correlations between specific energy content and water content, very accurate predictions of one component can be mad from the measurement of the other (Lambert and Dutil 1997a).

Knowing that lipid content of a liver is linked to energy reserves it might be possible to save both time and money by simply measuring the water content of a liver and thereby get a fairly good estimation of condition.

5.2. HSI and lipids

Specific liver energy content increases as HSI increases (Fig. 3), similar to other reports (Lambert and Dutil 1997a). HSI gives a fairly good estimate of the proportion of fat in the liver and consequently the energy reserves of the fish (Auðunsson 1999).

This positive correlation between the hepatosomatic index and the proportion of lipids in the liver is not linear but shows very similar pattern for deep water fish ($R^2 = 0.666$) and shallow water fish ($r^2 = 0.602$) (Fig.3). The lipid content rises fast at lower HSI and slows down as the HSI goes up. So the biggest fish does not necessarily have the biggest and fattest liver and therefore the best condition. Cod with HSI < 5% and thereby low lipid energy content is in poorer condition than cod with HSI > 10% and high lipid energy reserves.

5.3. Energy

The results show clearly that bigger fish have bigger liver and store more amounts of energy reserves than smaller fish (Fig. 4). The strong correlation between the energy per gram of body weight and HSI (Fig. 5) shows how good the index is to predict about condition of a cod by looking into the energy content of the liver.

5.4. Difference between depths

There is not a significant difference in HSI between depths (ANOVA, $p = 0.243$). However there is a significant difference in K between deep (>200 m) and shallow (<200 m) (ANOVA, $p > 0{,}001$).

Dispite the fact that there is not a significant difference in HSI between depths (Fig. 6), there is a slightly higher mean HSI for deep water fish (6.27±2.83) than for shallow water fish (5.49±3.70) and that is according to reported by Pardoe (2008).

For K the results are opposite, there is a significant difference in K between depths, mean K is higher in shallow water (0.79±0.11) than deep water (0.74 ± 0.08), same as Pardoe (2008, 2009) reported.

This small difference in HSI might be due to lower metabolic costs in shallow water, and/or higher concentrations of food supply within temperature boundaries that the deep water cod are more likely to enter (Pardoe et al. 2008).

Other studies have reported higher mean HSI for deep water fish than for shallow water fish although the difference was not significant in this study. One explanation for that difference might be that the energetic cost of feeding migrations are likely to be higher for deep water fish so that they might be unable to maintain muscle protein energy stores to the same extent as shallow water fish (Pardoe & Marteinsdóttir 2009). However, the deep water fish benefits from more stable food supply than shallow water fish and perhaps also more lipid rich (Pálsson & Thorsteinsson 2003). Deep water fish might then be more able to maintain higher short term energy reserves in the form of liver lipids and that would be the reason for the observed bathymetric variation in HSI (Pardoe & Marteinsdóttir 2009).

5.5. Seasonal difference in HSI, K and energy

The results for seasonal difference are the same as Mello and Rose reported (2005) and Pardoe (2008), that the condition of the Icelandic cod, observed both by K and HSI, varies significantly between seasons. During spawning the cod is in its poorest condition (low HSI yield). When the cod has spawned it feeds intensively and its condition gets better. Late winter (fourth season) cod is in its top condition (Mello & Rose 2005, Auðunsson 1999). However HSI and K show different seasonal results, almost opposite.

The fact that there is no correlation between HSI and K tells us that K is a poor predictor on condition because condition is supposed to reflect the amound of energy stored by a fish. A big stocky fish can eider have a high or low HSI.

Fish caught in shallow water have higher mean K than fish caught in deep water. That indicates that deep water fish has relatively more muscle than shallow water fish but because there is no difference in HSI between shallow and deep this difference is not due to the reason that they are storing extra energy (lipids) in their liver.

The relationship between liver energy content and HSI, for samples of cod captured at different times of the year, demonstrates utility of condition indices as tools to follow yearly variations in the energy reserves of cod stocks (Lambert and Dutil 1997a).

6. Conclusions

These results show that condition is lead by both bathymetric and spatial changes which support both hypotheses that were given in the beginning.

The Icelandic cod stock has do deal with heterogeneous conditions throughout the annual cycle. However there are other things effecting cod condition which are not discussed here, such as age, sex, maturity stage, and environmental factors; temperature and food availability.

One thing is important to keep in mind; the environmental conditions in the locations the cod was caught in were not necessarily those that had the most influence on their condition.

The metrics HSI and K are not equivalent and therefore they cannot be used separately to measure condition. They show different results for bathymetric trends and opposite seasonal difference. HSI is a better metric for condition while K reveals how stocky the fish is. The simultaneous use of the condition factor and HIS gives insight into the short- and long-term responses of cod to environmental conditions. Available energy reserves in cod are very compartmentalized, with most of the protein being located in the muscle and most of the lipids in the liver (Lambert and Dutil 1997a). The condition factor (K) will therefore not respond at the same rate as the HSI to changes in environmental conditions. Therefore differences in HIS between depths for cod with similar values of condition factor (K) could indicate differences in short term feeding intensity between locations (Lambert and Dutil 1997a).

During the liver analysis there was especially one thing we considered a lot, the storage method of the liver samples and if it decreases the samples quality. The liver samples had been stored frozen for two and a half year in plastic bags witch in some cases were open and full of ice. Some of the liver samples were therefore damaged. This might affect the water and fat content results in the way that water content increases. Therefore I would propose an experiment that would test whether storage affects the samples. Other errors that occurred during the liver analysis are most likely due to our lack of experience with the analysis. The first stage in the liver analysis is important, that is the stage where the samples are taken from freezer and start thawing and some of the fat leaks from it. Therefore I think it is important to process the sample before that happens.

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Appendix A

Appendix 1 : Substances and implements used for the liver analysis.

Consumables
Silica gel orange (2,5 kg, Groco, 65006200) as a drying agent in the desiccant.
Sand washed with acid and dried before use (Merck 1.07711).
Petroleum ether. Boiling point at 40-60 °C
Boiling stones
Cotton
Sand (acid washed)
Equipment
Aluminum can with lid
Analyzing scale with the precise of 1mg
Heating cabinet adjusted on 100°c
Desiccant.
Porcelain bowls and glass sticks
Analyzing scale
Analyzing scale with the precision of 1 mg
Heating cabinet adjusted to 103.2°c
Aluminum cub
Filtering thimble: Thimbles Single Thickness, from Tecator no 15220045, internal diameter 33mm, and external length 80mm.
2050 Soxhlet Avanti Automatic System machine for distilling
Desiccator with sufficient drying substances

I have learned a great deal from our lab work. Some things I learned during our lab work but some things after the lab work ended and we started looking into the results. One of the most important thing I learned was how extremely important it is to be well organized. One thing I learned after the lab work ended was that it is important to note down everything that comes into your mind during the lab work. I now know that I should have noted down more thoroughly the condition on each liver sample. For example all unusual things noticed and if there were parasites (and the amount of them).