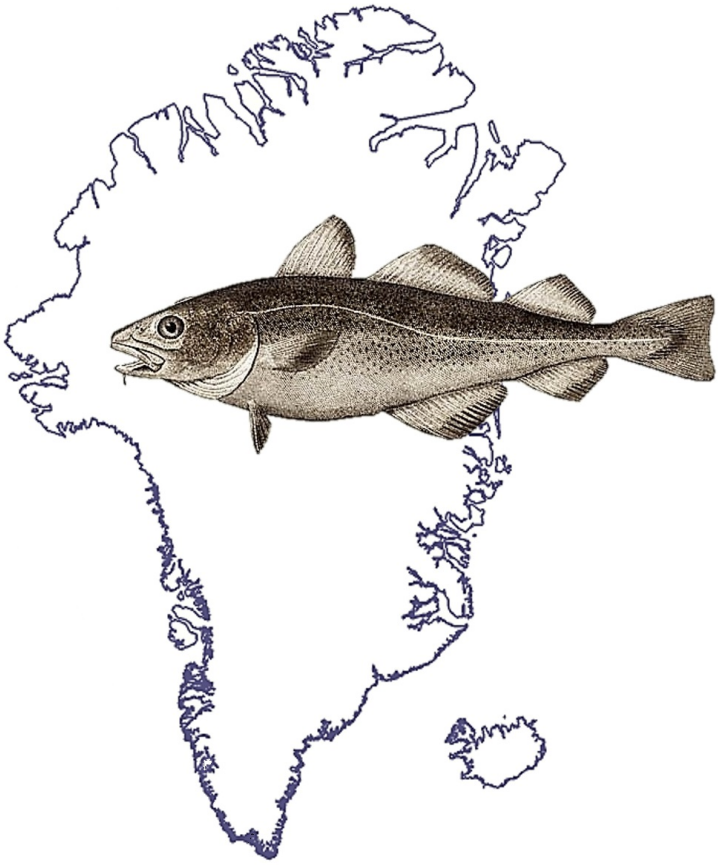
Regional influences on condition and reproductive outcome of female Atlantic cod (*Gadus morhua*) in Greenland waters



M.Sc. Thesis in Biological Oceanography

by

Ina Stoltenberg

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Abstract

Greenland cod stocks were among the largest of the world but collapsed in the 1990s. In Greenland waters Atlantic cod lives in an environment that is influenced by arctic and subarctic climate, undergoing annual and decadal fluctuations, which is strongly impacted by climate change. Different current systems and water bodies from the cold polar region and the warmer North Atlantic encounter and mix in this region, providing habitats with different food sources and abundances. Differences in condition of cod can be seen along the east Greenland coast but the reasons for that are still not fully understood. Total lipid contents and fatty acid composition of livers and gonads of fish have shown significant correlations with its condition, spawning times, hatching success, larval survival and fecundity. Therefore, these parameters can be used as a complement to classic proxies used in fisheries biology to determine fish condition and reproductive output and to establish a connection between diet, condition and reproductive output. Thirty-two mature female individuals of 75-90 cm were sampled from three areas subject to different environmental conditions off the East and Southwest Greenland coasts Total lipid, fatty acid and stable isotope analysis were conducted. Additionally classical condition indices, liver macro-parasites and liver tissue degradation were measured. Two trophic regions were determined, where cod fed either majorly on benthic or on pelagic prey sources. Total lipid content of livers and gonads reflected the results of hepatosomatic indices and gonadosomatic indices. Fatty acid compositions of gonads were dependent of the sampling site. Mesopelagic fish and crustaceans as food sources promoted an enrichment in important polyunsaturated fatty acids and a better condition of female cod. Total lipid contents of liver and gonads, as well as fatty acid compositions of gonads indicated a difference in maturity stages of female cod between the sampling sites. According to large differences in condition and liver health of female cod and fatty acid amounts of gonads between sampling sites, it can be assumed that the habitat plays an important role for the reproductive outcome of Atlantic cod in Greenland waters.

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Glossary

ARA Arachidonic acid

BS Bottom salinity

BT Bottom temperature

δC δC Stable Isotope ratio of 13C to 12C

DHA Docosahexaenoic acid

δN δN Stable Isotope ratio of 15N to 14N

EGC East Greenland Current

EGCC East Greenland Coastal Current

EPA Eicosapentaenoic acid

GSI Gonadosomatic index

HSI Hepatosomatic index

IC Irminger Current

K Fish condition factor

MUFA Mono unsaturated fatty acids

ParaIndex Parasite index

PUFA Poly unsaturated fatty acids

SFA Saturated fatty acids

SSSal Sea surface salinity

SST Sea surface temperature

SWInd Shannon-Wiener Index of stomach content

TFA Total fatty acids in ng/mg dry weight

TFGonads Total lipid content of gonads

TFLiver Total lipid content of liver

1 Introduction

1.1 Motivation

The cod stocks in Greenland waters once were among the largest in the world (Sundby 2000). Landings increased in the early 20th century and peaked in the 1960s with a catch size of up to 460,000 t per year (Figure 1.1). In the early 1970s, the off-shore stocks collapsed due to a combination of high fishing pressure and a decrease in temperature (Buch et al. 1994) and could never recover completely. Even though the collapse was severe and Greenland's economy is largely dependent on fisheries (Hamilton et al. 2003), there is still little known about food web interactions and the impact of oceanography on condition of Atlantic cod in Greenland waters. Timeseries data of condition and stomach content analysis revealed distinctive patterns of cod condition and prey preferences along the East Greenland coast (Werner et al. 2018) that were consistent over time and might be influenced by the oceanography and environmental features in these areas. So far there are no studies from this area, which specializes on large, mature female fish, which have a prominent role when it comes to reproduction and resilience of stocks (Marteinsdóttir & Steinarsson 1998; Scott et al. 1999; Berkley et al. 2004; Hixon et al. 2013). Additionally, there are no studies using methods like fatty acid profiles and stable isotopes of cod in Greenland waters that give an idea about larger time scale food web interactions, compared to stomach content analysis. Fatty acid profiles have the potential not only to give insight into food web interactions but also into food quality, temperature adaption, condition and the reproductive outcome of an individual (Dalsgaard et al. 2003, Tocher et al. 2003, Arts and Micheal T. 257-280 in Lipids in Aquatic Ecosystems 2009, Røjbek et al. 2014). Together with fatty acid analysis, stomach contents and stable isotopes provide an intresting tool to link oceanography, food web interactions and fish physiology like condition and reproductive outcome.

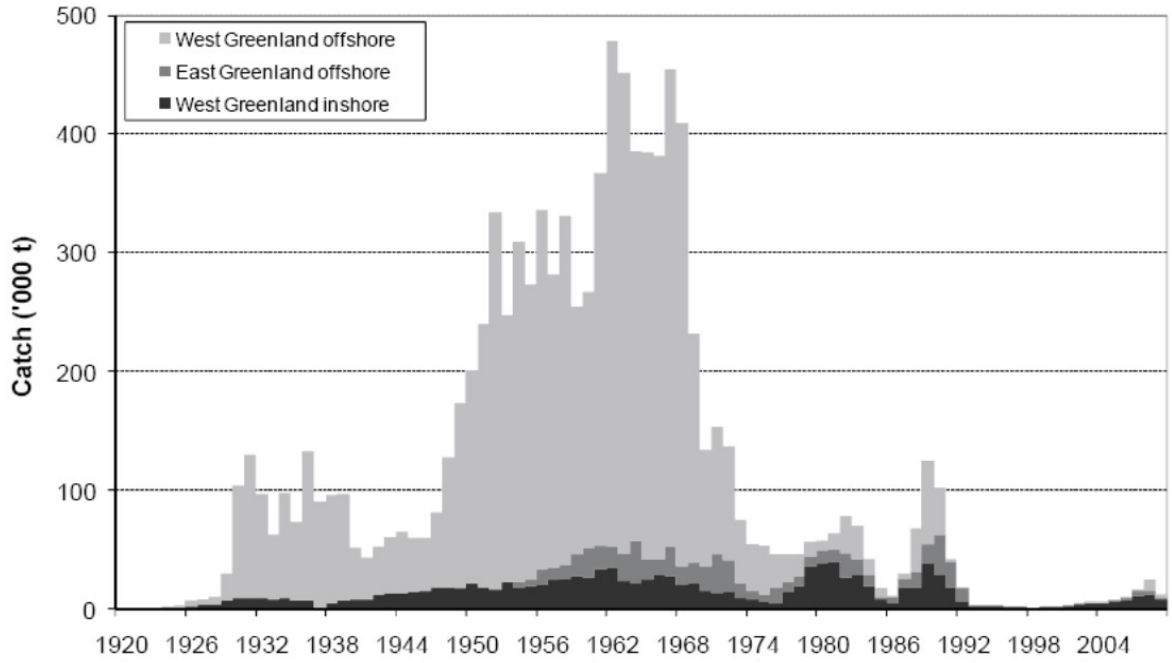


Figure 1.1: Atlantic cod landings in Greenlandic waters since 1920, divided in the three different stocks from this area (ICES Advice Book 2 2009)

1.2 Atlantic cod in Greenland waters

Atlantic cod (*Gadus morhua*) (Picture 1.2) is a widely dispersed demersal fish species in the North Atlantic where it is ecologically and economically highly important (Ottersen et al. 2006) and therefore of great interest in several research fields. Since the cold arctic waters represent the northern-most distribution range of this species (Hansen 1949, Buch et al. 1994), growth, recruitment and the productivity of the populations here do strongly depend on temperature (Rätz et al. 1999, 2003, 2005; Brander et al. 1995). The oldest reports of Atlantic cod in the waters around Greenland date back to the 16th century and its occurrence and abundance underwent large natural fluctuations since then (Schmidt 1931). From the late 1920s on fishing became an additional driver for changes in numbers (Figure 1.1). Today, cod in Greenland waters are thought to consist of three different stocks. Two offshore stocks in East and West Greenland and the inshore stock in the large and distinct fjord systems along the west coast (ICES 2018).

Picture 1.2: Female Atlantic cod (*Gadus morhua*) caught along the East Greenland coast in autumn 2017

All stocks show different migratory behavior (Figure 1.3) but at least for the offshore stocks similar egg drift patterns. In some irregularly occurring years of high recruitment in the waters around Iceland, there is also an influence of recruits from Iceland stocks (Begg et al. 2000, Wieland et al. 2002).

Eggs and larvae from East Greenland and Iceland stocks are drifting with the East Greenland current (Figure 2.1) from the spawning grounds in East Greenland and Iceland south-westwards, along the coast to West Greenland where they settle on offshore banks and migrate back as soon as they become mature (Rätz et al. 2005, Bonanomi et al. 2016, ICES 2018). The majority of individuals from stocks in the fjord systems are however sedentary (Hansen 1949, Storr-Paulsen et al. 2004). The stock on West Greenland offshore banks is nowadays comparably small (ICES 2018) and specimens are migrating along the West Greenland coast (Storr-Paulsen et al. 2004), causing a distinct age pattern along the coast of the island, with a larger proportion of older and better conditioned specimens in East Greenland and younger ones in south-west Greenlandic waters (ICES 2015). As a regional effect, cod in the waters around Greenland is in general (with some exceptions) of less good condition compared to more southerly populations (Rätz et al. 2003) and is showing an enhanced fluctuated recruitment. Since the arctic waters represent its northern-most range of distribution (Hansen et al. 1949, Buch et al. 1994), these effects are partly temperature dependent (Rätz et al. 1999; Ottersen et al. 2006).

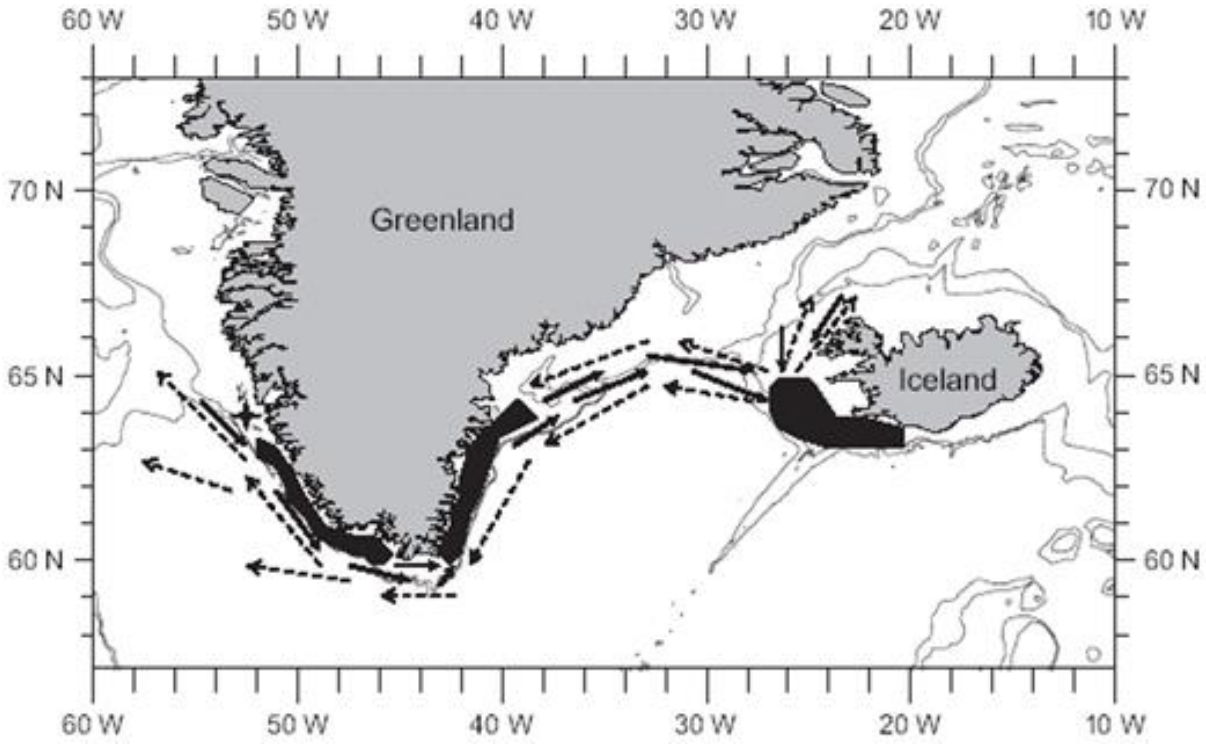


Figure 1.3: Migrations patterns of Atlantic cod around Greenland. Dashed arrows are indicating larval drifts with the Irminger, East Greenland and West Greenland Current. Black arrows are indicating homing migration of adult cod. (Rätz et al. 2005)

1.3 Lipids and fatty acids

The major role of lipids in fish is the storage of metabolic energy in form of ATP, that is provided by β-oxidation in mitochondria (Sargent et al. 1989, Froyland et al. 2000). One of the constituents in lipids are fatty acids. Fatty acids play an important role in every living organism from bacteria to plants to metazoans and hence fish. They are ubiquitous and involved in many main functional and structural traits of live, like cell membranes, energy storage (Tocher et al. 2003) and hormone production (Johnston et al. 1985). In fish they are the preferred source of metabolic energy used for growth, movement and therefore migration and reproduction (Tocher et al. 2003). Fatty acids mainly consist of a carbon chain that is either saturated (Saturated Fatty Acids = SFAs) and containing no double bonds or unsaturated containing one (Mono Unsaturated Fatty Acids = MUFAs) or more double bonds (Poly Unsaturated Fatty Acids = PUFAs and Highly Unsaturated Fatty Acids = HUFAs) plus a carboxyl group (Figure 1.4) (Tocher et al. 2003).

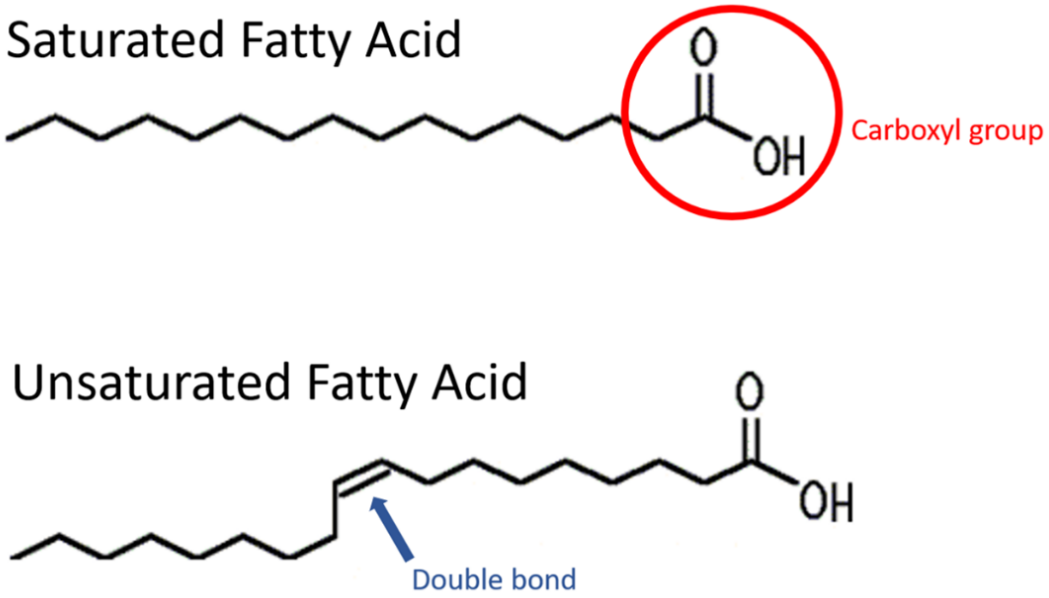


Figure 1.4: Schematic of the chemical structure of fatty acids. On top a saturated fatty acid (SFA) with no double bond, the carboxyl group is indicated by the red circle. At the bottom a mono unsaturated fatty acid (MUFA) with a cis double bond

Most natural fatty acids have an even number of carbon atoms and their structure and function e.g. the melting point, depend on the length of the carbon chain, the number and kind of double bonds (cis or trans) and their position within the carbon chain (Tocher et al. 2003). The predominant SFAs occurring in animal lipids are C16:0 and C18:0, they can be produced de novo by every animal (Sargent et al. 1989). The predominant MUFAs in lipids are C18:1n-9 and C16:1n-7. Whereas fish lipids, especially in high latitudes are also very rich in 20:1n-9 and 22:1n-11, originating from zooplanktonic wax esters from mainly calanoid copepod species (Sargent and Henderson, 1995). In fish the main PUFAs are Arachidonic acid (20:4n-6) and its precursor Linoleic acid (18:2n-6) and Eicosapentaenoic acid (20:5n-3) and Docosahexaenoic acid (22:6n-3) and their precursor Linolenic acid (18:3n-3). These precursors are essential fatty acids for all vertebrates and therefore fish, and can later on be elongated and desaturated to the PUFAs mentioned before (Tocher et al. 2003). This means that fish including cod are incapable of producing these PUFAs *de novo*. Cod liver is in general very rich in these PUFAs and therefore widely used to produce dietary supplements in form of fish oil capsules for humans. PUFAs have a crucial role for organisms. They are important structural parts of the cell membrane and therefore involved in cell communication and transport of matter as well as in the maintenance of membrane fluidity (Singer and Nicolson 1972) under changing temperatures (Farkas et al. 1981, Arts and Michael 2009). A subset of these PUFAs, the eicosanoids can act as precursors for hormones involved in several pathways including reproduction (Johnston et al. 1985; Dalsgaard

et al. 2003). Since marine food webs are rich in lipids unlike freshwater systems, these PUFAs are usually obtained directly from the food source instead of being anabolized from their precursors (Tocher et al. 2003). High lipid amounts in food are even suppressing a *de novo* fatty acid production (e.g. Shimeno et al. 1995 and 1996). By this mechanism the fatty acids are channeled up the food web (Dalsgaard et al. 2003). Depending on the group of phytoplankton dominating the first trophic level of the food web and also depending on environmental influences, fatty acid composition can vary significantly between individuals and areas (Dalsgaard et al. 2003). In the marine realm latitudinal differences in fatty acid profiles of copepod species were already found (Kattner and Hagen 2009). Additionally, climate and climate change can have an impact on plankton community composition and their fatty acid profiles (Dalsgaard et al. 2003, Kattner and Hagen 2009). Therefore, regional influences, like food sources and abiotic factors might also be manifested in the fatty acid profiles of cod in Greenland waters.

1.4 Importance of female fish condition and its linkage to lipid compositions

A keystone for the recovery of fish stocks is the spawning stock biomass, its age structure and the reproductive outcome of the individual fish, as well as the general condition (Kjesbu et al. 1991; Marteinsdóttir & Steinarsson, 1998; Berkley et al. 2004; Hixon et al. 2013; Rätz et al. 2002; Ottersen et al. 2006). Especially females and their condition have an important role for the survival of the offspring (Marteinsdóttir & Steinarsson, 1998; Scott et al. 1999). It is widely known that larger and older females (BOFFFFs) have the highest egg qualities and egg quantities (Berkley et al 2004; Hixon et al. 2013). Their spawning times are extended and batch numbers are higher compared to younger and more unexperienced individuals (Hixon et al. 2013). This is causing a higher survival and therefore larger contribution of offspring to recruitment from older females, due to an enhanced chance of matching spawning times with good environmental conditions (Hedgecock 1994). Hence maternal effects are of great importance for the health of fish stocks (Marteinsdóttir & Steinarsson, 1998) and the evaluation of longterm consequences of fisheries, as they have the potential to buffer extreme environmental fluctuations (Hixon et al. 2013). General condition, energy storage and resource allocation can be considered as some of these maternal effects. Cod is a lean fish, meaning the fat storage is basically located in the liver instead of muscles or around inner organs (Lambert & Dutil 1997b). These liver fat storages are then allocated to the gonads before spawning, causing a negative correlation of Hepatospmatic index (HSI) to Gonadosomatic index (GSI) (Røjbek et al. 2012). Especially the fatty acid composition of this fat storages, that is driven by the diet of the individuals (Dalsgaard et al 2003, Tocher et al. 2003, Røjbek et al. 2014), can later have an influence on the quality

of the offspring (Sargent et al. 1999). Røjbek et al. (2012, 2014) found a correlation between the ratios of important PUFAs Arachidonic acid, Eicosapentaenoic acid and Docosahexaenoic acid with the fecundity, the hatching success and survival of the larvae and the maturity stage of female cod. Whereas a total reduction of the fat content can force females to skip spawning in certain seasons (Rideout et al. 2006) and is related to a reduction in hormones that are involved in the gonadal development (Cerdá et al. 1994, Matsuyama et al. 1994). A shift in the diet and hence the fatty acid composition of Atlantic cod in the Baltic Sea was correlated with a shift in maturity and spawning times (Tomkiewicz et al. 2010) which can lead to a miss match between spawning times and environmental conditions suitable for the development of eggs and larvae.

1.5 Feeding patterns and stable isotopes

Different habitats in the marine realm are dictated by different abiotic and biotic environmental factors, such as current systems, temperature and salinity gradients or varying depth profiles, as well as predators or competitors, parasites, prey abundance and composition. In Greenland waters, cod feeds in pelagic and benthic habitats. Its most important prey species are capelin (*Mallotus villosus*), krill (*Meganyctiphanes norvegica*), benthic crabs (Majidae), redfish (*Sebastes* sp.), mesopelagic fish, amphipods (Hyperiidae and Gammaridae) and northern shrimp (*Pandalus borealis*) (Nielsen & Andersen 2001, Hedeholm et al. 2016, Werner et al. 2018). Importantly, diet composition is a strong predictor of condition, energy storage and lipid content in cod (Lie et al. 1988, Jobling 1988). While stomach content analysis show short-term feeding patterns, stable isotopes contain information about diet preferences weeks to month prior to sampling (Hobson 1999). Hence, stable isotope analysis can be used to examine food web interactions and trophodynamics. Stable isotope signatures are dependent of the source of isotopes (e.g. terrestrial or marine) and their fractionation (Peterson and Fry 1987). Most often Carbon and Nitrogen isotopes are used for this purpose. The mean fractionation from one trophic level to the next is about 3.4‰ for δ15N and 0.4‰ for δ13C (Post 2002). Carbon isotopes can also be used to determine benthic or pelagic food sources (Dunton 1989, Hobson 1994, Agurto 2007). By setting a source baseline, which is in marine pelagic systems preferably phytoplankton, and adding the information about fractionation of nitrogen isotopes, trophic levels of individuals can be determined (Post 2002) and a food web can be reconstructed. Since the isotope signature is shaped by a diversity of biogeochemical processes which differ between regions, stable isotope analysis can also be used to examine migration patterns and link individuals to a certain area they formerly occupied (Hobson 1999). For this study an isotope baseline is missing, which limits the explanatory power of the analysis. Nevertheless, the signatures are still able to display differences between fish, which are generated by different diets and different biogeochemical processes, in each habitat.

1.6 Oceanography

The fatty acid composition of an individual is largely influenced by its diet composition (Dalsgaard et al. 2003, Tocher et al. 2003; Jobling & Leknes 2010, Røjbek et al. 2014) and can as well as the general condition be influenced by the environment, hence the habitat or region that it lives in (Sargent and Henderson 1995). The samples for this study were collected from different habitats along the east and south-west coast of Greenland, separated by distance and climate regimes as well as current systems and differing in their depth profiles (Figure 2.1, Table 3.4). Sample collection started on the Dhorn Bank with sampling site 1 (Figure 2.1). The northern most sampling station is mainly of arctic influence. The mixture between the warmer and saltier Irminger Current and the East Greenland Current (Rudels et al. 2002) is providing high production and food abundance in this area. The short distance to the continental slope, plus the influence of the Irminger Current are linking sampling site 1 to the mesopelagic food web. Following the low saline and cold East Greenland Current southwards reaching Kleine Bank at sampling site 2 (Buch 2002), where samples were taken within a resident eddy system between the east Greenland current (EGC) and the east Greenland coastal current (EGCC) (Figure 2.1). The primary production in this area seems to be decoupled from the surrounding area with an earlier peak in spring blooms and inter annual changes in zooplankton communities (Fock et al unpublished, Stippkugel 2018). Ending at the southern tip of Greenland at sampling site 3, around the corner of Cape Farwell that is dominated by warmer North Atlantic waters (Buch et al. 1994), keeping this region mainly ice free and representing subarctic conditions (Buch 1990,2000) and also having the shallowest sampling depth (Table 3.4).

1.7 Importance and research objectives

Habitat can have a a strong influence on the condition (Pardoe et al. 2008) and reproduction of fish (Kjesbu et al. 1991), especially at the extremities of their distribution ranges, where recruitment success can be highly sensitive to environmental fluctuations (Berkley et al. 2004). This sensitivity towards extreme environmental fluctuations is even more enhanced in populations, which experience high fishing pressure, that is disturbing the age structure in spawning stock biomass (Berkley et al. 2004). Well-conditioned females can positively influence the buffering capacity towards environmental fluctuations and hence increase the recruitment success in these environments (Ottersen et al. 2006, Hixon et al. 2013). Regional effects on the condition and reproductive outcome of female Atlantic cod should therefore be considered in stock management, since the populations in Greenland waters still show a lack of resilience and could never recover from the severe collapse in the late 20th century (ICES 2018a).

The different regional influences of oceanographical and biological origin in the waters around Greenland, indicate the existence of regional differences in food abundance and feeding behavior (benthos vs. pelagic). This is causing differences in the general condition of cod (Werner et al. 2018) and will probably also cause differences in its total lipid content as well as differences in the fatty acid composition of the individuals and hence differences in the reproductive outcome of cod in Greenland (Røjbek et al 2014). In order to reveal the existence of regional differences in condition and reproduction of female Atlantic cod in Greenland waters, that has been and still is suffering from high fishing pressures, this study will investigate potential differences in feeding preferences by looking mainly at total lipid contents of livers and ovaries as well as the fatty acid composition of the later and different ratios in stable isotope enrichments.

2

Methods

2.1 Sampling

Samples were collected during the annual German Bottom Trawl Survey in Greenland with the FFS Walther Herwig III of the Thünen Institute for Sea Fisheries from 06.10.2017 to 17.11.2017 (WH410). Three different sites in East and South Greenland were sampled (Figure 2.1, Table 3.4).

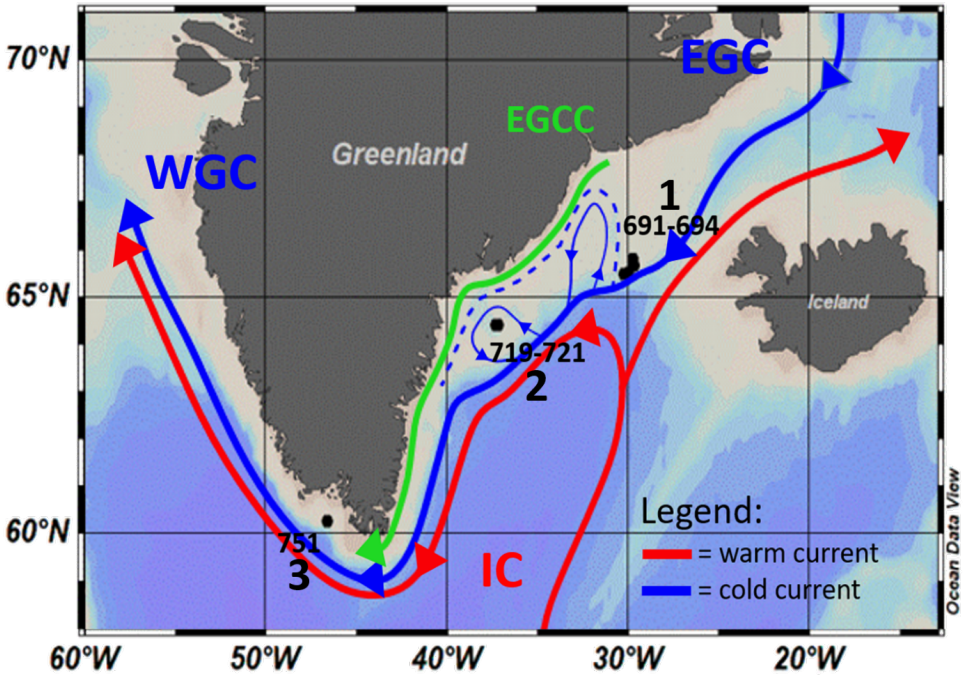
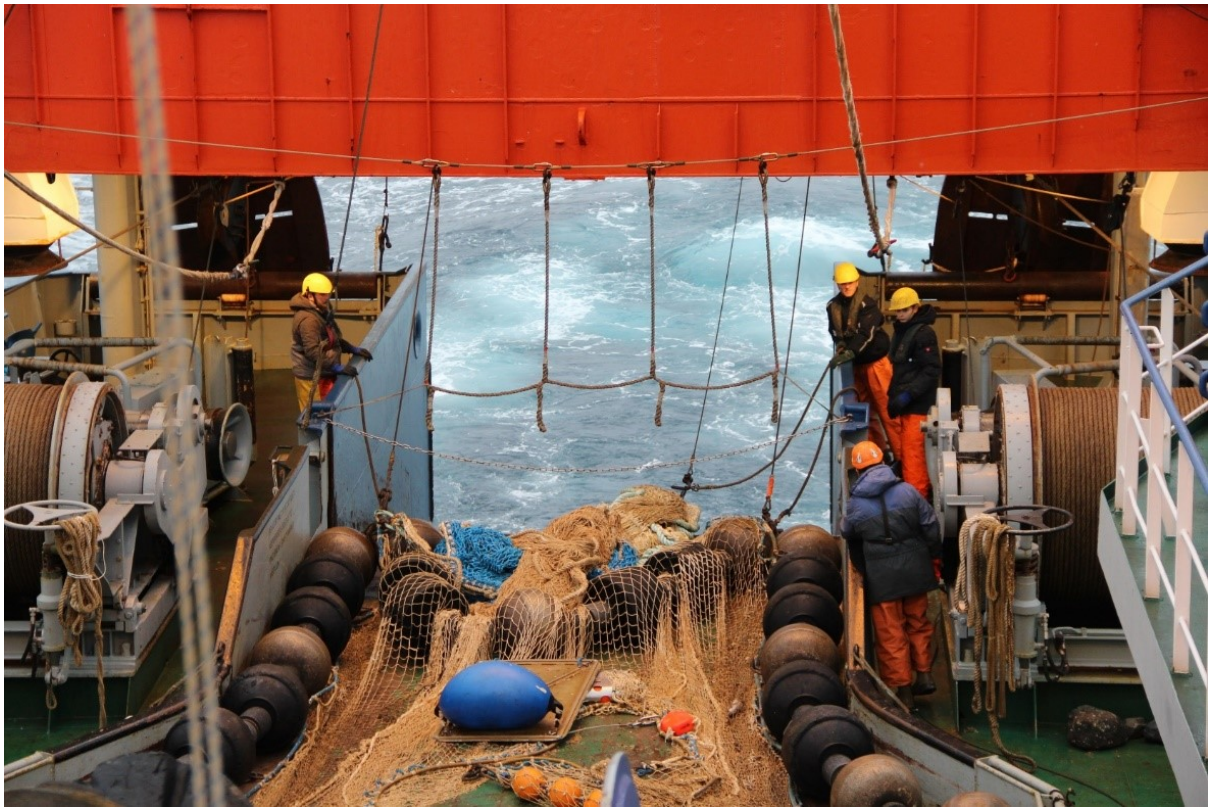


Figure 2.1: Schematic Greenland current systems, sampling stations and sites. Solid lines show observed paths of the East Greenland current (EGC), East Greenland coastal current (EGCC), and the Irminger current (IC), while dashed lines indicate possible flow paths induced by bathymetric or wind effects. WGC= West Greenland Current. Adapted from Sutherland and Pickart (2008)

In order to collect only mature female cod, a size range of 75-90cm was chosen. At least 10 specimens were sampled per site. A survey bottom trawl net (BT140 bobbin gear) (Picture 2.2) with pony otter boards and a cod end mesh size of 20mm was used to collect the samples (Supplementary).



Picture 2.2: Deploying the BT140 (bottom trawl) net in Greenland autumn 2017

Right after the catch, fish were slaughtered and total, gutted, gonad and liver weight, as well as total length were collected as part of the biological sampling protocol on board. The otoliths were dissected from the head and kept in paper bags for age determination. Stomachs were dissected from the abdominal cavity and stored in plastic bags kept on ice, until final freezing at minus 30°C. The gonads and livers were kept on ice until the final tissue sampling for total lipid content and fatty acid composition could be done. Gonad tissue was always sampled from the right lobe, even though ovaries of Atlantic cod are presumed to be homogenous (Witthames et al. 2009). Liver tissue was always sampled from the smallest lobe, after carefully removing the macro liver parasites, to make sure only pure liver tissue was sampled. The tissue samples were collected then in 2.5 ml cryo-tubes and stored in liquid nitrogen (-196°C). Tissue samples for stable isotopes were taken from the left side of the dorsal muscle behind the head and kept in cryo-tubes at minus 30°C.

2.2 Lipids and fatty acids

2.2.1 Total lipid content of livers and gonads

The extraction of lipids was done by a modified version of the methods described in Bligh and Dyer (1959) and Folch and colleagues (1956). Subsamples of liver and gonad tissue were freeze dried for at least 24 hours in a Christ Alpha 1-2 LDplus freeze-dryer, with a condenser temperature around -63°C and a pressure of about 0.36 mbar. Afterwards the tissue was extracted three times for 24h at -20°C, using a 1:1:1 Chloroform/ Dichloromethane/ Methanol (CHCL3/ CH2CL2/ CH3OH) extraction mix. The total fat content was then determined gravimetrically by subtracting the weight of the extracted tissue from the tissues dry weight.

2.2.2 FAME (Fatty Acid Methyl Ester) analysis

The extraction of lipids was done by a modified version of the methods described in Bligh and Dyer (1959) and Folch and colleagues (1956). By using this method, all fatty acids from all lipid fractions are extracted and after using the following steps esterified and quantified. A supplementary assignment to the lipid fractions is not possible and would need prior separation of the lipid extract in silica columns. Fatty acid methyl esters were measured for gonad tissue samples.

Extraction and internal-standard additions

Wet weights of all gonad tissue subsamples were taken. Subsequently the tissue samples were freeze dried for about 24 hours in a Christ Alpha 1-2 LDplus freeze-dryer, with a condenser temperature around -63°C and a pressure of about 0.36mbar. Gonad samples were afterwards grinded to a powder by using a mortar. Dried sub samples were weight into glass vials and the lipids were extracted with an addition of 3 ml CHCL3/ CH2CL2/ CH3OH Chloroform/Dichloromethane/Methanol (1:1:1) for at least 12 hours at -20°C. 100μL of C19:0 FAME (c=22.03$\frac{ng}{\mu L}$) and C21:0 (c=30.08$\frac{ng}{\mu L}$ and 30.09 $\frac{ng}{\mu L}$) as a fatty acid were added as internal standards.

Clean up

The extracted lipids were transferred to a separation funnel and the glass vials were spilled 2 times with 2 ml of the CHCL3/ CH2CL2/ CH3OH (1:1:1) solvent mixture. The chloroform layer was then separated by adding 2.25ml of 1M KCL solution. The lower layer was kept in a conical flask. The upper layer was rinsed 2 times with dichlormethane. NaSO4 was added to the conical flasks to avoid any water in the extract. Afterwards the extract was transferred to a centrifuge tube (2 times of re-extraction with 1.5ml dichlormethane).

Evaporation and pre-preparation

The extract was cooled down for one hour at -20°C. Subsequently the extract was reduced to total dryness in a rotary film evaporator (Heidolph Laborota 4000 efficient). The extract was re-dissolved with 100μL Chlorofrom (CHCL3) and transferred into a glass cocoon (Two times of rinsing the centrifuge tubes with 100μL CHCL3). Finally, the solvent was again removed by using the rotary film evaporator.

Esterification

After evaporation 100 μL of Toluene and 200 μL of 1% H2SO4 in CH3OH (50 ml CH3OH + 0.233 μl of concentrated H2SO4; 97%) was added to the cocoon. Afterwards the cocoon was flushed with N2, closed and heated up to 50°C for at least 12 hours by using a sand bath.

Re-extraction and analyses

After an addition of 300 μL of 5% sodium chloride solution (2.5 g NaCl in 47.5g Milli-Q water) the esters were then extracted in 3 portions of 100 μL n-hexane into a new glass cocoon. The solvent was reduced until dryness by using the rotary film evaporator again. The extract was re-dissolved with n-hexane to a final volume of 100 μL. 1μl of the final extract was analyzed by using a fast gas-phase chromatograph (Thermo ELECTRON CORPORATION Trace GC Ultra) coupled with an autoanalyzer (Thermo SCIENTIFIC AS 3000). The injection was splitless on a capillary column and hydrogen was used as carrier gas. An external standard of Supelco® 37 Component FAME Mix (supplementary) with a concentration of 40ng C19 per 1μl and a Bacterial Acid Methyl Ester (BAME) Mix (supplementary) were run three times and once before every set of analysis. Peak identification was done by retention times, comparing the external standard to the actual sample. Concentrations of fatty acids were afterwards calculated based on the peak area of the C19:0 internal standard. The internal C21:0 standard was used to check for the quality of esterification. For statistical analysis and plotting of data, the total quantities of fatty acids in Nanograms were chosen, to gain an overview about the actual energy storage and resource stocks of the gonads. Together with the percentage values depletions or enrichments in certain fatty acids or fatty acid groups can be determined.

2.3 Condition factors and liver health

2.3.1 Condition factors

Because gadoid fish species (Lloret, Shulman & Love, 2013), such as cod, store largest parts of their energy in the liver, the hepatosomatic index (HSI) as percentage contribution of liver weight to gutted fish weight, was chosen as main index of condition and energy storage (Lambert

& Dutil 1997b). Furthermore, HSI is more sensitive to diet variability and can better reflect spatial differences of diet (Jobling et al. 2010, Pardoe et al. 2008). The morphometric condition factor K, Hepatosomatic index (HSI) and Gonadosomatic index (GSI) were calculated for each individual using the following equations:

$$K = \frac{W}{L^3}$$

$$HSI(Hepatosomaticindex) = \frac{liverweight}{guttedweight} * 100$$

$$GSI(Gonadosomaticindex) = \frac{gonadweight}{guttedweight} * 100$$

2.3.2 Liver parasites and tissue

During sampling and parasite dissection, differences between the consistence of liver tissues were recognized. These differences might affect liver physiology and therefore, a liver tissue index was developed to document these differences. In the laboratory livers were defrosted in a warm water bath. The index was determined by using the following criteria (Table 2.1, Picture 3.3, Picture 3.4, Picture 3.5), before dissecting all parasites from the tissue.

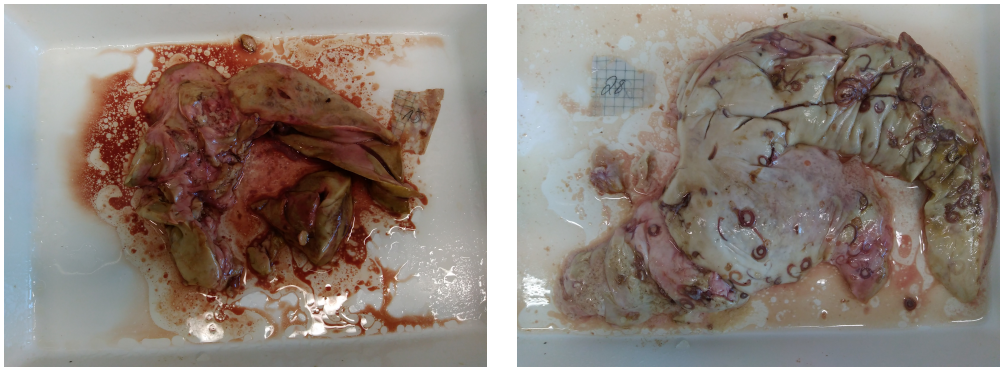
Table 2.1: Criteria to determine the liver tissue degradation

Tissue Index	Criteria
0	Tissue not degenerated, solid/hard
1	Low degeneration, mostly solid/hard tissue
2	Medium degeneration, hardly any solid/hard tissue left
3	Strong degeneration, no solid/hard tissue left
X	Ulceration

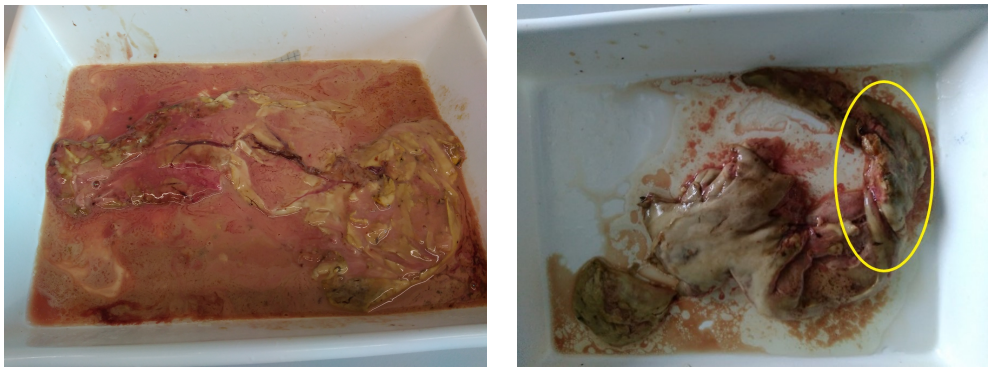
After determination of the liver tissue index, all macro liver parasites (Picture 4.4) were dissected carefully with forceps and weighted. The Parasite index represents the percentage contribution of liver macro parasites to liver weight and was calculated by using the following equation:

$$ParasiteIndex = \frac{TotalParasiteWeight}{Liverweight} * 100$$

Picture 2.3: Liver tissue index 0. Liver tissue is totally solid



Picture 2.4: Liver tissue index 1 (left) and liver tissue index 2 (right). Liver tissue index 1 still has large solid parts (right site) and only little degradation (middle). Liver tissue index 2 only has small solid parts left (lower part of the liver) the rest is mainly fluid and only held together by the outer membrane of the liver. This liver also shows a high macro parasite loading



Picture 2.5: Liver tissue index 3. No solid tissue parts are left. Full degradation of the tissue. Yellow circle at the right picture indicates an ulceration with encapsulated parasites

2.4 Feeding patterns and stable isotopes

Stomach content analyses were made, in order to gain an overview of the feeding preferences of the individual fish of different habitats. The stomachs were defrosted in a warm water bath. Full stomach and empty stomach weights were taken before the content analysis. The stomachs were cut open by using scissors. The content was then sorted and the ingested organisms were if possible, identified down to species level, counted and weight. Fish prey was identified by their otoliths, using the Otolith Atlas by Campana (2004) and our own reference collection. Invertebrates were identified by using the following literature (Schneppenheim & Weigmann-Haass 1986; Baker et al. 1990; Hayward & Ryland 1990a, b). Digestion rates and filling degree were determined using the following categories (Table 2.2, Table 2.3):

Table 2.2: Criteria to determine the degree of filling of stomachs

Filling Degree	Criteria
0	Stomach is empty, stomach wall is thick, distinct gastric rugaes
1	Stomach is partly filled, stomach wall is thick, distinct gastric rugaes
2	Stomach is filled, stomach wall is thinner, some parts smooth with no rugaes
3	Stomach is fully filled, stomach wall is thin, no gastric rugaes

Table 2.3: Criteria to determine the digestion rate of prey species dissected from stomachs

Digestion Rate	Criteria
1	No signs of digestion, net feeding
2	Small signs of digestion
3	Medium signs of digestion
4	Strong digestion, organism is only identifiable by special features left (e.g. otoliths)
5	Full digestion, organism is not identifiable anymore

Table 2.4: Prey categories to determine prey origin and prey index

Prey Categories	Prey Orignin
1	Benthic
2	Unkown
3	Pelagic

A Prey Index was calculated by using the following prey categories and equation to determine differences in the food preferences of each fish (Table 2.4):

$$PreyIndex = \frac{PreyCategory * PreyWeight}{TotalWeightStomachContent}$$

The closer the prey index is to the value of a prey category the more the individual was preying on prey from this certain category. To determine differences in the diversity of the prey, the Shannon-Index, which takes into account both the number of species and its proportion, was calculated for each stomach by using the following equation:

$$H' = \sum_{i=1}^{S} p_i ln p_i$$

$$H' = diversity$$
$$p_i = weightofeachspecies$$
$$S = numberofspecies$$

In addition to the stomach content analysis, which reflect short term diet patterns, stable isotope analyses were performed to have a better understanding of the trophic interactions and long-term feeding preferences of the fish. Therefore, the muscle tissue was freeze dried for at least 12 hours in a Christ Alpha 1-2 LDplus freeze-dryer, with a condenser temperature around -63°C and a pressure of about 0.36mbar. Afterwards tissue was grinded to a fine powder by using a mortar. Tissue powder was weighted in tin capsules (3.2x4mm, HEKAtech GmbH, Wegberg/Germany), using a Sartorius micro-balance with a resolution of 10-4mg. A sample amount of 0.04-0.06mg was used for analysis (Hansen et al. 2009). Between every step all tools were cleaned by using fuzz free cleaning tissues and Acetone. The analysis was done by a high sensitivity elemental analyzer (CE INSTRUMENTS EA1110) coupled with an isotope mass spectrometer (DeltaPlus Advantage, Thermo Fisher Scientific). Acetanilide (C8H9NO) was

used as internal standard and measured in between samples to calibrate the measurements. The ratios were calculated by the following equation (Hansen et al. 2009):

$$\delta \mathrm{X} = (\frac{Rsample}{Rstandard} \text{ - } 1) * 1000$$

X = δ15N or δ13C
R = 15N:14N or 13C:12C

2.5 Oceanography

All hydrographic data were collected using a CTD probe (Seabird 911+ carousel). Not all of the fishing stations had actual CTD data. Therefore, data from the closest CTD station was chosen by using the following distance equation:

$$Distance = 2 * 6371000 * arcsin\sqrt{sin(\frac{LAT2 * \pi}{180}) - (\frac{LAT1 * \frac{\pi}{180}}{2})^2 + \sqrt{\cos(\frac{LAT2 * \pi}{180}) * cos(\frac{LAT1 * \pi}{180}) * \sin(\frac{LONG2 * \pi}{180}) - (\frac{LONG1 * \frac{\pi}{180}}{2})^2}}$$

LAT1 = Latitude sampling station
LAT2 = Latitude corresponding station
LONG1 = Longitude sampling station
LONG2 = Longitude corresponding station

2.6 Statistical analysis

All statistical analysis was done with R version 3.3.1 (2016-06-21)(R Development Core Team (2008)). Data selection was done using the dplyr package (Wickham et al. 2017). Statistical analysis was divided in a univariate and a multivariate part. For the univariate part all measured variables were tested separately for significant differences between the sites by using two non-parametric tests. Fatty acids were tested as individuals and once in groups (SFAs, MUFAs, PUFAs, TFAs). A Kruskal-Wallis test was done for every variable to check for general significant differences between the sites. All variables that were significantly different in the Kruskal-Wallis

testing were then subsequently analyzed by using a post hoc pairwise Wilcoxon-rank-sum test, that revealed which sites were significantly different from each other. As the first part of the multivariate statistical analysis a PCA (Principal Component Analysis) was used to visualize the existing differences between the sampling sites and to determine the main drivers for differences. Therefore, all measured fatty acids and fatty acid groups plus the explanatory variables for site were used as variables in this PCA. To know which of these variables to prefer for the PCA a similarity percentage (SIMPER) analysis was done initially using the vegan package (Oksanen et al. 2018). The SIMPER function performs pairwise comparisons of groups of sampling units and finds the average contributions of each species to the average overall Bray-Curtis dissimilarity. The PCA was done by using the R packages FactoMineR (Le et al. 2008) and factoextra (Kassambara et al. 2017) and interpreted by using the following biplot-rule adapted from Leyer & Wesche (2007). A PCA is using a similarity matrix of variables based on the Pearson correlation coefficient, to extract an axis, here called dimension, that is explaining the most variance in the data. The second axis or dimension is then generated by the maximum amount of variance left and is orthogonal to the first axis or dimension. This continues until all variance is represented in a dimension. The less dimensions it takes to explain the most variance in the data, the better. The variables itself are represented as vectors, originating from the middle of the newly formed coordinate plane. On the basis of their length and position to each other and to the dimensions, the correlation between the variables itself and between the variables and the axis can be displayed. The correlations between variables and axes is additionally described by loadings. They reach from +1 to -1 and equal therefore the Pearson correlation coefficient. If a variable has a high loading on one axis or dimension, it increases (if it is a positive loading) or decreases (if it is a negative loading) strongly with this axis. The correlations among the variables can in simple words be displayed by the angle Îś in which they are position to each other. An angle below 90° is a positive correlation, an angle of 90° corresponds with no correlation at all and above 90° is a negative correlation. To integrate the samples, in this case the individual fish, in this coordinate plane, every value of the variable for each sample is multiplied by the axis loading of the variable. These products are then summed up for every variable and transferred as a coordinate into the plane. As a second step of the multivariate statistical analysis, two Redundancy Analysis (RDA) were done, using the vegan package (Oksanen et al. 2018) in R. For the first RDA model the fatty acid composition in nanograms was used as the response matrix. For the second RDA model fatty acid groups were used. Explanatory variables were chosen using a SIMPER analysis of all condition indices, hydrographical data and all other parameters e.g. liver macro parasites and stable isotope analysis, that were taken and significant in the Kruskal-Wallis test. Because fatty acid composition is mainly dependent on the diet, a second SIMPER analysis was done for stomach analysis data. The most influential species of both outputs, were then used as the explanatory matrix for both of the models. By using the anova.cca

function that is performing an ANOVA like permutation test for the RDA the significance of constrains was assessed. Because the total values of fatty acids have a large margin from 0 to above 300 ng/mg fourth root transformation of the data was done before the analysis.

3 Results

3.1 Lipids and fatty acids

3.1.1 Total lipid content of livers and gonads

The total fat content of gonads is the highest at sampling site 3 (Figure 3.1), even though the difference is not significant. The lowest mean fat content in gonads was found at site 2. The highest fat content in livers was found at sampling site 1 and is decreasing then gradually towards sampling site 3. The difference between total fat content of livers at site 1 and 3 is significant (Kruskal-Wallis chi-squared = 8.4688, df = 30, p-value = 0.01449).

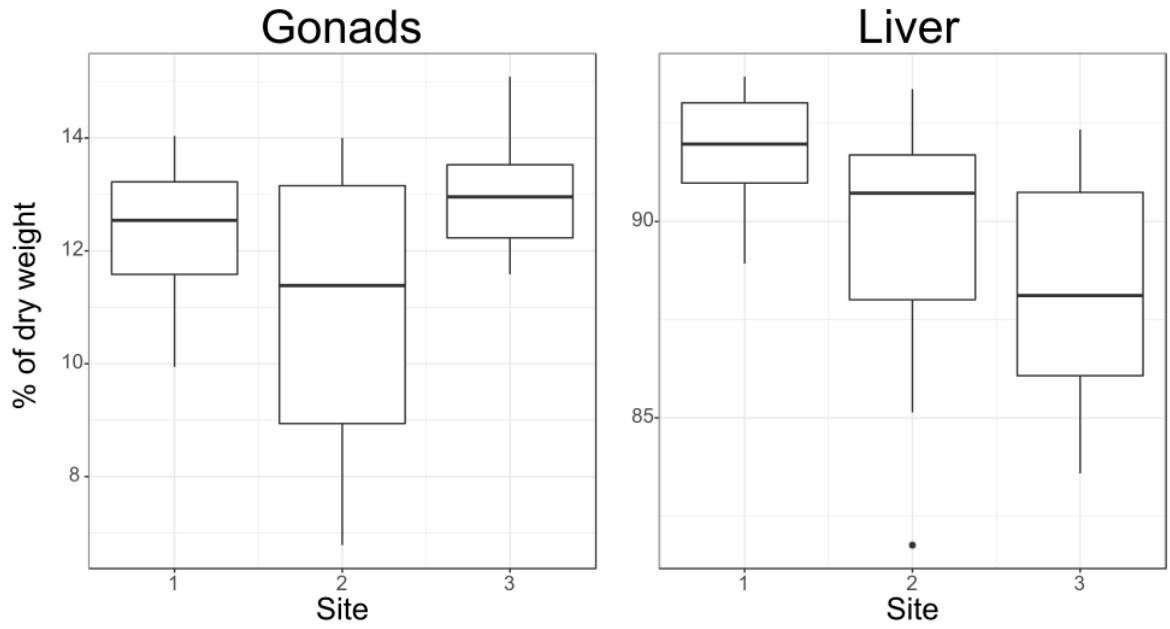


Figure 3.1: Total lipid contents of gonads (left) and livers (right). On the x axis are the three sampling sites. The y axis shows the fat content in percent of dry weight

3.1.2 Fatty acid profiles of gonads

Overall site 1 showed highest total amounts for gonad fatty acids in all 4 groups of fatty acids and highest values for Arachidonic acid and Docosahexaenoic acid (Figure 3.2). Significant are

the differences in the EPA:ARA ratio. Where site 3 has significantly higher values compared to both other sites (Kruskal-Wallis chi-squared= 12.808, df= 30, p-value= 0.001655, Pairwise Wilcoxon 3_1 p-value= 0.0078; 3_2 p-value= 0.0022). Between the amount of MUFAs of site 1 and both other sites, where site 1 has significantly larger amounts (Kruskal-Wallis chi-squared= 8.3226, df= 30, p-value= 0.01559, Wilcoxon 1_2 p-value= 0.028; 1_3 p-value= 0.027). The difference between total amount of gonad fatty acids (TFA) is significant in a Kruskal Wallis test (Kruskal-Wallis chi-squared= 6.5135, df= 30, p-value= 0.03851), but the post hoc Wilcoxon test is insignificant for the combination of all sites. Nevertheless, also other groups of fatty acids show trends of differences between the sites. The amount of ARA, is similar between site 1 and 2 with a mean of 12.83$\frac{ng}{mg}$ dry weight and 12.4$\frac{ng}{mg}$ and relatively lower at site 3 with a mean of 10.72$\frac{ng}{mg}$ dry weight. Looking at EPA on the other hand site 3 has the highest mean value (106.62$\frac{ng}{mg}$ dry weight) resulting in the highest EPA:ARA ratio of all sites (9.97). And site 2 has the lowest mean value for EPA (87.29$\frac{ng}{mg}$). For DHA site 1 has the highest mean value with 123.1$\frac{ng}{mg}$ dry weight and site 2 and 3 have similar means of 100.46 and 108.17$\frac{ng}{mg}$ dry weight. The saturated fatty acids are more or less the same at all sites, site 1 is slightly higher than the other two. This is the same for the PUFAs.

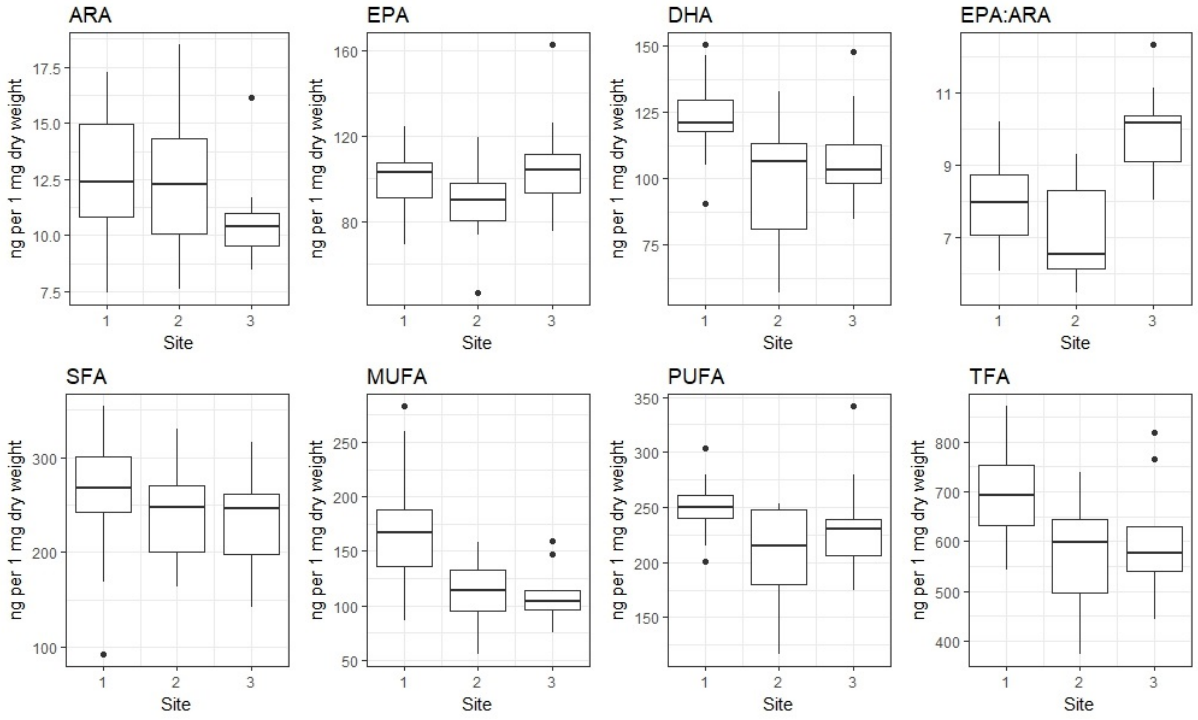


Figure 3.2: Box and whisker plot of the mount of the most important gonad fatty acids and fatty acid groups in ng/mg dry weight. The three sampling sites are displayed on the x axis. The y axis shows fatty acid amounts in ng/mg dry weight. Arachidonic acid (ARA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), poly unsaturated fatty acids (PUFA), total fatty acids (TFA)

The dominance of site 1, having the highest total values of gonad fatty acids in all four groups (SFAs, MUFAs, PUFAs, TFAs), is not pronounced in the percentage values for gonad fatty acids in proportion of the total fatty acid amount (TFA) (Figure 3.3). Only differences between Eicosapentaenoic acid and MUFAs are significant (Kruskal-Wallis EPA: chi-squared = 13.475, df = 30, p-value = 0.001186; Kruskal-Wallis MUFA chi-squared = 12.055, df = 30, p-value = 0.002411). Eicosapentaenoic acid values are highest at site 3 (Pairwise Wilcoxon EPA: 3_1 p= 0.00042, 3_2 p= 0.01026), as well as the differences between EPA:ARA ratios of site 3 and both other sites, as these stay the same as for the total values. Despite of having outliers the difference between site 1, which has the highest percentage contribution of MUFAs to total fatty acids, and both other sampling sites are significant (Pairwise Wilcoxon MUFA: 1_2 p= 0.0449, 1_3 p= 0.0025). In total contrast to the total values discussed before, PUFAs have the highest contribution to total fatty acids at site 3. And also, the values for Arachidonic acid shifted compared to the total values.

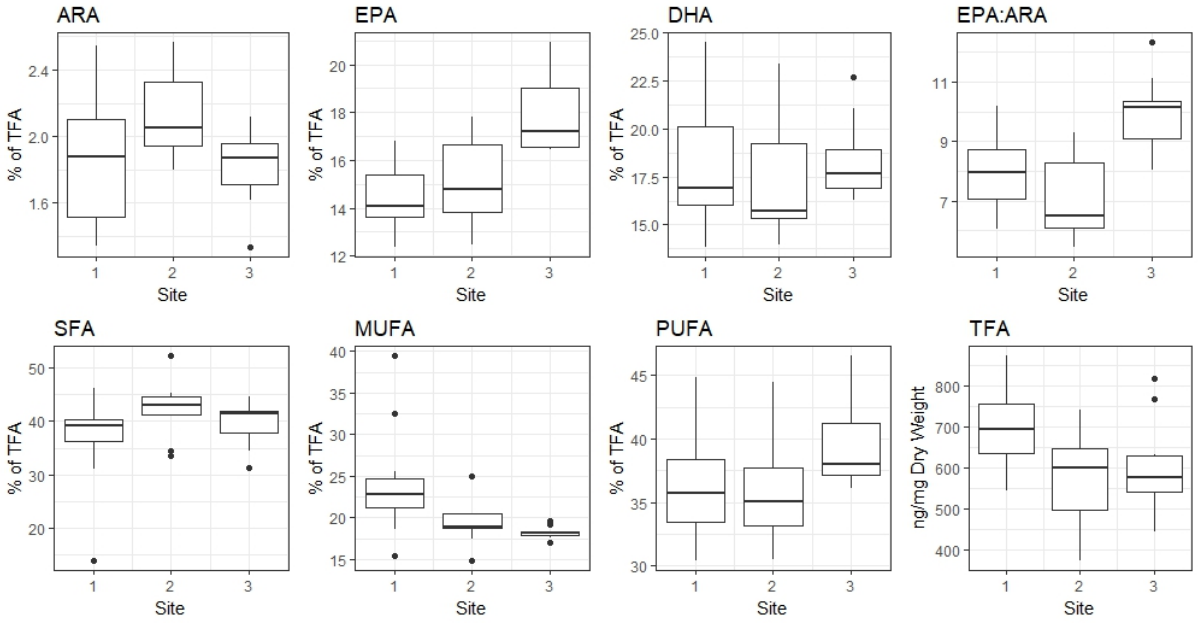


Figure 3.3: Box and whisker plot of the percentage contribution of the most important gonad fatty acids and fatty acid groups to total fatty acids (TFA). The three sampling sites are displayed on the x axis. The y axis shows fatty acid contribution in percent of total fatty acids. Arachidonic acid (ARA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), poly unsaturated fatty acids (PUFA)

3.2 Condition indices and liver health

3.2.1 Condition indices

Table 3.1: Condition indices and fish parameters. Mean Hepatosomatic index (HSI), Gonadosomatic index (GSI), condition factor K, length in centimeters, age in years and weight in grams per site are listed with standard deviation. (Site 1 n= 12; Site 2 n= 10; Site 3 n= 10)

	Site 1 n = 12			Site 2 n = 10			Site 3 n = 10		
Age	6.167	±	0.898	6.111	±	1.197	7.273	±	0.962
Weight	5990.583	±	838.386	4782.500	±	829.480	5709.545	±	602.002
Length	84.750	±	3.982	81.600	±	3.878	84.000	±	3.286
K	0.979	±	0.059	0.873	±	0.081	0.959	±	0.067
HSI	10.462	±	1.973	5.813	±	2.269	5.362	±	1.275
GSI	2.355	±	0.333	1.361	±	0.366	2.304	±	0.979

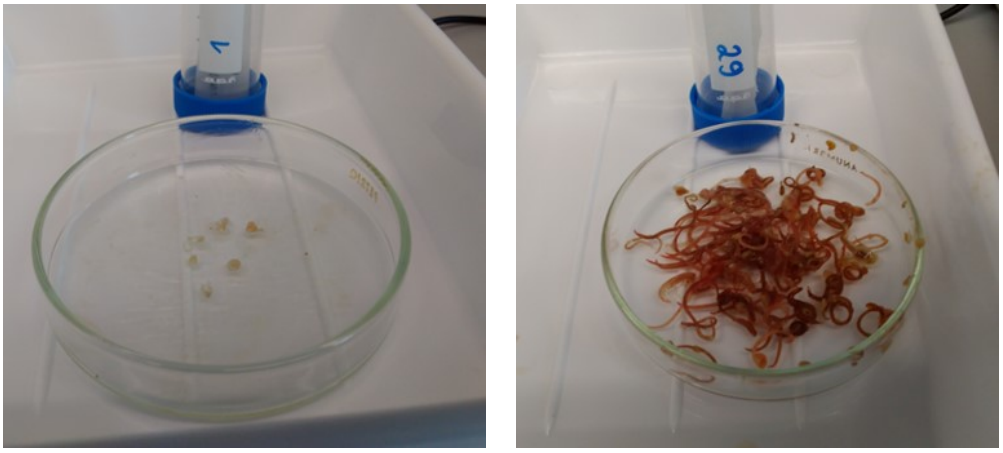
Fish caught at site 2 were on average 3 cm shorter than at sites 1 and 3 (Table 3.1). Individuals at site 3 are about the same size, but one year older than individuals from site 1 and 2, which indicates a faster growth of individuals at site 1. The condition factor K is about 10 percent lower at site 2 compared to both other sites, but the difference was not significant in a Kruskal Wallis test. Site 1 and 3 show similar mean GSI values, the differences between GSI of site 2 and both other sites is significant (Kruskal-Wallis chi-squared = 17.899, df = 30, p-value = 0.0001298, Pairwise Wilcoxon 1_2 p-value= < 0.001, 2_3 p-value= 0.00049). Whereas the values for HSI are lower than at site 1 and even lower than at site 2 (Kruskal-Wallis chi-squared = 19.008, df = 30, p-value = < 0.001, Pairwise Wilcoxon 1_2 p-value= 0.00031, 1_3 p-value= < 0.001, 2_3 p-value= 0.73936).

3.2.2 Liver parasites and tissue

Site 3 showed by far the highest amounts of macro liver parasites in proportion to the liver weight (mean parasite index of 0.62) and highest degrees of liver degeneration (Table 3.2). The parasite index between sites differs significantly (Kruskal-Wallis chi-squared = 14.152, df = 30, p-value = 0.000845). The difference of the tissue indices between sites was insignificant in a Kruskal Wallis test, but showed significant differences between site 3 and both other sites in a post hoc Wilcoxon pairwise test (3_1 p-value= 0.0003; 3_2 p-value= 0.005).

Table 3.2: Mean parasite indices and liver tissue indices per site are listed with standard deviation (Site 1 n= 12; Site 2 n= 10; Site 3 n= 10)

	Site 1			Site 2			Site 3		
Parasite Index	0.040	±	0.020	0.082	±	0.085	0.619	±	0.494
Tissue Index	0.083	±	0.276	0.600	±	1.020	2.300	±	0.640



Picture 3.4: 6 Liver macro parasites from an individual of site 1 (left) and an individual of site 3 (right)

3.3 Feeding patterns and stable isotopes

Prey indices between sites differed significantly (Figure 3.5, Table 3.3) (Kruskal-Wallis chi-squared = 22.766, df = 30, p-value = < 0.001). The prey composition of site 1 was mainly dominated by mesopelagic fish, a mix of crustaceans of which the habitat origin could not be determined properly and *Themisto libellula* a pelagic amphipod (Figure 3.6). This is supported by prey indices of site 1 (average prey index= 2.5) (Table 3.3), which intend a prey composition of an unknown origin with a tendency to pelagic origin, driven by consumption of mesopelagic fish and *Themisto libellua*. Mesopelagic fish were only preyed at Site 1, with exception of two fish at site 3 (Fish ID 25, 26). Same for *Themisto libellua* with the exception of two fish at site 2 (Fish ID 15, 17). Site 2 has a prey composition and an average prey index of 2.87, that suggests a pelagic food spectrum, being dominated by capelin (*Mallotus villosus*) and Euphausids. In contrast site 3 shows a clearly benthic dominated prey composition with an average prey index of 1.28 (Table 3.3). Main diet items at site 3 were benthic crabs (*Hyas* sp.), sea cucumbers (Holothuria) and brittle stars (Ophiuroidea). Four individuals of site 3 also had a proportion of pelagic food (Euphausiids, Hyperiidae and mesopelagic fish) in their stomachs (Fish 23, 24, 25, 26) (Figure Stomach content).

FishID	Site	Prey Index
1	1	1.718
2		2.633
3		2.874
4		2.535
5		2.378
6		2.667
7		2.979
8		2.409
9		2
10		2.382
11		2.849
12		2.856
13	2	2.950
14		2.961
15		2.996
16		2.906
17		2.819
18		2.46
19		3
20		2.886
21		2.779
22		2.954
23	3	1.169
24		2.199
25		1.694
26		1.054
27		1.108
28		1.115
29		1.117
30		1
31		1.298
32		1.019

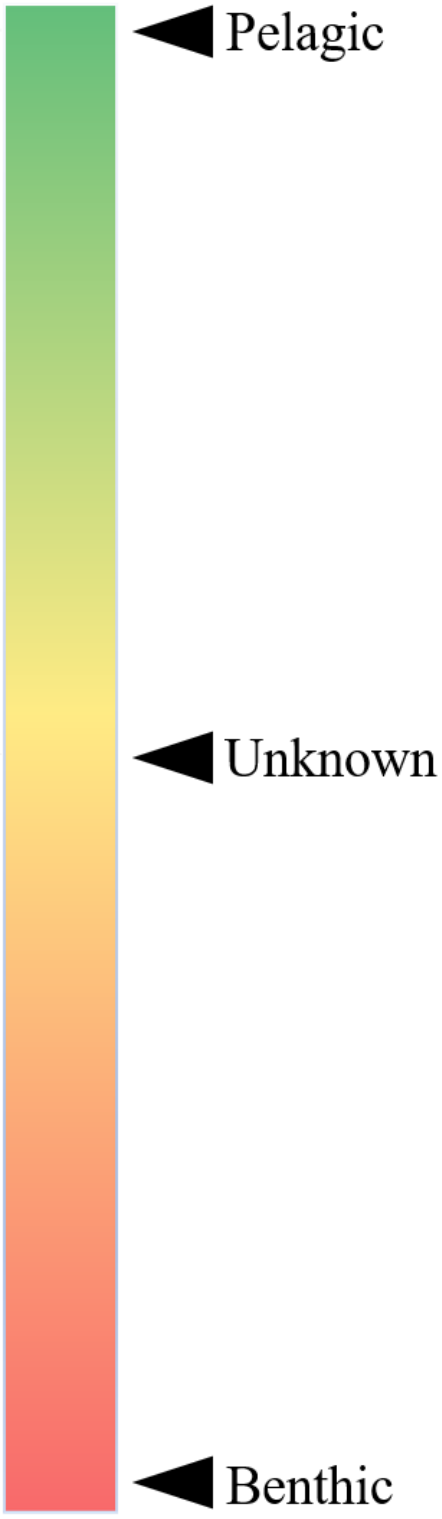


Figure 3.5: Prey indices for every individual fish and site. Colours indicate the main prey origin

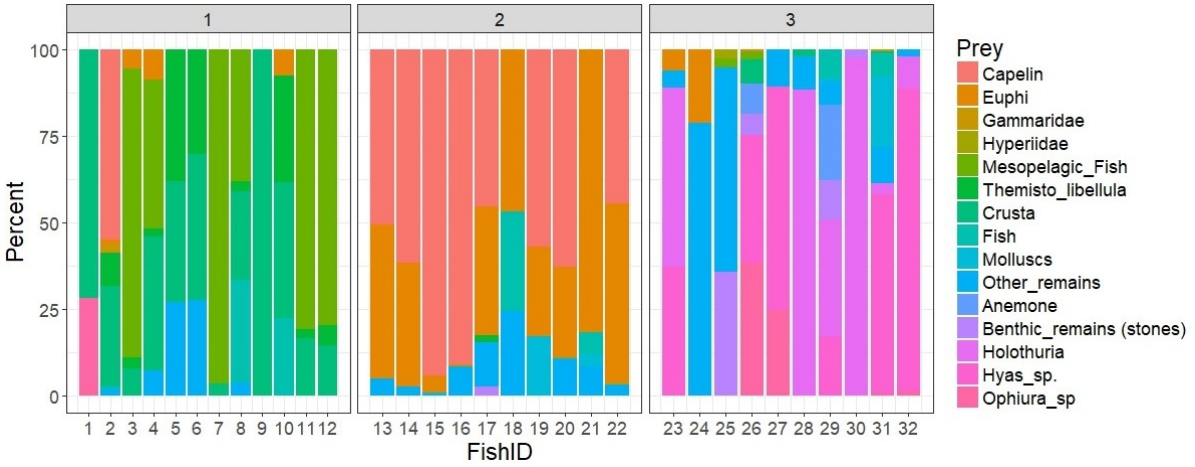


Figure 3.6: Species contribution (%) to the total stomach content weight of each individual. Unidentifiable, highly digested matter is combined as other remains. The Taxon Molluscs contains mainly Bivalvs, Gastropods and in some cases Theutids. Hard matter like stones and sand were combined as benthic remains. Colours and prey species are sorted by prey origin, pelagic on top, benthic on the bottom

A SIMPER analysis revealed capelin, crustaceans, euphausiids, mesopelagic fish, *Hyas* sp. and sea cucumbers as the most influential species (Supplementary). Site 1 shows the highest diversity in the prey composition with an average Shannon index of 1.19 for the stomach content analysis compared to 0.74 at site 3 and 0.72 at site 2 (Table Shannon and Prey index). Stomach content diversities are significantly different from each other (Kruskal-Wallis chi-squared = 8.2384, df = 30, p-value = 0.01626).

Table 3.3: Mean Shannon index of stomach contents per site and mean prey indices per site are listed with standard deviation. (Site 1 n= 12; Site 2 n= 10; Site 3 n= 10)

	Site 1			Site 2			Site 3		
Shannon Index	1.185	±	0.415	0.718	±	0.367	0.738	±	0.439
Prey Index	2.523	±	0.360	2.871	±	0.153	1.277	±	0.362

The stable isotope data show a clear seperation of site 3 from sites 1 and 2, with lower negative values for δC. Site 1 and 2 had similar values for δC and δN and are overlapping (Figure 3.7). This is in consistency with the prey composition data from the stomach analyses, where site 1 and 2 are both driven by a pelagic food spectrum and site 3 by benthic feeding (Agurto 2007). Three individuals from site 3 (Fish 24, 25, 26) are within the pelagic cluster of sites 1 and 2. Interestingly, these three individuals were among the four fish with pelagic diet at site 3. One individual however from site 2 (Fish 22) is found in the benthic cluster of site 3 (Figure 3.7), but has no sign of benthic prey in the stomach content (Figure 3.6).

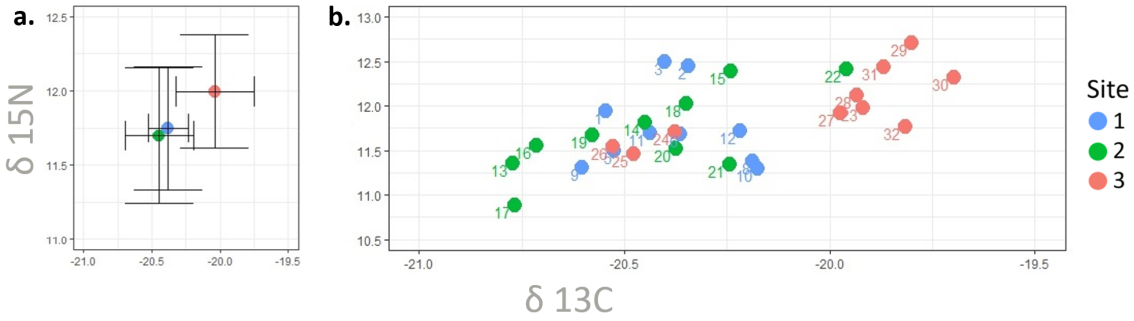


Figure 3.7: Stable isotope ratios of 30 fish, data for fish 4 and 7 were not available. On the x axis δC13 and on the y axis δN15. Sites are indicated by colour. a. is showing mean ratios of the sites, the error bars are showing the standard deviation. b. is showing the stable isotope signature for each individual. Numbers resemble FishID

3.4 Oceanography

Temperatures and salinity were obtained from corresponding stations with a maximum distance of ca. 31 km (Supplementary). Depth measurements were obtained from the net probe attached to the fishing gear. Sampling site 1 is the deepest station of all (Table 3.4). Site 3 is about 200 meters shallower than site 1 and the shallowest station of all. Because the data for site 1 was obtained from two different corresponding stations it shows a high variability in bottom (BT) and sea surface temperature (SST) within the site and minor variability in sea surface salinity (SSS). All other sites hat only one corresponding station. Low sea surface temperatures cooccur with lower sea surface salinities. The bottom salinities are around 35 PSU for all stations and are independent from bottom temperatures.

All measured parameters were significantly different between the sites (Kruskal-Wallis, Depth: chi-squared = 28.595, df = 2, p-value = < 0.001; BT: chi-squared = 22.589, df = 6, p-value = < 0.001; SST: chi-squared = 8.7891, df = 6, p-value = 0.01234; BS: chi-squared = 29.817, df = 6, p-value = < 0.001; SSS: chi-squared = 22.589, df = 6, p-value = < 0.001)

Table 3.4: Sampling sites and station properties. All sites with their sampling stations and number of sampled individuals are shown. Mean depth, sea surface temperature (SST) in degrees Celsius, bottom temperature (BT) in degrees Celsius, sea surface salinity (SSS) in practical salinity units (PSU) and bottom salinity (BS) in PSU are listed

			Site 1			Site 2		Site 3
Station	691	692	693	694	719	720	721	751
Depth [$\oslash m$]	325	351.5	373.5	383	188.5	159	156.5	92
BT [°C]	1.037	1.037	4.757	4.757	4.429	4.429	4.429	5.574
SST [°C]	0.622	0.622	7.858	7.858	5.207	5.207	5.207	1.677
BS [PSU]	34.842	34.842	34.969	34.969	34.762	34.762	34.762	34.553
SSS [PSU]	32.443	32.443	34.773	34.773	34.311	34.311	34.311	31.952
Individuals Sampled				12♂			10♂	10♂

3.5 Regional influences on fatty acids

All variables included in this plot, were determined as the most influential species by using a SIMPER analysis. The first two dimensions explain 87.7% of the variance (Figure 3.8). All three sites differed in their biotic and abiotic patterns and are separated in their own clusters (Figure 3.9). The difference between site 1 and both other sites is driven by depth and MUFAs, being the deepest sampling station and having the highest total amounts ($\frac{ng}{mg}$ dry weight) and percentage contributions of MUFAs. The depth of the sampling station and the amount of PUFAs are not correlated. Total fatty acids, saturated fatty acids and C16:0 are subsets of each other and positively correlated. Individuals from site 2 and 3 have less amounts of all of the latter, whereas most individuals from site 1 are enriched.

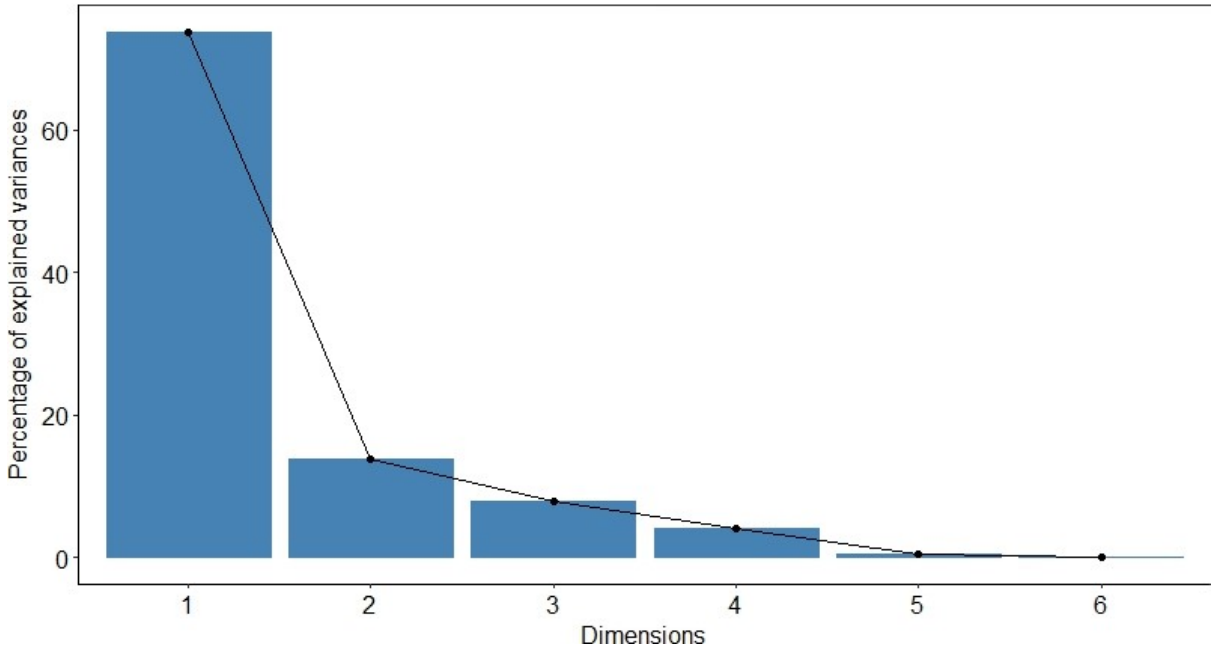


Figure 3.8: Scree stack plot of the principal component analysis (PCA). The x axis shows the dimensions and the y axis shows the percentage of explained variance

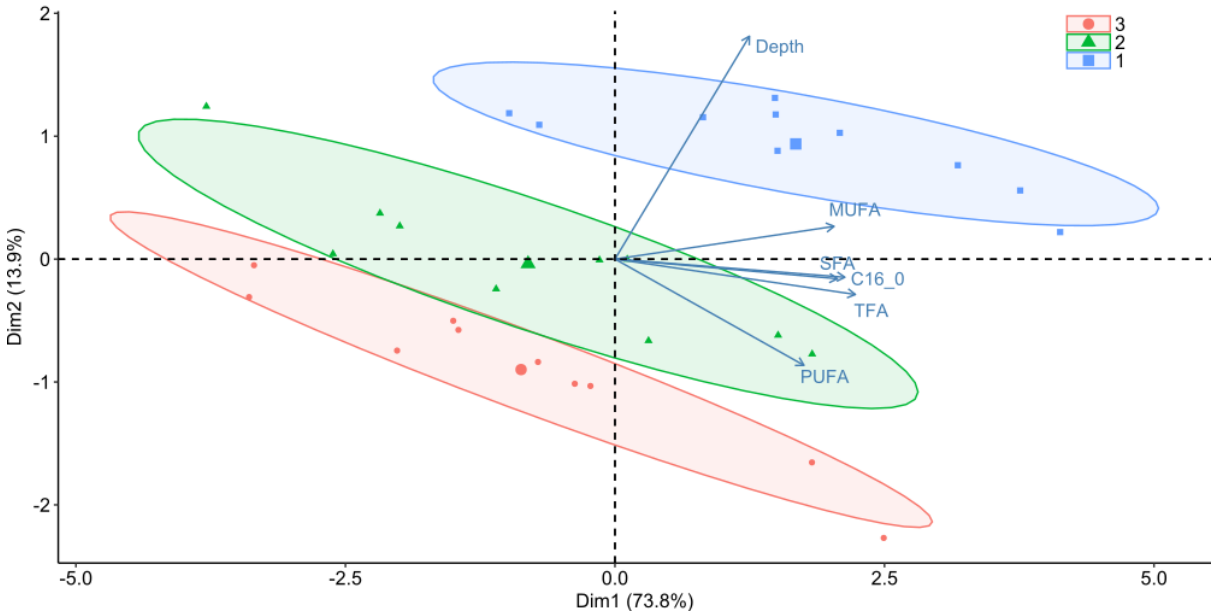


Figure 3.9: Principal component analysis of all parameters with data from 30 individual fish. Fish 4 and 7 are missing, due to a lack of stable isotope data. On the x axis dimension 1 and the percentage contribution of the dimension to the explained variance. On the y axis dimension 2 and the percentage contribution of the dimension to the explained variance. The single data points resemble the individual fish, colours indicate the sampling site. The vectors show the explanatory variables and their correlation between each other and the dimensions

The ANOVA function shows a significance for crustaceans (p-value= 0.042)(Supplementary), but overall no significance for any of the RDA axis. Sea cucumbers (Holothuria) and *Hyas* sp. are positively correlated and share a quadrant (Figure 3.10). They show no correlation with euphausiids and capelin and a low negative correlation with crustaceans and mesopelagic fish and a high negative correlation with depth. Euphausiids and capelin share a quadrant and have a low negative correlation with depth and a high negative correlation with mesopelagic fish and crustaceans. Mesopelagic fish and crustaceans share a quadrant, both are also positively correlated with depth which has a quadrant of its own. Mesopelagic fish and crustaceans are associated with enrichments in Docosahexaenoic acid, Eicospentaenoic acid, C18:1n7, C16:1, C20:1n9c and C14:0. Capelin and euphausiids are depleted in these fatty acids. Depth is associated with an enrichment in C16:0, C18:0, and C18:1n9c. Sea cucumbers and *Hyas* sp. are associated with a depletion of these fatty acids.

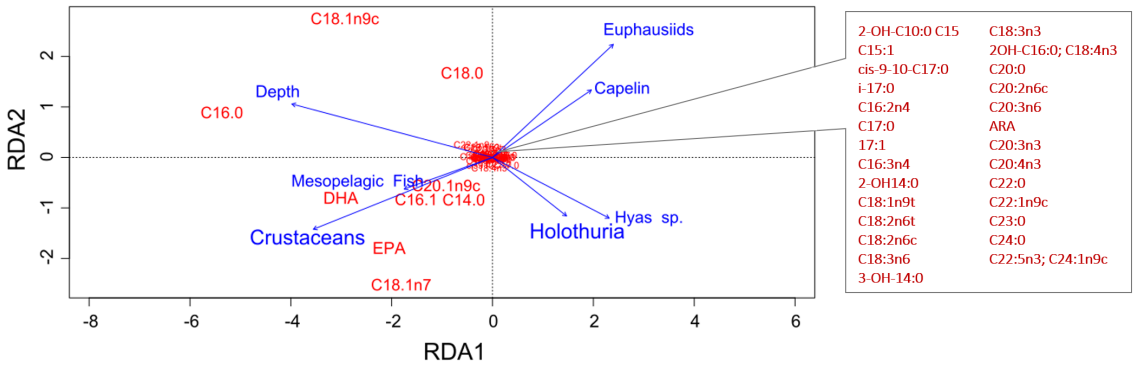


Figure 3.10: Redundancy analysis (RDA) for explanatory variables that were chosen by a prior SIMPER analysis and all fatty acids

The ANOVA function shows a significance for crustaceans (p-value= 0.03), but overall no significance for any of the RDA axis (Supplementary). Euphausiids and capelin share a quadrant and are negatively correlated with mesopelagic fish and show almost no correlation with depth, crustaceans, sea cucumbers and *Hyas* sp. (Figure 3.11). Depth and crustaceans share a quadrant and are positively correlated with mesopelagic fish and negatively correlated with *Hyas* sp. and sea cucumbers. *Hyas* sp. and sea cucumbers share a quadrant. They show no correlation with mesopelagic fish, which has a quandrant on its own. Depth and crustaceans are associated with saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in their quadrant and are enriched in these groups of fatty acids. Mesopelagic fish joins total fatty acids (TFAs) and poly unsaturated fatty acids (PUFAs) in its quadrant and is associated with an enrichment in both of these fatty acid groups. *Hyas* sp. and sea cucumbers are associated with high Eicosapentaenoic acid (EPA) to Arachidonic acid (ARA) ratios and are linked to a depletion in SFAs and MUFAs. Euphausiids and capelin are linked with a depletion in PUFAs and TFAs.

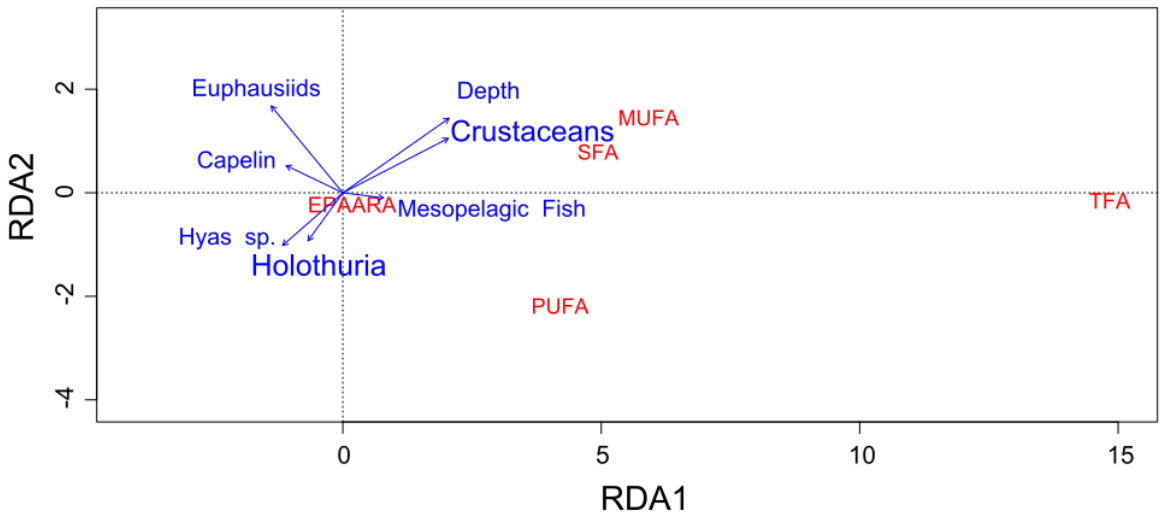


Figure 3.11: Redundancy analysis (RDA) for explanatory variables that were shosen by a prior SIMPER analysis and all fatty acid groups plus the Eicosapentaenoic acid (EPA) to Arachidonic acid (ARA) ratio

4 Discussion

4.1 Lipids and fatty acids

Fatty acids are, due to their different chemical constitution, to a variate extent sensitive towards high temperatures, light and oxygen. Exposure to one or more of these factors enhance degradation and change fatty acid compositions (Myashita 2018), which should be considered in discussion. In order to prevent any potential sampling bias, fish tissue was processed as fast as possible and kept on ice during the whole procedure. Afterwards samples were stored continuously at at least -80°C, to minimize potential sources of errors. Total lipid contents of livers and therefore energy storage of cod, are highest at site 1 and decrease consecutively at the other two sites. These results are consistend with a previous study (Werner et al. 2018), where high condition of cod was linked to mesopelagic feeding in the area at and around site 1 (Dhorn Bank) (Figure 2.1). Total Lipid contents of gonads show a different pattern. In contrast to liver lipid contents, highest lipid contents in gonads were found at site 3. Even though the differences of gonad lipid content between sites are not significant, the combination of low liver lipid content and high gonad lipid content at site 3 indicates, that females at site 3 have a different maturity stage than females from the other sampling sites and low energy storages and bad condition were already linked to a shift in spawning times of Baltic sea cod (Tomkiewicz et al. 2010) and might also be possible for cod in Greenland waters. Consitent with high total lipid contents of livers, the total fatty acid (TFA) amounts (in Nanogram) in gonads are highest in individuals from sampling site 1. This also accounts for total amounts of all sub groups of fatty acids (SFAs, MUFAs and PUFAs), which showed highest values at site 1. Digging deeper in the important fatty acid group of PUFAs, the patterns become more complex. Total amounts of Arachidonic acid are lowest at site 3 and highest at site 1. Total amounts of Docosahexaenoic acid are highest at site 1. Amounts of Eicosapentaenoic acid are more balanced between the sites, but were highest at site 3. Low ARA values and high EPA values at site 3, result in the highest EPA:ARA ratio at site 3. Both of these patterns can also be seen in the percentage contributions of fatty acids to the total fatty acid amounts of gonads. Røjbek and colleagues (2012) who looked at lipid dynamics and reproductive cycles of Baltic Sea cod, found similar patterns in females, that were close to spawning. In their studies EPA levels stayed more or less constant over the year, but ARA levels were strongly decreasing towards spawning. This resulted in increased

EPA:ARA ratios towards spawning. EPA:ARA ratios of site 3 are similar to Baltic Sea cod ratios of EPA:ARA in the triacylglycerols fraction, in the second phase of spawning (VI) (Tomkiewicz et al. 2002, Røjbek et al. 2012). Additionally, egg EPA:ARA ratios similar to what was found in gonads at site 3, were later on linked to reduced fecundity, hatching success and larval survival in Baltic sea cod (Røjbek et al. 2014). Comparing the total amounts of fatty acids to the percentage contribution of fatty acids, site 3 has the lowest PUFA amounts in nanograms, but the highest percentage contribution of PUFAs to total fatty acids and hence low SFA and MUFA contribution to total fatty acids. In Baltic Sea cod Røjbek and colleagues (2012) found high SFA percentage contribution to TFA at early maturation stages and a selective incorporation of PUFAs towards spawning. In addition to the results found in lipid contents of gonads and liver, this strongly indicates, that females at site 3 differ in their maturity stages from females of sites 1 and 2. Nevertheless, a 3-weeks sampling delay between site 1 and sampling site 3, should be kept in mind as it might contribute to the differences in maturity stages between sites. Since sampling site 3 is located at the tip of southern Greenland, were the two Greenland offshore stocks (west and east) meet and overlap (ICES 2015, ICES 2018a), different spawning times could be related to different stock origins of the fish. Cod from the west offshore stock have their peak spawning in March and April and fish from the east offshore stock are reported to spawn until end of June to July (ICES 2005).MUFAs and SFAs are the preferred source of energy and essential fatty acids like PUFAs are rather stored than used directly for energy production (Müller-Navarra et al. 2008). Low total amounts of PUFAs and high percentage contribution of PUFAs, as well as low MUFA:PUFA ratios, can also be an indication for a bad nutritional condition of individuals.

4.2 Condition and liver health

Condition factor K is almost the same for site 1 and 3, but significantly lower at site 2. But as discussed by Lloret, Shulman & Love (2013), HSI is the better choice of condition index for gadoid species like cod and is therefore used to discuss the condition of cod in this study. HSI is the highest at the deepest sampling site 1, which is in consistency with earlier results of Werner and colleagues (2019) and with the results for total lipid contents of livers in this study. The results do also reflect the ones of the liver lipid content. In addition to these patterns, which show highest energy storages at site 1, age, length and weight data show a faster growth of individuals at site 1. The results go along with results from Pardoe and colleagues (2008) which found a positive correlation with depth (>200m) and HSI of cod in the waters around Iceland. Mean HSI of sites 2 and 3 are close to the 5% threshold below which a rapid decline in liver energy content can be observed (Lambert & Dutil 1997a,b; Dutil & Lambert 2000) GSI patterns reflect, the results of lipid contents in gonads and also the results for condition factor K. GSI values were lowest for individuals at site 2 and almost the same for individuals caught at sites 1 and 3. Liver

health, for which the parasite and tissue indices were used as indicators, was by far the best at site 1 and the poorest at site 3, where individuals showed highest degradation of liver tissue and the highest amounts of macro parasites compared to liver weight. Degradation of liver tissue might influence the liver physiology and its capacity to store proper amounts of energy, which might be one of the reasons for lower liver lipid contents, samller liver sizes and lower amounts of total fatty acids at site 3. Higher parasite loadings might be influenced by either a higher parasite concentration in the waters of sampling site 3, or the bad condition of individuals at site 3, which makes them more susceptible to parasite infections. Or it is a combination of both. Higher concentrations of parasites in the water can be linked to high seal populations, since seals are an important host in the cycle of fish parasites (Zuo et al. 2018). Nevertheless, a literature research gave no indication of larger seal populations in south Greenland, compared to the rest of Greenland. Yet it is also conceivable, that current regimes and eddy systems might trap and enlarge the concentration of parasites in the water. Overall fish caught at site 1 showed superior condition and health compared to individuals from sites 2 and 3.

4.3 Feeding patterns and stable isotopes

Overall feeding patterns of the individuals examined match the patterns of stomach contents described in literature (Chapter 1.5) (Nielsen & Andersen 2001, Hedeholm et al. 2016; Werner et al. 2018). A limitation in stomach content analysis is the correct identification of prey species, which is impeded by digestion. Species with harder parts like otoliths, or heavy exoskeletons can be identified more easily and outlast longer than other species (Haywood 1995). Which is why some species might be over or under estimated in the proportional weight contribution to total stomach content. Stomach sampling always took place at the same time as length and weight measurements on board and as soon as possible after catch. Stomachs were kept on ice until final storage at -20°C, to avoid further digestion. Species that could not be identified precisely were always assigned to the next higher taxon, to avoid misidentification. Even though baselines for exact trophic level determinations are missing, the stable Isotope signatures are able to reveal two different trophic regions, that are also displayed in the redundancy analysis. One that is characterized by pelagic prey sources and larger depth, which includes sampling sites 1 and 2 and one that is characterized by benthic prey sources and shallower waters at sampling site 3. Using the results from the SIMPER analyses all three sampling sites can be linked to specific traits, that characterize the habitat. Sampling site 1 is associated with large depths, crustaceans and mesopelagic fish as prey sources. Site 2 is linked to consumption of euphausiids and capelin and site 3 is associated with shallow depth and benthic prey of Holothurians and *Hyas*. In the redundancy analysis larger depth and benthic prey are negatively correlated, whereas there is a positive correlation between larger depth and mesopelagic fish and

crustaceans. Depth is therefore, probably the reason for a pelagic prey preference like *Themisto libellua* and mesopelagic fish at site 1 and capelin and euphausiids at site 2. Sampling site 1 is the deepest site and close to the continental margin (Table 3.4, Figure 2.1). The warmer Irminger current and the cold arctic East Greenland current have an opposing flow at this sampling site. The close distance to the continental margin and the current systems might be a reason for more mesopelagic fish in stomach contents of female cod caught at site 1. Individuals that had mesopelagic fish in their stomachs, had no sign of capelin and only in two cases a small proportion of euphausiids in stomach contents. This is also displayed in the biplot of the redundancy analysis, were consumption of mesopelagic fish is negatively correlated with consumption of caplin and euphausiid. Mesopelagic fish can have lipid contents twice as high as capelin and can have even higher lipid contents than euphausiids (Falk-Petersen et al. 1986). This might indicate a selective foraging of individuals, with a preference for more lipid rich prey sources, but might also be due to the absence of capelin and euphausiids at site 1 and mesopelagic fish at site 2. Whereas the absence of mesopelagic fish at site 2 is more plausible, due to shallower. Abundance data of prey species and feeding experiments would be needed to fully answer this question. Sampling site 3 has the shallowest depth of all sites. Only individuals from this site had benthic species in their stomachs. Nevertheless, four individuals of site 3 had also pelagic food items in their stomach and 3 of these individuals had a stable isotope signature similar to the pelagic signature of sites 1 and 2. This might be explained by either two different feeding specialization at the same site or individuals migrate between the sites and therefore, switch their stable isotope signatures. Due to the absence of an isotope baseline, an answer to this question cannot fully be given.

4.4 Oceanography

Hydrographical data for this study could not be obtained directly from sampling station and had to be taken from stations nearby. But since the largest distance between sampling station and corresponding station was about 31 km (supplementary), the data can still be used to determine and proof existing differences of hydrographical regimes between the sampling sites. Salinities and water temperatures are, as expected, significantly different between the sites and show that the sampling sites are subject to different oceanographic regimes. Low sea surface temperatures and salinities at two stations within site 1 and at site 3 show an influence of the cold polar East Greenland Current at these sites Nevertheless, to determine the effect of temperature and salinity on fish condition and reproduction, long-term data, like seasonal means are needed. Therefore hydrographical data, except from depth, was not used to discuss the biotic patterns.

4.5 Regional influences on fatty acids

Regional abiotic different oceanographic and bathymetric regimes, are present between the three sampling sites, shaping the biotic parameters in these areas (Figure 3.9). The redundancy analysis revealed an enrichment of three saturated fatty acids, Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0) and four mono unsaturated fatty acids, Palmitoleic acid (C16:1), Vaccenic acid (C18:1n7), Oleic acid (C18:1n9c), Gadoleic acid (C20:1n9c), as well as two essential poly unsaturated fatty acids, Docosahexaenoic acid and Eicosapentaenoic acid, with larger depth and consumption of mesopelagic fish and crustaceans, all of which are strongly related with sampling site 1. Mesopelagic fish are especially associated with Gadoleic acid, Palmitoleic acid and Myristic acid in the redundancy analysis, which match the findings of Baby et al. 2014 and Falk-Petersen and colleagues 1986. Except from the PUFAs none of these fatty acids are linked to special physiological traits in fish. They are rather common fatty acids in lipids in general (C18:1n9c), in animal lipids (C14:0) and in fish oil (C18:1n7, C20:1n9c) and are mainly used as energy storage (Tocher et al. 2003), which underlines again the importance of mesopelagic fish for lipid storage. DHA and EPA on the other hand, are essential fatty acids and crucial for fish physiology. Consumption of crustaceans were the only significant explanatory variable in both RDAs and associated with an enrichment in DHA and EPA, both of which are essential PUFAs. Crustaceans are related to high concentrations of astaxanthins (Czeczuga 1976), which are important antioxidants in cod (Miki et al. 1982, Grung et al. 1993). Species that were summarized under the taxon crustaceans, were mainly deep-red shrimp like crustaceans and are therefore likely to have high astaxanthin concentrations. Due to their chemical constitution, especially PUFAs are sensitive against free radicals and peroxidation (Sargent et al. 2002). Astaxanthins can protect PUFAs against free radicals and help to preserve them. This might contribute to higher amounts of EPA and DHA in individuals that preyed on crustaceans. Larger amounts of capelin and euphausiids in stomach contents, which characterize sampling site 2, are linked to a depletion of several of the storage fatty acids mentioned before and a depletion of EPA and DHA and PUFAs in general. This is contrary to what is known about capelin and euphausiids. Both are known to be rich in lipids (Falk-Petersen et al. 1986, Tocher et al. 2003) and especially capelin is known to be a high quality food source for cod (Sherwood et al. 2007; Pardoe et al. 2008). Not only top predators like cod, but also lower trophic level species are at least partly dependent on diet fatty acids. They are channeled up the food web (Dalsgard et al. 2003, Tocher et al. 2003) and therefore the quality mainly dependents on the initial source of fatty acids as well as on the reprocessing of fatty acids on lower trophic levels. The initial source of fatty acids, at least in pelagic systems, is phytoplankton. The composition of fatty acids is dependent on the composition of phytoplankton communities and can additionally be altered by temperature, climate and latitude (Dalsgaard et al. 2003, Tocher et al. 2003, Kattner

and Hagen 2009), so there is a high regional influence on fatty acids. Sampling site 2 is located within a resident eddy system between the East Greenland Current and the East Greenland Coastal Current (Sutherland & Pickart 2008). This hydrographical feature decouples the primary production of sampling site 2 from adjacent areas, resulting in earlier spring blooms (Fock et al. unpublished) and might also cause differences in the phytoplankton community composition at this sampling site. The system is controlled by climatic influences and shows inter annual fluctuations in zooplankton community compositions (Stippkugel 2018). This might include a shift from lipid rich zooplankton species to species with a lower total fat and fatty acid content. That in turn would cause a bottom-up effect, reducing the fat storages and changing the fatty acid profiles of capelin and euphausiids, which prey on these zooplankton species and would therefore also affect cod in this area. Copepods are known to be an important source for PUFAs (Tocher et al. 2003, Dalsgaard et al. 2003), therefore lower levels of PUFAs in fish gonads in this sampling area might be explained by a shift in zooplankton community composition due to an overall habitat effect. A second explanation might be a trait off between foraging energy and the energy level of the food source. The larger the fish become, the more energy they need to forage on fast pelagic fish (Sherwood et al. 2007) and might therefore loose more energy to forage on capelin than they gain from capelin. So capelin might be of good nutritional quality especially for smaller individuals, but not for cod in the size class that was sampled (Sherwood et al. 2007). Sea cucumbers (Holoturia) and *Hyas*, which were the most important diet items at sapmling site 3, are linked, as discussed before (Chapter 4.1) to high EPA:ARA ratios and a depletion in SFAs and MUFAs (C16:0, C18:0 and C18:1n9c). This contributes to the assumption, that females caught at site 3, started selective incorporation of PUFAs into the gonads and that females at site 3 might had a general lack of fatty acid energy storage. Considering these results benthic prey can be regarded as low-quality food for cod in Greenland waters, which goes along with studies from other areas (e.g. Atkinson & Wacasey 1976, Norrbin & Båmstedt 1984), that showed low organic and caloric contents for benthic invertebrates.

5

Conclusion and outlook

Fish caught at sampling site 1 showed by far the best condition, had the highest energy storage and the best fatty acid composition in their gonads. Fish caught on both other sites were of poorer condition, had smaller energy storage and lower PUFA contents in their gonads. In addition, fish caught at site 3 showed strikingly poorer liver health, than fish from both other sites. The importance of female cod condition for reproductive outcome was pointed out already 20 years ago by Marteinsdóttir and colleagues (1998). The results of this study indicate an advantage in reproduction of cod living and foraging at site 1. Fish living in the waters around sampling site 2 and 3 are likely to have a lower fecundity and in general a bad reproductive outcome (Kjesbu et al. 1991, Lambert & Dutil 2000). Low energy levels could trigger females to skip spawning (Rideout et al. 2006, Tomkiewicz et al. 2010) and increase post-spawning mortality for females at site 2 and 3 (Lambert & Dutil 2000). Mesopelagic fish had a positive influence on the lipid and energy storage as well as on fatty acid content and profiles of gonads of Atlantic cod off the East Greenland coast, as well as on the general condition of cod. Higher contents of total fatty acids and especially of health supporting PUFAs in the gonads of Cod feeding on mesopelagic fish, will most likely have a positive influence on the reproductive outcome of the individuals in this area. Habitats, in which cod can feed on mesopelagic fish, seem to be of high value for the maintenance of good conditioned individuals that produce healthy and vital offspring and should therefore be under special protection. Fisheries of cod in these areas but also bycatch of mesopelagic fish should be carefully managed to keep these valuable habitats intact. Capelin that is promoted as a lipid rich, good quality food source for cod and fish in general (e.g. Tocher et al. 2003 and Sherwood et al. 2007), is at least compared to mesopelagic fish and in Greenland waters of less nutritional value for large female cod. Female cod at sampling site 3 showed a poor conditional state, health and quality of gonad fatty acids, which are strong indicators for reduced reproductive capacities of individuals at this site (Marteinsdóttir et al. 1998). Fishing pressure at this site should be adapted. The fact that low fat storage and bad condition are linked to a shift in spawning times and enhanced atresia in cod (Tomkiewicz et al. 2010), plus the findings of this study, that indicate different maturity stages of female cod at site 3, might support a separation of the cod stocks in this area from the rest of the East Greenland stock in the future. This should be further monitored and integrated in fisheries management.

Further analysis of food web parameters like fatty acid composition and stable isotope signatures of prey sources and organisms of lower trophic levels from the same spatial and temporal origin are urgently needed to shed more light on food web interactions and their consequences on cod condition and reproduction. Fatty acid analysis of the liver fat storage, which unfortunately could not be done within the frame work of this thesis, would have provided important additional data and should be done in the future. Especially because the fatty acid profiles gained from gonads in a state of post to pre-spawning are of less information for reproductive outcome, compared to gonads shortly before spawning. Since satellite data only give proxies for primary production and phytoplankton biomass, actual phytoplankton samples from spring blooms should be taken to further determine the dominant groups of phytoplankton and fatty acid food web markers which can help to link diet composition to condition and reproduction of cod. Genetic information of fish could help to reveal stock origin. In addition, a higher resolution of maturity stages by using a different maturity determination key (e.g. Tomkiewicz et al. 2002) could help to reveal a potential stock separation.

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Appendix

Table 5.1: Output Kuskar-Wallis Test; Site 1 n=12, Site 2 n=10, Site 3 n=10

	Chi-Squared	df	p-value
TFGonads	3.8265	30	0.1476
TFLiver	8.4688	30	0.01449
HSI	19.008	30	7.45E-05
GSI	17.899	30	0.0001298
Preyindex	22.766	30	1.14E-05
SWInd	8.2384	30	0.01626
SSSal	22.589	30	1.24E-05
SST	8.7891	30	0.01234
ParaIndex	14.152	30	0.000845
BT	22.589	30	1.24E-05
Bsa	29.817	30	3.35E-07
Depth	28.595	30	6.17E-07
K	11.07	30	0.003946

Table 5.2: Output Kuskar-Wallis Test; Site 1 n=12, Site 2 n=10, Site 3 n=10

	Chi-Squared	df	p-value
ARA %	4.8083	30	0.09034
EPA %	13.475	30	0.001186
DHA %	2.1278	30	0.3451
EPAARA %	13.318	30	0.001282
MUFA %	12.055	30	0.002411
SFA %	3.6703	30	0.1596
PUFA %	5.4051	30	0.06703
ARA ng	4.4206	30	0.1097
EPA ng	4.1058	30	0.1284
DHA ng	4.3535	30	0.1134
EPAARA ng	12.808	30	0.001655
MUFA ng	8.3226	30	0.01559
SFA ng	4.6684	30	0.09689
PUFA ng	4.56	30	0.1023
TFA ng	6.5135	30	0.03851

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Declaration

Statutory declaration/ Eidesstattliche Erklärung

I declare that I have developed and written the enclosed Master Thesis completely by myself, and have not used sources or means without declaration in the text. Any thoughts from others or literal quotations are clearly marked. The Master Thesis was not used in the same or in a similar version to achieve an academic grading or is being published elsewhere.

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