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Icelandic blue mussels – A valuable high quality product

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Gæðakræklingur er gulls ígildi

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Report summary

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Ágrip á íslensku:

Til þess að íslensk kræklingarækt geti vaxið og dafnað er mikilvægt að framkvæma grunnrannsóknir varðandi öryggi og gæði á ferskum íslenskum kræklingi sem geta nýst framleiðendum við markaðssetningu og sölu afurðanna.

Tilgangurinn með þessu átján mánaða langa rannsóknarverkefni var að safna upplýsingum um öryggi og gæði kræklings (*Mytilus edulis*) í markaðsstærð (> 45 mm) sem ræktaður er við strendur Íslands.

Samtals var þrettán kræklingasýnum í markaðsstærð safnað á fjórum mismunandi ræktunarstöðum við landið (Hvalfirði, Breiðafirði, Álftafirði og Eyjafirði) á mismunandi árstímum. Kræklingur í markaðstærð fékkst ekki á Eskifirði og þessi ræktunarstaður var því útilokaður frá verkefninu. Í staðinn voru tekin sýni oftar á hinum ræktunarstöðunum fjórum en upphaflega var ráðgert. Kræklingi var safnað af ræktunarlínum og tími og staðsetning skráð. Þyngd, lengd og holdfylling var mæld. Kræklingurinn var kyngreindur og kynþroskastig áætlað í hverju sýni.

Í þessu verkefni var safnað umtalsverðum upplýsingum um næringarefnainnihald (prótein, vatn, fita, aska) auk lífvirkra efna s.s. selens, sinks, karótíníða og fitusýrusamsetningar í kræklingi frá mismunandi ræktunarstöðum og á mismunandi árstímum. Sömuleiðis voru mæld óæskileg ólífræn snefilefni (blý, kvikasilfur, kadmíum, kopar, nikkel, arsen, króm og silfur) í öllum sýnum. Einnig var unnið að því setja upp og prófa hraðvirkrar mæliaðferðir til að mæla þrjár tegundir þörungaeiturs þ.e.a.s. ASP, PSP og DSP. Mæliaðferðirnar voru bestaðar gagnvart þeim tækjabúnaði sem til staðar er hjá Matís og einnig mæld viðmiðunarsýni (þ.e. kræklingur með þekktu magni þörungaeiturs) til að meta gæði mælinganna. Prófuð voru tvenns konar hraðvirk próf sem til eru á markaði til þess að meta hvernig þau reynast við þörungaeitursmælingar í kræklingi. Annars vegar voru prófuð svokölluð Jellet próf og hins vegar ELISA próf. Niðurstaðan er sú að bæði prófin eru tiltölulega einföld í notkun, hins vegar er nauðsynlegt er að prófa þau á aðeins fleiri sýnum, en gert var hér, til að leggja betra mat á það hvernig best væri að nýta þau í gæðaeftirliti með kræklingarækt. Nauðsynlegt er að gera sér grein fyrir takmörkunum þessara hraðvirku prófana þar sem þau munu ekki koma algerlega í staðinn fyrir mælingar með viðurkenndum rannsóknaraðferðum. Þessar prófanir, gætu aftur á móti, fækkað þeim sýnum verulega sem send eru til viðurkenndra mælinga, þar sem ekki væru send sýni þegar forprófanirnar sýna að þörungaeitur er til staðar og ekki fengist leyfi til að uppskera krækling.

Niðurstöðurnar benda til þess að íslenskur kræklingur hafi ákjósanlega næringarefnasamsetningu sem þó er háð náttúrlegum árstíðabreytingum. Fjölbreytagreining (PCA) sýnir að kræklingur inniheldur hærra hlutfall af fitu og próteini á vorin (maí og júní) líklega vegna þess að kræklingurinn er að undirbúa hrygningu á þessum árstíma. Snemma að hausti minnkar hlutfall próteins á meðan magn óþekktra efna eykst. Á þessum árstíma er hrygningu að ljúka, ef ekki lokið. Greiningin sýnir einnig veika jákvæða fylgni milli próteins og fitu, en sterka neikvæða fylgni milli próteins og óþekktra efna.

Styrkur þungmálma (kvikasilfurs, blý, kadmíums) var almennt lágur, en í nokkrum tilvikum var styrkur kadmíums þó hærri en leyfilegt er samkvæmt íslenskum og Evrópusambands reglugerðum (1 mg/kg). Mikilvægt er því að fylgjast með styrk kadmíums í íslenskum kræklingi áður en hann fer á markað. Niðurstöður fitusýrugreininga sýna að íslenskur kræklingur inniheldur umtalsvert magn af omega-3 fitusýrunum EPA (C20:5n3) og DHA (C22:6n3) auk Palmitoleate (C16:1n7), sem allar eru þekktar fyrir jákvæð áhrif á heilsu.

Niðurstöður verkefnisins sýna að íslenskur kræklingur er samkeppnishæfur varðandi næringarefna samsetningu og inniheldur auk þess jákvæð lífvirk efni. Þessar niðurstöður munu tvímælalaust nýtast kræklingaræktendum við markaðskynningar og skipulagningu varðandi uppskeru og sölu kræklingsafurða.

Lykilorð á íslensku:	*kræklingur, næringarefnainnihald, lífvirk efni, þungmálmar, þörungaeitursmælingar*

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

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Summary in English:

In order to enable the Icelandic blue mussel industry to grow, market and sell their product, there is a critical need to perform some fundamental studies.

The purpose of this eighteen months long research project was to investigate the quality and value of Icelandic blue mussels (*Mytilus edulis*) grown at different growing sites of Iceland. A total of 13 samples were collected from blue mussel culture sites around Iceland (Hvalfjörður, Breiðifjörður, Álftafjörður and Eyjafjörður). The Eskifjördur sampling site was excluded from the project due to the lack of market sized blue mussels and resulted in sampling from growing lines of four different culture sites. The mussels were characterised according to location, time of year, weight, length, meat yield and reproductive status.

This report summarises the considerable amounts of data obtained regarding the chemical composition of Icelandic blue mussels, including trace metals (lead, cadmium, copper, zinc, mercury, arsenic, selenium, chrome, nickel and silver), nutrients (moisture, protein, lipid and ash content) and bioactive components (carotenoids and fatty acid profile). In addition, the presence of common algal toxins in blue mussels was investigated and concluded that further work will be needed to optimise the rapid assays tested for measuring algal toxins i.e. PSP and DSP toxins. The results obtained need to be further verified by using standard addition procedures or with certified reference material. It is important to keep in mind that these rapid tests for PSP and DSP only provide screening results. Further testing with reference analytical methods will be required to confirm the results from these rapid tests before the mussels are harvested and sold on market. The rapid tests are suitable for quality control and decision making regarding whether or not it is safe to harvest the mussel crop or if the mussels should be harvested later after purification in the ocean.

The results obtained here indicate that Icelandic blue mussels compose well balanced nutritional and trace element levels. A moderate seasonal variation pattern was observed in all measured nutritional parameters. A principal component analysis (PCA) showed that mussels contained higher proportion of fat and protein during spring (May-June). In the autumn the proportion of protein reduced while the proportion of other unknown substances increased. The PCA analysis also revealed a weak positive correlation between protein and fat and a strong negative correlation between protein and other unknown substances. Heavy metal concentrations were generally low. However, elevated levels of cadmium were measured in mussel samples from certain culture sites, which in some cases exceeded the maximum EU limits (1 mg/kg) for cadmium in bivalve molluscs. The fatty acid profile revealed significant levels of omega-3 polyunsaturated fatty acids such as Eicosapentaenoic (EPA, C20:5n3) and Docosahexaenoic (DHA, C22:6n3) as well as Palmitoleate (C16:1n7), all recognised for their health beneficial effects.

This fundamental information proves that Icelandic blue mussels is a market competitive product of high quality and will greatly aid in developing the Icelandic mussel industry and in making the best choices considering growing, harvesting , marketing and selling their products.

English keywords: *blue mussels, chemical composition, bioactive components, heavy metals, algal toxin measurements*

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1. Introduction

The mussel culture industry is relatively new in Iceland (Gunnarsson et al., 2005; Thorarinsdottir et al., 2007). From the year 2000 the number of experimental mussel farmers has increased and in late 2009 they were 17 (Pálsson et al., 2009).

Studies outside of Iceland have demonstrated that seasonal variations and life cycle of the mussel may influence the nutritional composition, quality and shelf-life of blue mussels (Krzynowek and Wiggin, 1979; Lemaire et al., 2006), which in turn can have a significant impact on their value and marketability. Icelandic blue mussels comprise a meat yield above market requirements all year round, except just after spawning. The meat yield depends on the annual gamteogenic cycle, spawning and nutrient reserves, which accumulate in the mantle tissue during summer and autumn. The gametes (eggs and sperm), which are mainly made up of protein are also located in the mantle. In Icelandic waters the mussels usually start gametogenesis in late autumn, immediately after spawning, using energy reserves for this purpose. As food supply increases in early spring the individuals become fully ripe and spawn during summertime until late autumn (Thorarinsdottir and Gunnarsson, 2003). Spawning initiation is however dependent on sea currents and temperature and food availability. In some cases gametogenesis is delayed until spring when food availability as phytoplankton has increased, since stored energy reserves are only used for non-reproductive metabolic requirements during winter time (Thorarinsdottir, 1996). This means that the nutritional composition of mussels might be different depending on the season of the year and this was one of the research questions that we wanted to investigate in this project. Although greatly needed, detailed information about composition and quality for different growing regions and seasons in Iceland is are presently not available. Analyses of blue mussels collected at different geographical locations have revealed excellent nutritional compositions as well as the presence of certain known bioactive components. For example are blue mussels low in dietary fat and cholesterol but contain an above average concentration of omega-3 fatty acids, which are well known for their heart health benefits, importance in brain development and immune enhancing effects. Mussels are also rich in zinc, which is important for the immune system as well as growth and brain health. In addition are blue mussels rich in carotenoids, which are potent antioxidants and can have a multitude of positive health effects (Kantha, 1989; Liaaen-Jensen, 1991).

Astaxanthin belongs to the carotenoids and is structurally similar to lutein, fucoxanthin and vitamin A. Besides pigmentation, carotenoids have been proposed to play a role in a number of important functions in crustaceans, such as a source of pro-vitamin A activity, increased stress tolerance and in various development and differentiation processes. Crustaceans obtain astaxanthin through metabolism of ingested carotenoids in their diet and studies have revealed that astaxanthin account for app. 95% of total carotenoids present in crustaceans, depending on its availability in the environment.

The cold waters of Iceland create a challenging environment for the mussels and it is know that such environments may promote biochemical changes in marine organisms. Therefore it is likely that Icelandic blue mussels could be a source of various interesting bioactive and health beneficial compounds.

The coastal zones around Iceland are generally not polluted and thus, provide outstanding conditions for mussel culture. The only health issue that has appeared at the Icelandic culture sites is that the level of cadmium has been measured above permitted EU limits (1 mg/kg wet weight) in the mussel flesh at some sites along the Icelandic coast. The origin of the cadmium is from natural sources but not as a result of pollution from human activities (Oddson et al., 2008). In addition, it is necessary to

measure lead and mercury in the mussel flesh since the levels of these heavy metals are also restricted in the EU food legislation. Furthermore, to ensure food safety, it is always necessary to test for marine algal toxins i.e. Amnesic Shellfish Poison (ASP), Diarrhetic Shellfish Poison (DSP) and Paralytic Shellfish Poison (PSP) in the mussel flesh before harvesting. ASP, DSP and PSP toxic syndromes primarily arise due to consumption of mollusc shellfish which have accumulated these toxins after filter-feeding on toxic algae. Today, the Icelandic blue mussels are tested for algal toxins abroad and it may take up to 5 days before results are received. Rapid assays for the determination of marine algal toxins in shellfish are now available on the market. These tests could be used to monitor the presence of algal toxins in the mussel flesh. In cases where the toxins are present, an accurate quantification of the amount of toxins also has to be carried out using reference quantification methods. The application of rapid assays to test for marine algal toxins would save a considerable amount of time during mussel harvesting, as the results could be available within one day instead of five. This would enable the mussel farmer to send the product to the market 3-4 days earlier than presently. This reduction in time of product delivery can make a great difference when the market demand for blue mussels is high. In addition, the rapid assays are considerably less expensive for the growers than the methods presently used. Thus, the establishment of rapid methods for measuring of algal toxins could support the present Icelandic blue mussel farmers, as well as enhance the expansion of the mussel industry in Iceland.

The committee, appointed by the Minister of Fisheries in December 2007, suggested that the authorities should support the growth and prosperity of the industry with general actions supporting infrastructure, research and increased services for the mussel industry. The objective should be to increase knowledge, improve organisation and reduce risk in order to make the mussel industry attractive to investors (Oddson et al., 2008). This study will contribute significantly to achieve these objectives.

Currently the production of Icelandic blue mussels is limited and the market is small. However, there are excellent conditions in Iceland to grow mussels and create a significant market for these products based on their quality and the clean environment they are grown in. At the same time as consumer demand for mussels is growing, the shellfish production in Europe has declined from 606.000 tonnes in 1998 to 470.000 tonnes in 2007 and consequently the European market has to import up to 2 tonnes of blue mussels from countries such as Canada, Chile and China. Thus there is clearly an unfilled demand for high quality mussels on the European market, which opens a great opportunity for Iceland to establish as a major player on the blue mussel market. For this to be realised, some fundamental studies are needed on Icelandic blue mussels to aid the industry in growing, marketing and selling the product. The fundamental information generated on Icelandic blue mussels in this project (composition and health promoting properties, safety and rapid response of potential accumulation of bio-toxins) will greatly aid the Icelandic mussel industry in maturing and making their best choices in growing, harvesting, marketing and selling their products. The objective of this project is to gather information regarding nutritional and bioactive components as well as chemical risk factors in Icelandic blue mussels.

2. Material and Methods

2.1 Sampling

The blue mussel samples were collected from growing lines at 2 m depth at 4 different growing sites. According to the project proposal the sampling sites should have been 5 and sampling to start in May 2010. However, the proposal was first granted in the middle of May 2010, the contract with AVS was signed early in June 2010 and the sampling started that same month. When the project started the Eskifjörður (East of Iceland) culture site was excluded from the project due to lack of market size blue mussels. In June 2010, a total of 100 blue mussels (40-50 mm) were sampled from growing lines in Breiðifjörður, Álftafjörður and Eyjafjörður. No mussels of proper size were available in Hvalfjörður at that time. An extra sample was collected in Eyjafjörður in August and in October 2010 samples were obtained from all 4 growing sites. In February 2011 samples were taken from Eyjafjörður and in May 2011 from all growing sites except Hvalfjörður which was sampled in June. A total of 13 samples of market size mussels were collected and analysed as part of this AVS project. Mussels were characterised according to location, time of year, meat yield (weight) and shell length. The meat yield was calculated both according to the European (weight of cooked meat x100/fresh whole weight) and the Canadian (weight of cooked meat x100/weight of cooked meat + shell weight) method. All sampling in Hvalfjörður and Breiðifjörður and the sampling in February 2011 in Eyjafjörður was carried out by staff from P2 (Hafró) in Reykjavík while staff from the Hafró branches in Ísajörður and Akureyri was responsible for the sampling in Álftafjörður and other dates in Eyjafjörður, respectively. The blue mussel growers assisted the researcher with the sampling. After sampling the samples were sent as soon as possible to P1 (Hafró) where they were measured and weighed and from there a subsample was sent to (Matís) in Reykjavík for measurements and analysis. The samples obtained in the project are listed in Table 1.

Table 1. Sampling sites and time of sampling.

	Samples obtained					
	June 2010	August 2010	October 2010	February 2011	May 2011	June 2011
Breidafjörður	x	-	x	-	x	
Álftafjörður	x	-	x	-	x	
Eyjafjörður	x	x	x	x	x	
Hvalfjörður	-	-	x	-	-	x

2.2 Sample preparation for chemical analysis at Matís

Each sample of mussel contained at least 50 individuals. During sample preparation care was taken to avoid any possible water loss prior to chemical analysis. This is important since the water content in the mussel flesh is very high and alterations can have major effects on the parameters measured. The mussels were weighed and their length (4-5 cm), height and width measured, after this the flesh and the shell were separated. Samples were homogenised and the sample material frozen in three separate containers for the various chemical analysis i.e. trace metal analysis (lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), mercury (Hg), arsenic (As), selenium (Se), chrome (Cr), nickel (Ni) and silver (Ag)), nutritional composition (moisture, protein, lipid and ash content), bioactive components (fatty acid profile and carotenoids) as well as algal toxin measurements.

2.3 Trace metal analysis

Inorganic contaminants and minerals (Cd, Cu, Zn, As, Se, Hg, Pb, Cr, Ni and Ag) in samples were determined by ICP-MS after mineralisation of the samples with closed vessel acid digestion.

Portions (up to 200 mg weighed to 0.1 mg) of freeze dried samples together with 3 ml HNO_3 and 1.5 ml H_2O_2 were transferred to 50 ml digestion bombs. Samples were digested in a Mars5 microwave oven (CEM, North Carolina, USA). The digested sample solutions were quantitatively transferred to 50 ml polypropylene tubes and diluted to 30 ml with Milli-Q water. The concentration of the different elements in these digests was determined by ICP-MS (Agilent 7500ce, Waldbronn, Germany) and 115In was used as internal standard (Gunnlaugsdottir H. et al., 2010).

2.4 Nutritional composition

Protein. AE 3. The sample is digested in sulphuric acid in presence of $CuSO_4$ as catalyst. There after the sample is placed in distillation unit, 2400 Kjeltec Auto Sampler System. The acid solution is made alkaline by use of NaOH solution. The ammonia is distilled into boric acid which is then titrated with H_2SO_4. The nitrogen content is multiplied by factor 6,25 to obtain % crude protein (ISO 5983, 2005).

Moisture. AE 4. The sample is heated in the oven at 103°C+/-2°C for 4 h. Percentage of moisture corresponds to the weight loss (ISO 6496, 1999).

Ash. AE 5. The sample is ashed at 550°C for 3 h, and the residue weighed (ISO 5984, 2002).

Fat. AE 1. The sample is extracted with petroleum ether on a 2050 Soxtec Avanti Automatic System apparatus, boiling range 40-60°C (AOCS Ba-3-38, 1997).

2.5 Fatty acid profiling

The first step of this procedure was a fat extraction based on Bligh and Dyer (Bligh and Dyer, 1959) fat extraction with Icelandic Fisheries Laboratories adaptation. The second step involves methylation based on AOCS Official Method Ce 1b-89 with minor adjustments. The fatty acid methyl esters (FAME) were then separated on a Varian 3900 GC equipped with a fused silica capillary column (HP-88, 100 m x 0.25 mm x 0.20 µm film), split injector and flame ionisation detector fitted with Galaxie Chromatography Data System, Version 1.9.3.2 software. The oven was programmed as follows: 100°C for 4 min, then raised to 240°C at 3°C/min and held at this temperature for 15 min. Injector and detector temperature are 225°C and 285°C, respectively. Helium is used as a carrier gas at the column flow 0.8 mL/min; split ratio, 200:1. The programme is based on AOAC 996.06. The peaks in the chromatograms were identified by comparison with known fatty acid methyl ester standards (Sigma Chemical Co, Ltd).

2.6 Carotenoids and astaxanthin analysis

The method used for estimation of astaxanthin was based on a method published by Tume et al., 2009. Briefly it was as follows; each tissue was weighed, finely chopped and extracted three times with 10 mL acetone at 4 °C, the mixture was allowed to stand for 1 h between each extraction. The mixture was spun down on a JA-25 centrifuge from Beckman Coulter in TS-5.1-500 bucket rotor at 4.000rpm for 10 min at 4°C and the acetone solvent extract transferred to a 30 ml measurement tube. The remaining muscle residues following this exhaustive extraction were essentially visually devoid of pigmentation. The pooled extracts were adjusted to a total volume of 30 mL with acetone and then 5 mL H_2O and 2,5 mL n-hexane were added, mixed and allowed to phase separate. The upper layer was removed and the lower layer was washed twice with 5 mL H_2O and 5 mL n-hexane.

The concentration of astaxanthin in the extracts was determined by measuring the absorbance at 474 nm, using a molar extinction coefficient of 2172 in a 1 cm cell. Astaxanthin standard was obtained from Sigma Chemicals.
The spectrophotometric quantification of astaxanthin is not as accurate as HPLC, but has been shown to yield a close estimation to results obtained with HPLC with UV detection. This variance in accuracy is thought to arise due to the presence of astaxanthin derivatives and other carotenoids in the samples which interfere with the spectrophotometric measurements.

2.7 Colour vision analysis of mussels

Machine vision technology is an analysis method which combines the technologies of photography and computer to create a colour space in order to specify, create and visualise colour. A computer describes colours as amounts of red, green and blue and a printer produces a specific colour in terms of the reflectance and absorbance of cyan, magenta, yellow and black inks on a printing paper (Ford and Roberts, 1998). This method could possibly be useful in estimating the quality of mussels regarding colour and meat yield.
The main colour spaces are RGB, CMYK, CIE, HSL and HSV. There are different uses and purposes for these colour spaces. For instance RGB (Red, Green and Blue) is used for electronic displays (LCD, plasma etc.) while CMYK (Cyan, Magenta, Yellow and Black) is used in printing. CIE (International Commission of Illumination) has two different colour spaces, the main one being L*a*b* which is mainly used in software applications, the other being L*u*v*. Both of them are attempts to linearize the perceptibility of unit vector colour differences.
In this experiment one of the main machine vision colour spaces, CIE L*a*b*, was used. The CIE colour spaces are based on human vision, which is useful when determining how to let a program operate and since it is nearly linear it is perfect for repetition when analysing colours of samples (Ford and Roberts, 1998).

2.8 Algal toxins

In addition to the mussel samples obtained from Icelandic culture sites in this project, a few mussel samples were received from Norges veterinærhøgskole. These samples were used as reference samples as they had previously been measured for PSP, DSP and ASP using conventional analytical methods i.e. LC-FLD for PSP, LC-MS/MS for DSP and LC-UV for ASP.
ELISA (Enzyme- Linked Immunosorbent Assays) **test kits** and receptor-based bioassay were used to detect and quantify concentrations of ASP, DSP, and PSP. . Three ELISA test kits were used; Saxitoxin (PSP) ELISA Microtiter Plate, Okadaic acid (DSP) ELISA Microtiter Plate and ASP ELISA Microtiter Plate, purchased from two different suppliers Biosense Laboratories, Bergen, Norway (ASP) and Abraxis LLC, Warminster, USA (DSP and PSP). Samples and tests were prepared and tests conducted according to instructions provided with the test kits by their producers.
Jellet rapid test (JRT) for PSP and DSP were obtained from Jellet rapid testing Ltd. Nova Scotia, Canada. These are qualitative (yes/no) tests that provide a visual determination of the presence of toxicity in shellfish tissue, phytoplankton or water samples. Extraction steps involved preparation of mussel samples by removing the tissue from the shell and blend about 120-150 ml until pureed and transfer it into the tube. 10 ml of 0.1 N HCI was added to the tube and placed in boiling water (note: this step is necessary for the JRT tests in all three cases). If the test is positive for the toxin, a single line appears in the window. If the test is negative, two lines appear in the window (for more details refer to www.jellet.ca).

3. Results

3.1 Sampling and measurements

Hvalfjörður: The sampling was carried out at 2 m depth (64°21´74-21°29´27) in October 2010 and June 2011. The mean shell length in the 2 samples taken in October 2010 was 49,8 mm and 43,4 mm respectively. Depending on the method used, the meat yield was 31,8% (European method) and 46,6% (Canadian method) in the one sample and 30,3% and 43,3% in the other (Figure1). In June the mean length was 53 mm and the meat yield had increased to 38,7% and 50,5% depending on the method used (Figure 1).

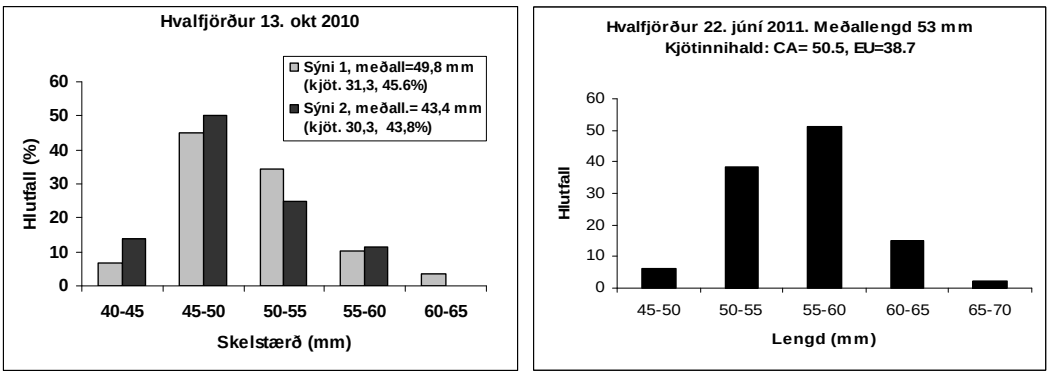

Figure 1. Shell length distribution, mean length and meat yield in samples from Hvalfjörður in October 2010 and June 2011.

Breiðafjörður: The sampling was carried out at 2 m depth at Króksfjarðarnes (65°27´205-21°56´95) in June and October 2010 and May 2011. The mean shell length in June was 58,9 mm and the meat yield was 34,3 and 46% depending on methods used (Figure 2). In October the mean shell length was 55,4 mm and the meat yield had decreased to 26,5% and 40,4%, because of spawning (Figure 2). In May 2011 the mean shell length had reached 61 mm and the meat yield 28,3% and 39,2% depending on the method used.

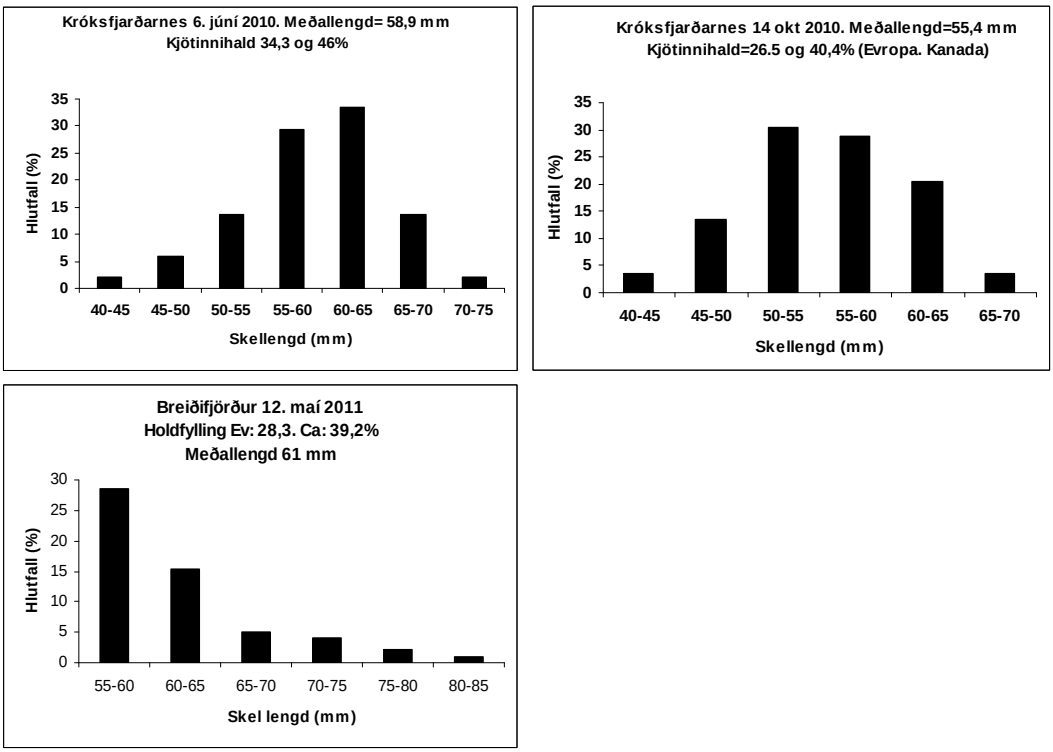

Figure 2. Shell length distribution, mean length and meat yield in samples from Breiðifjörður, in June and October 2010 and May 2011.

Álftafjörður: The sampling was carried out at 2 m depth in Álftafjörður (66°00´6-22°58´5) in Ísafjarðardjúp in June and October 2010 and May 2011.
In June the mean shell length of the samples was 61,8 mm and the meat yield was 29,2% and 46,6% depending on method (Figure 3). The samples from October were not measured for length or weight. In May 2011 the mean shell length had reached 63,4 mm and the meat yield was 28,3% and 46,7 % depending on the method used.

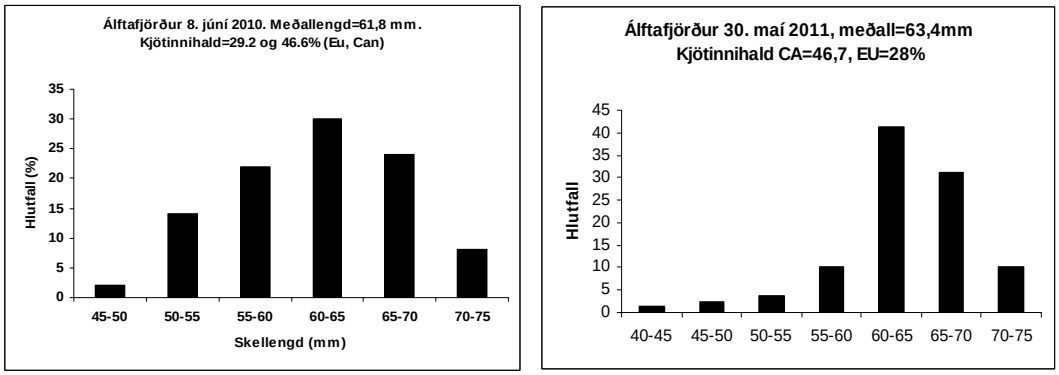

Figure 3. Shell length distribution, mean length and meat yield in samples from Álftafjörður, in June 2010 and May 2011.

Eyjafjörður: The sampling was carried out at 2 m depth at Hrísey in Eyjafjörður (66°58´6-18°22´0) in June, August and October 2010 and in February and May 2011. In June the mean shell length of the samples was 63 mm and the meat yield was 27,7% and 41,5% depending on method (Figure 4). In August the mean shell length was 60,1 mm (Figure 6) and the meat yield had decreased to 21,6% and 30% respectively, because of spawning. The samples from October were not measured for length or weight.

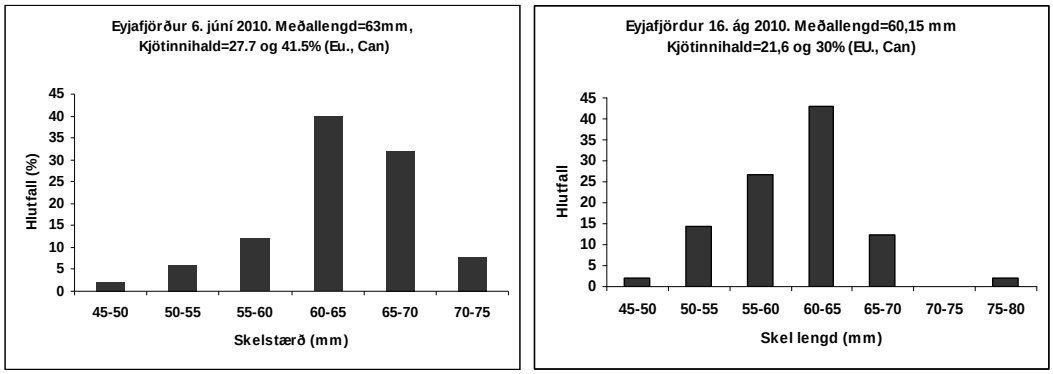

Figure 4. Shell length distribution, mean length and meat yield in samples from Eyjafjörður, in June and August 2010.

In February 2011 the mean shell length was only 48 mm because of sampling from lines with younger mussels than the year before. The meat yield was rather high for this time of the year, 24,2% and 35,4% depending on the method used (Figure 5). In May the mean length was about the same, 47,8 mm, and the meat yield had reached 38% and 51% depending on method used.

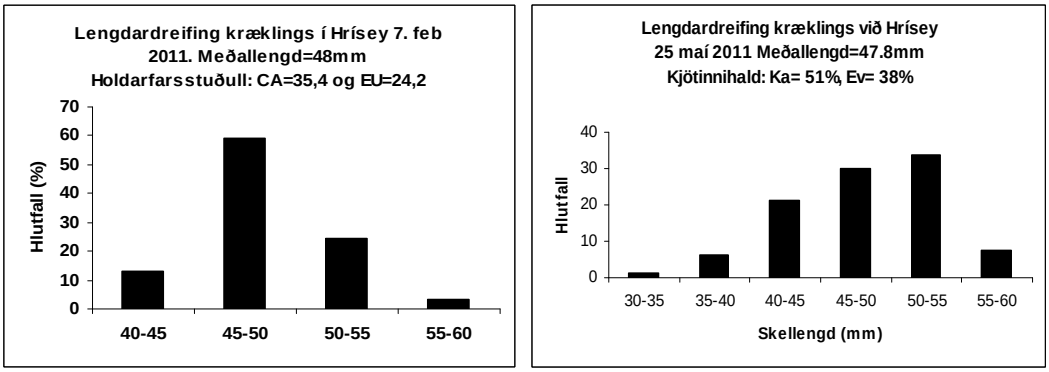

Figure 5. Shell length distribution, mean length and meat yield in samples from Eyjafjörður, in February and May 2011.

3.2 Nutritional composition

Analysis of protein, fat, ash and water were performed in all samples (Figure 6 and 8). The average values measured were 8,99% protein, 1,11% fat, 2,38% ash and 84,5% water.

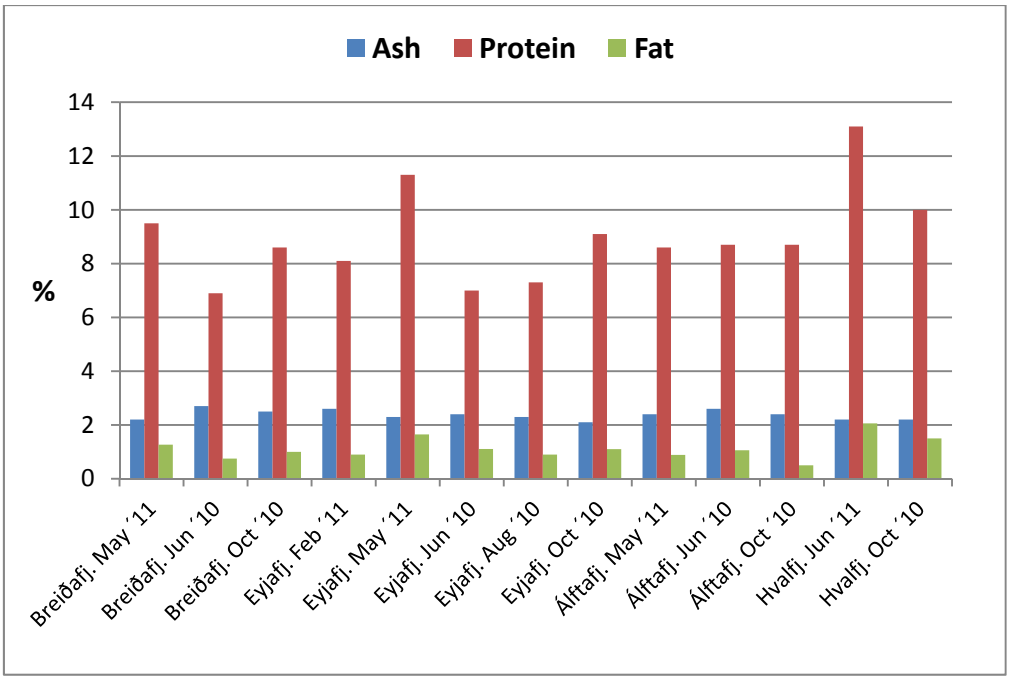

Figure 6. Nutritional composition of blue mussel samples from all growing sites, edible portion.

A moderate seasonal variation pattern was observed in all measured nutritional parameters and the principal components analysis (Figure 7) showed that mussels contained higher proportion of fat and protein during spring (May-June) months, in the autumn the proportion of protein is reduced while the proportion of other unknown substances increases. The PCA analysis also revealed weak a positive correlation between protein and fat and a strong negative correlation between protein and other unknown substances.

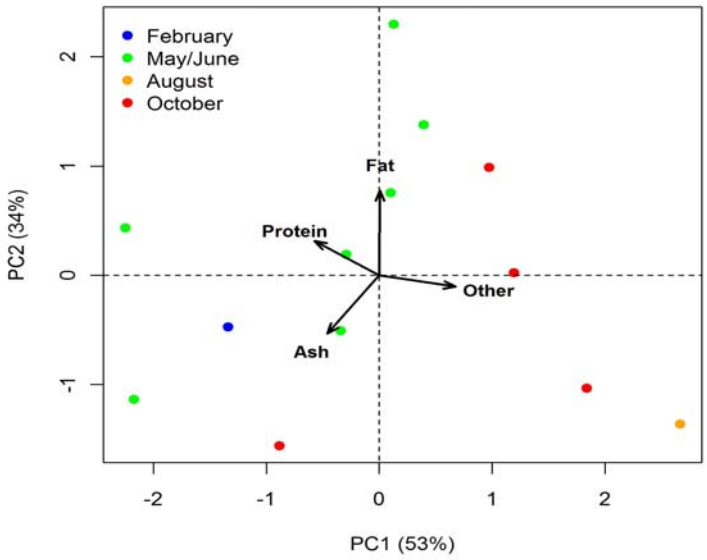

Figure 7. Principal Component Analysis (PCA) of variation in nutritional composition with respect to season.

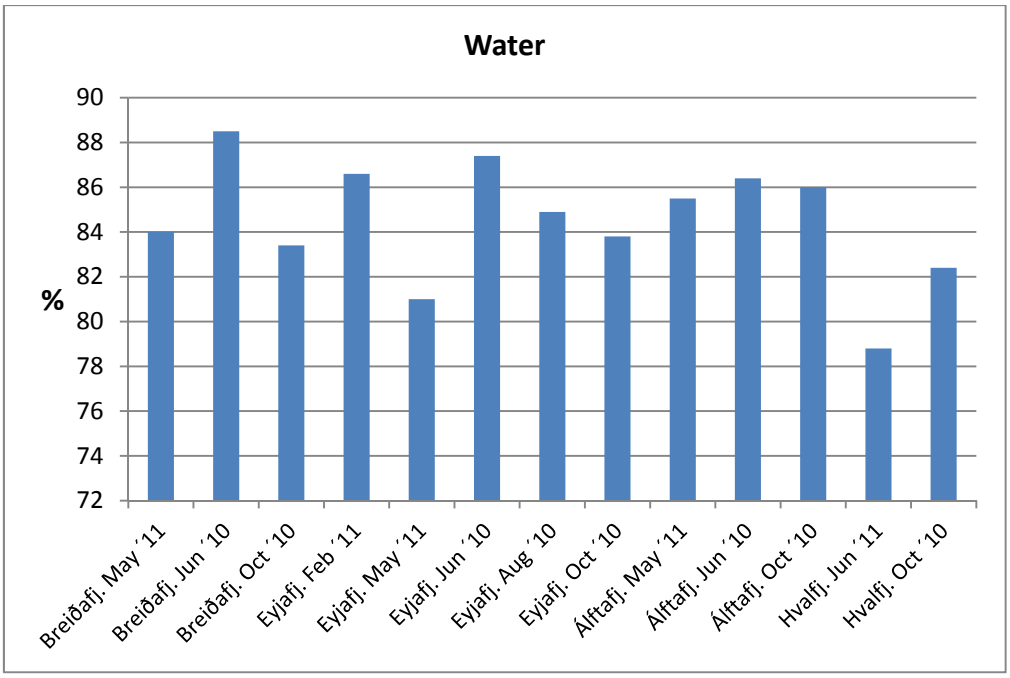

Figure 8. Water content in blue mussel edible portion from all sampling sites.

3.3 Bioactive components

Particular attention was given to analysis of zinc (Zn), for its brain function promoting activity and strengthening of the immune system as well as selenium (Se) which is known to work against mercury uptake in the brain. The results obtained are shown in Figures 9 and 10. In all samples from the four harvesting sites a slight increase in the measured amounts of these important trace elements were observed in the spring months (February-May).

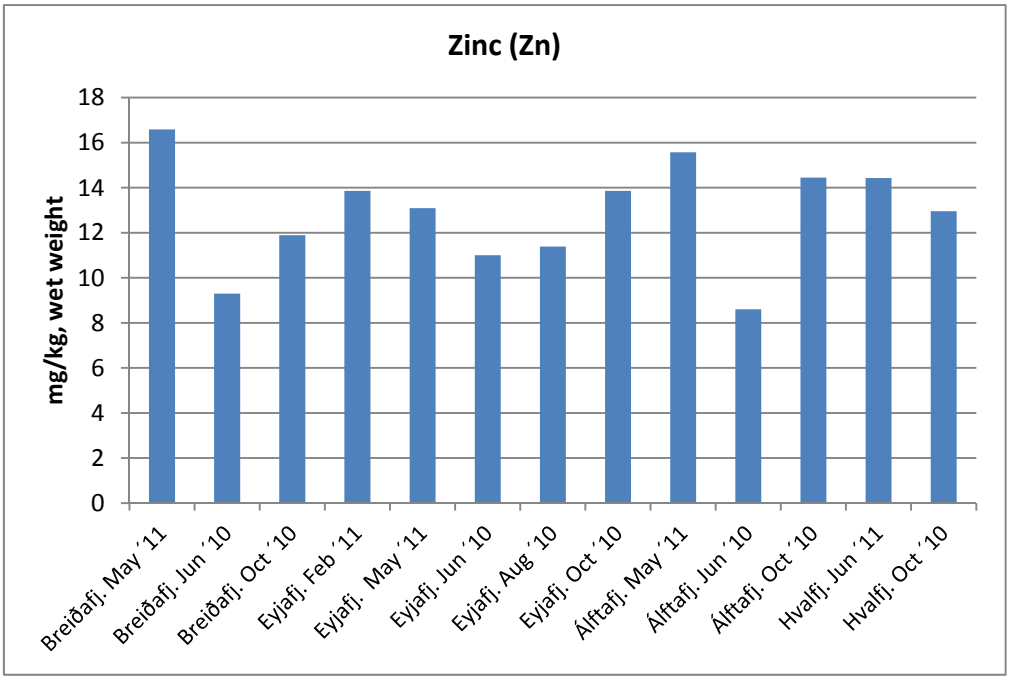

Figure 9. Zinc levels in blue mussel edible portion from all sampling sites.

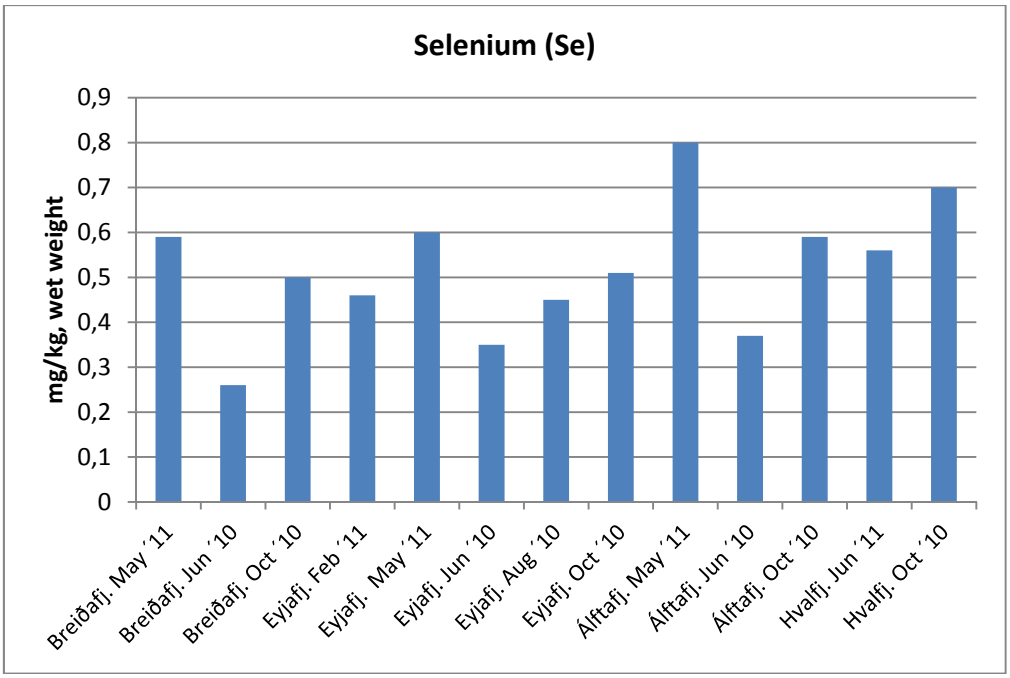

Figure 10. Selenium levels in blue mussel edible portion from all sampling sites.

The results obtained from the lipid extraction with Bligh and Dyer (an essential part of the fatty acid profile method) compared with the Soxlet extraction with ethyl ether were as expected, where the higher polarity of the Bligh and Dyer solvent mixture resulted in a more effective fat extraction from the mussel flesh in all cases.

The fatty acid profile of the mussel samples is illustrated in Figure 11 and the main results accumulated in Table 2. The results reveal high levels of poly-unsaturated fatty acids in all samples (41,5-46,6%, Table 2.), especially the omega-3 fatty acids EPA and DHA (C20:5n3 and C22:6n3), as well as significant amounts of palmitoleate (C16:1n7) (Figure 11).

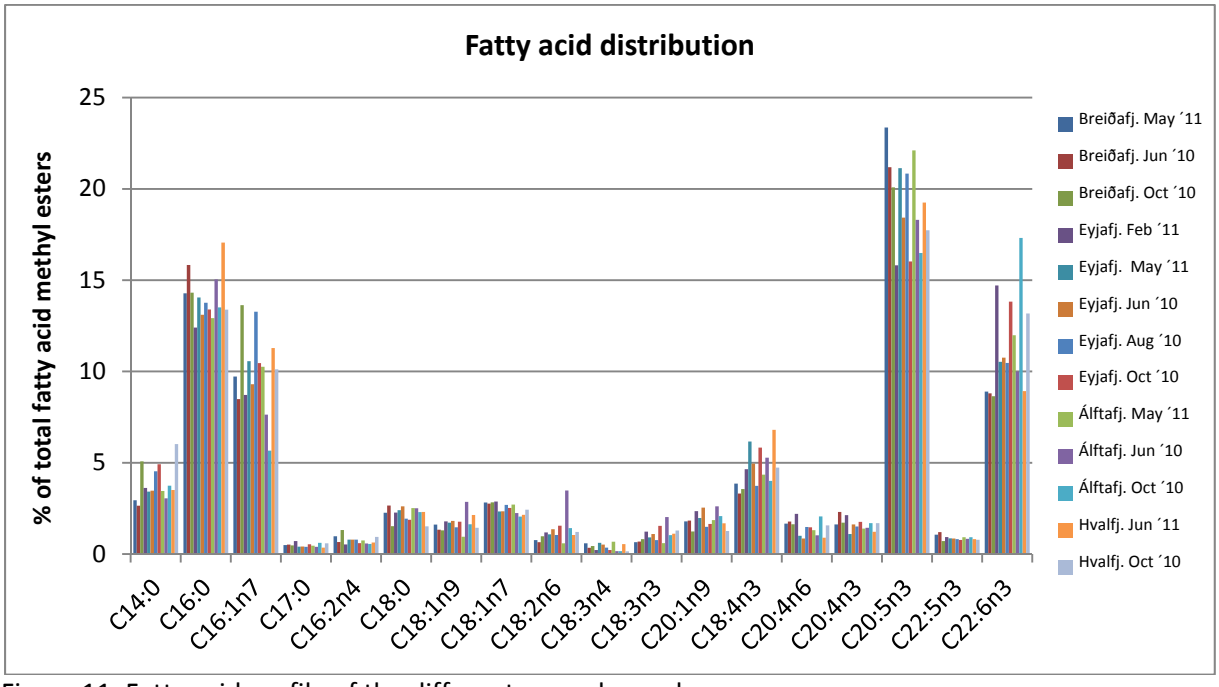

Figure 11. Fatty acid profile of the different mussel samples.

Table 2. Fatty acid distribution of the different mussel samples.

	Fatty acid distribution expressed in % of total fatty acid methyl esters				
	Saturated	*Mono-unsaturated*	*Poly-unsaturated*	*Trans*	*unknown*
Breiðafj. May ´11	20,6	16,4	44,2	0,6	18,3
Breiðafj. Jun ´10	22,3	16,0	41,6	0,4	19,7
Breiðafj. Oct ´10	22,3	20,6	41,5	0,5	15,1
Eyjafj. Feb ´11	20,0	16,4	44,0	0,4	19,1
Eyjafj. May ´11	20,9	17,2	44,9	0,3	16,7
Eyjafj. Jun ´10	20,4	17,9	42,4	0,5	18,9
Eyjafj. Aug ´10	21,4	20,4	42,8	0,4	15,1
Eyjafj. Oct ´10	21,6	18,1	44,5	0,6	15,2
Álftafj. May ´11	20,1	16,3	45,4	0,3	17,9
Álftafj. Jun ´10	21,8	16,8	44,3	0,4	16,7
Álftafj. Oct ´10	21,3	12,7	46,6	0,4	19,0
Hvalfj. Jun ´11	23,9	17,7	41,7	0,4	16,3
Hvalfj. Oct ´10	22,5	16,8	44,3	0,6	15,8

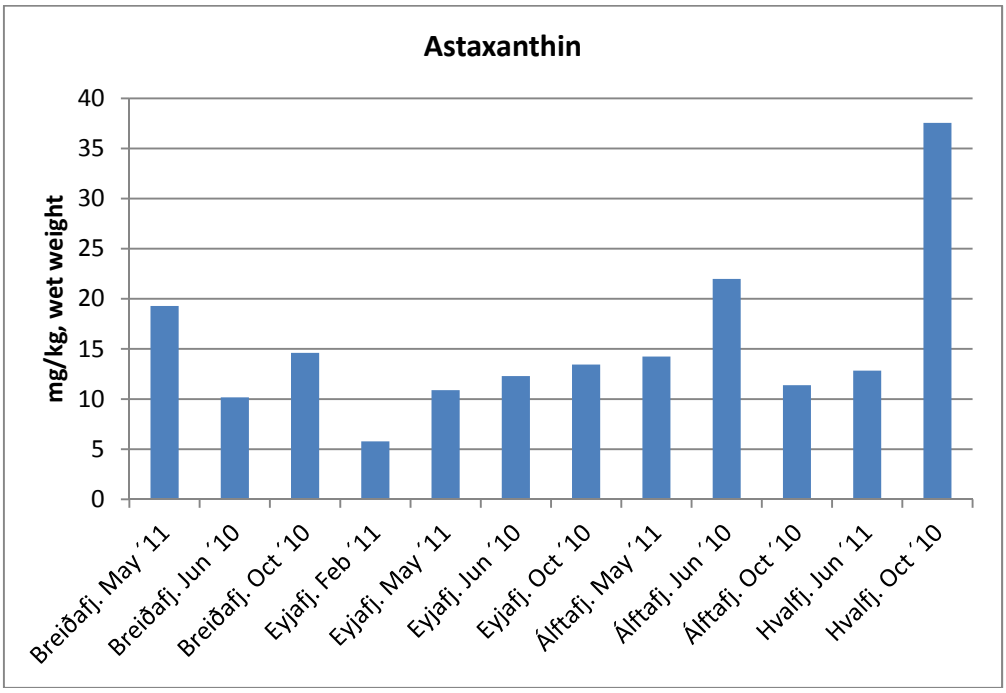

Figure 12. Astaxanthin levels in harvested mussel samples.

Astaxanthin was measured in all 13 mussel samples (Figure 12) and the average concentration was 15,4 mg/kg of wet weight. Although there was no obvious pattern, some moderate seasonal variations in measured carotenoids could be seen. The general trend appears similar as for the nutrients measured, with higher concentrations during spring months, before spawning and again during autumn months after spawning (Figure 7). Alterations in astaxanthin levels are therefore probably influenced by the same factors as other biochemicals in mussels, although a key factor for astaxanthin production is the algal availability in the local surroundings.

It was also studied how the astaxanthin concentration correlates with CIE L*a*b* values from the machine vision, where L* represents lightness of the colour, “a” is the redness vs. greenness and “b” is the yellowness vs. blueness of the mussel tissue. No correlation could be found between astaxanthin concentration and the L values (data not shown). As for “a” and “b” a minor trend was visible, but further studies with a larger sample set would be needed to confirm these findings.

3.4 Chemical risk factors

Market size mussel samples from all four harvesting sites were subjected to trace element analysis in order to evaluate levels of toxic elements with defined EU maximum limits (Pb, Cd, Hg), as well as other trace elements of concern (Figure 13 and 14).

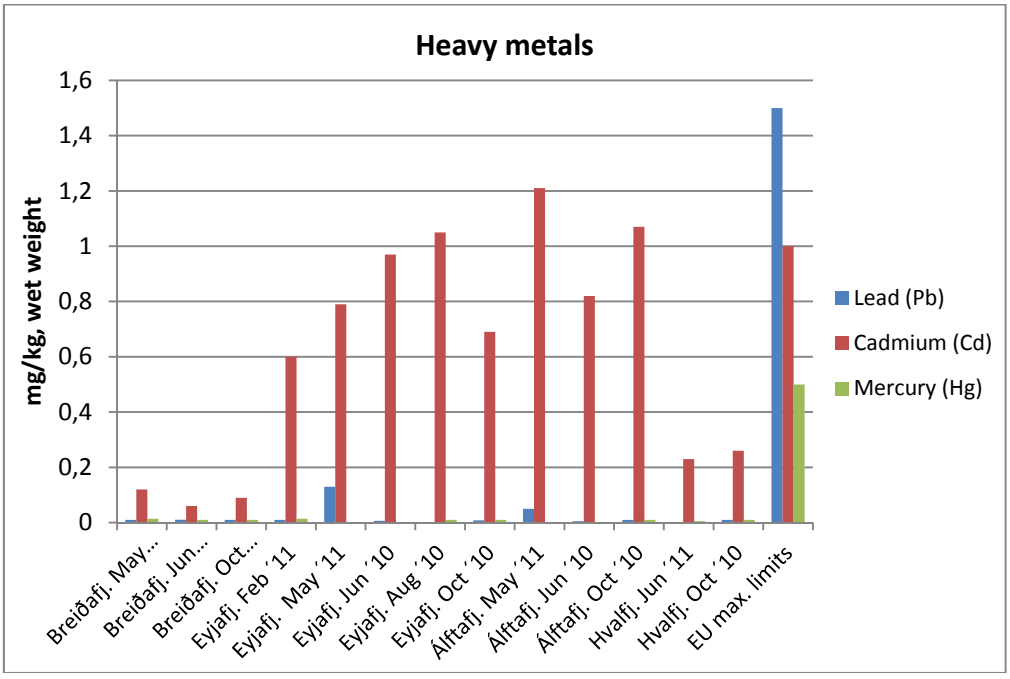

Figure 13. Levels of lead, cadmium and mercury expressed as mg/kg wet weight in the mussel flesh of all harvested samples.

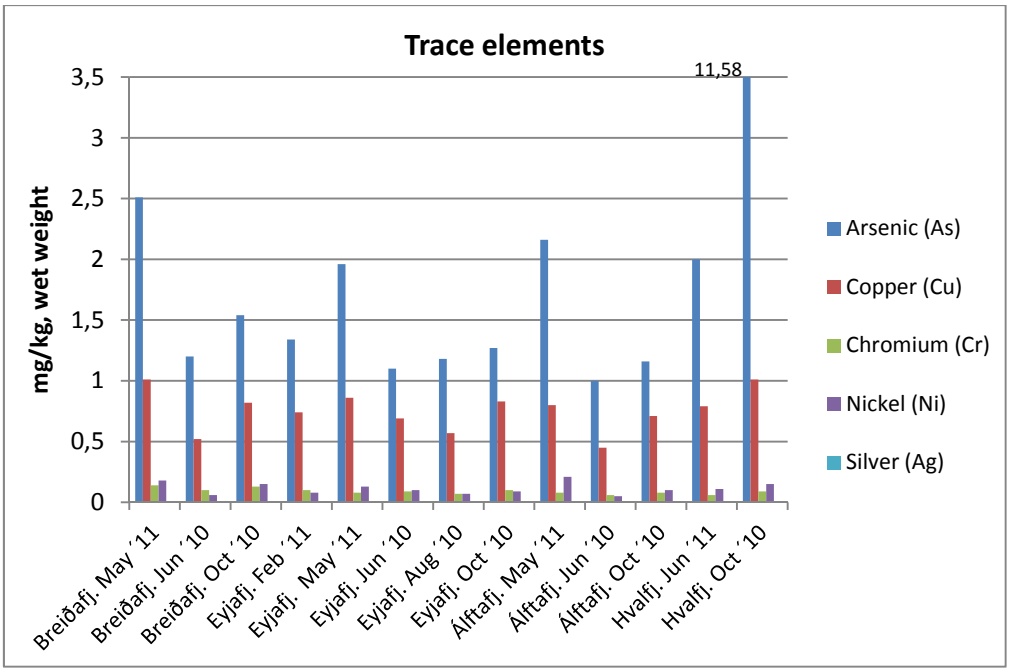

Figure 14. Levels of As, Cu, Cr, Ni and Ag expressed as mg/kg wet weight in the mussel flesh of all harvested samples.

These results indicate a seasonal variation in the concentration of heavy metals and other trace elements in the mussel flesh (Figure 13 and 14). Elevated levels of cadmium were found in market size mussels at Icelandic culture sites, which appears to be of particular concern for the mussel farmers in Álftafjörður and Eyjafjörður where in some months it might exceed the maximum EU limits (1 mg/kg wet weight) for bivalve mussels on the European market. Since this would render it illegal to export the product to EU or sell it on the Icelandic market, it is very important to follow closely the amount of cadmium in Icelandic market size mussels. Very low levels of mercury and lead were measured in the mussel samples and thus these heavy metals do not raise concerns. For one sample from Hvalfjörður a considerably higher level (11,58 mg/kg wet weight) of arsenic was measured than in the other mussel samples therefore the measurement was repeated and confirmed. Currently there is no maximum limit in the EU for arsenic in bivalve mussels so this single case does not necessary require any follow up.

PSP algal toxins:

A rapid commercially available ELISA assay (from Abraxis) to measure Saxitoxin (one of > 20 PSP toxins) has been tested to evaluate its potential as a screening assay for PSP in the edible portion of the blue mussel. To evaluate the rapid assays suitability for routine monitoring and quantitative analysis of PSP mussel samples were received from Norges veterinærhøgskole that had been measured previously for PSP using conventional analytical methods. The results from the Saxitoxin ELISA test measurements for all 13 samples taken as part of this research project are illustrated in Figure 15 and in all 13 cases the concentrations are under the EU regulatory limit of 800 µg/kg for PSP in mussel flesh.

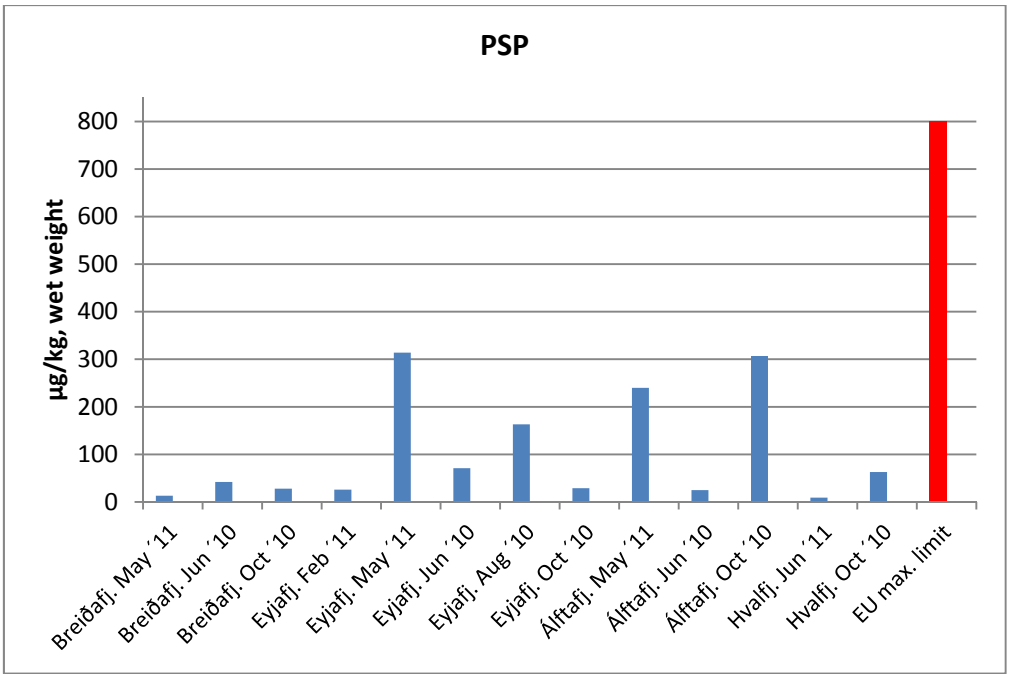

Figure 15. Levels of PSP/Saxitoxinexpressed as µg/kg wet weight in the mussel flesh of market size mussel samples taken at four different culture sites around Iceland.

One of the Norwegian samples, N422 was measured on three different occasions using the Saxitoxin ELISA test and results compared with the results obtained using liquid chromatography with fluorescence detection (LC-FLD). The results of the Saxitoxin ELISA test were considerably lower than those from Norway that used LC-FLD to measure the concentration of PSP (Table 3). The reason for this underestimation of the amount of PSP toxins measured with ELISA compared to LC-FLD could be that PSP is caused by a suite of chemically slightly different toxins and presently more than 20 different congeners of PSP toxins have been identified. The Saxitoxin ELISA test is based on recognition of one of these congeners i.e. Saxitoxin by highly selective antibodies but considerably lower sensitivity for other PSP-toxin analogues. If the PSP toxins in mussel sample N442 were primarily composed of other congeners than Saxitoxin this could explain the lower values obtained when the ELISA measurement was used compared to LC-FLD. Unfortunately, the details regarding the PSP toxin profile determined by LC-FLD for the N442 sample was not provided by Norges veterinærhøgskole and thus this conclusion cannot be confirmed. Nevertheless, the results obtained in this research project indicate that there is a risk for underestimation of the PSP toxin amount measured when the Saxitoxin ELISA test is used.

Table 3. PSP measured in mussel sample using ELISA assay from Abraxis and conventional method.

	µg/kg
N442_1, ELISA	175
N442_2, ELISA	155
N442_3, ELISA	358
N442, LC-FLD	690

The Saxitoxin ELISA test was easy to work with, and instructions clear. Depending on the number of samples to be measured the test procedure and the calculations of results can be performed within one working day.

Problems related to the ELISA test were the following;

1. In cases of high PSP concentration it might be necessary to further dilute the samples and re-run the test
2. Since the test is based on Saxitoxin recognition through selective Saxitoxin antibodies with low sensitivity for other PSP toxin congeners (> 20 different existing) there is a risk for underestimation of the actual PSP toxin amount.
3. Our results indicate that the Saxitoxin ELISA test gives semi-quantitative results and can indicate the risk of exceeding the maximum EU limits for PSP, however the repeatability of the test procedure was limited and was highly dependent on how well the prepared sample dilutions fitted on the standard curve for Saxitoxin. Further work needs to be carried out to optimise this ELISA assay for the measurement of PSP in Icelandic mussels and verify the results obtained.

As part of the evaluation of a rapid test for PSP a so-called Jellet test was also tried out. For this test a similar preparation of the mussel samples is required prior to running the test, but no laboratory equipment such as centrifuge or spectrophotometer is needed to carry out the test. The Jellet test is easy to perform and the only drawback of the test is that it does not give quantitative results, i.e. only positive or negative answer regarding the presence of PSP is obtained, and hence there is some risk for false positive or false negative results.

It is important to keep in mind that these rapid tests for PSP only provide screening results. Further testing with reference analytical methods will be required to confirm the results from these rapid tests before the mussels are put on the consumer market. The rapid tests are suitable for quality control and decision making regarding whether or not it is safe to harvest the mussel crop or if the mussels should be harvested later after purification in the ocean. These rapid tests can thus reduce the mussel growers analytical cost as the pre-screening will indicate whether the level of toxin present in the mussel flesh is likely to exceed the EU regulatory limit.

DSP algal toxins:

A rapid commercially available Okadaic acid (DSP) ELISA assay (from Abraxis) was used to measure DSP in blue mussel samples to evaluate if this assay could be used for screening of DSP in the edible portion of the blue mussel. As mentioned above mussel samples were received from Norges veterinærhøgskole that had previously been measured for DSP using conventional analytical methods. These samples were used as reference samples to evaluate whether the rapid DSP ELISA assay from Abraxis is suitable for routine monitoring and quantitative analysis of DSP in mussels. The results from the Okadaic acid (DSP) ELISA test measurements for the samples obtained in this research project are illustrated in Figure 16. The concentrations of Okadaic acid (OA) were generally higher than expected and in fact higher than the EU limit for OA (160 µg/kg) in 4 samples out of the 13 blue mussel samples investigated. DSP values measured with the rapid ELISA test were also higher compared with the results from Norway that were measured using LC-MS/MS for DSP (Table 4). In this case the test is based on Okadaic acid recognition by specific antibodies which are highly selective (100%) for Okadaic acid but considerably lower for Dinophysistoxins DTX-1 and DTX-2. Possibly interference caused by matrix effects could explain the overestimation of Okadaic acid observed in this study.

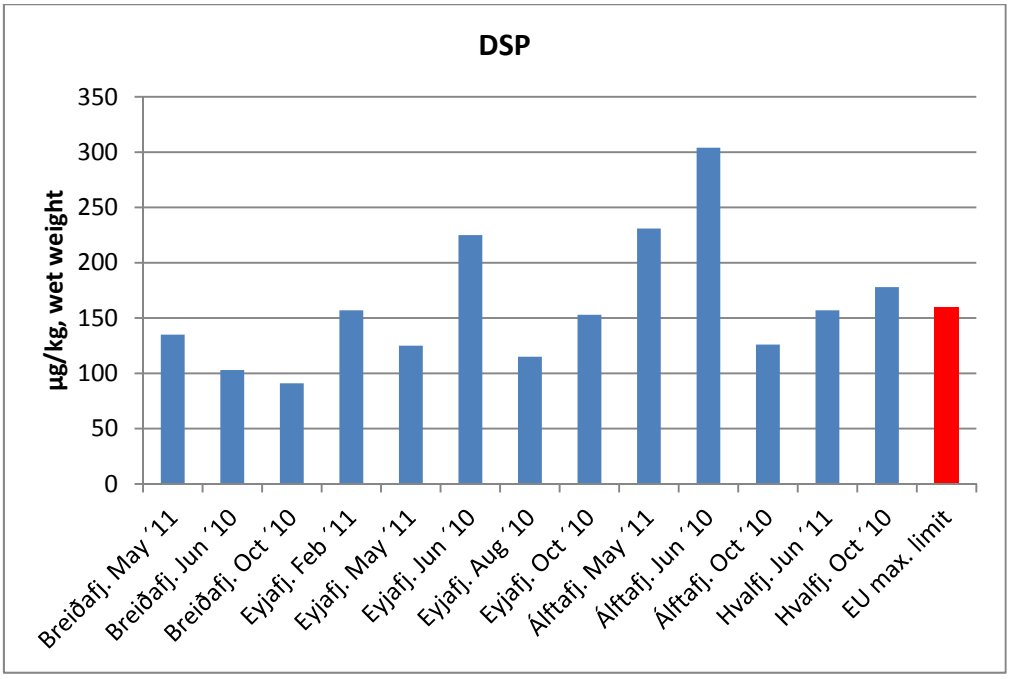

Figure 16. Levels of Okadaic acid (DSP) expressed as µg/kg wet weight in the mussel flesh of market size mussel samples taken at four different culture sites around Iceland.

Table 4. DSP measured in Norwegian mussel sample using ELISA assay from Abraxis and conventional analytical method.

	ELISA, µg/kg	LC-MS/MS, µg/kg
N2009/22	545	405
N2010/483	301	117

Same advantages and disadvantages as observed and reported for the Saxitoxin ELISA test from Abraxis are true for the Okadaic acid (DSP) ELISA assay (from Abraxis) to measure DSP (see detailed discussion above for PSP algal toxins) with the exception that the DSP test is based on recognition of Okadaic acid by specific antibodies.

A Jellet test for DSP was also tried out and similar observations were made regarding their applicability as for the PSP test (see above).

Further work needs to be carried out to optimise this ELISA assay for the measurement of DSP in Icelandic mussels and verify the results obtained. As for PSP it is important to keep in mind that these rapid tests for DSP only provide screening results. Further testing with reference analytical methods will be required before the mussels are put on the consumer market to confirm the results from these rapid tests.

ASP algal toxins:

Rapid commercially available ELISA assay for ASP from Biosense was also tested to evaluate whether this assay could be used for screening of ASP in the edible portion of blue mussels. The assay is primarily intended for use in routine monitoring of Domoic acid (DA), commonly related to amnesic shellfish poisoning (ASP).

Table 5. ASP measured in mussel samples using ASP ELISA assay from Biosense.

Sample	Abs.	Extract/solution (pg/mL)	Sample DA eqv. (mg/kg)	ASP measured with other methods (µg/kg)
Álftafj. Jun ´10	0,928	>Amax	To diluted	NA
Breiðafj. Jun ´10	0,875	40.289	To diluted	NA
Eyjafj. Jun ´10	0,835	83.511	To diluted	NA
2007/730 RSM	0,329	3.073.487	15,4	10,27
2009/1078 RSM	0,889	>Amax	To diluted	< 0,500

The results obtained using the ASP ELISA test showed that only one of the Norwegian reference samples contained measurable amount of DA, while all other samples were below the test detection limit. This is not surprising given that ASP has never been detected in Icelandic mussel samples. For this assay only one positive sample was available from Norway and the ASP measured with the rapid ELISA ASP test compared fairly well with the result from Norway that used conventional analytical methods i.e. LC-UV to measure the ASP concentration in the sample.

4. Discussion and conclusions

Mussels have been characterised according to location, time of year, meat yield, shell length and weight. The results of shell growth at the culture sites in the present study were variable, most probably due to the sampling. In Hvalfjörður the sampling was conducted in October and June and the growth in this period was 7 mm. The meat yield in October was 31% and spawning had probably not been completely finished, whereas in June the meat yield had reached its maximum (39%) and the shells were probably ready to spawn. These results are comparable to other studies from this site (Thorarinsdottir, 1996). In Breiðifjörður and Álftafjörður the shell growth was very sparse from June to May (2 mm) which is not comparable to other studies from these sites but the meat yield was. The meat yield in Breiðifjörður in June (34%) was probably reaching its maximum as spawning starts in July (Thorarinsdottir and Gunnarsson, 2003). The meat yield in Álftafjörður was 30% in June and spawning at this site is most likely from August through October (unpublished results). In Eyjafjörður on the other hand, samples were not collected from the same culturing ropes every time, and shell lengths therefore not reliable. However, the meat yield was convincing, 28% in June, spawning starting in July, main spawning in August and the meat yield 21% in the end of that month. After spawning the meat yield started to increase again and in February it had reached 24%, increasing steadily until May when it reached its maximum of 38% and the shells ready to spawn, even though spawning starts first in late summer. Our results also indicate that the nutritional composition is influenced by factors such as developmental stage and sex of the mussel.

Considerable amounts of data regarding chemical composition of Icelandic mussels have been gathered, including trace metals (lead, cadmium, copper, zinc, mercury, arsenic, selenium, chrome, nickel and silver), nutrients (moisture, protein, lipid and ash) and bioactive components (carotenoids and fatty acids).

Further work needs to be carried out to optimise the rapid ELISA assays for PSP and DSP toxin measurements and verify the results obtained e.g. by using standard addition procedures with certified reference material.

Our results indicate some seasonal variations in nutritional composition and trace element levels, but more data would be needed to confirm this correlation. Elevated levels of cadmium were measured in mussel samples from certain culture sites and some of these samples actually exceeded the maximum EU limits (1 mg/kg wet weight) for cadmium in bivalve molluscs, indicating the need for further investigation and monitoring.

The average lipid content on a wet weight basis was 1,11% in the Icelandic market size mussels which is similar to lipid content values reported from earlier studies, which ranged from 1,2 to 1,8% in blue mussels (Murphy et al., 2002). Lipids have been shown to be involved in spawning-related biochemistry in marine species, where the lipid contents may be expected to be at its highest just prior to spawning and at its lowest just after spawning periods (Mclean and Bulling, 2005). In this study, the results for blue mussel lipids showed a slight seasonal trend for the different sampling times, although the relatively few samples and infrequent sampling can make it difficult to detect any larger changes in lipid levels. From the PC analysis some correlation could be drawn between season and nutritional composition, where higher contents of fat and protein appear to be related to the spring and summer months whilst energy reserve components are predominant during the autumn months.

The fatty acid profile of the blue mussel samples revealed interesting results where apart from the presence of common saturated fatty acids found in plants and animals (Myristic C14:0, Palmitic C16:0 and Stearic C18:0), there are significant levels (average values of 19,3% and 11,4%, respectively) of the health beneficial omega-3 poly-unsaturated fatty acids Eicosapentaenoic (EPA, C20:5n3) and Docosahexaenoic (DHA, C22:6n3). The results obtained in this study are similar to earlier findings (Murphy et al., 2002) with slight differences in individual lipid class composition, which probably reflects variations in the composition of their algal and phytoplankton diet, as well as the developmental stage and sex of the mussel. In addition was the newly discovered lipokine Palmitoleate (C16:1n7) found in relatively high amounts in the Icelandic mussels with an average value of 9,9% compared to 3,3% reported by Murphy et al. 2002. Lipokines are a new class of hormones which have demonstrated several health beneficial actions such as increasing the response of muscle tissue to insulin, regulating the liver's handling of fats, reducing build-up of harmful fats and inflammation mediators normally produced by adipose tissue (Cao et al., 2008).

From the limited number of samples investigated in this study it is not possible to draw any definite conclusions, however, will these promising results provide a great aid and encouragement to the maturing Icelandic mussel industry. This report also determined that Icelandic blue mussels is a product of high quality in comparison to the market standard and can be a valuable source for Icelandic economy.

5. Acknowledgement

This research received financial support from AVS R&D Fund of Ministry of Fisheries in Iceland, also Matis, Marine Research Institute, Nesskel, Norðurskel, Vesturskel and SR4 contributed to the financing of the project. All employees of these companies and institutes are acknowledged for their contribution to the project. Norges veterinærhøgskole in Oslo is also gratefully acknowledged for providing reference mussel samples.

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