Masters thesis

Quantitative Microbiological Risk Assessment of *L. monocytogenes* in Blue Mussel (*Mytilus edulis*)

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Department of Natural Resource Sciences

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UNIVERSITY
Fisheries Training programme

Declaration

I hereby wish to declare that this work is done by me and was not done and submitted at any place before.

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Md. Murad Mufty

It is hereby confirmed that this master thesis is satisfactory to M.Sc.-degree from the faculty of Business and Science, department of Natural Resource Science.

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Prof. Hjorleifur Einarsson PhD

Dedication

I would like to dedicate this work to my beloved wife Bosra Khanam, our two daughters Rostiana & Raftah and my mother Peara Begum, for their support and influence in my life and work especially in favour of uncomplaining and missing my company during the entire study period.

Abstract

Aims

Mussels are generally regarded as high-risk products; they are likely to be contaminated with indigenous and non-indigenous pathogens and toxins. The aim of this study was to conduct a quantitative microbiological risk assessment (QMRA) of *L. monocytogenes* in the blue mussel (*Mytilus edulis*), following the guideline provided by Codex Alimentarius (1999).

Methods

The study was based on prevalence of illness (Listeriosis) and by consumer survey on blue mussel consumption in Iceland, also by collecting data on *L. monocytogenes* contamination in blue mussels and its environment, simulation and prediction of bacterial growth in the mussels. A quantitative risk assessment model was developed to assess the risk in healthy and susceptible population. Different assumptions were made to describe the variables of the model by probability distribution and mathematical models. Monte Carlo simulations of the model were run to estimate the number of cases in healthy and susceptible populations.

Results

Contradictory to results from abroad no *L. monocytogenes* were found in blue mussels collected locally in Iceland. Consumption of mussels in Iceland is low compared to other countries. From the three risk management measures simulated, shelf life reduction and hygiene improvement with triangle distribution for the both healthy and susceptible population groups was the most effective in reducing the number of cases of illness. Bases on research data, few risk management options were provided to minimize the defined risk.

Conclusion

This work showed that the risk of acquiring Listeriosis from the consumption of locally grown Blue mussels is minimal. The fresh Blue mussels were found save to consume however other hazards might pose other risks

Keywords: *Listeria monocytogenes, Mytilus edulis,* QMRA, Risk assessment, Simulation, Iceland.

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List of Papers

This thesis is based on the following three papers, referred to in the text by their respective Roman numerals –

Paper I. Prevalence of *L. monocytogenes* in blue mussel (*Mytilus edulis*) in Iceland

Paper II. Consumption pattern of blue mussel (*Mytilus edulis*) in Iceland

Paper III. Survival and growth of *L. monocytogenes* in blue Mussel (*Mytilus edulis*) in Iceland

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Glossary

AIDS	:	Acquired immune deficiency syndrome
BAM	:	Bacteriological Analytical Manual
BGLB	:	Brilliant Green Lactose Bile
BHI	:	Brain Heart Infusion
bp	:	base pair
CAMP	:	Christie, Atkins, Munch-Petersen
CFU	:	Colony Forming Unit
DSMZ	:	Deutsche Sammlung von. Mikroorganismen und Zellkulturen GmbH
DSP	:	Diarrhetic Shellfish Poisoning
EC	:	European Commission
EC (broth)	:	*Escherichia coli* (broth)
EEA	:	European Economic Area
EU	:	European Union
FAO	:	Food and Agriculture Organization
FDA	:	Food and Drug Administration
GHP	:	Good Hygiene Practice
GMP	:	Good Manufacturing Procedures
HACCP	:	Hazard Analysis Critical Control Point
LM	:	*Listeria monocytogenes*
LST	:	Lauryl Sulfate Broth
MPN	:	Most Probable Number
NA	:	Nutrient Agar
NB	:	Nutrient Broth
OCLA	:	Oxford Chromogenic *Listeria* Agar
PCA	:	Plate Count Agar
PCR	:	Polymerase Chain reaction
PI	:	Probability of Illness
ppm	:	Parts Per Million
PSB	:	Paralytic Shellfish Poisoning
QMRA	:	Quantitative Microbiological Risk Assessment
RA	:	Risk Assessment
RASFF	:	Rapid Alert System for Food and Feed
RTE	:	Ready To Eat
SCVPH	:	Scientific Committee on Veterinary Measures relating to Public Health
SS	:	Serving Size
SSSP	:	Seafood Spoilage and Safety Predictor
TSYEA	:	Tryptone Soy Yeast Extract Agar
TSYEB	:	Tryptone Soy Yeast Extract Broth
UNAK	:	University of Akureyri, Iceland
UNU-FTP	:	United Nations University-Fisheries Training Programme
WHO	:	World Heath Organization

1. Introduction

Food safety is of prime importance for consumers, authorities and producers. According to the current European Food laws (Regulation (EC) 178/2002) food shall not be placed on the market unless it is safe. It is primarily the responsibility of food producers to ensure food safety and to minimize any risk associated with consumption of a given food item by using systematic approach to identify hazards associated with a given product and process, implement appropriate control measures and apply general Good Manufacturing Practices (GMP). Risk assessment is the scientific basis for risk management and safety criteria for foods.

Risk assessment (RA) is the determination of quantitative or qualitative value of risk related to a concrete situation and a recognized thread called hazard and should be undertaken in an independent, objective and transparent manner. Risk assessment is a scientifically based process consisting of four steps (FAO 1999);

Hazard identification,
Exposure assessment,
Hazard characterisation and
Risk characterisation

Risk assessment is a careful examination of what, in one's surroundings, could harm people, so that one can weight up whether he has taken enough precautionary measures or should do more to prevent harm (HSE, 2010). In public health, risk assessment is the process of estimating, either qualitatively or quantitatively, the probability of a harmful effect to individuals or populations from certain human activities (EHRA, 2002). The presence of specific chemicals, biochemical agents, pathogenic bacteria, viruses etc. are not allowed unless it can be shown that they don't increase the risk of death or illness above an acceptable level. Risk assessment is thus important throughout all steps of food production from field to fork not at least in the seafood processing industries. Risk assessment is an addition to the traditional safety precautionary measure like HACCP, GMP, and GHP.

Risk assessment is an objective foundation for the development of a science-based system of preventative controls. The objective of risk assessment is to characterize the nature and graduation of the risk to human health associated with hazards, and to make clear the degree of scientific certainty of the data and the assumptions used to develop the estimates (Adeel *et al.*, 2004).

The aim of a microbiological risk assessment for a particular pathogen or group of pathogens is to estimate the level of illness that may be expected in a target population from a product or group of products. The risk assessment process involves the description of a food production system and the identification of possible points of failure that could increase the risk for a particular hazard. Its most important objective is to provide information that will help the producers as well as each intervenient in the farm-to-fork continuum (production to consumers table) to determine whether a pathogen is, or could be, a significant hazard in their production system and how best to prevent the hazard being realised.

Risk assessment is increasingly used as a scientific process to assess the potential for adverse health effects to occur and as a basis for management of unacceptable risks. The importance of estimation of the risk of eating a certain food (based on factual information or experience) is increasingly considered. Food authorities and consumers associations need to support their decisions of whether the risk is small enough, and may accept the risk and inform the consumer, or to implement actions to reduce the risk to a tolerable level. For many foods, the level of food safety generally accepted reflects the history of their safe consumption by humans and is generally considered safe, provided that care is taken during primary production, processing, storage, handling and preparation. But for certain higher risk pathogens and certain health and susceptible group of persons, considerations on safety have made the Codex Alimentarius Commission (Codex Alimentarius,1999) to propose, research on these risks using risk assessment process. The aim of a risk assessment approach is to estimate the level of illness that may be expected on a target population from a product or group of products.

The move to increased consumption of ready-to-eat (RTE) foods demands changes in food handling and storage practices by food manufacturers, distributors, preparers and consumers to minimize microbial contamination.

One hazard associated with RTE foods is *Listeria monocytogenes*. It is commonly found in food processing, distribution, and retail environments also in the home and it can grow in many foods at refrigeration temperatures.

Shellfishes are important potential sources of food borne illness. Because of their way of feeding they concentrate bacteria present in their frequently polluted surrounding aquatic environment therefore pathogens like *Listeria* may be regularly present in this product. Recent efforts by industry and regulatory agencies led to a substantial reduction in the incidence of Listeriosis, but it continues to be considered a major public health issue. However, further reductions are being difficult because of the unique challenges associated with controlling these pathogens and changes in the ways foods are processed, distributed, prepared, and consumed today.

At present, more foods are bought already prepared from retail establishments where adequate food safety measures may not be in place to control or prevent microbial contamination. Food items like shellfish that are traditionally eaten raw or very lightly cooked present an increased health risk (Garret & Hudak-Roos, 1991).

FAO and WHO (FAO/WHO, 2001) have been gathering information concerning RA to seafood from several pathogens but there is not much information on *Listeria.*

In this context and taking into consideration the lack of data at European level on the risk of consuming *Listeria* contaminated traditional food items, this project intention was to collect information, generate data, and propose a predictive risk model in order to contribute to extend the knowledge on the risk to public health and food safety.

2. Background

In order to conduct a risk assessment of *L. monocytogenes* in blue mussel it is essential to get previous results from similar tasks. Much effort has been made both in academy and in industry during recent decades to assess the quantitative risk of pathogens in foods for the consumer safety followed by Codex Alimentarius both in natural contamination studies and with inoculation studies of risk assessment in seafood.

Both FAO/WHO (2001) and FDA/FSIS (2001) had carried out QMRA on *L. monocytogenes* in RTE foods. Many RTE foods were found to cause sporadic and small outbreaks of Listeriosis. Rocourt *et al*. (2003) made an opinion on FAO/WHO risk assessment process after the FAO/WHO *Listeria* risk assessment team adapted and expanded the risk assessment of *L. monocytogenes* in RTE foods from national level to international level. The FAO/WHO (2004a) risk assessment of *L. monocytogenes* in RTE foods summarized that the ability of RTE foods to support growth of *L. monocytogenes* resulting in increased risk of Listeriosis on a per serving basis by 100 to 1000 fold over what the risk would have been if the food did not support growth. Similarly the ability of a product to support growth within its shelf-life can increase substantially the risk of that product being a vehicle for food borne Listeriosis.

Growth of *L. monocytogenes* in food is a function of the storage time, storage condition, and rate of growth in specific foods. FDA (2003) conducted risk assessment of *L. monocytogenes* of foods from the contamination data obtain from retail to consumption. The square root exponential growth rate (EGR) model was used because of its simplicity and general acceptance as indicated by the documented use in the microbiology literature (Ratkowsky *et al.,* 1982). Augustin *et al.* (2005) suggested a cardinal and square root type of model could be used for the growth probability prediction of *L. monocytogenes* in dairy, meat and seafood products.

Recently, Carrasco *et al.* (2010) conducted a probabilistic quantitative microbiological risk assessment model of *L. monocytogenes* in RTE lettuce salads in Spain using Monte Carlo simulations to estimate the number of cases in low-risk and high-risk populations and suggested four risk management measures, among them one found best from

simulated results which was the injection of a mixture of gases into packets at manufacture (CO_2 about 5.5%, O_2 about 3% and N_2 for the balance). Pouillot *et al.* (2009) also used a second-order Monte Carlo simulations to develop a model of risk assessment of *L. monocytogenes* in cold smoked salmon in France.

'A dose-response relationship describes how the likelihood and severity of adverse health effects (the responses) are related to the amount and condition of exposure to an agent (the dose provided)' (EPA, 2012). Typically, as the dose increases, the measured response also increases. The second step of risk assessment process is exposure assessment, the relation between exposure levels (dose) and frequency of illness gives the outcome of risk assessment step which is the severity of doses/ exposures. McLauchlin *et al.* (2004) tried to find out the relationship between exposure levels and frequency of illness from a review on QMRA of *L. monocytogenes* done in England and Wales, together with incidence data on different age and risk groups for human *L. monocytogenes* infections. McLauchlin found that the majority of cases of human Listeriosis are food-borne with multiple and complex routes of infections. Buchanan *et al.* (1997) developed a dose-response curve for *L. monocytogenes* based on prevalence data on cold-smoked salmon in Germany. Based on dose-response studies from Buchanan *et al.* (1997), FAO/WHO (2001) and FDA/FSIS (2001) concluded that the minimal infectious dose, i.e. low level of organism (100 cfu/g or less) are very unlikely to cause disease. FAO/WHO (2001) concluded that if *L. monocytogenes* were kept below 1000 cfu/g at the point of consumption, then 99% of all Listeriosis cases would be eliminated (Huss *et al.,* 2003). The key findings of FAO/WHO (2004a) risk assessment of *L. monocytogenes* in RTE foods was that the vast majority of Listeriosis cases are associated with the consumption of foods that do not meet the current standards for *L. monocytogenes* for food, whether the standard is zero tolerance or 1000 cfu/g. Elisa & John (2000) suggested changing the regulatory policy on *L. monocytogenes* towards risk analysis scheme, the policy should include the level of consumption, epidemiology, dose response, and the virulence, biology, and ecology of the organism.

3. Objectives

From the information given above it can be assumed that shellfish is a high-risk product and as is grown close to shore, it is likely to be contaminated with pathogens including *L. monocytogenes*. In order to be able to provide the market with safe products the shellfish industry is in need of scientific data, to conduct a thorough risk assessment. Therefore the main goal of this study was to collect relevant information in order to perform a quantitative risk assessment of *L. monocytogenes* in blue mussel in Iceland.

The study was divided into following tasks:

- Determination of the prevalence and sources of *L. monocytogenes* in shellfish with special reference to the fresh live blue mussel (*Mytilus edulis*).

- A consumer survey on shellfish consumption especially blue mussel consumption in Iceland and to quantify consumer practices relating to the purchase, transport, storage, and preparation of fresh produce, with emphasis on practices that affect safety.

- Estimation of the survival and growth of *L. monocytogenes* in blue mussels from purchase to consumption.

Furthermore the purpose of this study was to find out the most possible risk pathway from the processing steps until consumption and to assess the risk of illness after consuming *L. monocytogenes* contaminated blue mussel.

Based on the developed model, the best risk management options will be proposed in order to minimize the possible risk from *L. monocytogenes* in mussels.

4. Risk Assessment – Methodology & Data Sources

This work follows the general outline of risk assessment given by Codex Alimentarius (Codex Alimentarius, 1999) i.e. Hazard identification, Exposure assessment, Hazard characterization and Risk characterization. In this work a probabilistic QMRA of *L. monocytogenes* in the blue mussel in Iceland was conducted. The QMRA covers all steps along the food chain up to consumption. The QMRA model was built in Microsoft Excel and was simulated by using Vose ModelRisk (Vose ModelRisk Standard 4.0). A sensitivity analysis of the developed model was done to select appropriate Risk Management (RM) measures.

The support information was considered during the modelling are the risk pathways (Food handling process) (figure 3), processing time, weight of sample package, % of contaminated package, initial concentration in the package, r values (single hit probability of illness from one pathogen) and number of serving. It was assume that all contaminated package will make ill and the contaminated package was contaminated with same concentration. We considered for a particular strains of *Listeria* spp. (*L. monocytogenes* serovar 1/2a, DSM 20600).

Data for this work was collected both from literature and from own research. Thus data was collected as follows:

4.1. Own data collection

4.1.1. Human incidence of Listeriosis

The up-to-date Listeriosis incidence was collected from the website of Icelandic directorate of health (Director of Health, S. E., 2012), Centers for Disease Control and Prevention (CDC, 2012.) and European Food Safety Authority (EFSA, 2012).

4.1.2. Prevalence & enumeration of *L. monocytogenes* in blue mussel & its environment (Paper I)

A Review on prevalence of *L. monocytogenes* in seafood, especially in shellfish worldwide and in Iceland was completed from the support of previous published available literature and documents. The enumeration study of *L. monocytogenes* was included 43 live fresh blue mussel samples from the four blue mussel producers of Iceland (figure 11, paper I), retail shops in Akureyri and 46 swab samples from the largest blue mussel producing company - Norðurskel ehf, Sjávargata, Hrisey, Akureyri, Iceland.

4.1.3. Consumption pattern of shellfish and mussels in Iceland (Paper II)

A consumer survey on shellfish especially blue mussel consumption was conducted in Iceland supported by RHA (The Research Centre of the University of Akureyri, Iceland). From the consumption survey we found the time delays from the retail purchase to consumption in home, frequency of consumption, quantity of serving etc. The survey respondents were included 619 people from Iceland among the age group from 17 to 65+ years old.

4.1.4. Growth studies of *L. monocytogenes*

The growth studies were inoculation studies of *L.monocytogenes* in mussels and "in silico" growth forecast studies. The inoculation study was performed in live mussels, in contaminated water and in mussel meat. The pH level was measured by pH electrode (ThermoFisher, USA, Waltham MA), water activity measurement was measured with Aqua Lab (Series 3TE, USA, Pulman, Washington), salt was estimated by using Kohler theory (Kreidenweis *et al.,* 2005). Growth rate / doubling time (μ) was estimated using Seafood Spoilage and Safety Predictor (SSSP v.3.1., 2009) by using a fixed initial concentration (cfu/g) of *L. monocytogenes*. Temperature (ºC) values used were as found in during retail in the shops. The growth rate was found to vary mainly with storage temperature.

4.2. Hazard identification

Codex Alimentarius (1999) defined hazard identification as "the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods".

Despite the fact that Listeriosis is associated with only a few virulent strains, all strains of *L. monocytogenes* were assumed as pathogenic to humans in this work. In this sense, McLauchlin (1997) stated that "in the interests of public safety and for considerations for public health purposes, all *L. monocytogenes*, including those recovered from food should be regarded as potentially pathogenic." In USA the presence of any *Listeria* spp. is regarded as a presence of a pathogenic strain. In this study the presence of L. *monocytogenes* strains were considered as potential hazard.

4.2.1. General Hazard Identification

Seafood is generally regarded as very save and highly nutritious. It provides protein of high nutritional quality; it is high in omega-3 essential fatty acids and low in saturated fatty acids. However it is a highly perishable food item and there are always chances for presences or contamination of hazards.

The Rapid Alert System for Food and Feed (RASFF, 2009) gives a good overview of hazard associated with seafood. From the figure below (figure 1) it can be seen that chemical hazards are the most frequent (48%) but microbial hazards counts for 10%.

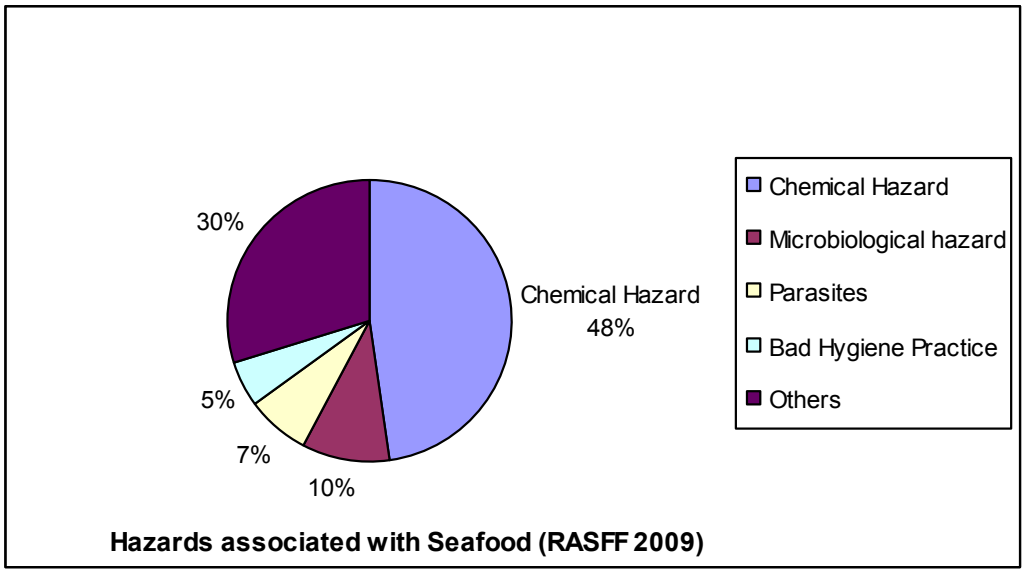

Figure 1: Hazard association with seafood, from January-July 2009 (RASFF 2009)

A total of 35 notification received because of microbiological contamination by the RASFF 2009, *L. monocytogenes* notified in 13 (35%) consignments, *Escherichia coli* in 11 (31%) and *Salmonella* spp. in eight (22%) consignments (figure 2). Fin fish, shellfish and the fish feed is being contaminated with these pathogens. From RASFF 2009, it is has been seen that *L. monocytogenes* was reported more frequently in 2009 compared to previous years, because of a rise in notifications relating to processed fish.

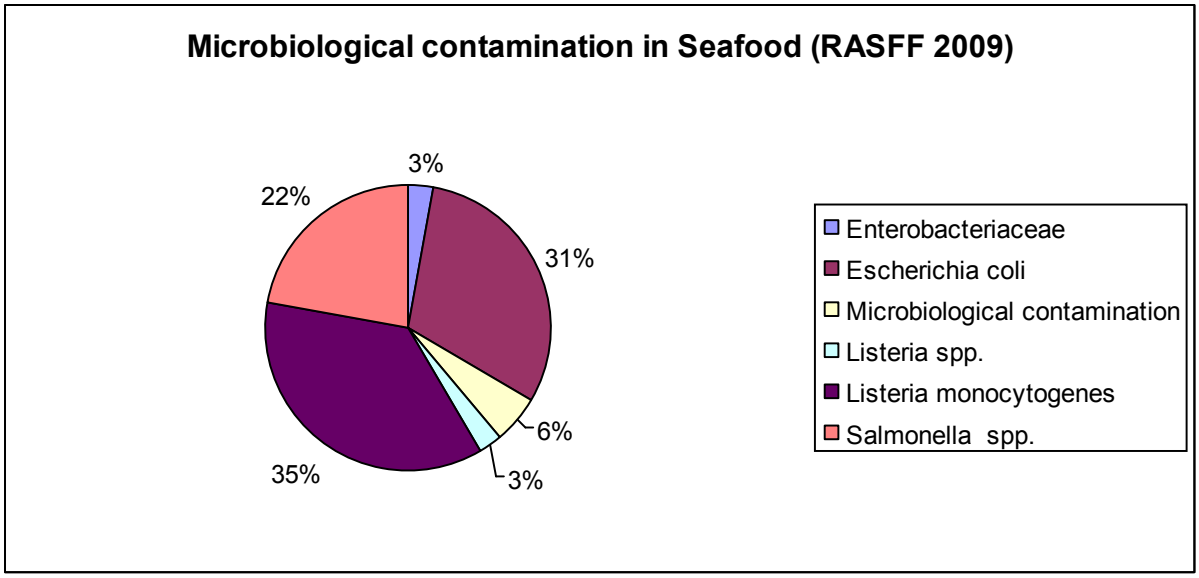

Figure 2: Microbiological Contamination of Seafood, from January to July 2009 (RASFF 2009)

Seafood frequently associated with food borne infection or food intoxications in seafood (table 1 and 2, Paper I). Seafood borne diseases from molluscan shellfish are typically: *Vibrio parahaemolyticus,* viruses and shellfish poisoning (table 3, Paper I).

Although not involved in food borne seafood diseases *Listeria* is commonly isolated from shellfish. Thus from 120 live shellfish samples that were collected from nine littoral sites in Brittany (western France) revealed *Listeria* spp. in 55% of samples, a much higher rate than the previous, infrequent, recorded data (Monfort *et al.,* 1997). *L. monocytogenes* was isolated from 4% of the bivalve molluscs samples although with very low contamination levels (less than 100 mpn/g) (Pinto *et. al.,* 2006). The natural occurrence of *L. monocytogenes* in shellfish was analysed over a period of 18 months. Sixty-one samples of 14 different species of molluscan shellfish were collected from local Lisbon markets (Portugal); *L. monocytogenes* was isolated from eight samples (13.1%) (Pereira *et al.,* 2001).

4.2.2. Hazard identification in Icelandic Seafood

Most of the fish caught in Icelandic waters comes from the open sea and thus free from pathogenic bacteria. It has also very low levels of chemical contaminants. Reports of pathogenic bacteria come from processed fish. A six-year period of bacteriological analysis of Icelandic cold water shrimp (*Pandalus borealis*) was done from 1988-1993; 7913 samples of shrimp from 26 Icelandic factories were analysed. Some 70% of the samples had less than one coliform bacterium per gram and 99.9% of the samples had less than one faecal coliform bacterium per gram. *Staphylococcus aureus* was detected in less than 0.2% of the samples. *Listeria* spp. was found in 270 of 3331 samples examined or 8.1%. Species identification was done on 49 of the 270 positive samples. The proportion of *L. monocytogenes* was found to be 26.5% (Valdimarsson *et al.,* 1998).

Hartemink & Georgsson (1991) tested a total of 128 samples of seafood from the Icelandic market for the presence of *L. monocytogenes* and other *Listeria* species which included raw, smoked and dried fish, frozen shellfish and shrimps and several fish salads. All these fish foods are normally consumed without cooking or heating. *Listeria* spp. was present in 56% of the samples of raw fish, 29% of the smoked fish, 9% of the shrimps and 32% of the salads. All products sampled had been processed and packed in Iceland, mostly for the domestic market.

Listeria spp. was detected in 12.5% and *L. monocytogenes* was detected in 11.2% of the Icelandic cooked peeled shrimp (*Pandalus borealis*), sampling was from final, semi final products and from shrimp-processing environment from 1998 to 2001 (Gudmundsdóttir *et al.* 2006).

There is almost no border notification yet from RASFF until July 2009. The only border notification reported for Iceland is too high content of colour into the black lumpfish roe (RASFF 2009).

4.2.3. Listeriosis

L. monocytogenes is widely spread in nature; it is available in soil, marine sediments and water. From the early 1900s, it was believed that *L. monocytogenes* caused illness in farm animals only. In 1980s, it became clear that *L. monocytogenes* is the vector that causes human Listeriosis (Iain *et al.,* 2006). A healthy individual is either unaffected by *L. monocytogenes* or suffer only a mild flu, but the highest risk is for cancer patients, drug and alcoholics, pregnant women and AIDS victims. However in large outbreaks "healthy" individuals also become ill. Severe Listeriosis can cause meningitis, abortions, septicaemia, some of which may lead to death. The highest risk of acquiring Listeriosis is from RTE products that do not require further cooking at home. *L. monocytogenes* has been isolated from raw fish, cooked crab, raw and cooked shrimp, raw lobster, surimi and smoked fish. Moreover, during the treatment of foodstuffs, even under conditions of good manufacturing practice, it appears impossible to achieve the total absence of *L. monocytogenes.* When there is evidence of *L. monocytogenes,* the food must be classified as harmful to health.

L. monocytogenes has specific characteristics that increase its importance as a food borne organism. It is able to grow at $0^{\circ}C$, and may thus grow well in refrigerated foods. It is able to survive harsh environments, drying and salting. Furthermore, *L. monocytogenes* is able to grow at low oxygen concentrations, and even without available oxygen, giving the organism an advantage in vacuum-packed foods.

The Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) agreed to keep the criteria for concentration of *L. monocytogenes* in food below100 cfu/g at the end of shelf life (SCVPH 1999; The EU commission regulations No. 2073/2005 of 15 November, 2005 and (EC) No. 1441/2007 of 5 December, 2007).

In the case of *L. monocytogenes* (LM), two types of adverse health effects are expected; one severe known as Listeriosis (Hof, 1998) and the other which is characterised by temporary and self limiting gastro-intestinal complaints (Dalton, 1997 and Aureli,1998). For Listeriosis, the clinical characteristics are well known as the high risk groups. In pregnant women, infections with LM can cause abortion and stillbirth, and in infants,

elderly people and persons with a weakened immune system it may lead to septicaemia and meningitis. The significance of foods as the primary root of transmission for human exposure to LM was recognised during 1980s. Outbreaks and sporadic cases of Listeriosis have been predominantly associated with RTE foods. In 2001, FAO and WHO initiated international risk assessment work on LM in RTE foods (fresh milk, ice-cream, fermented meats and cold-smoked fish). The report (2004) concluded "The risk assessment provides a valuable resource for risk managers in terms of the issues to be considered when managing the problems associated with *L. monocytogenes*". The referred report also suggests that most cases of Listeriosis result from the consumption of high number of LM in RTE foods which do not meet the suggested criteria of 0.04 or 100 cfu/g and the probability of becoming ill after eating the food pathogen was also greater for members of "susceptible" population groups.

4.3. Exposure assessment

Exposure assessment is defined by Codex Alimentarius (1999) as the "qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant." A realistic representation of the exposure of the target population (Iceland in this work) to *L. monocytogenes* was provided. For this, it was necessary to gather data regarding (1) population, (2) consumption, and (3) *L. monocytogenes* status (prevalence and concentration) in the food (blue mussel) at the time of consumption. These data were adequately combined following a mathematical and statistical approach.

Population

The QMRA of *L. monocytogenes* in blue mussel status was based on the Icelandic population. The Statistics Iceland (2011) reported a population size of 319,090 inhabitants in the 1st January, 2011. Out of total population about 27% is under age group 65+ years (both male and female) and 2% found pregnant. However, it was assumed that children under two years old do not consume blue mussel. A distinction between low-risk (healthy) and high-risk (susceptible) populations were made with the aim of a more accurate assessment of the risk. For this purpose, the fractions reported by FAO/WHO (2004a) of the total population corresponding to high-risk individuals were

applied to the Icelandic population. FAO/WHO (2004a) stated that, among adults, high-risk groups should include adults over 65-years old, pregnant women, and individuals with impaired immune systems and certain medical conditions, such as cancer and recent organ transplantation.

Consumption pattern of Blue mussel in Iceland

At the present time human nutrition shifting habits favour raw, slightly cooked and RTE food items intake which greatly enlarge the risk of consuming contaminated foods with refrigeration resistant pathogens like *Listeria* spp. The changed life pattern of European consumer's demands more ready to eat products, and the trend is toward increased consumption of fresh and lightly preserved seafood. In Iceland blue mussel have a great economic and social importance as a very fresh food item. The way of being prepared this food item is very susceptible to being contaminated with different microbial pathogens starting on their production but also along the commercialization circuit or industrial preparation until being consumed in households or restaurants. The blue mussels are generally eaten lightly cooked. The consumers prefer fresh live mussel for their meal, they make it light-cook before consumption. The meat is normally removed to eat after cooking the mussels in steam or boiling water.

Status of L. monocytogenes in Blue mussel at the time of Consumption

The status of *L. monocytogenes* in a food is defined by the prevalence and concentration of the pathogen in the food at the time of consumption. The QMRA model describes changes in both parameters from manufacture at the factory to the time of consumption. Figure 3 shows general processing steps of blue mussel. Steps before manufacture were not considered in the model as it was assumed that there are no controllable factors influencing the status of *L. monocytogenes* in blue mussel. Prevalence and concentration of *L. monocytogenes* in blue mussel was the initial inputs of the QMRA model, where 'Prev' is the prevalence of contaminated heads of blue mussel and N_0 is the initial concentration of *L. monocytogenes* in raw produce (figure 3). Both prevalence and concentration of *L. monocytogenes* in blue mussel were measured by previous studies, quantitative microbiological analysis and by a probability distribution. The three key processes were modelled: retail, home storage and consumption. The concentration

values of the pathogen in all three steps were modelled by Vose ModelRisk software (Vose ModelRisk Standard 4.0) for subsequent calculations. The temperature profile was considered during all steps of modelling. A correlation between time and temperature of storage and processing was established.

4.4. Hazard characterization

Codex Alimentarius (1999) defined hazard characterization as the "qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard. For the purpose of microbiological risk assessment, the concerns relate to microorganisms and/or their toxins."

The following exponential growth modelling (as because if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth) were used to get the subsequent concentration of *L. monocytogenes* in further steps-

$Log(N_t) = Log(N_0) + \mu t$ or $N_t = N_0 + e^{\mu t}$, where $Log(N_0) / N_0$ = initial concentration/ exposure (cfu/g), μ = growth rate/ doubling time, t = time (hours) of growth.

We used the following exponential dose-response modelling for random distribution- $P_{ill}=1-e^{-rD}$, where, r = single hit probability of illness from 1 pathogen, D= Dose (cfu) and, P_{ill} = probability of illness from a single pathogen.

4.5. Risk characterization

According to Codex Alimentarius (1999), risk characterization is "the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment." The risk of Listeriosis in the Icelandic population was estimated by integrating the results from the previous steps, as shown in figure 3. The number of cases of Listeriosis among low-risk and high-risk populations was be simulated (10,000 iterations) for the uncertainty realizations of 'Prev' obtained in the exposure assessment above. At each uncertainty calculation, a random value of P (uncertainty variable) was assigned. The probability of illness for an individual exposed assumed to follow a random distribution and was described according to the exponential dose-response model- $P_{ill}(D;r)=1-e^{-rD}$.

4.6. Risk assessment model(s)

For risk assessment both deterministic and stochastic model were used for dose-response modelling. Most variables in the models are represented by probability distributions. Deterministic models don't provide insight in the uncertainty of the results but stochastic model include uncertainty and variability. In the stochastic model both the uncertainty and variability can be represented by probability distribution.

The concentration of *L. monocytogenes* varies with delay of time during blue mussel processing and storage, temperature variations, pH and water activity changes. For our risk assessment study we kept the temperature, pH and water activity at a fixed value in our predict modelling, 'time' was the variable used to get the predict growth during consumption which will revealed the risk of illness in Icelandic population after consuming *L. monocytogenes* infected fresh live blue mussel. The concentration of *L. monocytogenes* varies with time during retail to consumption periods.

Uncertainty in the stochastic model was estimated by both beta and binomial distribution. A VoseBinomial (1, P_{ill}) was applied to get the random number of illness where mean illness were calculated by VoseSimMean () simulation (Monte Carlo). And the risk per serving was calculated by multiplying mean illness with the beta distribution of prevalence.

Uncertainty and variability were combined by calculating the mean probability of illness (Mean P_{ill}) by Vose Mean () (Variability) and prevalence (beta distribution of Uncertainty).

Instead of a fixed distribution for the prevalence (uncertainty) we used Vose Percentile () to distribute the prevalence into different cumulative probability (Vose percentiles). From the percentile we calculated the median cases (50%) which were our final output from individual scenario of variability and uncertainty.

In our modelling we used one baseline modelling where all inputs were based on our available data (sample package weight, r values, prevalence, number of serving in Iceland population), the pert distribution were used to get the probability of illness; from

the probability of illness, the risk per serving and/or cases of illness were retrieved by two ways- from the beta distribution of prevalence and from the binomial distribution of probability of illness. Based on these two outputs of cases of illness we compared other three alternative scenarios:

- Shelf life reduction during retail and home storage (pert distribution).

- Shelf life reduction during retail and home storage and hygiene improvement during processing (pert distribution).

- Shelf life reduction during retail and home storage and hygiene improvement during processing (triangle distribution).

VoseSimPercentile () distribution was use to estimate the uncertainty of different probability distribution of each alternatives, and then the median (average risk estimation) was collected to get the best output of the distribution having the minimum risk of illness. We compared the risk using different control measures into the two major population groups healthy population ($r=5.34 \times 10^{-14}$) and susceptible population ($r=5.85 \times 10^{-12}$) (FAO/WHO, 2004b)

The exponential deterministic model and four other types (triangle, pert, beta and binomial) of distribution with stochastic model were used to get the best fit for our risk determination study.

4.7. Sensitivity Analysis

According to Codex Alimentarius (1999), sensitivity analysis is "a method used to examine the behaviour of a model by measuring the variation in its outputs resulting from changes to its inputs." Different variables of input were introduced into the developed mathematical model like time and temperature changes in different steps of the product situation. The sensitivity analysis method was applied through Vose ModelRisk Standard software, with this tool, several inputs steps were kept fixed, and a simulation was performed at each step.

5. Results

5.1. The risk pathway

In Iceland, the blue mussels are being cultured using the 'longline culture' technique. The mature (2-3 years age) mussels are collected from the culture area (bay), the mussels are separated from the socks, cleaned, washed & sorted (graded) by size with automatic grader. After grading, the mussels are kept into natural seawater for about 30 minutes to absorb seawater into the mussel shell (figure 3).

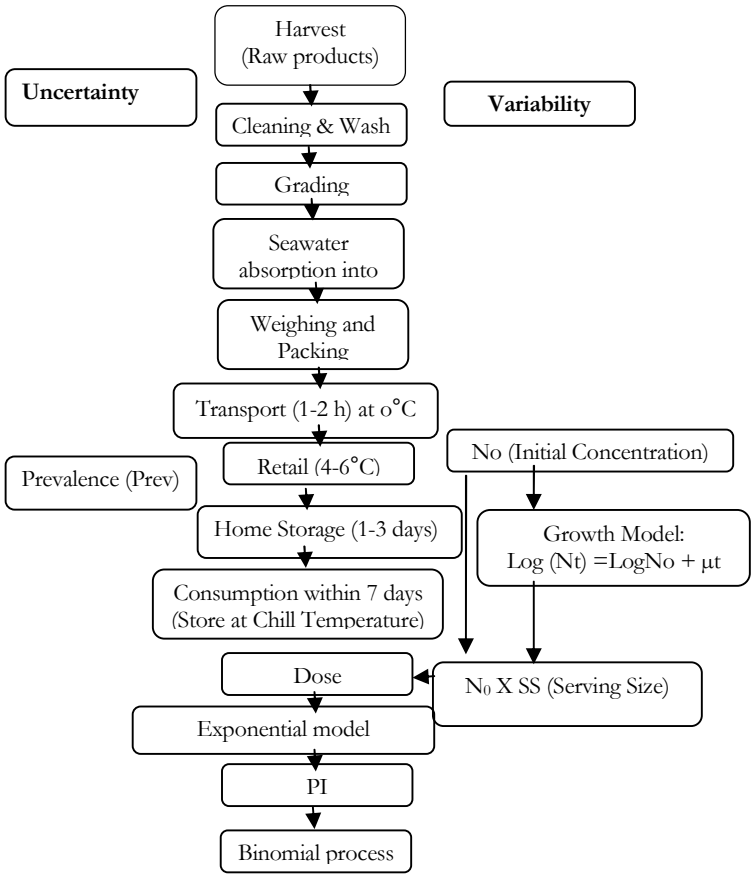

Figure 3: Risk pathways of live fresh blue mussels for Listeria spp. in Iceland

After seawater absorption, the mussels are weighted and packed according to the buyer demand (one kg packet for local sell in Iceland) and kept in 0^0C temperature until transport. The packed mussels are transported by refrigerated van with controlled chill temperature. The transportation time varies between one to 12 hours. The retail store displays the product with the controlled temperature (4^0C to 6^0C). After purchasing the consumers mostly prefer to eat the fresh mussel within the day, sometimes they preserve

into the refrigerator (4^0C to 8^0C) at home for one to three days. The shelf life of live fresh blue mussels is about one week in chill/refrigerated temperature, but it remains consumable-fresh until 12^{th} days. Almost the entire live fresh blue mussels' production is sold directly to the local market in Iceland. The temperatures used to process and store live fresh blue mussel are insufficient to inactivate the growth of *L. monocytogenes*. There are no steps in blue mussel production until consumption that would reduce or eliminate the pathogen. The possible contamination of final product of fresh live blue mussel by *Listeria* spp./*L. monocytogenes* might be due to contamination from the environment or/and during processing. For our risk assessment process we analysed the end product samples from the mussel producing companies and from the retail stores and also the swab sampling from the different production surfaces of Norðurskel ehf.

5.1.1. Duration from retail to consumption (Paper II)

From the consumption survey we found the following time delays (table 1) from the retail purchase to consumption at home.

Table 1: Consumption delay from purchase to consumption

Number of days	Number of respondents	%
0	29	27.88
1	29	27.88
2	12	11.54
3	3	2.88
5	1	0.96
7	1	0.96
10	1	0.96
Do not know	28	26.92

Most of the consumer prefers to eat mussels within three days after purchase. About 28% prefer to eat the same days of buying and 28% prefer to eat just after one day of purchasing, about 11.54% like to have it within two days of purchasing.

5.2. Human incidence of Listeriosis and population characteristics

The first alleged connection of the bacterium with Listeriosis disease was in 1929 and the first outbreak connected with food was in Canada in 1981, where at least 41 individuals were infected and seven died, in that outbreak the infection was traced to cabbage contaminated with infected sheep manure when used as a fertiliser (Halligan, 1991). In USA the largest outbreak happened in 2002 with 54 illnesses and eight deaths, in France there were two outbreaks recorded from October 1999 to March 2000, the first one was traced to a rillettes (Páté-like meat) but in the second outbreak of Listeriosis it was strongly linked to jellied pork tongue (De Valk *et al.*, 2001). In the United Kingdom a small outbreak of Listeriosis was traced to prepacked sandwitches (Dawson *et al.*, 2006), a Listeriosis epidemic occurred in Finland between June 1998 and April 1999 that was due to *L. monocytogenes* (serotype 3a) in butter (Maijala *et al.*, 2001) and in a small area in northwest Switzerland in 2005 there was a report of 10 cases of Listeriosis that were traced to soft "tomme" cheese (Bille *et al.*, 2006). In 2009 there were 2.77/million cases of Listeriosis observed in USA (figure 3, Paper I). The number of Listeriosis cases in humans slightly decreased in 2010 in EU countries as in previous years (figure 4).

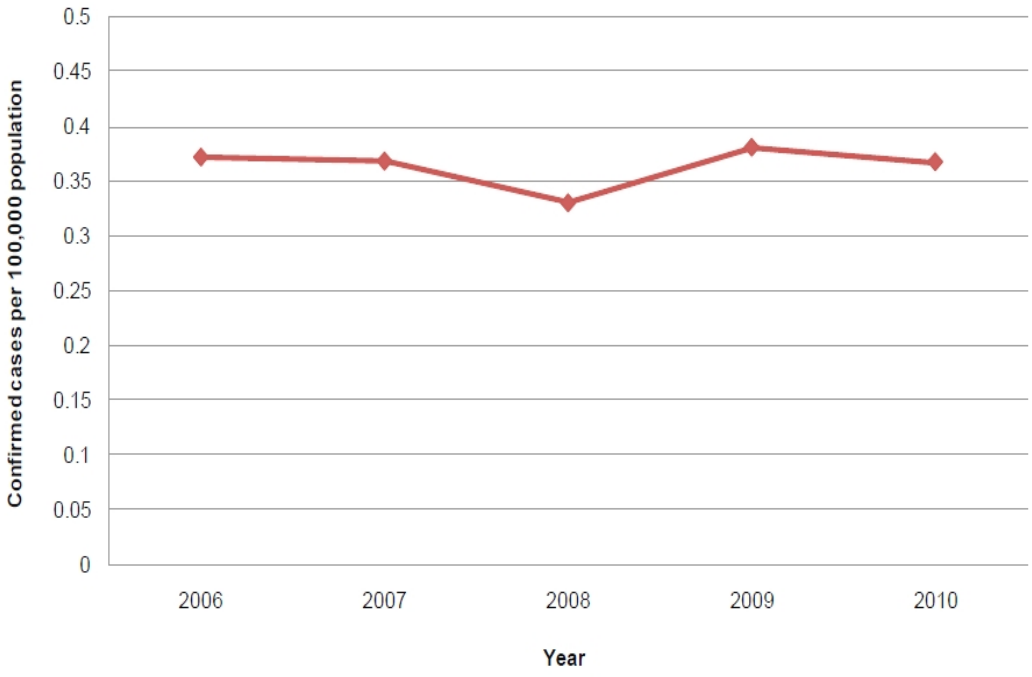

Figure 4 : Notification rates of reported confirmed cases of human Listeriosis in 25 Member States[1] during 2006-2010 (EFSA, 2012).

There are nine Listeriosis cases observed in Iceland during 1997 to 2010, the highest cases found in 2007 (4 cases), the immediate one case observed in 2010 (figure 5).

[1] Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden and United Kingdom

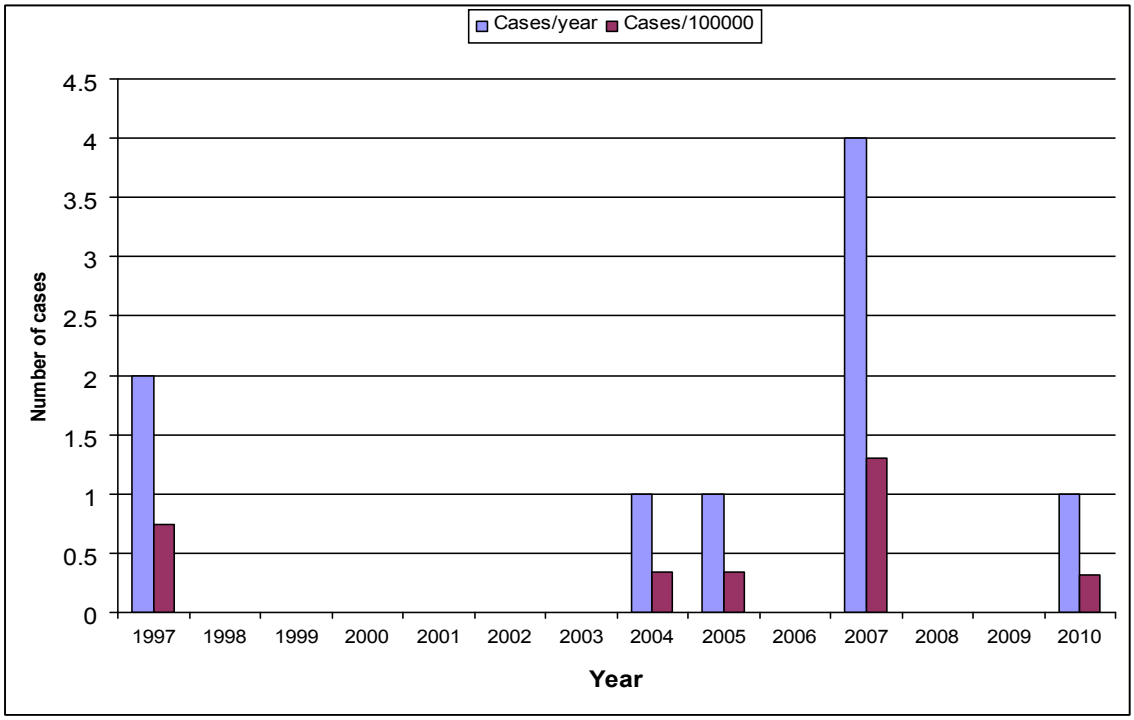

Figure 5: Number of confirmed cases of Listeriosis per 100.000 in Iceland

5.3.Prevalence of *L. monocytogenes* worldwide(Paper I)

There are several etiological agents that can be transmitted to human body through seafood consumption; *L.monocytogenes* is considered one of the most important pathogens in terms of public health and disease (Zarei et. *al*., 2012). *Listeria* spp. and *L.monocytogenes* have been isolated from seafood on a regular basis since 1987 (Embarek, 1994).

Some of the prevalence study indicated that the incidence of *Listeria* spp. and *L.monocytogenes* more frequent in raw or/and fresh seafoods compare to frozen and processed seafood. Zarei et. *al*. (2012) found *L. monocytogenes* from raw/fresh fish and shrimp samples, but found absent in frozen products. Monfort *et al.* (1998), Dhanashree *et al.* (1999) reported *Listeria* spp. and *L. monocytogenes* from fresh live shellfish. Thimothe *et al.* (2004) found higher quantity of *Listeria* spp. and *L. monocytogenes* in raw fish samples than to finished products. Inoue *et al.* (2000) isolated *L. monocytogenes* from raw seafood samples.

Shellfish has been found a career and source of transmit *L. monocytogenes* to human as shellfish is a choice of favourite food item. Beleneva and Maslennikova (2002), Halit and Kapllan (2010), Pinto *et al.* (2006), Brett *et al.* (1998), Laciar and de Centorbi (2002), Laciar *et al.* (1998), Pereira *et al.* (2001), Maria (2000), Destro *et al.* (1994), Ripabelli *et al.* (undated), Heinitz *et al.* (1998), Hofer and Ribeiro (1990), Satoko *et al.* (2005), Jeyasekaran *et al.* (1996), Cordano and Rocourt (2001) have been isolated *L. monocytogenes* from shellfishes.

Mussel has been found a dominated shellfish as a source of *Listeria* spp. and *L. monocytogenes* compare to other kinds of shellfish. Laciar and de Centorbi (2002) isolated *Listeria* spp from mussel; Brett *et al.* (1998), Pinto *et al.* (2006), and Laciar *et al.* (1998) has isolated *L. monocytogenes* from mussel; Halit and Kapllan (2010) isolated *L. monocytogenes* from mussel/ bivalve mollusk (*Mytilus galloprovincialis*); Beleneva and Maslennikova (2002) has isolated eight *L. monocytogenes* strains from blue mussels (*Mytilus edulis*).

The microbiological compliance of *L. monocytogenes* stated that it cannot be found in 25 g of RTE foods intended for infants or patients and should not exceed 100 cfu/g at the end of shelf life (Commission Regulation (EC) No.1441/2007, 2007). A quantitative prevalence study by Pinto *et al.* (2006) found >100 cfu/g of *L. monocytogenes* in mussels, crustaceans, molluscan shellfish and fish, Huss *et al.* (2000) found <100 cfu/g of *L. monocytogenes* from cold-smoked salmon; whereas Uyttendaele *et al.* (2009) found >100 cfu/g of *L. monocytogenes* from smoked fish. Inoue *et al.* (2000) has counted >100 cfu/g of *L. monocytogenes* from raw seafoods.

5.4.Prevalence of *L. monocytogenes* in Iceland (Paper I)

There were very limited prevalence studies of *Listeria* spp. and *L. monocytogenes* in seafood in Iceland found. Valdimarsson *et al.* (1998) and Hartemink and Georgsson (1991) found *Listeria* spp into shrimps. Gudbjörnsdóttir *et al. (*2004) found both *Listeria* spp. and *L. monocytogenes* into cooked shrimp, raw salmon and cod; samples from five seafood processing plants of Faroe Islands, Finland, Iceland, Norway and Sweden. Gudmundsdóttir *et al.* (2005) has isolated *Listeria* spp. and *L. monocytogenes* from cold smoked salmon and its processing facilities; the incidence of *L. monocytogenes* into the final cold-smoked salmon product was found less than to intermediate products.

5.5. Results from Incidence and enumeration of *L. monocytogenes* identification in blue mussel and its environment (Paper I)

5.5.1. Blue mussel sample analysis

No *Listeria* spp were detected from the blue mussel samples from the four locations (figure 11, Paper I) of Iceland (appendix 2, paper I).

5.5.2. *Listeria* spp. / *L. monocytogenes* identification from microbiological analysis of swab samples

No *Listeria* spp were detected from the swab samples from the largest blue mussel producing company Norðurskel ehf, Sjávargata, Hrisey, Akureyri, Iceland (appendix 3, paper I).

5.5.3. *Listeria* spp / *L. monocytogenes* identification from PCR analysis of swab samples

The swab samples collected from the Norðurskel ehf, were detected no *L. monocytogenes* (figure 17, paper I) from the PCR analysis.

5.6.Consumer survey of blue mussel in Iceland(Paper II)

Data from the nationwide fresh live blue mussel consumption surveys were used to provide estimates of exposure to *L. monocytogenes* via distribution of consumption (Appendix 2 and 3, Paper II).

5.7. Growth study of *L. monocytogenes* in blue mussel in Iceland (Paper III)

5.7.1. *L. monocytogenes* growth in blue mussels

The growth of *L. monocytogenes* in live blue mussel found a very limited growth or no growth after 2 hours, but in most cases it found a decrease of growth after 168 hours (7 days) from 2 hours (figure 3 and 4, Paper III). The pathogen grown well into depurated water after 2 hours (figure 5 and 6, Paper III). There was an uneven growth of *L. monocytogenes* observed into blue mussel meat; Out of eight samples, two samples were found an increase of growth after two hours; but all samples grows well after 96 hours, while reduction of number of *L. monocytogenes* observed after seven days (figure 7, paper III).

5.7.2. Growth of *L. monocytogenes* of blue mussels using 'Growth Predictor'

The 'Growth Predictor' (Growth Predictor, 2012) showed an exponential growth of *L. monocytogenes* from zero hour to 168 hours (7 days) in four different temperatures (1ºC, 4ºC, 7ºC and 15ºC) (figure 8, paper III) using the found physical properties of blue mussel.

5.8. Risk assessment of *L. monocytogenes* in blue mussel in Iceland

5.8.1. Physical properties of blue mussel

The highest pH level of the blue mussel sample was found 6.8 and lowest found 6.2 and the average was 6.5 with a standard deviation of 0.167, the temperature during pH measurement varied from 20.1°C to 26.0°C with the average of 23.1°C. The highest water activity measured was 0.99 and lowest was 0.98 with a standard deviation of 0.00519, the temperature during the water activity measurement varied from 19.8°C to 23.7°C.

Salt content (NaCl) was found to be 1.8% in the water phase of blue mussel meat.

5.8.2. Growth Modelling

The growth rate / doubling time (μ) was obtained from the Seafood Spoilage and Safety Predictor (SSSP v.3.1. 2009) by introducing the following values -

The initial concentration (cfu/g) of *L. monocytogenes* = 10 (Average concentration obtained from review of prevalence in shellfish)

NaCl in water phase % = 1.8

pH = 6.5 (average pH value of blue mussel found in Iceland).

Smoke components-Phenol (ppm) =10 (default value from SSSP v3.1)

Storage period (days) =7 (maximum average time period from retail store to consumption/shelf life of fresh blue mussel sold in Iceland)

Temperature (^{0}C) during retail sell in the shop = 2/5/7/10

The output from the growth models prediction software is (table 2) –

Table 2: Calculation of growth rate form the growth forecaster (SSSP v3.1) software

Sl. No.	pH	NaCl in water phase %	L. monocytogenes initial cell level (cfu/g)	Storage period (day)	Storage temp (^{0}C)	Growth rate/ Doubling time (μmax (1/h))
1	6.52	1.8	10	7	2	0.0073
2	-do-	-do-	-do-	-do-	5	**0.0192**
3	-do-	-do-	-do-	-do-	7	0.0303
4	-do-	-do-	-do-	-do-	10	0.0516

From the following exponential growth modelling the exposure during consumption obtained -

$Log(N_t) = Log(N_0) + \mu t$

or $N_t = N_0 + e^{\mu t}$, where $Log(N_0) / N_0$ = Initial concentration/ Exposure (cfu/g).

μ = growth rate/ doubling time.

t = time (hours) of storage

$Log(N_t)/N_t$=concentration/ exposure (cfu/g) after the time t.

The output from the exponential growth modelling are (table 3) –

Table 3: Exponential growth modeling of Listeria monocytogenes

Processing steps	Log(N_0)	(µmax (1/h))	t	Log(N_t)
Retail	1		24	1.200
			48	1.600
			72	1.800
			96	2.001
			120	2.201
			144	2.401
Consumption		0.0192 (at 5^0C)	168	2.601

Having the fixed values for initial concentration, pH, NaCl in water phase % and temperature during storage from retail to consumption we can obtain the exponential growth of *L. monocytogenes* with the change of time (figure 6).

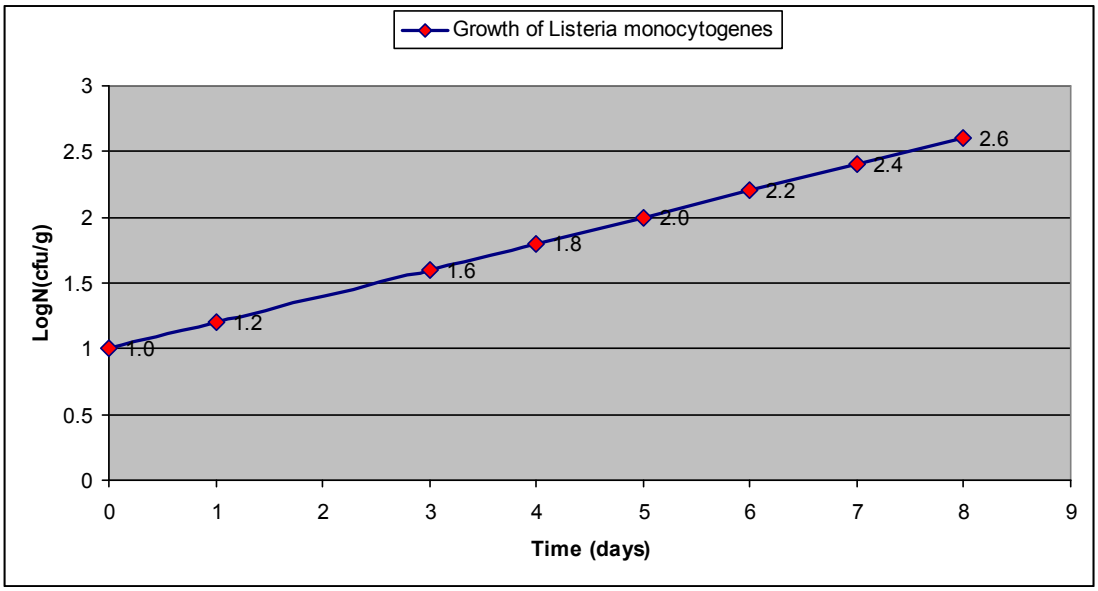

Figure 6: Exponential growth of L. monocytogenes with the change of time.

5.8.3. Deterministic model

In the deterministic model all variables are represented by a single number. At deterministic modelling we used dose-response modelling. A dose is the number of colony forming unit (cfu) consumed at one limited time-period. At consumption of a dose, the probability of illness is given by a dose-response relationship. In dose response modelling transform the amount of exposure into the probability to become infected at consumption of a serving. The determination of the relationship between the magnitude of exposure (dose) to a microbiological agent and the severity and/or frequency of

associated adverse health effects (response). The deterministic dose-response models estimate the probability of each organism in the food being individually capable of causing illness in the consumer.

From the consumption survey we found that Icelandic people eat about 15 mussels (70% respondents prefer <12 mussels and rest of them prefer >12 mussels, 15 mussel is as average) per meal. The average weight of meat of 15 marketable mussels is 150 g. In the dose-response modelling we can consider that the average unit of consumption is 150 g/ serving.

We know, dose (n) (cfu) = Exposure (cfu/g) X Sample package (g).

Where, Exposure (cfu/g) = the initial concentration (N_0 exposure (cfu/g)) of the live fresh blue mussels in the retail = 10 cfu/g.

We used the following exponential dose-response modelling for random distribution –

$$P_{ill} = 1 - e^{-rD}$$

Where, r = Single hit probability of Illness from 1 Pathogen

$= 5.85 \times 10^{-12}$(*L. monocytogenes* for susceptible population (FAO 2004b)).

$= 5.34 \times 10^{-14}$ (*L. monocytogenes* for healthy population (FAO 2004b).

D = Dose (cfu) and

P_{ill} = Probability of Illness from a single pathogen.

From the prevalence study (appendix 1 and table 7, Paper I) we know that 2% to 56% of our products (various shellfishes) are being contaminated with *Listeria* spp.

So, we can get the % of illness by following equation –

% of Illness = P_{ill} X % of contaminated packages

Where, Pill = Probability of Illness from a single pathogen.

% of contaminated package = from (appendix 1, Paper I)

If we know the number of serving we can get the cases per number of serving from the following equation –

Cases/37,789* serving = % of Illness X 37,789

(* Number of serving in Icelandic population of the age group 20-65+ years for the year 2010, From the Survey result; Paper I)

The exponential dose-response modelling ($P_{ill}=1-e^{-rD}$) were used for random distribution, where $r=5.34x10^{-14}$ for healthy population and $r=5.85x10^{-12}$ for susceptible population. Keeping the initial concentration (N_0) constant (10 cfu/g) during the retail, the exponential output of cases were found $9.08x10^{-9}$ cases/37,789 serving which increases with time in home storage and consumption. The log value of exposure and cases were plotted in the figure 7, there is always a risk to increases the cases of illness with the exposure delay in home storage and consumption, even if the percentages of contamination packages is being fixed (2%; the minimum prevalence value taken from appendix 1, Paper I). If the initial concentration increases, the cases of illness during consumption will increase as well (figure 6). The number of cases and exposure always will be higher for susceptible population group than to healthy population (figure 7).

Table 4: Estimation of risk by deterministic model

Processing steps	**Time (hour)**	**Exposure No (cfu/g)**	**Sample package (g)**	**Dose (n)(cfu)**	**Probability of illness ($p=1-e^{-rn}$)**	**% of contaminated packages**	**% of illness**	**No. of serving**	**Cases/5668 serving or Cases/Year**
Retail	96	10	150			2			
Home storage	48	158.76							
Consumption	24	251.69		37753.01	$2.02x10^{-9}$		$4.03x10^{-11}$	37,789	$5.8x10^{-7}$

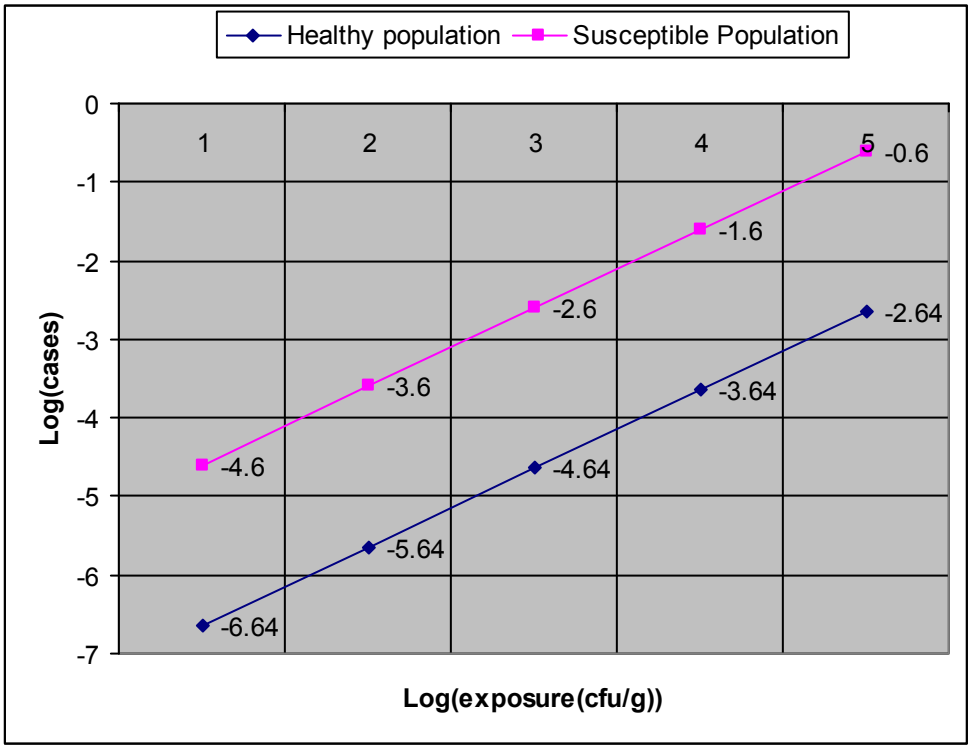

Figure 7: Comparison of risk between healthy and susceptible population

5.8.4. Stochastic model

In the stochastic model all or some variables in the model are represented by probability distributions. Deterministic models don't provide insight in the uncertainty of the results but stochastic model include uncertainty and variability. In the stochastic model both the uncertainty and variability can be represented by probability distribution.

We assume that the initial concentration (N_0 exposure (cfu/g)) of the fresh live blue mussel during retail store is 10. Here we used both the VosePert (0,10,1000) and VoseTriangle(0,10,1000) distribution, where the minimum, most likely and maximum values are 0, 10 and 1000 respectively. In the pert and triangle distribution there are three points distribution- minimum, most likely and maximum. The pert distribution has a tail, it helps to get the better output when the most likely values distributed over the tail region. Finally, we observed the comparative scenario of pert and triangle distribution. For the uncertainty measurement we used beta distribution. If the given

sample size ‘n’ has ‘s’ success then p (probability) follows a Beta(s+1, n-s+1) distribution.

If we consider that 2% (755.78 out of 37,789 serving) of our product (fresh live blue mussel) are contaminated with *L. monocytogenes*. Then, VoseBeta (755.78+1, 37,789-755.78+1) from the beta distribution, we can get random distribution from each simulation.

The initial concentration (N_0 cfu/g) of *L. monocytogenes* in blue mussel (10 cfu/g) were distributed by both triangle and pert distribution with a minimum, most likely and maximum values 0, 10 and 1000 respectively.

5.8.4.1. Variability in stochastic model

The concentration of *L. monocytogenes* varies with delay of time during blue mussel processing and storage, temperature variations, pH and water activity changes. For our risk assessment study we kept the temperature, pH and water activity at a fixed value in our predict modeling, time is the variable used to get the predict growth during consumption which will revealed the risk of illness in Icelandic population after consuming *L. monocytogenes* infected fresh live blue mussel. The concentration of *L. monocytogenes* varies with time during retail to consumption periods.

The triangle distribution shows that, there is only about a 5% chance of falling the value of N_0 below 29 cfu/g and 5% probability of exceeding the value from 787 cfu/g (figure 8); where in the pert distribution the minimum and maximum values are 12.35 cfu/g and 466.08 cfu/g with 90% confidence interval (figure 9), so the pert distribution giving the better closer look of the probability distribution compare to triangle distribution.

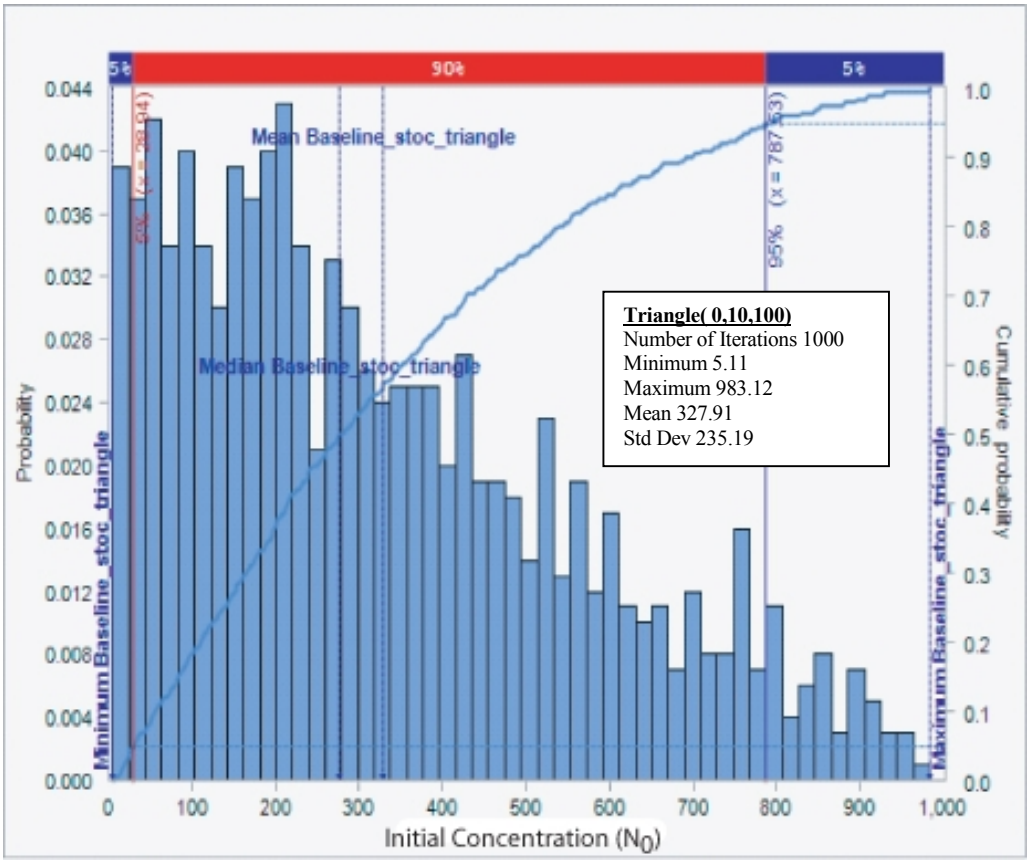

Figure 8: Triangle distribution of Initial concentration (N_0)

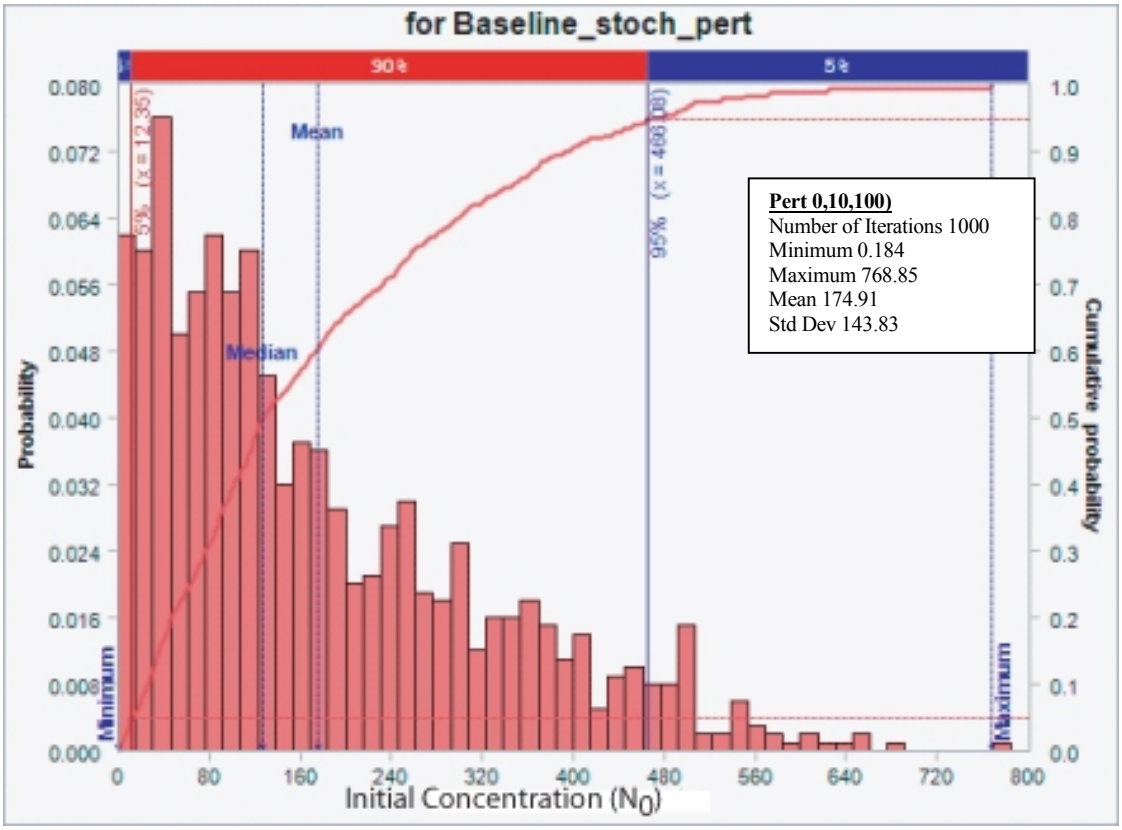

Figure 9: Pert distribution of initial concentration (N_0)

The initial concentration during slicing/packaging is 10 cfu/g

We used the pert distribution keeping the value 10 cfu/g most likely.

The output of the pert distribution is the initial concentration N_0 (cfu/g).

We used the following exponential growth modelling to get the subsequent concentration of *Listeria* spp. in further steps-

$Log(N_t) = Log(N_0) + rt$, where the concentration of *L. monocytogenes* increase with time (t).

$= 0.8934 + (5.34X10^{-14})X168,$

$= 0.8934$

So, $N_t = 7.823$ cfu/g (The concentration of growth after time t)

where, 0.8934 = $Log(N_0)$ from a random simulation (Monte Carlo),

$5.34x10^{-14}$ = r for healthy population,

168 = t for time in hours from retail to consumption

The consumption amount of fresh live blue mussel for each meal is 150g.
Then the Dose (D) is = N_t X 150 cfu.

= 1173.466 cfu

The probability of Illness (Pill) = $1-e^{-rD}$

$= 6.27X10^{-11}$

Mean illness = VoseSimMean() of Pill (figure 10)

$= 1.36X10^{-9}$

Cases of illness/ 37,789 serving = $1.36X10^{-9}$ X 37,789

$= \mathbf{5.14X10^{-5}}$

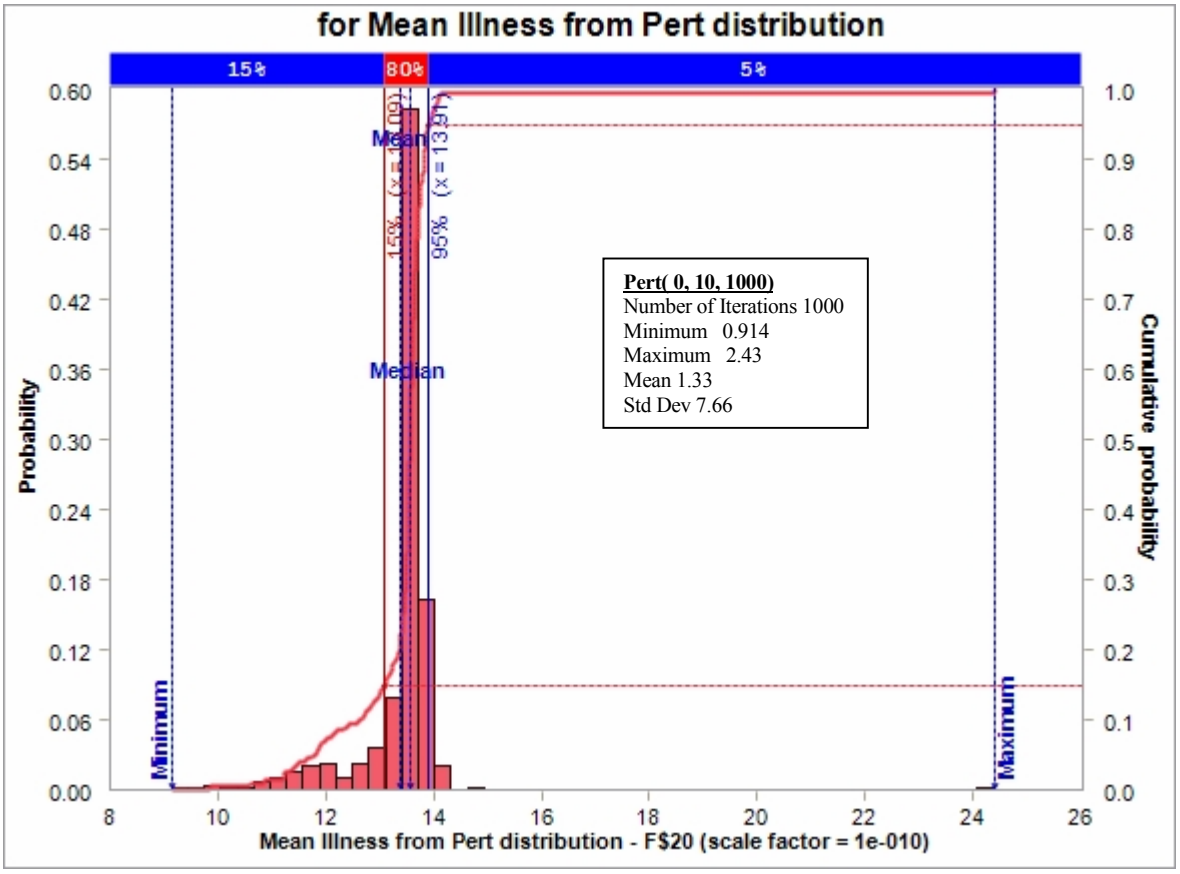

Figure 10: Mean Illness from the pert distribution

5.8.4.2. Uncertainty in stochastic model

Uncertainty by beta distribution

From the prevalence study (appendix 1 & table 7, Paper I) we found the minimum contamination of shelfish is 2%., means 755.78 serving out of 37,789 (total number of serving of fresh live blue mussel in Iceland) serving of fresh live blue mussel are being

contaminated in Iceland by *Listeria* spp. So, our uncertainty is 0.02 for deterministic model but for stochastic model we used the beta distribution.

The prevalence (2%) of contamination of blue mussel by *Listeria* spp is simulated by beta distribution. VoseBeta(755.78+1, 37,789 -755.78+1), from this beta distribution, we got random distribution from each simulation where 2% (755.78 out of 37,789) serving are being considered contaminated by *Listeria* spp.
So, VoseBeta(755.78 +1, 37,789 -755.78 +1) = 0.020 (from a random simulation)

Uncertainty by binomial distribution

The probability of illness (P_{ill}) = $1.31X10^{-9}$ (exponential P_{ill} from pert distribution)
VoseBinomial (1,P_{ill}) = 0 (from the random simulation).
Mean Illness = VoseSimMean (VoseBinomial (1,P_{ill}))= 0 (from the random simulation).

5.8.4.3. Combination of Uncertainty and variability

We have the doses from different processing steps to until consumption which are the variabilities. From the doses we calculated the probability of illness –
$P_{ill}=1-e^{(-r*D)}$, where r is the single hit probability of illness, and D is dose (cfu).
Then we calculated the mean probability of illness (Mean P_{ill}) by Vose Mean ().
Finally we combined both the variability and uncertainty-
Mean Risk per serving = Mean P_{ill} X Prevalence (beta distribution),
Here Mean P_{ill} = Variability,
Prevalence (beta distribution) = Uncertainty.

From beta distribution
We have the mean probability of illness (Mean P_{ill}) derived from the beta distribution of Probability of illness ($P_{ill}=1-e^{(-r*D)}$). So, we calculated the risk per serving by following-
Risk per serving = Mean P_{ill} (VoseSimMean)X Prevalence (beta distribution)
= $1.36X10^{-9}$ X0.020
= $2.45X10^{-11}$
Cases of illness/37,789 serving= $1.01X10^{-6}$

From binomial distribution

We have the mean probability of illness (Mean Illness) derived from the binomial distribution of the Probability of illness ($P_{ill}=1-e^{(-r*D)}$). So, we calculated the risk per serving by following-

Risk per serving = Mean Illness (VoseSimMean of Binomial) X Prevalence (beta distribution)

= 0X0.020

= 0

Cases of illness/37,789 serving= 0

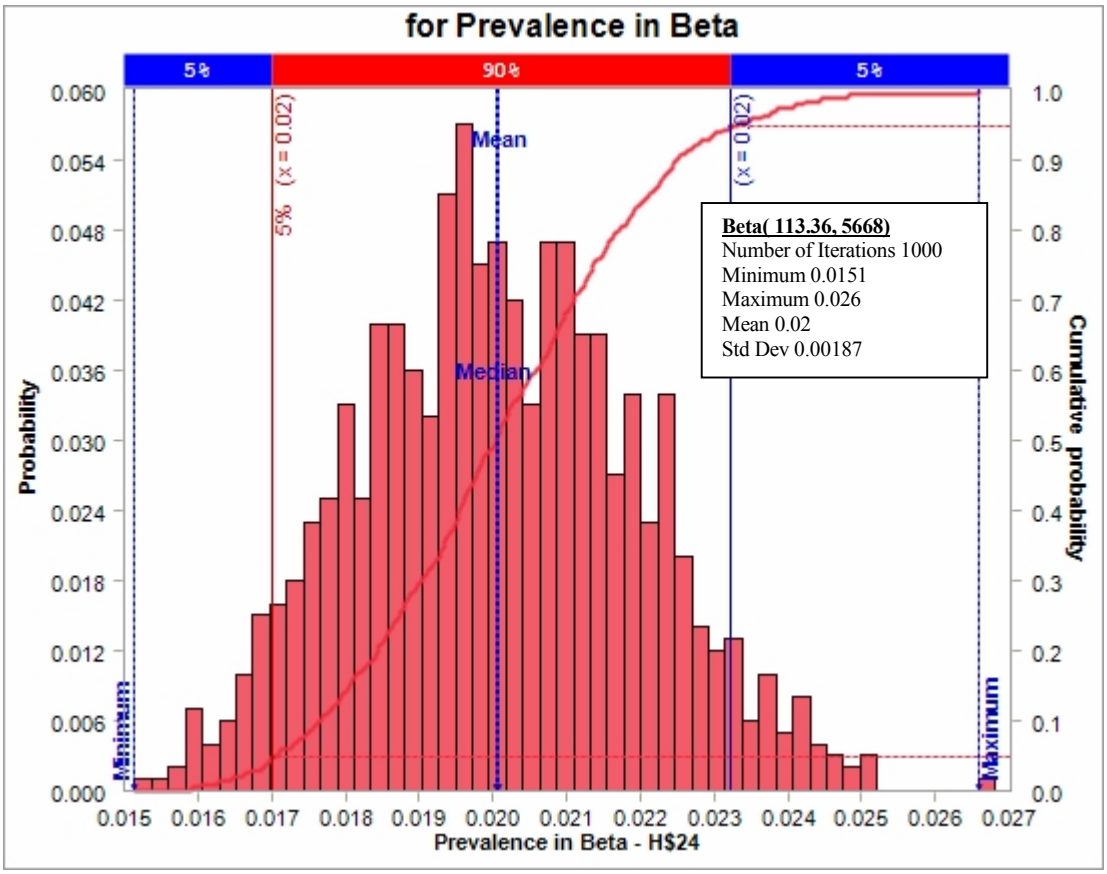

Figure 11: Cumulative probability of uncertainty (prevalence) in beta distribution

5.8.4.4. Distribution of uncertainty using Risk Percentile ()

Instead of a fixed distribution for the prevalence (uncertainty) we used Vose Percentile() to distribute the prevalence into different cumulative probability (Vose percentiles).

From the percentile we calculated the median cases (50%) which were our final output from individual scenario of variability and uncertainty.

From the prevalence (uncertainty) of beta distribution we found -

VoseBeta (755.78 +1, 37,789 -755.78 +1) = 0.020 (from a random simulation), this output of prevalence were distributed in different percentiles by using VoseSimPercentile(). And the number of cases from each percentiles were manipulated from both pert and pert and binomial distribution (figure 12)-

Number of cases (Risk (P_{ill}))= Prevalence from each VoseSimPercentile()X cases of illness/37,789 serving (from variability of pert distribution) and

Number of cases (Binomial (P_{ill})) = Prevalence from each VoseSimPercentile()X cases of illness/37,789 serving (from uncertainty of binomial distribution).

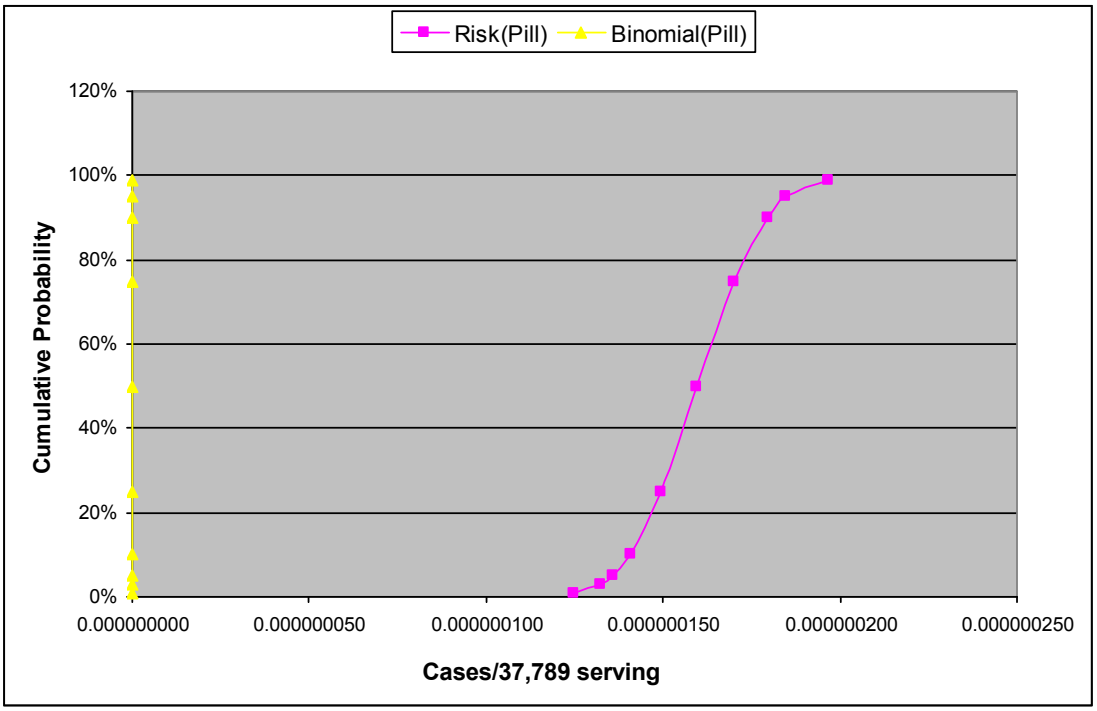

Figure 12: Probability distribution of prevalence using Vose Percentile().

5.8.4.5. Uncertainty measurements of different control measures

In our modelling we used one baseline modelling where all inputs were based on our available real time data (sample package weight, r values, prevalence, number of serving in Iceland population), the pert distribution were used to get the probability of illness; from the probability of illness, the risk per serving and/or cases of illness were retrieved by two ways- from the beta distribution of prevalence and from the binomial distribution of probability of illness. Based on these two outputs of cases of illness we compared other three alternative scenarios -

Alternative 1- Modelling using pert distribution for initial concentration after the reduction of shelf life during retail and home storage.

Alternative 2- Modelling using pert distribution for initial concentration after the improvement of shelf life during retail and home storage and hygiene during processing.

Alternative 3- Modelling using triangle distribution after the improvement of shelf life during retail and home storage and hygiene during processing.

Baseline - Modelling using pert distribution for initial concentration after the actual duration of shelf life during retail and home storage.

Using the VoseSimPercentile() we distributed the uncertainty of different probability distribution of each alternatives, and then we collected the median (average risk estimation) to get the best output of the distribution having the minimum risk of illness. We compared the median of different control measures- 'shelf life reduction', 'shelf life reduction and hygiene improvement' and compared the scenarios to get the best risk option to improve the quality of the product and to reduce the risk of illness.

The three alternatives- 'shelf life reduction using pert distribution', both 'shelf life reduction' and 'hygiene improvement' using pert distribution and both 'shelf life reduction' and 'hygiene improvement' using triangle distribution shown that 'shelf life reduction' and 'hygiene improvement' using pert distribution is the best option to follow because it gives least cases of illness (table 5,6 and figure 13,14).

Table 5: Uncertainty measurement of different control measures in healthy population.

Models	No. of cases-Risk (Pill)				
	Median			Confidence Interval	
	50%	5%	95%	50%-5%	95%-50%
Baseline (Stochastic,Pert)	1.57579E-07	1.35682E-07	1.82186E-07	2.18967E-08	2.46079E-08
Stochastic,Pert,Time	1.57224E-07	1.33306E-07	1.84294E-07	2.39183E-08	2.70701E-08
Stochastic,Pert,Time, Hygiene	**2.11697E-08**	**1.8231E-08**	**2.47465E-08**	**2.93868E-09**	**3.57678E-09**
Stochastic,Triangle,Time, Hygiene	3.3369E-08	2.8541E-08	3.90121E-08	4.82801E-09	5.64313E-09

Table 6: Uncertainty measurements of different control measures in susceptible population

Models	No. of cases-Risk (Pill)				
	Median			Confidence Interval	
	50%	5%	95%	50%-5%	95%-50%
Baseline (Stochastic,Pert)	1.79213E-05	1.52395E-05	2.09845E-05	2.68185E-06	3.06319E-06
Stochastic,Pert,Time	1.64368E-05	1.39666E-05	1.9195E-05	2.47016E-06	2.75825E-06
Stochastic,Pert,Time, Hygiene	**2.41934E-06**	**2.06691E-06**	**2.82597E-06**	**3.5243E-07**	**4.06636E-07**
Stochastic,Triangle,Time, Hygiene	3.71704E-06	3.17587E-06	4.31921E-06	5.41163E-07	6.02176E-07

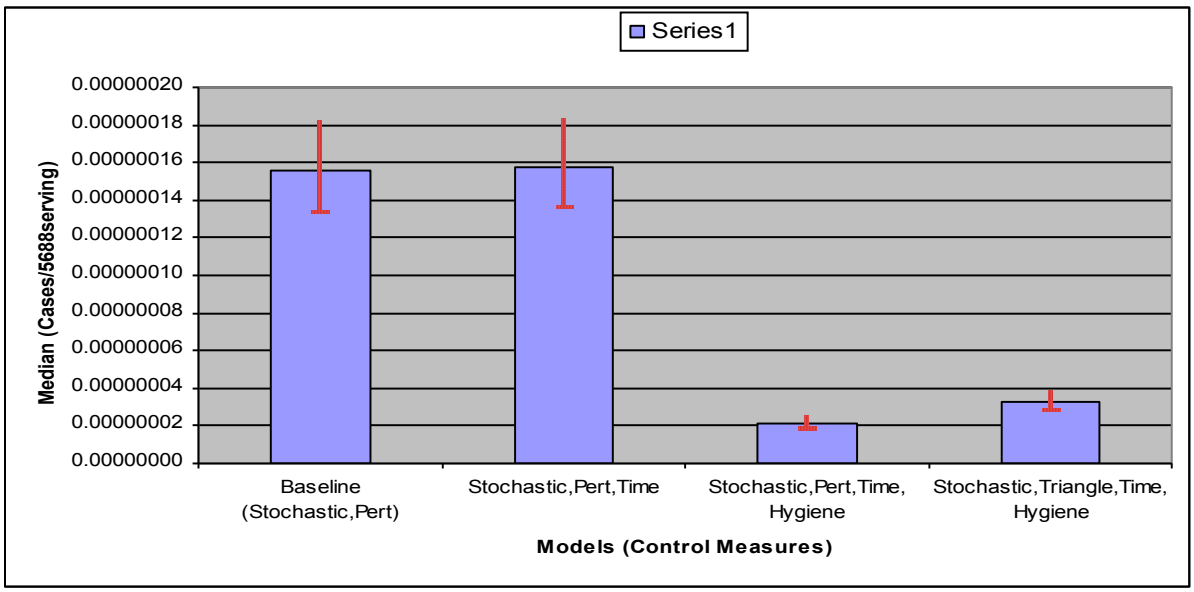

Figure 13: Comparative risk of different control measures in healthy population groups.

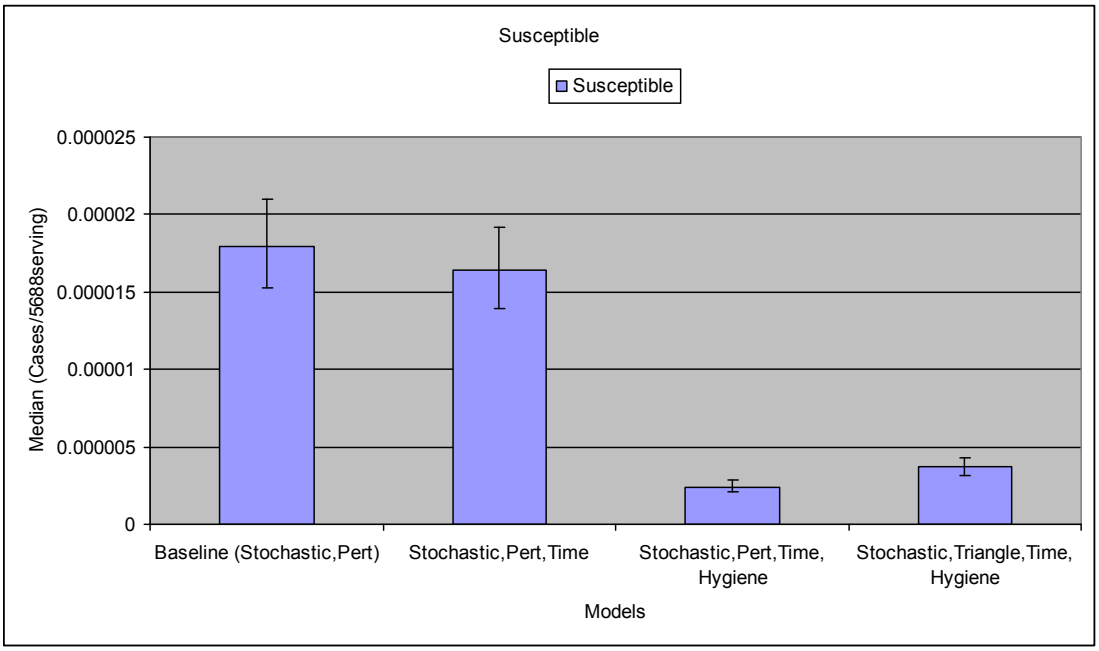

Figure 14: Comparative risk of different control measures in susceptible population groups.

5.8.4.6. Comparative risk of different population group

We compared the risk of illness using different control measures into the two major population groups: healthy population ($r=5.34 \times 10^{-14}$) & susceptible population ($r=5.85 \times 10^{-12}$).

A comparative risk (cases of illness) of two population groups shown that susceptible population (young and old aged people) group has more risk of Listeriosis illness than healthy population (figure 15).

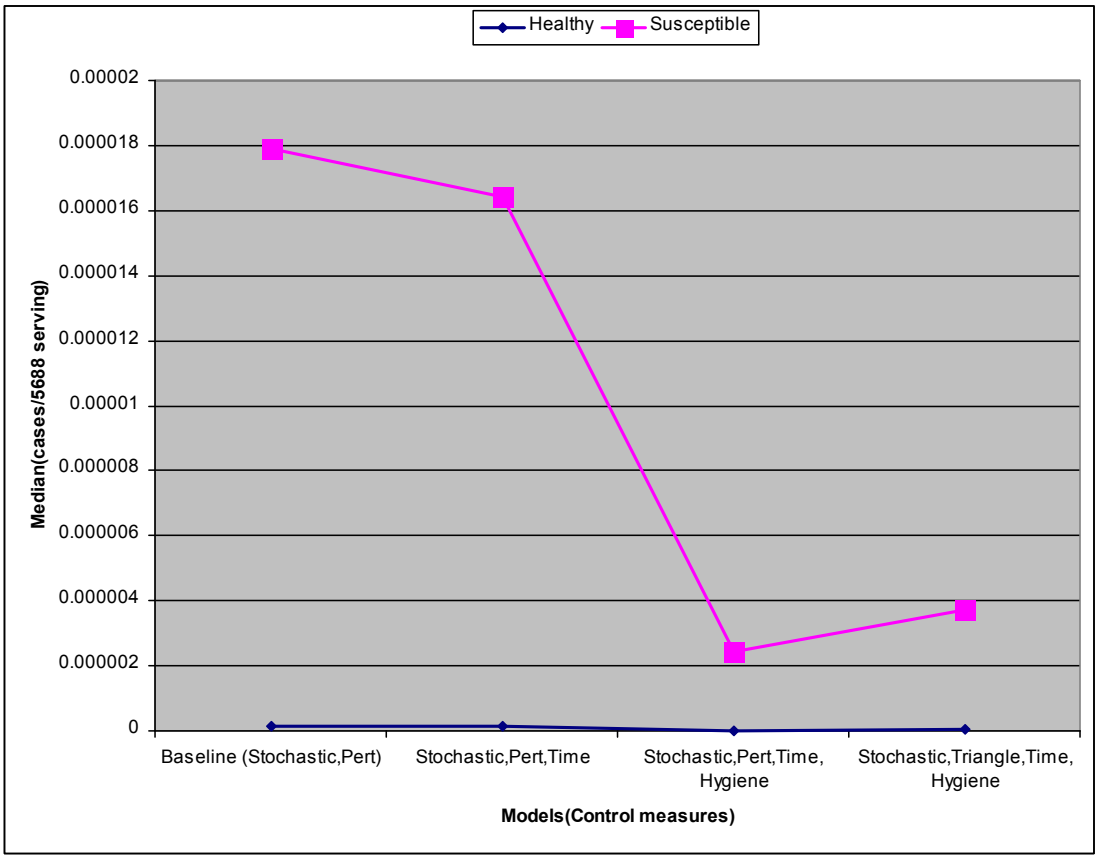

Figure 15: Comparative cases of illness between healthy and susceptible population

5.8.4.7. Comparative output of different model-distribution pairs

The exponential deterministic model and four other types (triangle, pert, beta and binomial) of distribution with stochastic model were used to get the best fit of our risk determination study.

A comparative output of different distribution (figure 16) of healthy population shown the best fit model to use; regardless of high number of cases for triangle distribution with stochastic model might be best robust because it shown the maximum number of cases compare to other distribution and model pairs.

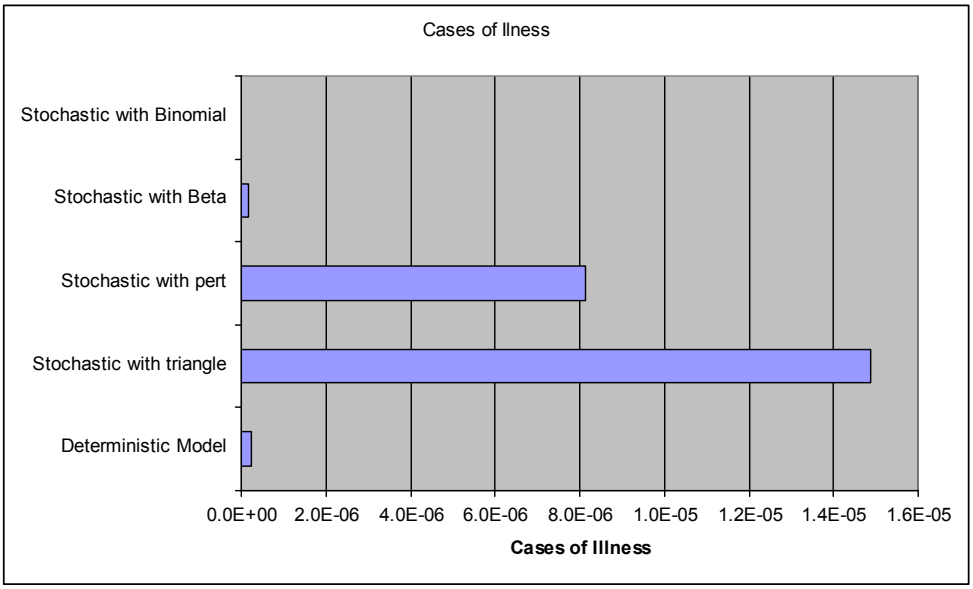

Figure 16: A comparative output of cases of illness of different distribution used for healthy population group.

6. Discussion

This risk assessment study included data from the available scientific literature and data obtained during the course of this work. The data was used for the development of exposure assessment and dose-response models to predict the relative public health impact of food borne *L. monocytogenes* from the consumption of fresh live blue mussel.

The developed QMRA model of *L. monocytogenes* in blue mussel was based on some initial input of fixed values (initial concentration, prevalence, pH, water activity, temperature, number of serving, number of population in Iceland), the only variable was 'time' during retail, home storage and consumption. The simulation was run using different probability distributions (triangle, binomial, beta), different shelf life (time), two dissimilar r values (for healthy and susceptible population) and few control measures to reduce exposure of *L. monocytogenes* until consumption of blue mussel.

The developed risk assessment model accounts for the uncertainty and variability associated with exposure of *L. monocytogenes* from blue mussel consumption and of susceptibilities to this hazard on the basis of data obtained in Iceland specifically during 2010 - 2012. The results obtained from QMRA models should not be in contradiction with epidemiological data observed in the same area during the same period of time. In Iceland infections happened by *L. monocytogenes* are rather rare; the highest number of confirmed cases was found four in 2007, about 0.00125% of total population (Director of Health, S.E., 2010). The prevalence of *L. monocytogenes* in blue mussel in Iceland was found to be 0%. The prevalence of *L. monocytogenes* was found from 2% to 55% among seafood worldwide (appendix 1, Paper I) and from 8.1% to 56% in fishes and shellfishes in Iceland (table 7, Paper I), these information motivated to move forward for an essence consumer survey of the target species (blue mussel) in Iceland. Blue mussel is being an occasional item with a preference of 1-6 times/year, having 150 g mussel meat for each meal, the quantity of meat served in one year is 5668 kg for the whole Iceland with a consumption rate of 0.028 kg/capita/year. Fresh live blue mussels are popular elsewhere because of its tastes and freshness. Icelandic Fisheries Minister

committee suggested increasing knowledge, improving organization and reducing risk in order to make the mussel industry attractive to investors (MFA, Iceland, 2008).

In this microbial risk assessment, the infectious unit was set as a single microorganism. The microbiological analysis for the presence of *L. monocytogenes* in blue mussel sample from the four locations (figure 11, paper I) of Iceland, from the retails stores in Akureyri and swab sampling from the company Norðurskel ehf, showed no presence of L. monocytogenes (appendix 2 & 3, figure 17, Paper I). The figure (figure 3) shows the processing flow diagram of live fresh blue mussels where we assume that the possible *L. monocytogenes* contamination might be from the environment or/and from the processing steps. Food chain was modelled from retail to consumption. During storage at retail, transport and storage at home, the time and temperature operating in these stages permitted the growth of *L. monocytogenes* until reaching the concentration in packages at the time of consumption. Throughout the model, the variables dealing with concentration and prevalence of *L. monocytogenes* represented the variability and uncertainty respectively. We used the average (10 CFU/g) concentration (variability) (from appendix 1 & table 7, Paper I) and minimum (2%) prevalence (uncertainty) in all the modelling. At the time of consumption, the amount of L. monocytogenes cells ingested, the dose, depends on Initial Concentration (N_0) and Serving Size (SS) (figure 3). The simulated N_0 distribution of *L. monocytogenes* can be observed in figure 8 (triangle distribution) and figure 9 (pert distribution). Figure 8 reveals a concentration of high dose (787.63 CFU/g) with 95% confidence interval. The simulated beta distribution of uncertainty (prevalence) can be observed in figure 11, having a very low dose of prevalence (near to given prevalence 0.02). The simulated mean PI distribution can be observed in figure 10. The risk (cases of illness) retrieved from the combination of variability and uncertainty using both beta and binomial distribution. The simulated distribution of uncertainty using Vose Percentile () can be observed in figure 12, where the number of cases (risk) obtained from both variability and uncertainty. From the percentiles we retrieved the medians for three different control measures and compared with confidence intervals of cases of illness; gave the best control measures to minimize risk, medians values were used to represent the expected of the estimated risk values.

This study suggested few processing and storage guidelines according to our alternation modelling (Alternative 3 - Modelling using triangle distribution) with the following corrective actions-

- Reduce the shelf life from 168 hours (7 days) to 120 hours (5 days) during retail storage.

- Improve the hygiene condition which will decrease the numbers of L. monocytogenes during the processing steps (washing/grading). We considered that the concentration decrease to 100 cfu/g from 1000 cfu/g because of better hygiene improvement (we used maximum value of 100 cfu/g in pert distribution during retail storage).

- Improve the workers efficiency (quicker processing time).

In the deterministic model a comparative risk between healthy and susceptible population showed (figure 7) that susceptible population group is at more risk. If we consider the healthy population group for Listeriosis illness and its control measure the mean cases is $1.52X10^{-6}$/37,789 serving. The mean cases of illness for susceptible population is $1.67X10^{-4}$/37,779 serving. The susceptible population group for the Listeriosis is old age (65+ years) and child/infant (0-5 years) age group those who are less immune response to Listeria illness compare to healthy mid-age population group. The message from the risk manager desk based on this study would be that the susceptible population group should not eat fresh live blue mussel. As the cooking / heating of fresh live of blue mussel is not enough to kill *L. monocytogenes.*

In this study, we demonstrated modelling procedures of risk estimation of Listeriosis, leaving aside the absolute risk estimates until more realistic models are available. There were some limitations can be identified in this QMRA model-

-The prevalence and concentration of L. monocytogenes in blue mussel were taken from foreign source.

- Cross-contamination during retail, home storage and consumption were not considered.

- Low-risk and high-risk populations have the same consumption pattern.

- It was assume that all strains isolated from food have the same potential to cause Listeriosis.

The risk assessment performed has unavoidable limitations due to the scarcity of data and uncertainty about parameter values. The results of this risk assessment could change, improved as a result of the availability of new information, changes in scientific approaches and data.

7. Conclusion

It is concluded that blue mussels from Icelandic waters are save and the risk of acquiring Listeriosis after consumption of blue mussels is close to 0.

A model is mimicking of real world situations, helps to understand the subject represented although local data was missing. The model developed on 'Quantitative Microbiological Risk Assessment of *L. monocytogenes* in fresh live blue mussel' is based on several assumptions which make the model weak and unstable. But the expert opinions and assumption might be the best possible truly scientific, the probability distribution of assumption plays to go throw into the most true outcome.

There are several possible pathways of contamination of fresh live blue mussel are from harvest area to consumption. Lacking of elimination steps for *L. monocytogenes* in live fresh blue mussel production and after processing can further amplify the numbers. The exponential growth modelling gave the best possible risk reduction options along the risk pathway.

The stochastic model gave more probabilistic outcome of risk assessment process compare to deterministic model because of randomization and distribution of sampling throughout the whole population.

The best control measures from the risk analysis output are 'shelf life reduction and hygiene improvement for both healthy and susceptible population groups.

Despite of important uncertainty association with the predictions, this model provides a scientific base for risk managers and risk business operators to gain a better understanding of the prevention of Listeriosis due to blue mussel consumption.

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9. Appendix

Appendix 1

Risk analysis using Excel and Vose ModelRisk in Microsoft Excel< Model_Risk_L_monocytogenes_blue_mussel >

[End of main Paper]

Paper I. Prevalence of *Listeria monocytogenes* in blue mussel (*Mytilus edulis*) in Iceland

I

Abstract

Aims

There are no prevalence studies on *Listeria monocytogenes* in blue mussel (*Mytilus edulis*) in Iceland. This study was conducted to review prevalence studies worldwide and to try to determine prevalence of *L. monocytogenes* in blue mussels in Iceland

Methods

A literary review on prevalence of *L.monocytogenes* in blue mussel was conducted be reviewing latest available articles published in journals and books. A microbiological study on prevalence of *L. monocytogenes* in blue mussel was conducted using both traditional microbiology and Polymerase Chain Reaction (PCR) analysis.

Results

No *L. monocytogenes* was found in blue mussel in Iceland during our study period from retail to consumption chain. However the literature showed that there is a significant level of *L. monocytogenes* contamination in mussels and other shellfish elsewhere.

Conclusion

During our study period the blue mussel found free from *L. monocytogenes* contamination. A further study should try to elucidate the difference in bacterial ecology between Icelandic and foreign mussels

Significance and Impact of study

The study was limited by irregular and limited number of samples; the sampling location might not represent the Icelandic blue mussel resources as a whole.

Keywords: Prevalence, microbiology, *Listeria monocytogenes, Mytilus edulis,* Iceland.

1. Introduction

There is an increasing concern of *L. monocytogenes* and its implications since the last 30 years; since 1975, food borne human Listeriosis outbreaks have been reported in industrial countries in Europe, North America and Oceania (Laciar and de Centorbi, 2002). *L. monocytogenes* is considered one of the most important pathogens in terms of public health and disease (Zarei et. *al*., 2012). RTE (Ready to Eat) live-fresh blue mussel is being popular in around Europe because of its delicious taste and flavour, meanwhile this trendy nature of consumption attitude bringing a new corridor of food borne diseases like Listeriosis.

The recent increasing rate of Listeriosis in USA (figure 3), some EU countries (figure 6,7, 8 and 9) and the recent reported case in Iceland (figure 10) encouragred on a prevalence study among the RTE shellfish specially blue mussel in Iceland.

2. Objective

The aims of this study were to investigate the prevalence and sources of *L. monocytogenes* in shellfish with special reference to the fresh live Icelandic blue mussel (*Mytilus edulis*) production chain and to improve the safety of its production. The study has the following sub objectives-

- Review on prevalence of *L. monocytogenes* in seafood, especially in shellfish worldwide.
- Review on prevalence of *L. monocytogenes* in seafood product of Iceland.
- Review on human Listeriosis in USA, EEA countries and in Iceland
- Investigate for the possible source of *L. monocytogenes* in the blue mussel production chain.
- Estimate the prevalence of *L. monocytogenes* in blue mussel in Iceland.

3. Prevalence of seafood hazards, sources and significances

3.1. Definition

The word ‘prevalence’ defined as the quality or state of being prevalent, the degree to which something is prevalent; specially the percentage of a population that is affected with a particular disease at a given time (Merriam-Webster online Dictionary, 2011). Here in this study our consideration was the prevalence of infected heads of shellfish infected by *L. monocytogenes*.

3.2. Hazards in seafood (hazard identification)

Codex Alimentarius (1999) defined hazard identification as “the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods”. Despite the fact that Listeriosis is associated with only a few virulent strains, all strains of *L. monocytogenes* were assumed as pathogenic to humans in this work. In this sense, McLauchlin (1997) stated that “in the interests of public safety and for considerations for public health purposes, all *Listeria monocytogenes*, including those recovered from food should be regarded as potentially pathogenic.” In the USA the presence of any *Listeria* spp. is regarded as a presence of a pathogenic strain. The available *L. monocytogenes* strains will considered as potential hazard in the selected products of Iceland.

3.2.1. General hazard Identification

Seafood is generally regarded as very save and highly nutritious. It provides protein of high nutritional quality; it is high in omega-3 essential fatty acids but low in saturated fatty acids. However it is a highly perishable food item and there are always chances for it to be contaminated with adverse hazards.

The hazards associated with seafood are categorized into biological, chemical and physical type’s hazards; table 1 is a comprehensive list of those hazards. The hazards listed in table 1 can further be divided into indigenous and non-indigenous

(contaminants) hazards. As the indigenous hazards are an inherent part of the raw material they must receive a special attention. Example of indigenous hazards in fish is *L. monocytogenes* and *Clostridium botulinum* type E and an example of non-indigenous hazard is *Salmonella.*

Table 1: List of Hazards Association with Seafood (Seafood HACCP Alliance, 2010)

Type of Hazards		Name of Hazards
Biological Hazards	Bacterial Pathogens	*Bacillus cereus* *Campylobacter jejuni* *Clostridium botulinum* *Clostridium perfrigens* *Pathogenic Escherichia coli* *Listeria monocytogenes* *Salmonella spp.* *Shigella spp.* *Pathogenic Staphylococcus aureus* *Vibrio cholerae* *Vibrio parahaemolyticus* *Vibrio vulnificus* *Yersinia enterocolitica*
	Viral Pathogens	Hepatitis A Virus Norwalk Virus
	Parasites	Anisakis simplex Pseudoterranova decipiens Diphyllobothrium latum Roundworms (*Anisakis* spp., *Pseudoterranova* spp., *Eustrongylides* spp. and *Gnathostoma* spp.), Cestodes (*Diphyllobothrium* spp.) Trematodes (*Chlonorchis sinensis*, *Opisthorchis* spp., *Heterophyes* spp., *Metagonimus* spp., *Nanophyetes salminicola and Paragonimus* spp)
Chemical Hazards	Marine Biotoxins	Amnesic Shellfish Poisoning (ASP) Diarrhetic Shellfish Poisoning (DSP) Neurotoxic Shellfish Poisoning (NSP) Paralytic Shellfish Poisoning (PSP) Paralytic Shellfish Poisoning (PSP) Ciguatera Fish Poisoning (CFP) Gempylotoxin, Scombroid Toxin, Tetrodotoxin
	Histamin	*Morganella morganii,* *Klebsiella pneumoniae,* *Proteus vulgaris* *Hafnia alvei*
	Other Marine Toxins	Gempylotoxin Scombroid Toxin (Histamine) Tetrodotoxin (puffer fish)
	Other Chemical Hazards	Aquaculture Drugs e.g. Nitrofurazone (Semicarbazide) Allergens Chemical Contaminants Food Additives Glass Metal Fragments
Physical Hazards		Metal Fragments Bones Plastic

Important source of information regarding hazards associated with seafood is the Rapid Alert System for Food and Feed (RASFF). As can be seen in figure 1 notification by RASFF regarding hazards in seafood are mostly associated with chemical hazards. But the microbiological, parasitic and some other concerns like poor hygiene practice, alternation of organic characters for poor processing systems, damaged packing, bad temperature control from farm to folk and few physical hazards also observed (RASFF 2009). From January-July 2009, there were total 359 RASFF notification declared, which included alerts and information notifications and border rejections; out of 359 RASFF, 172 (48%) were chemical hazards, 35(10%) were microbiological hazards, 26(7%) were parasitic hazards, 19(5%) was bad hygiene practice and the rest 107 (30%) was involved with other hazards like altered organic characters, package damage, fake or unravelling, absent or fraudulent of health certificate, unauthorized establishment, thawing, corrosion of metals, glass or bone fragments with the final products etc.

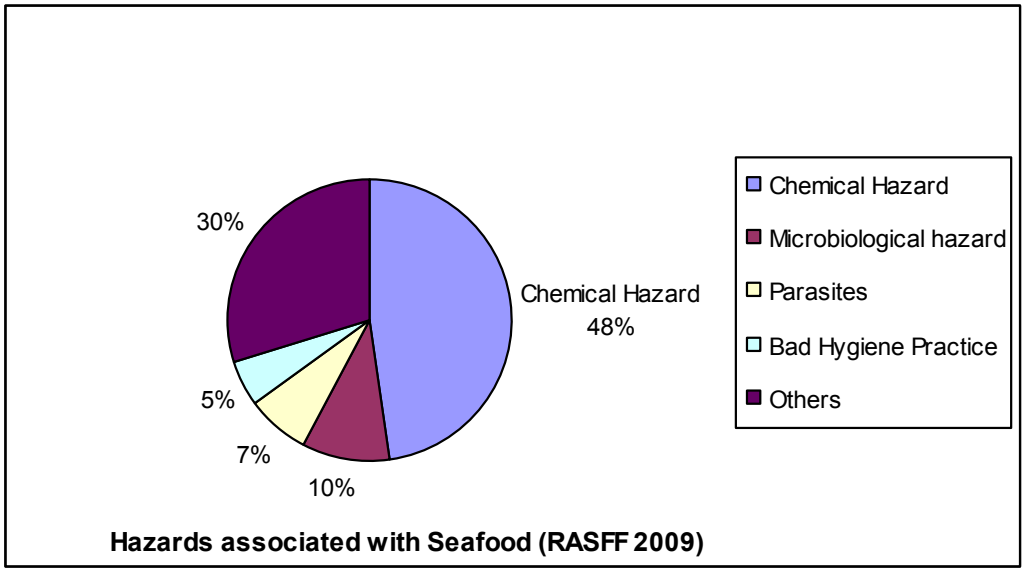


Figure 1: Hazard association with seafood, from January-July 2009 (RASFF 2009)

3.2.2. Microbiological hazard

Disease causing bacteria or pathogenic bacteria can be divided into two groups, indigenous and non-indigenous bacteria. Indigenous bacteria are commonly found in the environment of the organism, for instance bacteria in the living habitats of cod. In case of fish and pathogenic bacteria, the sea temperature has a selective effect on the microbial biota in the sea and the pathogenic bacteria that can be found in the fish.

Bacteria belonging to this group are for instance some strains of *Clostridium* and *Vibrio*, *Aeromonas hydrophila, Plesiomonas shigelloides* and *L.monocytogenes.* Non-indigenous bacteria, on the other hand reach the seafood during processing, for example *Salmonella, Shigella, E. coli* and *Staphylococcus aureus*, which are common contaminating bacteria (Huss, 1994).

The majority of cases were caused by Scombroid and Ciguatera intoxication (table 2). Also, several outbreaks of botulism were recorded as were more than 300 cases of Salmonellosis.

Table 2: Seafood borne diseases traced to "fish" in the USA from 1990 to 1998, outbreaks and cases for which the etiological agent has been identified (Huss et al. 2003).

Agent	**Outbreaks**					**Cases**				
	total	**%**	**Hawaii**	**Florida**	**Alaska**	**total**	**%**	**Hawaii**	**Florida**	**Alaska**
Scombroid	131	50	46	10	0	759	47	287	55	0
Ciguatera	98	37	73	16	0	394	24	260	82	0
Botulism	14	5	1	0	10	43	3	3	0	30
Salmonella	11	4				305	18			
Haff disease[1]	2	1				6	-			
S. aureus	1	-				2	-			
E. coli O157	1	-				3	-			
V. cholerae	1	-				26	2			
C. perfringens	1	-				25	2			
Norwalk	1	-				37	2			
Tetrodotoxin	1	-				3	-			
"chemicals"	1	-				58	4			
Total	263	100				1661	100			

1. *Haff disease is an unexplained rhabdomyolysis (the breakdown of muscle fibres with leakage of potentially toxic cellular contents into the systemic circulation) in a person who ate fish in the 24 hours before onset of illness.*

Diseases causing and toxin producing pathogens are not only found in fish, but also in shellfish and molluscs as previous tables (table 2) shown.The table 3 showing the number of outbreaks and cases from "molluscan shellfish" in USA from 1990 to 1998.

Table 3: Seafood borne diseases traced to "molluscan shellfish" in the USA from 1990 to 1998. Outbreaks and cases for which the etiological agent has been identified (Huss et al. 2003)

Agent	**Outbreaks**		**Cases**	
	total	%	total	%
V. parahaemolyticus	18	27	733	22
Norwalk/ virus	15	23	2175	66
PSP / TOXIN	14	20	92	3
Salmonella	6	9	183	6
Scombroid	2	3	4	-
Ciguatera	3	5	5	-
Shigella	2	3	17	0.5
Campylobacter	2	3	6	-
V. vulnificus	1	-	2	-
V. alginolyticus	1	-	4	-
C. perfringens	1	-	57	2
Giardia	1	-	3	-
Total	66	100	3281	100

A total of 35 notification received because of microbiological contamination by the RASFF 2009, *L. monocytogenes* notified in 13 (35%) consignments, *Escherichia coli* in 11 (31%) and *Salmonella* spp. in eight (22%) consignments (figure 2). Fin fish, shellfish and the fish feed is being contaminated with these pathogens. From RASFF 2009, it is has been seen that *L. monocytogenes* was reported more frequently in 2009 compared to previous years, because of a rise in notifications relating to processed fish.

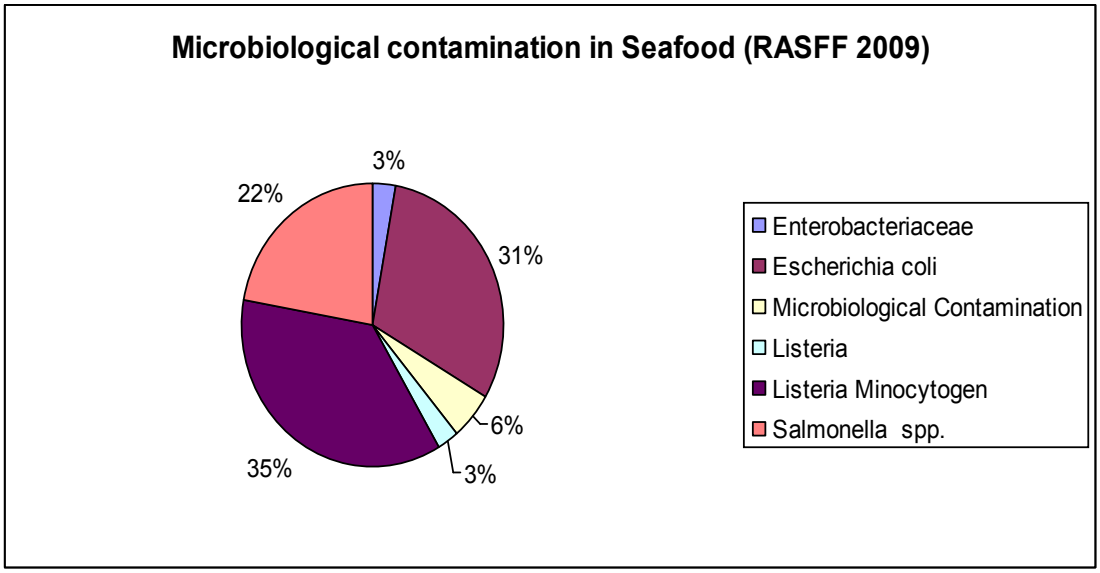


Figure 2: Microbiological Contamination of Seafood, from January to July 2009 (RASFF 2009)

Table 4 below shows the country of origin, product and hazard leading to the notification showing that shellfish is often involved and *Listeria, Escherichia coli* and *Salmonella* are most frequent hazards.

Table 4: List of Microbiological Hazards Association with Sea Foods (RASFF 2009)

Country of Origin	No. of Notification	Products	Hazards
Belarus	1	Herring and Rainbow trout (*Oncorhynchus mykiss*)	*Listeria*
	1	Herring fillets	*L. monocytogenes*
Ecuador	1	Tuna	Microbiological contamination
Greece	1	Live clams (*Venus verrucosa*)	*Escherichia coli*
	2	**Live mussels (*Mytillus galloprovincialis*)**	***Escherichia coli***
Germany	1	Fish meal	*Salmonella spp.*
	1	Soy bean meal	*Salmonella spp.*
Italy	3	Live clams (*Tapes semidecusatus*)	*Escherichia coli*
Peru	1	Fish meal	*Salmonella spp.* *Enterobacteriaceae*
	3	Smoked salmon (*Salmo salar*)	*Listeria monocytogenes*
Poland	1	Frozen breaded Saithe fillets	*Listeria monocytogenes*
	1	Smoked mackerel	*L. monocytogenes*
	1	Prawn	*L. monocytogenes*
Spain	**1**	**Mussels**	***Escherichia coli***
	1	Fish meal	*Salmonella spp.*
	1	**Bivalve molluscs**	***Salmonella spp.***
Turkey	1	Clams (*Donax trunculus*)	*Escherichia coli*
	3	Live clams	*Escherichia coli*
	1	Frozen shrimps (Penaeus spp.)	*Salmonella spp.*
	4	Frozen *Pangasius hypophthalmus*	*L. monocytogenes*
	2	Frozen fish	*L. monocytogenes*
Vietnam	1	Frozen fish	Microbiological contamination
	1	Frozen crustaceans	*Salmonella spp.*
	1	Frozen white clams (*Meretrix lyrata*)	*Salmonella spp.*

3.2.3. Sources of hazards

The table below (table 5) shows outbreaks and cases of food borne diseases in the United States of America (USA) between 1993 and 1997 and the relatively high percentage of fish and shellfish borne outbreaks and cases (Huss *et al.,* 2003).

Table 5: Food implicated in food-borne disease in the USA from 1993-1997 (Huss et al., 2003)

Food	Outbreaks		Cases		Deaths	
	Number	%	Number	%	Number	%
Meat	66	2.4	3205	3.7	4	13.8
Pork	28	1.0	988	1.1	1	3.4
Poultry	52	1.9	1871	22	0	0.0
Other meat	22	0.8	645	0.7	2	6.9
Shellfish	**47**	**1.7**	**1868**	**22**	**0**	**0.0**
Fish	140	5.1	696	0.8	0	0.0
Egg	19	0.7	367	0.4	3	10.3
Dairy products	18	0.7	313	0.4	1	3.4
Ice cream	15	0.5	1194	1.4	0	0.0

Bakery goods	35	1.3	853	1.0	0	0.0
Fruits and vegetables	70	2.5	12369	14.4	2	6.9
Salads	127	4.6	6483	7.5	2	6.9
Others	66	2.4	2428	2.8	0	0.0
Several foods	262	9.5	25628	29.8	1	3.4
Total known foods	967	35.2	58908	68.5	16	55.2
Total unknown food	1784	64.8	27150	31.5	13	44.8
Total	2751	100.0	86058	100.0	29	100.0

In table 6 shows the number of outbreaks and cases for the period between 1990 and 1998 (Huss *et al.,* 2003). It is clear from both these tables that although fewer outbreaks are associated with shellfish than fish consumption, more individuals get ill. A total of 1661 cases were caused by consumption of fish.

Table 6: Number of outbreaks and cases related to seafood in the USA from 1990 to 1998, listed are only outbreaks for which an etiological agent has been identified (Huss et al. 2003).

Seafood group	Outbreaks	Cases
Fish	263	1661
Molluscan shellfish	66	3281
Other shellfish	8	148
Total	337	5088

3.2.4. Sources of Listeria contamination in seafood worldwide(appendix 1)

Contamination sources of *Listeria* in food products can be divided into two main categories that are environmental or natural sources and processing sources. *L. monocytogenes* can be found all over in the environment, both in fresh and seawater, decaying vegetation, soil, sewage sludge and silage (Adams and Moss, 2005) in addition it has also been found in humans and a variety of animals. The bacterium has been found in more than 37 mammalian species, both domestic and in wild animals, in at least 17 bird species and some species of fish and shellfish (FDA, 2009).

There are several places in the processing of RTE foods that can cause a cross contamination of *Listeria*. The nature of the processing contamination can be of various reasons and at various stages of the production. The contamination can for instance come from a equipment before packaging like slicers, dicers, shredders, blenders or other equipment that are used after heating treatment or decontaminating and before packing of the product. The contamination source can be conveyors or collators that are

used for assembling or arranging the product before it is packed and spiral freezers or blast freezers. It can occur while filling or packaging or from solutions that are used in chilling food, from hand tools, gloves and aprons. From food storage containers that the food is kept in while waiting for further processing or packaging like tubs, baskets or from the racks used transporting the product after it has been packed and is ready for human consumption (Tompkin *et al.*, 1999).

In last decade *Listeria* spp. and *L. monocytogenes* has been isolated from kinds of shellfishes and other fishes (Appendix 1):
Beleneva and Maslennikova (2002) isolated eight *L. monocytogenes* strains from mussels (*Mytilus edulis*) from the Trinity Bay of the Gulf of Peter the Great, the Sea of Japan. Halit and Kapllan (2010) *L.monocytogenes* from live bivalve mollusk (*Mytilus galloprovincialis*). *Listeria* spp. and/or *L. monocytogenes* have been isolated from mussel by Pinto *et al.*, (2006), Brett et *al.*, (1998), Laciar and de Centorbi (2002) and Laciar *et al.* (1998).

Shellfishes including crustacean's shrimps, molluscan shellfish, squid and other kinds has found as the source of *Listeria* spp. and/or *L. monocytogenes.* Monfort *et al.* (1998) identified *Listeria* spp. from live shellfishes. Pereira *et al.* (2001) found both *Listeria* spp. and *L. monocytogenes* from shellfish samples. Maria (2000) also found both *Listeria* spp. and *L. monocytogenes* from shrimp (*Penaeus brasiliensis*). Dhanashree *et al.* (1999) reported *Listeria* spp. and *L. monocytogenes* from the fresh shellfish in tropics. Heinitz *et al.* (1998) isolated *L. monocytogenes* from smoked shellfish samples.

Listeria spp. and/or *L. monocytogenes* also isolated from raw, frozen fin fishes and also from fish roe. *L. monocytogenes* was found in raw fish, shellfish and fish roe collected from retail stores in Tokyo, Japan (Satoko *et al.,* 2005). 65 samples of fish and shellfish were obtained from fish markets and processing factories in Mangalore, India. *L. monocytogenes* was detected in 17.2% of finfish and 12.1% of shellfish (Jeyasekaran *et al.*, 1996). The contamination of *L. monocytogenes* was found in 11.6% of the crustaceous shellfish (268 samples) in Santiago, Chile (Cordano and Rocourt 2001). Ten cold smoked salmon processing plants were visited a number of times in Denmark; 110, 70, 160 samples were collected; *L. monocytogenes* were found 0.9%, 6% and 41% samples respectively (Huss *et al.*, 2000). Gombas *et al.* (2003) found *L. monocytogenes*

in smoked seafoods. *L. monocytogenes* was isolated from smoked salmon (Inoue *et al.*, 2000). Smoked fish in Belgium found a high risk of *L.monocytogenes* (>100 cfu/g) of *L. monocytogenes* (Uyttendaele *et al.*, 2009). The prevalence of *L. monocytogenes* in cold-smoked salmon was 34-43% and in cold-smoked halibut was 45-60% from the naturally contaminated seafood in Denmark (Jørgensen and Huss, 1998). A survey analysis was done in Italy during 2001- 2002 for the presence of *L. monocytogenes*, 6% samples were positive with *L. monocytogenes* out of total 3160 samples of fish and fish products (Busani *et al.*, 2005). 25 different fish species from Portugal were examined for *L. monocytogenes* where 12% samples were positive (Mena *et al.*, 2004). A survey of 50 retail pre-packaged portions of marine fish (conger, swordfish, sole, grouper and whiting) was conducted in Spain; the incident of *L. monocytogenes* was 10% (Herrera *et al.*, 2006). A variety of 252 ready to cat fish (halibut) and meat products were analyzed for *L. monocytogenes* in Belgium. *L. monocytogenes* were detected from smoked halibut was 33.33% (Van *et al.*, 2004).

3.2.5. *Listeria* hazard hazards in Icelandic Seafood

Most of the fish caught in Icelandic waters comes from the open sea and thus almost free from pathogenic bacteria. It has also very low levels of chemical contaminants. Reports of pathogenic bacteria come from processed fish. A six-year period of bacteriological analysis of Icelandic cold water shrimp (*Pandalus borealis*) was done from 1988-1993; 7913 samples of shrimp from 26 Icelandic factories were analysed. Some 70% of the samples had less than one coliform bacterium per gram and 99.9% of the samples had less than one faecal coliform bacterium per gram. *Staphylococcus aureus* was detected in less than 0.2% of the samples. *Listeria* spp. was found in 270 of 3331 samples examined or 8.1%. Species identification was done on 49 of the 270 positive samples. The proportion of *L. monocytogenes* was found to be 26.5% (Valdimarsson *et al.*, 1998).

There is almost no border notification yet from RASFF until July 2009. The only border notification reported for Iceland is too high content of colour into the black lumpfish roe (RASFF 2009).

Salmonella has being identified from fish meal in Iceland. The presence of toxic algae has stopped harvesting of mollusks in Iceland (personal communication from Hjörleifur Einarsson, Professor, University of Akureyri, Iceland).

Hartemink & Georgsson (1991) tested a total of 128 samples of seafood from the Icelandic market for the presence of *L. monocytogenes* and other *Listeria* spp. which included raw, smoked and dried fish, frozen shellfish and shrimps and several fish salads. All these fish foods are normally consumed without cooking or heating. *Listeria* spp. was present in 56% of the samples of raw fish, 29% of the smoked fish, 9% of the shrimps and 32% of the salads. All products sampled had been processed and packed in Iceland, mostly for use on the domestic market.

Listeria spp. was detected in 12.5% and *L. monocytogenes* was detected in 11.2% of the Icelandic cooked peeled shrimp (*Pandalus borealis*), sampling was done from final, semi final products and from shrimp-processing environment from 1998 to 2001 (Gudmundsdóttir *et al.,* 2006).

During 1998/1999 a total of 1180 seafood samples were collected from five seafood processing (cooked shrimp, raw salmon, raw cod) plants of Faroe Islands, Finland, Iceland, Norway and Sweden. 14.3% samples were positive with *Listeria* spp. and 13.1% were positive with *L. monocytogenes* (Gudbjörnsdóttir *et al.*, 2004).

A total of 647 samples (125 from cold smoked salmon and 522 from its processing environment) were tested during 1997-1998 and 2001 from Iceland. *Listeria* spp. was infected with 15.1% samples and *L. monocytogenes* were infected with 11.3% samples but the incidence of *L. monocytogenes* in cold-smoked salmon final products was only 4% (Gudmundsdóttir *et al.*, 2005). The table (table 7) below summarise of these findings-

Table 7: Prevalence of L. Monocytogenes in Sea food in Iceland

Source	Country	Seafood types	No. of Samples	Prevalence (%)	Comments
Valdimarsson *et al.*, 1998	Iceland	Shrimp (*Pandalus borealis*)	3331	8.1%	*Listeria* spp
Hartemink and Georgsson, 1991	Iceland	Raw, smoked and dried fish, frozen shellfish, shrimps, and fish salads	128	56%(raw fish) 29%(smoked fish) 9% (shrimp) 32% (salads)	*Listeria* spp
Gudmundsdóttir *et al.* 2006	Iceland	Cooked peeled shrimp (*Pandalus borealis*)		12.5% 11.2%	*Listeria* spp *L. monocytogenes*
Gudbjörnsdóttir *et al.* 2004	Nordic	cooked shrimp, raw salmon, raw cod	1180	14.3% 13.1%	*Listeria* spp *L. monocytogenes*
Gudmundsdóttir *et al.* 2005	Iceland	Cold-smoked salmon Processing environment	647	15.1% 11.3%	*Listeria* spp *L. monocytogenes*

3.3. *Listeria* and Listeriosis

3.3.1. *Listeria monocytogenes*

Listeria is a Gram positive rod shaped (0.4-0.5μm × 0.5-2.0μm) bacterium that is facultatively anaerobic, non-spore forming, catalase positive and oxidase negative. *L. monocytogenes*'s optimal temperature is between 30 and 35°C, but it can grow over a wide range of temperatures or from 0 to 42°C which enables multiplication in refrigerated food. The bacterium can tolerate a quite high salt concentration and is able to grow in 10% sodium chloride and survive for a year in 16% at pH 6.0 (Adams and Moss, 2005). *Listeria* genus consists of a few species, but only *L. monocytogenes* is considered to be pathogenic to humans. The other species are *L. innocua, L. ivanvii, L. seeligeri, L. Welshimeri* (Halligan, 1991) and *L. grayi* (Rocourt *et al.*, 1992). *L. monocytogenes* has been associated with various food products for instance several milk products (cheeses, ice cream, raw milk and supposedly pasteurized fluid milk), raw vegetables, fermented raw-meat sausages, all kinds of raw meat, raw and cooked poultry and seafood (FDA, 2009).

3.3.2. Listeriosis

In humans *L. monocytogenes* are the cause of Listeriosis. Listeriosis is most likely to develop in very young, pregnant women, elderly people or individuals with compromised immune system. The symptoms of Listeriosis differ from being a mild flu-like illness to meningitis (or meningoencephalitis) or encephalitis and in pregnant women the *Listeria* is likely to cause influenza-like symptoms with fever, headache and even symptoms from gastrointestinal system and the bacterium has been linked to spontaneous abortion and stillbirth, which probably be attribute to trans-placental foetal infection (Adams and Moss, 2005). Incubation time of Listeriosis can vary from three and up to 60 days, and because how long the incubation time can be it is often hard to track the source of the infection. Because of how hard it can be to trace the source the majority of Listeriosis cases are documented as single cases, not outbreaks, nevertheless a few outbreaks have been documented (Yde *et al.*, 2010)

3.3.3. Frequency of Listeriosis in human

Listeriosis worldwide

USA

About 1,600 cases of Listeriosis illness is being estimated each year in USA, with 1,400 hospitalizations and 250 deaths, on average 2.4 outbreaks per year were reported from 1998-2008, before 2011, the largest outbreak occurred in 2002 with 54 illnesses, eight deaths, and 3 fetal deaths in nine states. An outbreak of 108 cases of Listeriosis was traced to *Listeria* infection in meat (Mead *et al.*, 2006).

Although human *Listeria* infections are nationally notifible, some cases may not be recognized through public health surveillance, in part because some *Listeria* isolates may not be forwarded or reported from clinical laboratories to state public health laboratories. Additionally, although invasive Listeriosis is a serious disease for which patients would be expected to seek medical care, it is likely that some cases of infection, especially those that involve miscarriages and stillbirths, may not be diagnosed (CDC, 2012). The annual reported of laboratory-confirmed cases of human Listeriosis in the United States from 2007 to 2009 are given in figure 3. There was an increasing rate of Listeriosis observed in 2009 (2.77 cases/million) compare to 2008 (2.49 cases/million).

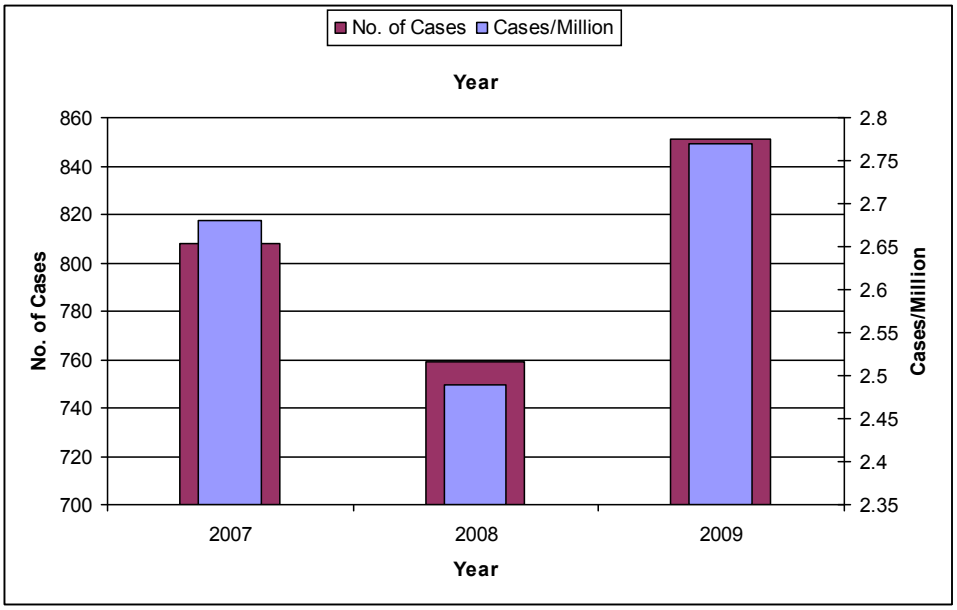


Figure 3: Reported Listeriosis cases in humans in US during 2007 to 2009 (CDC, 2012)

EEA countries

1,692 confirmed human cases were reported in 2010 among EEA (European Economic Area) countries, figure 4 shows reported Listeriosis confirmed cases in humans during 2007 to 2010 in EEA countries including Switzerland. Significant increasing trends in Listeriosis notification rates from 2007 to 2010 were noted in Austria, Latvia and Spain, while statistically significant decreasing trends were noted in Belgium, the Czech Republic, Luxembourg, and Slovakia.

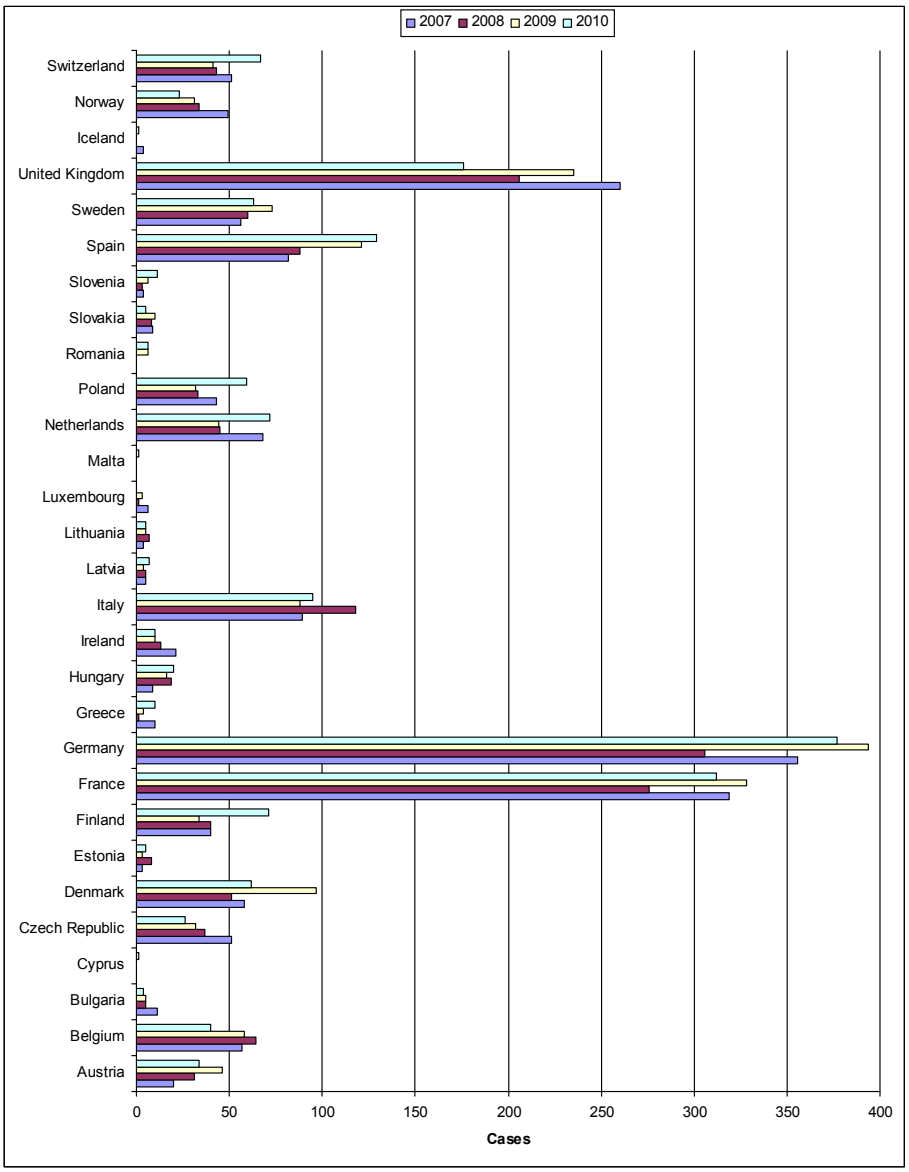


Figure 4: Reported Listeriosis cases in humans in EEA countries during 2007 to 2010 (EFSA, 2012)

L. monocytogenes was rarely detected above the legal safety limit (100 cfu/g) from RTE foods at retail. The overall EU notification rate in 2010 was 0.35 cases per 100,000 population, with the highest country-specific notification rates observed in

Finland (1.33 cases per million population) followed by Denmark and Spain (1.12 cases per million population) (figure 5). The notification rate was highest in those aged over 65 years (1.21 cases per million population), covering 60.2 % of all reported cases, while 6.7 % of cases were detected in the age group 0-4 years and the majority of these cases (96.3 %, N=108) were in infants (age <1 year). Of those, 87 cases were infected in 2010 with *L. monocytogenes* via suspected food, and 43 cases were pregnancy-associated. Of the cases infected via consumption of contaminated food, cheese was mentioned as the suspected vehicle for 13 cases, milk and fish for one case, while for the remaining cases no information on the food source was provided. The outcome of the disease was known for 1,063 confirmed cases (66.3 %) (figure 5) (EFSA, 2012).

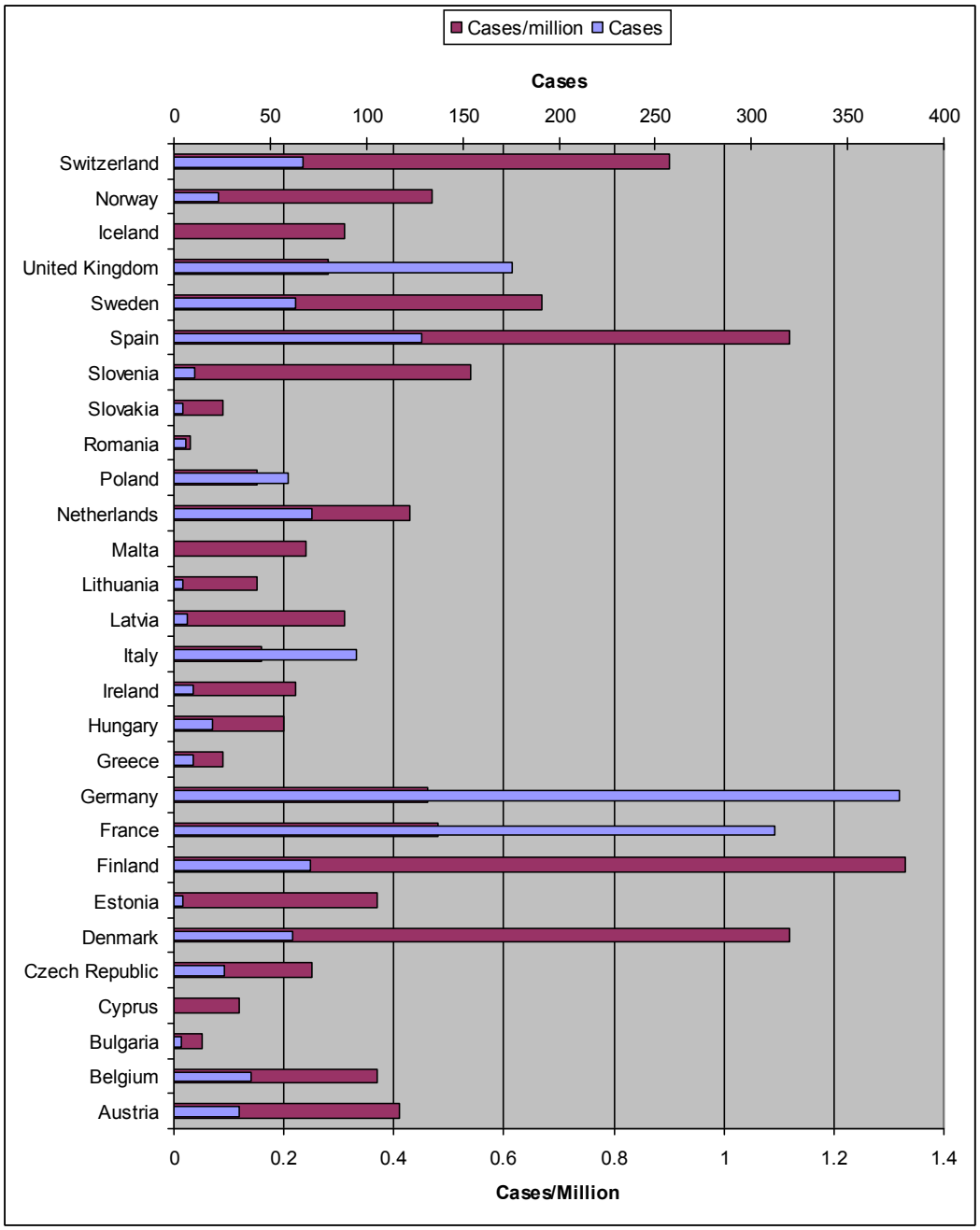


Figure 5: Reported Listeriosis cases in humans in EEA countries in 2010 (EFSA, 2012)

Reported cases of Listeriosis in Europe ranged from 0-7.5 cases per million inhabitants in 2002. The countries that had the highest reported Listeriosis cases were those that had a statutory notification of the disease (Hedberg, 2006). In many countries Listeriosis cases have been increasing for instance in Netherlands (Hedberg, 2006), Germany (Koch and Stark, 2006), Denmark (Kvistholm *et al.*, 2010) and in the UK (Gillespie *et al.*, 2010). In Germany (figure 6) the number of have increased from 0.62 per 100,000 inhabitants (217 cases) in 2001, when Listeriosis became mandatory notifible, to 0.62 per 100,000 (519 cases) in 2005. In Denmark (figure 7) the cases have increased from

about 0.6 per 100,000 inhabitants to about 1.0 per 100,000 in 2008 and in 2009 the cases were almost 1.8 per 100,000 (Kvistholm *et al.*, 2010) and in the UK the number of cases have increased from approximately 110 cases in the 1990 to about 200 cases in 2007 (Figure 8) (Gillespie *et al.*, 2010). In France on the other hand the cases of Listeriosis per million inhabitants decreased from 4.5 in 1999 to 3.5 cases in 2003 (figure 9) (Goulet *et al.*, 2006).

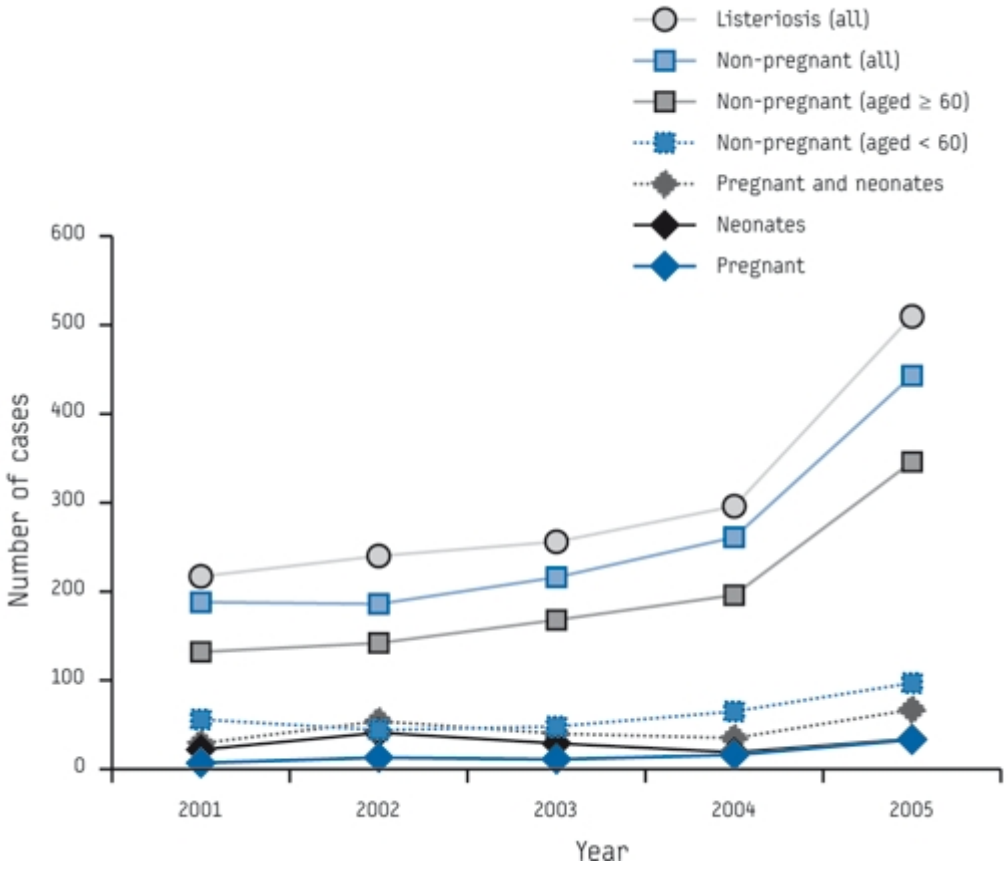


Figure 6: Annual cases of Listeriosis in Germany from 2001 to 2005 (Koch and Stark, 2006)

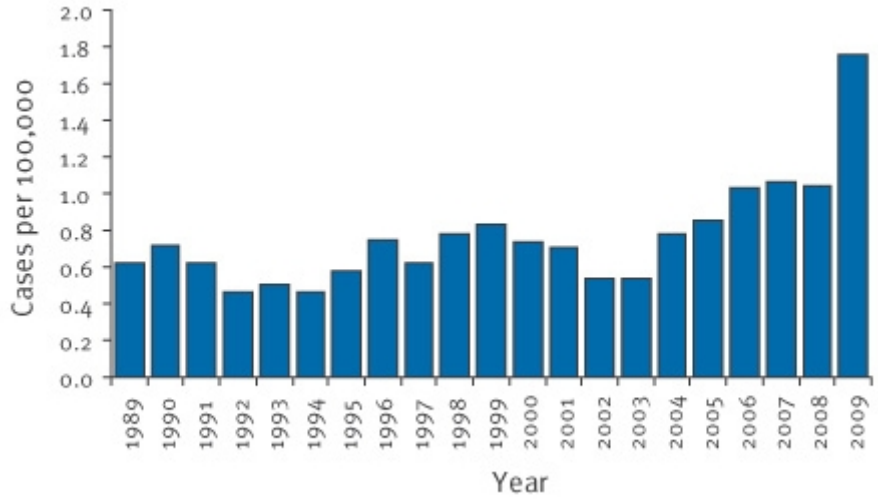


Figure 7: Annual incidence of Listeriosis per 100,000 inhabitants in Denmark from 1989-2009 (Kvistholm et al., 2010)

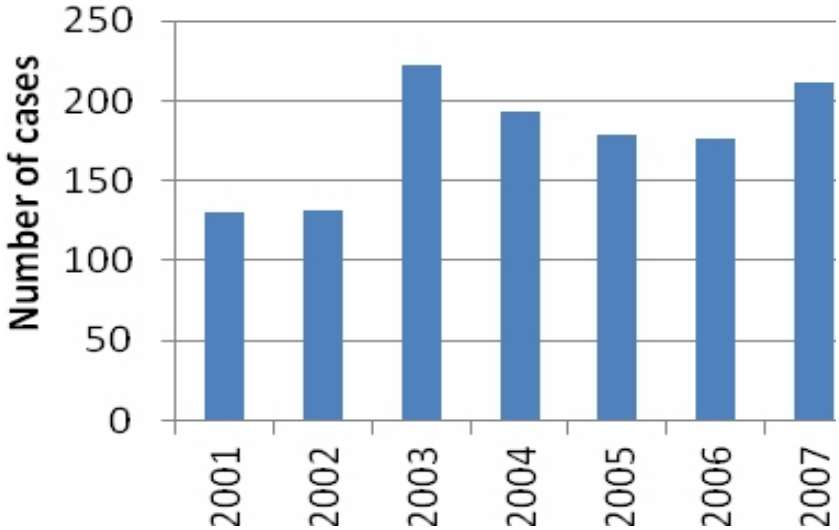


Figure 8: Annual cases of Listeriosis in the UK from 2001 to 2007 (Gillespie et al., 2010)

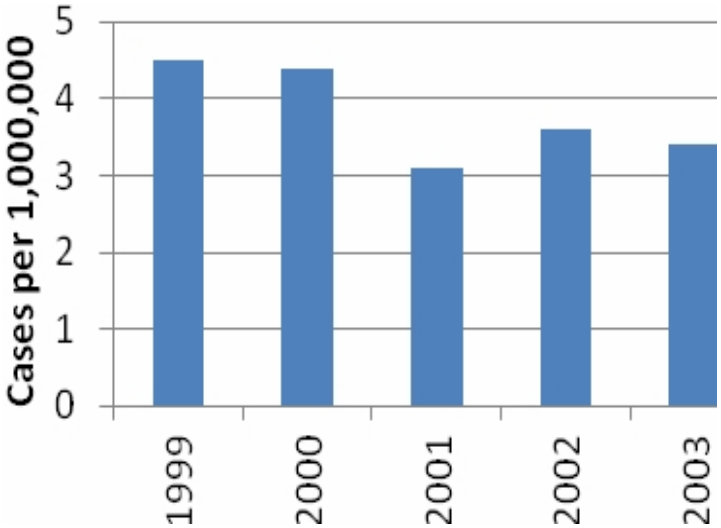


Figure 9: Annual incidence of Listeriosis per 100,000 inhabitants' in France from 1999-2003 (Goulet et al., 2006)

In Iceland infections by *L. monocytogenes* are rather rare. Figure 10 shows the confirmed cases of Listeriosis per 100.000 in Iceland from 1998 to 2007. The highest number of confirmed cases was in 2007 is four (1.25 per 100.000). From 1998-2003, 2006 and 2008-2009 no cases of *L. monocytogenes* were reported, in 2010 there was a case of Listeriosis observed (Director of Health, S. E., 2010).

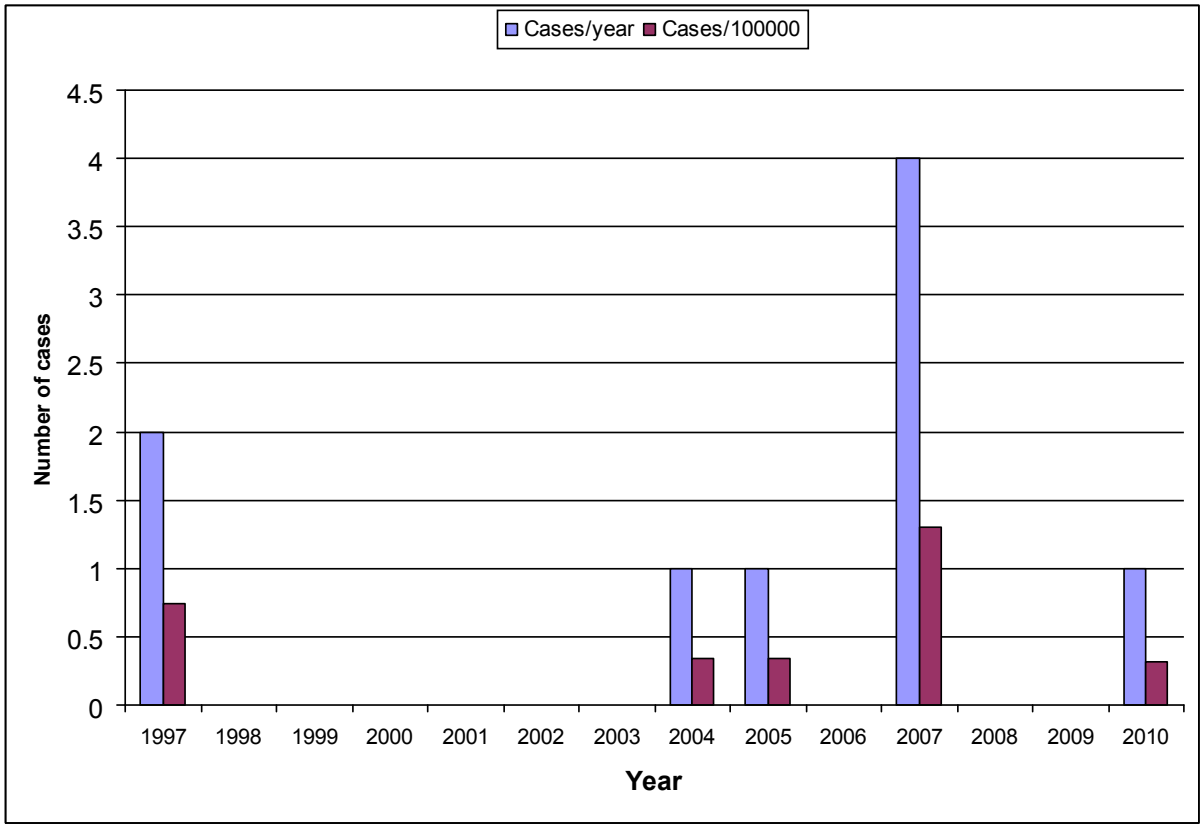


Figure 10: Number of confirmed cases of Listeriosis per 100.000 in Iceland

Despite of being quite severe Listeriosis is not a notifible disease in all countries, for instance in Netherlands and in the United Kingdom (Health Protection Agency, 2010) it is not necessary to report cases of Listeriosis to government authorities (Hedberg, 2006), while countries, like for instance Iceland (Regulation 420/2008, 2008), USA (CDC. 2010), Australia (Australian Government Department of Health and Ageing, 2005), New Zealand (Health Act 1956 No. 65, 2010), Canada (Public Health Agency of Canada, 2003), Germany (Koch and Stark, 2006), France (Goulet *et al.*, 2006) and Ireland (Iris Statute Book 2003, 2003) have a legally mandated reporting of the disease.

3.34. Regulations

The EU commission regulations No. 2073/2005 of 15 November, 2005 and (EC) No. 1441/2007 of 5 December, 2007 establish that *L. monocytogenes* cannot be found in 25 g of RTE foods intended for infants or patients. In RTE foods that are unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes the *L. monocytogenes* limits are 100 cfu/g at the end of the shelf-life time and finally for RTE food that are able to support the growth of *L. monocytogenes* that are not intended for infants or special medical purposes *L. monocytogenes* should be absence in 25 g of sample before the food has left the instant control of the food business operators, who has produced it but at the end of the shelf-life it should not exceed 100 cfu/g (Commission Regulation (EC) No. 2073/2005 and Commission Regulation (EC) No. 1441/2007).

4. Materials and Methods

4.1. Blue mussel (*Mytilus edulis*) Sample Collection

A total of 43 fresh live blue mussel samples (appendix 2) were collected from four different blue mussel processing factories (figure11) from Iceland; 28 samples were from Norðurskel ehf, Sjávargata, Hrisey where four samples were from the factory immediate after catch and 24 samples from the retail outlet (Hagkaup / Netto) in Akureyri, 11 samples from St2 ehf, Drangsnes, Drangsnesvegur, Western Fjords, two samples from Vogaskel ehf, Vaglasel, Greater Reykjavík Area and two samples from BioPol ehf, Skagaströnd, Norðurland vestra.

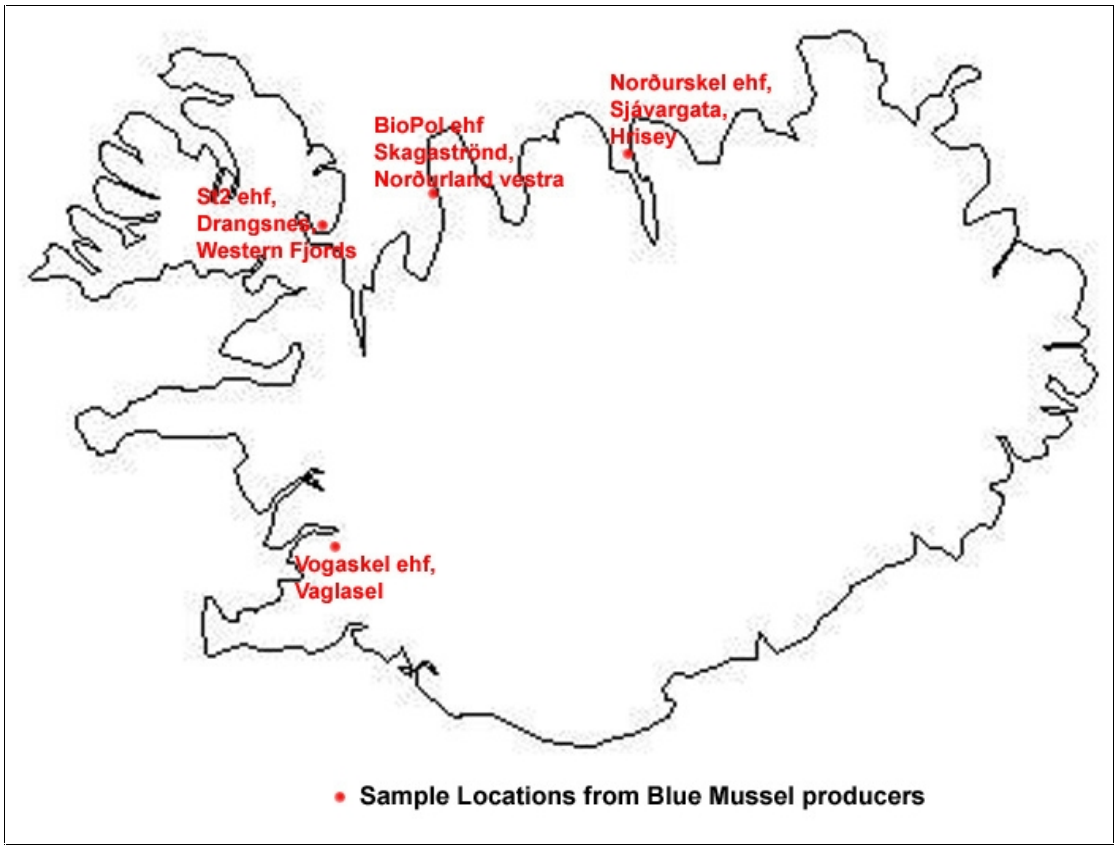


Figure 11: Sample locations of live fresh blue mussels from Iceland.

4.2. Swab Sample Collection

Forty six (46) swab samples (appendix 3) were collected from the largest blue mussel producing company Norðurskel ehf during December, 2011 and March, 2012. Samples were collected using aseptic techniques, transported to the laboratory on ice, and were started analysis earliest possible time. Swab samples were collected from different surfaces (floor, table top, machine surface, outdoor, fresh mussel tub etc) of the blue mussel processing industry. Move the swab, through a distance of 10x5 cm during the swabbing operation; rotated the swab against the overall wiping movement. Then stroke the area, in same direction three times, turning the swab slightly between strokes. Finally roll the swab once over the wiped area, but in the opposite direction, from that in which the original strokes were made. This was served to pick up whatever bits of cotton may be adhering to the surface, placed the swab immediately into a sterile bottle, pulled the stick free if the swab in the medium (1% peptone water) is to be transported, hold it under the same condition as swab samples are being transported below 5^0C until taken for analysis.

4.3. Microbiological analysis of Blue mussel Sample

4.3.1. Total Microbial number

The surface count plate method (spiral plate method: FDA-BAM, 2012a) was used to get the total microbial number into the blue mussel samples, gives reliable and consistent results. To measure the microbial number the fish was removed from the shell and put in a stomacher bag (BagPage, Interscience, France) along with sterile butterfilled buffer solution. The stomacher bag was then put in Lab-blender 400 (Seward Laboratory, England, Sussex) and mixed thoroughly. The solution was then diluted up to 10^4 and 50 µL from each dilution was spread on two PCA (Plate Count Agar) plates using an Eddy Jet spiral plating machine (IUL instruments).The plates were then stored at 15°C temperature for 4 days before counting the colonies.

4.3.2. MPN method for coliforms

The five tube MPN (Most Probable Number) method (FDA-BAM, 2012b) was used for detection of coliforms. The LST (Lauryl Sulfate Broth) tubes were then incubated in 35°C for 48 hours in circulating water bath and then from each positive LST tube, with

presumptive coliforms, a loop of the culture was put in both EC (*Escherichia coli*) broth (Difco) for detecting faecal coliforms and BGLB (Brilliant Green Lactose Bile) (Difco) for detecting coliforms. The EC tubes were incubated in 45°C for 24 hours but the BGLB tubes for 48 hour in 35°C in circulating water bath. The number of positive EC and BGLB tubes was then use to calculate the most probable number of coliforms and *E. coli* with a freeware MPN calculator (MPN Calculator, 2004).

4.3.3. L. monocytogenes strains

For positive control *L. monocytogenes* strain (DSM 20600) was used it was stored in nutrient broth (NB) at -20ºC between experiments. From the nutrient broth 1 ml of the culture was transferred to NA (Nutrient Agar) (Difco) and incubated at 37^0C for 24 h. From the nutrient broth 1ml or from NA a loop-full of *L. monocytogenes* colony was transferred to nine ml of 1/2 Fraser broth and Incubate at 30^0C for 24 h. Transfer 0.1 ml of the culture obtained from primary enrichment (1/2 Fraser broth) to a tube containing 10 ml of secondary enrichment medium (Fraser broth). Incubate the inoculated medium for 48h ± 2 h at 37^0C. From the primary & secondary enrichment medium inoculate a portion of the culture in the OCLA (Oxoid Chromogenic Listeria Agar) and oxford agar plate by means of a loop or glass rods. Incubate the plates at 37^0C and examination after 24 h and if necessary, after 48 h to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes*. From the OCLA and oxford agar plate a loop-full of *L. monocytogenes* strains were grown on 10 ml nutrient broth (Difco) for 24 h at 37^0C. The *L. monocytogenes* content of the nutrient broth was approx. 10^9 CFU/ml, from where 0.1 ml of the cultured nutrient broth was transferred to 10 ml 0.15% peptone water (Peptone Water) (Difco) solution which was 10^7 CFU/ml and a series of serial dilutions (10^5, 10^3, 10^2 and 10^1 CFU/ml respectively) of peptone water were made . From the peptone water dilutions (10^3, 10^2 and 10^1) 1ml of *L. monocytogenes* containing peptone water was inoculated to agar Listeria and oxford agar plate and was incubated at 37°C for 24 h. The concentration of cells were determined by viable counts on that OCLA and oxford agar plates. The prepared suspension of known concentration of *L.monocytogenes* strains were used as reference positive control.

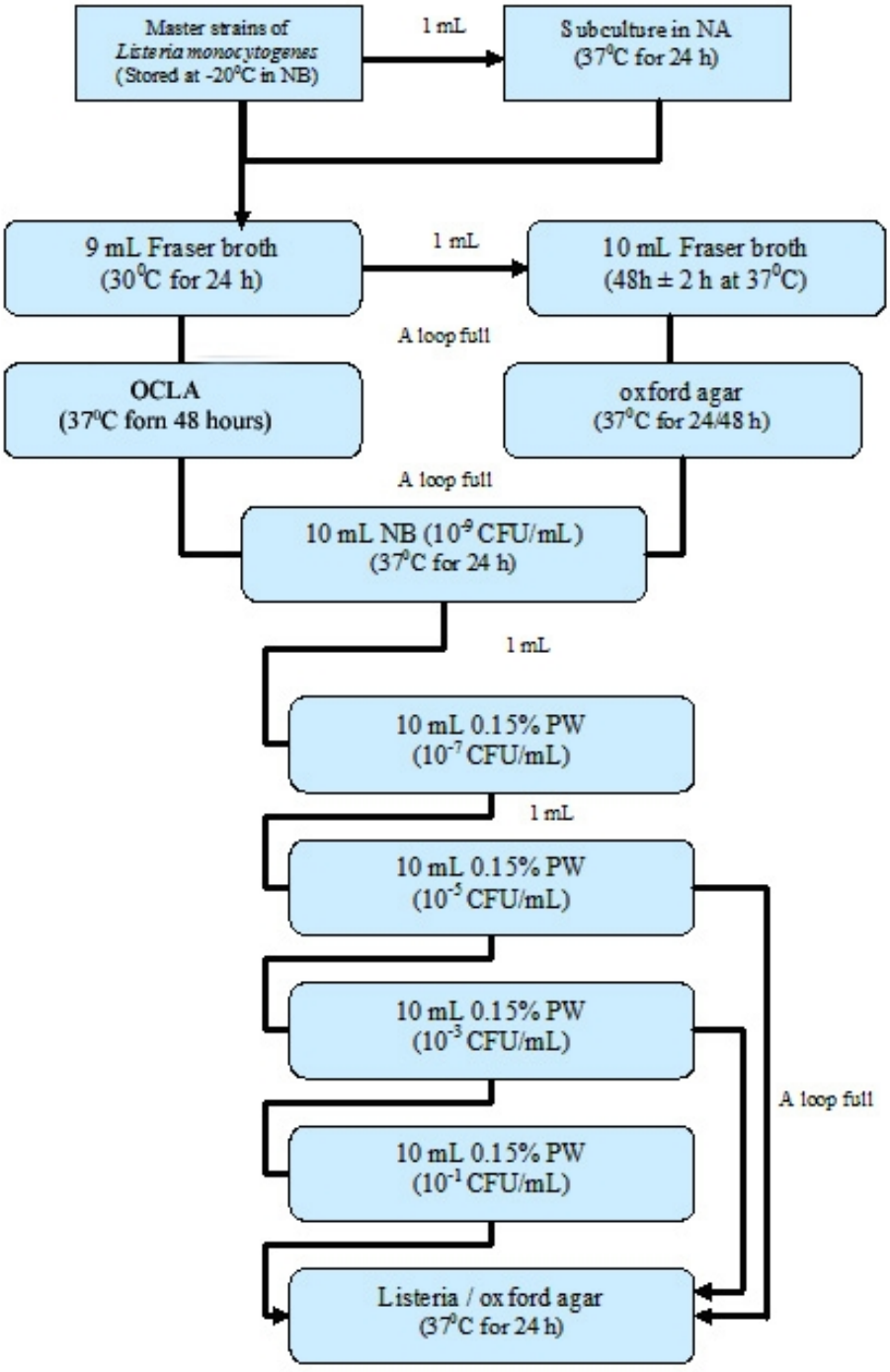


Figure 12: Flow diagram of the way of determination of L. monocytogenes strains concentration.

4.3.4. Detection and Enumeration of *L. monocytogenes* in Blue Mussel

For the detection and enumeration of *L. monocytogenes* in blue mussel the ISO 11290-1:2004(E) & ISO 11290-2:2004(E) was followed. For primary enrichment 25 gram sample of blue mussel put in stomacher bag along with 225 ml of 1/2 Fraser broth and Incubated the initial suspension of the test portion at 30^0C for 24 h. Transferred 0.1 ml of the culture obtained from primary enrichment to a tube containing 10 ml of secondary enrichment medium (Fraser broth), incubated the inoculated medium for 48h ± 2 h at 37^0C. From the primary & secondary enrichment medium inoculate a portion of the culture in the OCLA and oxford agar plate by means of a loop or glass rods, incubated the plates at 37^0C and examination after 24 h and if necessary, after 48 h to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes*. Typical colonies of *Listeria* spp grown on oxford agar for 24h are small (1mm) greyish colonies surrounded by black halos. After 48h, colonies become darker, with a possible greenish sheen, and are about 2 mm in diameter, with black halos and sunken centres, and the typical colonies of *Listeria* spp grown on OCLA are clearly visible blue/green colonies with *L. monocytogenes* colonies showing a distinctive opaque white halo. Sub-culture of the colonies of presumptive *L. monocytogenes* in particular agar plates, plated out and confirmation by means of appropriate morphological, physiological and biochemical tests. For the confirmation of the presence of *L. monocytogenes* the confirmatory tests-catalase test, gram staining, motility test, haemolysis test, carbohydrate utilization test and CAMP test was done according to ISO 11290-1:2004(E).

Enumeration method

Calculate for each of the plates the number of colonies of *L. monocytogenes* presents, using the following formula:
$a = (b/A)xC$, where

b is the number of colonies conforming to the identification criteria.
A is the number of colonies plated out for confirmation.
C is the total number of characteristic colonies enumerated on the petri-dish.

Calculate the number N of *L. monocytogenes* present in 1 ml or 1 gram of product, using the following formula:

$N = \sum a / \{V(n1+0.1n2)d\}$, where

∑a is the sum of the colonies of *L. monocytogenes* calculated after confirmation, on all the dishes retained at two consecutive dilutions, one of which at least contains at least 15 identified colonies;

V is the volume of the inoculum applied to each dish in millilitres.
n1 is the number of dishes retained at the first dilution;
n2 is the number of dishes retained at the second dilution;
d is the dilution factor corresponding to the first dilution retained.

4.4. Microbiological analysis of swab Sample for *L. monocytogenes* identification

The ISO 11290-1:2004(E) & ISO 11290-2:2004(E) was followed for the detection of *L. monocytogenes* in swab samples. The tubes contained the swab samples were filled with 10 ml of Fraser broth and inoculated for 48h ± 2 h at 37^0C. From enrichment medium inoculated a portion of the culture in the OCLA agar and oxford agar plate by means of a loop or glass rods. Incubate the plates at 37^0C and examination after 24 h and if necessary, after 48 h to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes*.

4.5. PCR analysis of Swab Sample for *L. monocytogenes* identification

30 swab samples were run in the PCR, *L. monocytogenes* strains (DSMZ 20600) was used a positive control, *Salmonella* serovar Montevideo and *E.coli* used negative control and no plate DNA used as blank sample.

4.5.1. Swab sampling for PCR analysis

L. monocytogenes might be contaminated to blue mussel from the naturally contaminated food contact surface of mussel processing company. The leading commercial manufacturer of fresh live blue mussel company Norðurskel ehf. was selected for surface swab collection, 30 cotton swab samples were collected, samples were collected using aseptic techniques, transported to the laboratory on ice, and were started analysis earliest possible time. Swab samples were collected from different surfaces of the blue mussel processing industry. The swab cotton was wet by 0.1%

peptone water, move the swab, through a distance of 10x5 cm during the swabbing operation; rotated the swab against the overall wiping movement. Then stroke the area, in same direction three times, turning the swab slightly between strokes. Finally roll the swab once over the wiped area, but in the opposite direction, from that in which the original strokes were made. This will serve to pick up whatever bits of cotton may be adhering to the surface. Placed the swab immediately into a sterile test tube contains 600 µl 0.1% peptone water (Oxoid, UK), pulled the stick free if the swab in the medium is to be transported. All the samples were transported to the laboratory in a cool box at 4^0C within one hour.

4.5.2. DNA extraction from the swab sample

The swab sample contains 300 µl peptone water (0.1%) was placed into a sterile 1.5 ml eppendorf tube and centrifuged in a microfuge for five minutes at 20,000g to extract any cells adhering to the swab. Afterwards, the swab was discarded and the filtrate (liquid) was returned to new 1.5 ml eppendorf tube. The sample was again centrifuged for five minutes at 20,000g and the supernatant was discarded. The pellet was suspended in 100 µl of 0.1 x TE buffer (1 mM Tris, o.1 mM EDTA) with 1 mg of lysozyme and incubated for 45 min at 37^0C. After incubation 1 µl of 10 mg/ml proteinase K and 1 µl of 10% sodium dodecyl sulphate (SDS) were added and incubated again for 30 min at 37^0C, then 100 µl of 10% chelex-100 (200-400 mesh, Bio-Rad) was mixed and incubated for 30 min at 56^0C and sample was vortexed for 10s and incubated again for 10 min at 100^0C. The sample was centrifuged for 5 min at 20,000g and the supernatant was transferred to a new eppendorf tube for PCR analysis. DNA concentration was assessed by fluorometer (Martin-Galvez, *et al.,* 2011) (figure 13).

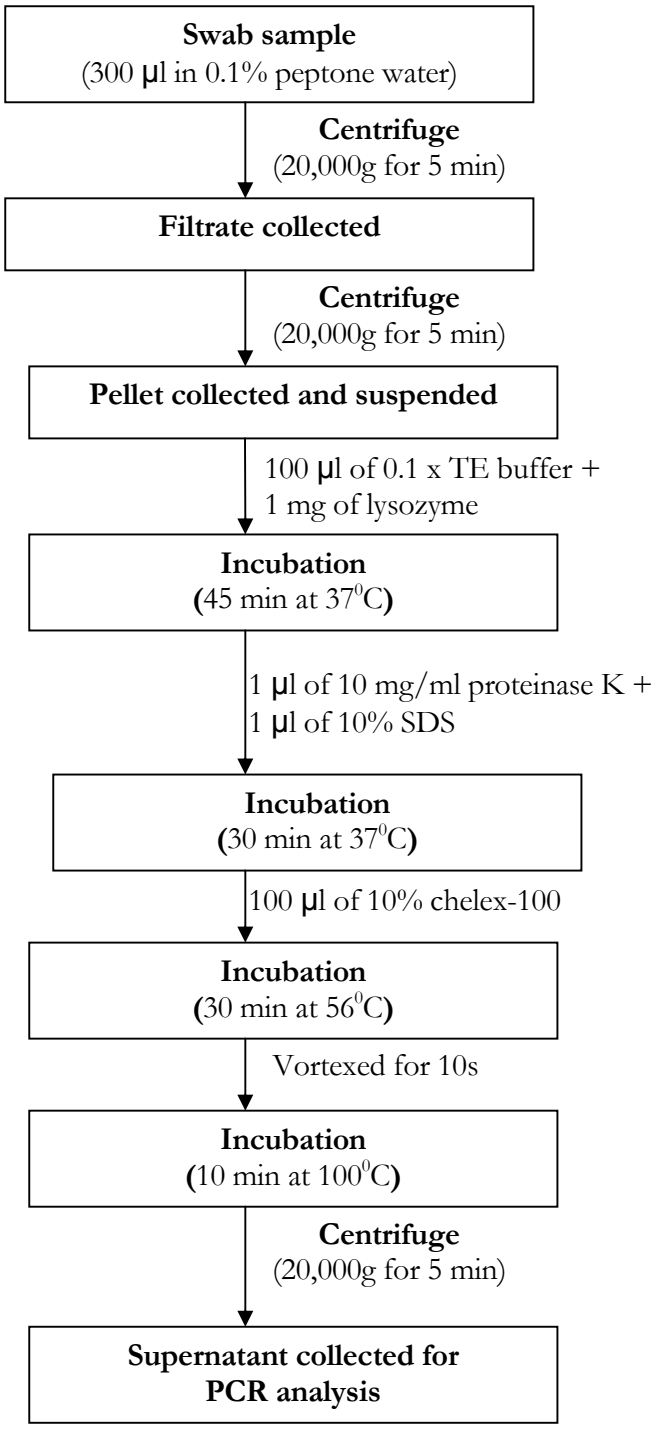


Figure 13: Flow diagram of DNA extraction from environmental swab sample.

4.5.3. Reconstruction of *Listeria* strains

The *L. monocytogenes* strains (DSMZ 20600) were diluted according to the instruction- The dried pellet of the strain into a double vial sealed vacuum ampoule, the tip of the ampoule was heated in a flame, soon after placed two or three drops of water onto the hot tip to crack the glass, carefully stricken off the glass tip, removed the insulation

material with a forceps and taken out the inner vial, the cotton plug was lifted, removed and kept under sterile conditions and flamed the top of the inner vial. 0.5 ml BHI (Brain Heart Infusion) broth was added and allowed the pellet to rehydrate for about 30 minutes, mixed the content gently with an inoculation loop and transferred the whole amount of the mixture to a test tube contained 5 ml BHI broth, streaked 100 µl of the suspension onto an agar plate, incubated for 18 hours at 37^0C.The concentration in the cell suspensions was confirmed by plate counts on BHI agar kept at 37 °C for overnight.

4.5.4. Optimization of PCR primer

A review was conducted from the early literature to get the best fit primer set for the detection of *L. monocytogenes* in swab samples (table 8).

Table 8: Review on L. monocytogenes Primers

Targeted gene sequence	Tm (^{0}C)	Amplicon size (bp)	Forward primer, Reverse primer Sequence (5′-3′)	Reference
ssrA		162	GCATCGCCCATGTGCTAC TCTACGAGCGTAGTCACCG	Justin , 2008
hly	**60**	**172**	**ACTTCGGCGCAATCAGTGA TTGCAACTGCTCTTTAGTAACAGC TT**	**Nari Lee *et al.*, 2011**
prfA	54	217	TCA TCG ACG GCA ACC TCG G TGA GCA ACG TAT CCT CCA GAG T	Sayed *et al.,* 2009
hly	62	106	GGGAAATCTGTCTCAGGTGATGT CGATGATTTGAACTTCATCTTTGC	Guilbaud *et al,*. 2005.

After the review and optimization study the primer set from Nari *et al.* (2011) found best fit (target gene sequence: hly, Tm(^{0}C): 60, Forward primer, Reverse primer Sequence(5′-3′): ACTTCGGCGCAATCAGTGA; TTGCAACTGCTCTTTAGTAACAGCTT and length of the gene was 172 bp).

4.5.5. Optimization of PCR reaction

Five different primer concentrations (table 9) were used to check the best fit and finally found 1 µl each (forward and reverse) works smoothly with the given reaction mixture. Four annealing temperature (58^0C, 59^0C, 60^0C, 61^0C) were used and found 61^0C the best fit for our reaction mixtures and selected primer set to use.

Table 9: Primer concentrations used for PCR optimization

Primer Concentration (µl), all are 0.5 µM	
Forward	**Reverse**
1.5	1.5
0.5	1.5
0.5	0.5
1	1
1.5	0.5

A reaction volume of 25 µl was used where 2 µl template DNA, 2 µl dNTP and 0.30 25 µl polymerage were used (table 10).

Table 10: Reaction mixture used for swab sample analysis

10x PCR Buffer *(Provides a final concentration of 2.5 mM $MgCl_2$)*	2.50 µl
dNTP	2.00 µl
Taq Polymerage (1 U)	0.30 µl
Forward Primer (3U/µl)	1.00 µl
Reverse Primers (3U/µl)	1.00 µl
Template DNA	2.00 µl
Water, nuclease-free	to 25 µl
Total Volume	**25 µl**

The thermal cycling condition was 95^0C for 15 min, followed by 50 cycles of 95^0C for 15 s, 61^0C for 20 s, and 72^0C for 30 s. A final extension of 72^0C for four minutes (Amagliani *et al.* 2006). All thermal cycling conditions was performed using a three-step cycling protocol. The PCR assay was run on 3% agarose gel electrophoresis with SYBR®Safe (Invitogen, UK) DNA Gel staining. The voltage and time of the gel electrophoresis was 70 Watt for 1.5 h. DNA moves towards positive anode due to the negative charges on its phophate backbone.

4.5.6. Preparation of agarose gel

Three (3) g of Agarose powder (Sigma, Germany) was mixed and homogenate with 200 ml of 0.5x TBE (Tris/ Borate/ EDTA); 50 ml of 10x TBE was mixed into one litre of distilled water to prepare 0.5x TBE and 5x TBE was prepared into a stock solution of 54 g of Tris base (TRIZMA-BASE, Sigma, USA), 27.5 g of boric acid and 20 ml of 0.5 M EDTA (Ethylenediaminetetraacetic acid)(p^H 8.0).

5. Results

5.1. Total Microbial Number from the blue mussel in Iceland

The total number of bacteria in 43 blue mussel samples during the whole experiment periods is represented in figure 14 and appendix 5. The total count was varied from 2 to 7 log cfu/g, the highest total bacterial count was in sample no. 33 (log 5.78 CFU/g) during winter (December, 2011), the lowest was found in sample no 22 (log 2.30 CFU/g) during summer (October, 2011), the average bacterial count was log 3.98, with a median value of 4.15 cfu/g and Standard Deviation 1.084. In two samples (sample 14 and 15) there are no results due to laboratory error. For the raw crustaceans the microbiological quality should be <log 5 cfu/g (satisfactory), log 5-log 6 cfu/g (acceptable) and ≥log 6 cfu/g (unsatisfactory) (Gilbert, *et al.* 2000). Out of 43 tested samples, 21 samples found below the satisfactory limit, only two samples (sample 12 and 33) found in between the accepted range. None of the samples found unsatisfactory level of bacterial counts (figure 15).

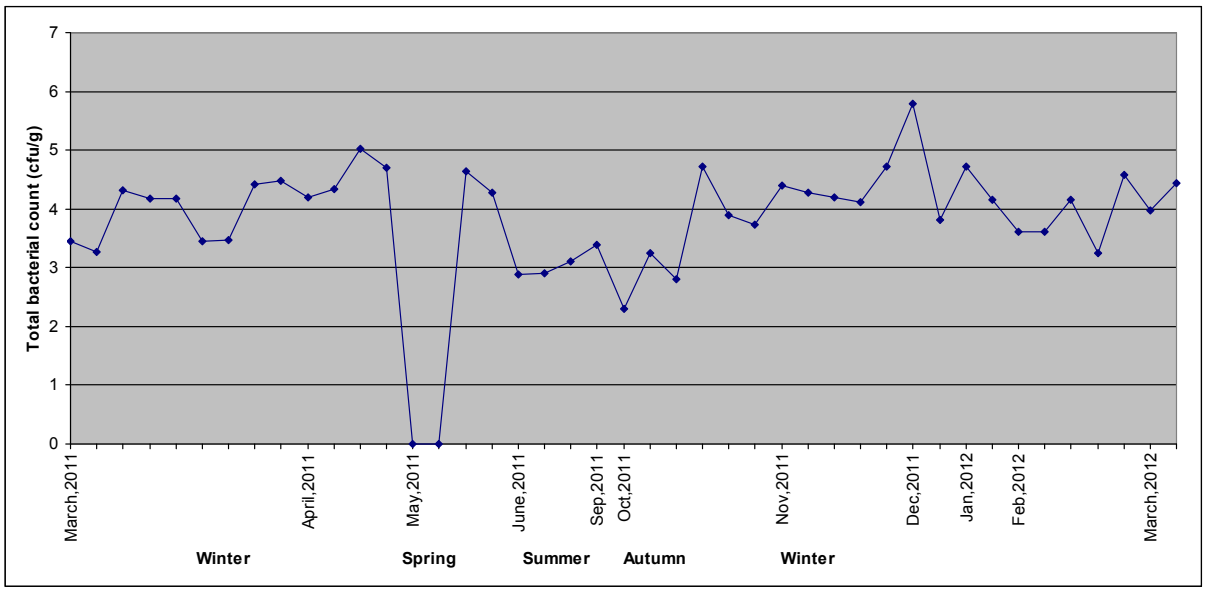


Figure 14: Total bacteria count (Log CFU/g) in mussel samples

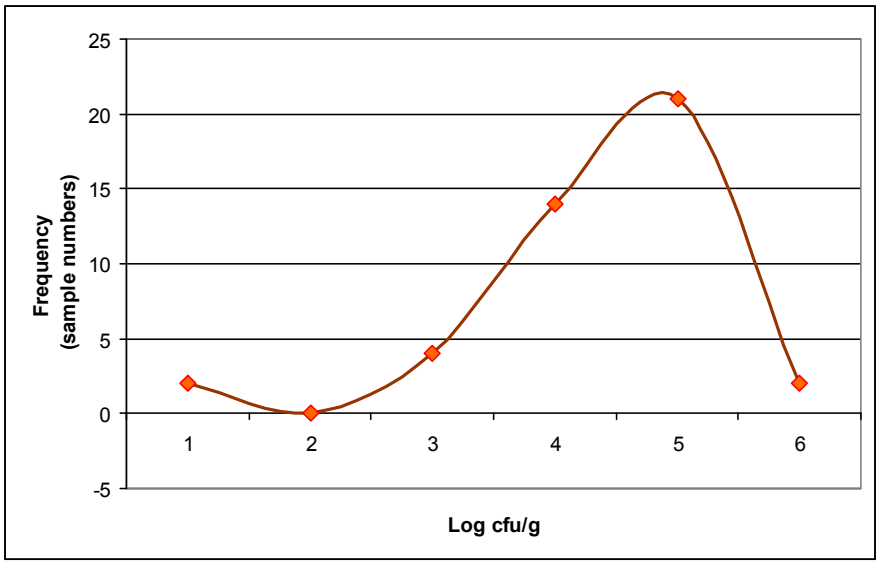


Figure 15: Frequency of total bacteria count (Log CFU/g) in mussel samples

5.2. Coliforms in the blue mussel in Iceland

The maximum number of coliforms was observed in sample no. 7 (1.4 MPN/g) and maximum number of E. coli was found in sample no 2 (0.78 MPN/g) (figure 16, appendix 6); out of all 43 samples only six samples were found positive for coliforms and three samples for E.coli with values ranging between 0.18 and 16 MPN/g. No samples are found >16 MPN/g which represents bad quality food products (figure 16). RTE foods should be free from E. coli O157 and other verocytotoxin producing E. coli (VTEC) (Gilbert, *et al.,* 2000). The maximum recommended bacteria counts for good quality fresh and frozen bivalve molluscs are 16 MPN/g(FAO, 2012).

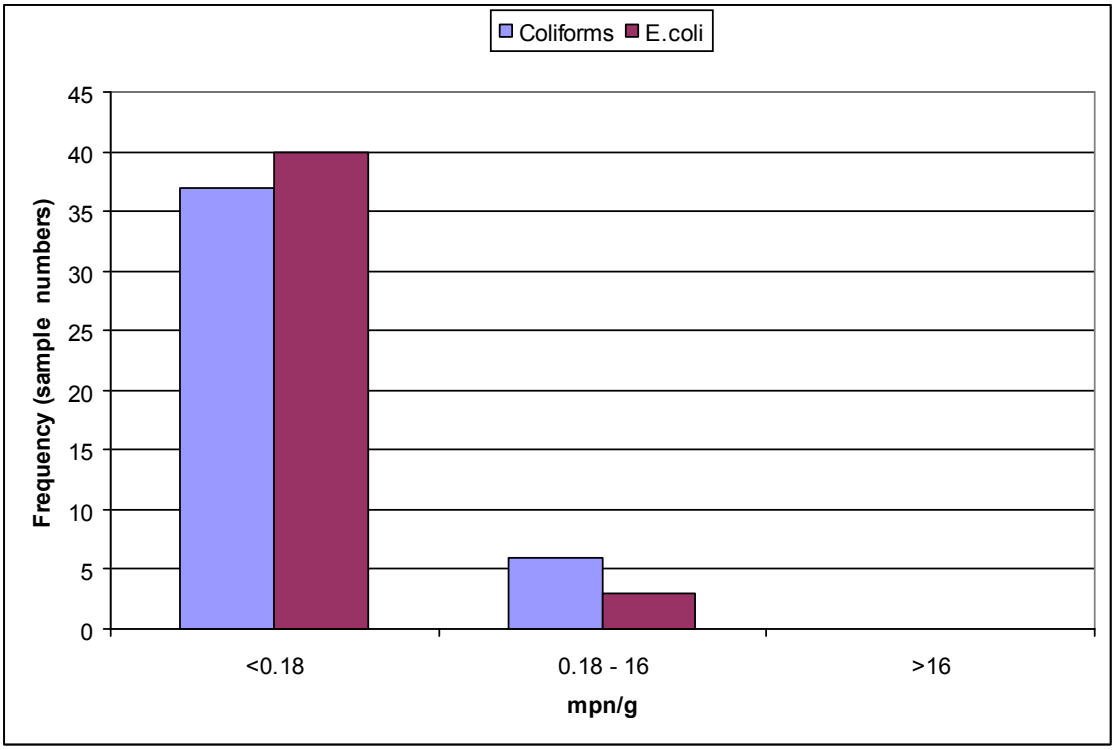


Figure 16: Results for coliforms from mussel samples

5.3. *Listeria* spp./*L. monocytogenes* results from blue mussel in Iceland

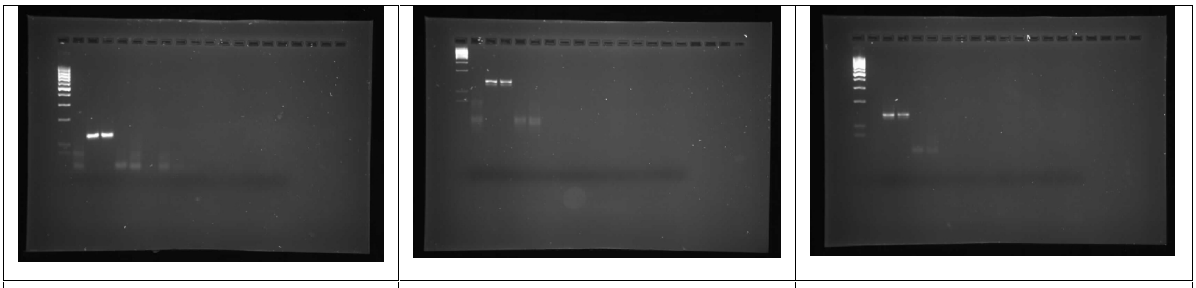
No *Listeria* spp were detected (appendix 2) from the blue mussel samples from the four locations of Iceland (figure 11).

5.4. Results from microbiological analysis of swab samples

No *Listeria* spp were detected (appendix 3) from the swab samples from the largest blue mussel producing company Norðurskel ehf, Sjávargata, Hrisey, Akureyri, Iceland.

5.5. Results from PCR analysis of swab samples

No *L. monocytogenes* was detected (figure 17) from the PCR analysis of 30 swab samples collected Norðurskel ehf. Only the two positive control (*L. monocytogenes*) showed positive band parallel (figure 18 (a, b and c) to 172 bp of the ladder. The negative control (*Salmonella* serovar Montevideo and *E.coli*) and blank samples and all the swab samples showed no band at all, provided that no *L. monocytogenes* present in the swab samples collected from the blue mussel producing company in Iceland.



(a) (b) (c)

Figure 17: PCR gel photos of 30 swab samples shows absence of L.monocytogenes (a: sample 1-10, b: sample 11-20, c: sample 21-30)

6. Discussion

Compared with other seafood, very few studies have been carried out on the prevalence of *Listeria* spp. in blue mussel. The review on prevalence reveals that the prevalence of *Listeria* spp. varies from 2% to 56% in shellfish (appendix 1 and table 7). *L. monocytogenes* is found more frequently than other pathogenic bacteria in seafood. (figure 2). In Iceland the *Listeria* spp. / *L. monocytogenes* have been found in shrimps (*Pandalus borealis*) but so far not in blue mussel.

The acceptable limit for *L. monocytogenes* as defined by the Food Law is less than 100 cfu/g at the end of shelf life of RTE food. Although several preventive measures are applied to minimize the risk *Listeria* infections Listeriosis is a wide spread problem in many countries, e.g. in USA 2.77 cases per million (2009), in Finland 1.33 cases/million (2010) and in Iceland 0.125 cases/million (2007) of Listeriosis have been reported. However in most countries there is a lack of quantitative data on Listeriosis.

Also quantitative data on number of *Listeria* in foods is lacking and when available, predominantly low numbers (<100 cfu/g) are reported, while a small portion of the *L. monocytogenes* positive samples contain >1000 cfu/g.

This study conducted microbiological analysis of 43 blue mussel samples, 46 swab samples from the largest mussel company in Iceland and 30 other swab samples for PCR analysis from the same blue mussel producing company. Listeria was found absent from all blue mussel and swab samples. The four representative sample locations (figure 11) may not truly be able to represent the blue mussel population in Iceland, but this prevalence study brings a breakthrough for further investigations for the presence of *L. monocytogenes* in Icelandic blue mussel. The swab sampling results (appendix 3, figure 17) from the Norðurskel ehf. is another prove of absence of *L. monocytogenes* in the processing areas of blue mussel companies, which also not representing the whole Icelandic blue mussel processing activities, even if still date Norðurskel ehf. is the largest blue mussel producer in Iceland.

The total microbial number and coliforms counts from the blue mussel samples reflect the background bacterial contamination of blue mussels. The count of total microbial number were found between 2.3 log cfu/g to 5.8 log cfu/g, coliforms between <0.18 mpn/g to 1.4 mpn/g and *E.coli* count between <0.18 mpn/g to 1.1 mpn/g. The total bacterial count and coliforms counts show that the mussels are safe for consumption, but the presence of other toxic or infectious agents is not excluded.

Although somewhat limited the sampling and microbiological and PCR analysis of blue mussel demonstrated that Icelandic live fresh mussel is free from *L. monocytogenes* contamination until retail shop.

7. Conclusion

The prevalence studies of *L. monocytogenes* in shellfish specially the blue mussel in Iceland were limited with the availability of shellfish, restriction on blue mussel harvesting due to shellfish poisoning on the culture ground in Iceland, only few farms were in operation, inadequacy supply to the supermarket for the consumer. The study found no *L. monocytogenes* in blue mussel; this drawback can't confirm the ultimate *Listeria* free blue mussel in and from Iceland. Because few studies (Valdimarsson *et al.*(1998), Hartemink and Georgsson (1991), Gudbjörnsdóttir *et al.* (2004) and Gudmundsdóttir *et al.* (2005)) on Icelandic fisheries water body reveals that *L. monocytogenes* present in seafoods in Iceland. Further study is recommended with an extended sampling area, sampling number and time duration.

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9. Appendix

Appendix 1: *Prevalence of L. Monocytogenes in Sea food worldwide*

Sl No.	Source	Country	Seafood types	No. Of Samples	Prevalence (%)	Concentration (MPN/g)	Comments
1	Beleneva and Maslennikova, 2002	Japan	Mussels (*Mytilus edulis*)	8 Strains	-		*L. monocytogenes*
2	Halit and Kapllan, 2010	Albania	Bivalve mollusk (*Mytilus galloprovincialis*)	78 98	5.1% 7.2%		*L. monocytogenes*
3	Pinto *et al.,* 2006	Portugal	Crustaceans, molluscan shellfish, fish and mussels	75	4%	<100	*L. monocytogenes*
4	Brett *et al.,* 1998	New Zealand	smoked mussels	-	-		*Listeria monocytogenes*
5	Laciar & de Centorbi, 2002	Argentina	Fish, squid and mussel	100	12%	>100	>100 MPN/g. *L. monocytogenes*
6.	Laciar *et al.,* 1998	Argentina	Fish, squid, mussels	50	2% 2%		*Listeria innocua* *L. monocytogenes*
7	Monfort *et al.* 1998	France	Live shellfish	120	55%		*Listeria* spp
8	Pereira *et al.*, 2001	Portugal	Shellfish	61	13.1%		*L. monocytogenes*
9	Maria, 2000	Brazil.	shrimp (*Penaeus brasiliensis*)	178	50% 18%		*Listeria* spp. *L. monocytogenes*
10	Ripabelli *et al.* undated	Italy	Crustaceans or molluscs	213	27%		*Listeria* spp.
11	Gudbjörnsdóttir *et al.* , 2004	Nordic	cooked shrimp, raw salmon, raw cod	1180	14.3% 13.1%		*Listeria* spp *L. monocytogenes*
12	Heinitz *et al.,* 1998	Canada Norway Philippines UK USA	Smoked finfish Smoked shellfish	1080	14%		*L. monocytogenes*
13	Maria, 2000	Brazil	Frozen shrimp	45	6.6% 8.8%		*L. innocua* *L. monocytogenes*

14	Dhanashree *et al.,* 1999	Tropics	fresh shellfish		12.0%		*Listeria* spp.
					4.0%		*L. monocytogenes*
15	Satoko Handa *et al.,* 2005	Japan	Raw fish, shellfish and fish roe	208	4.8%		*L. monocytogenes*
16	Jeyasekaran *et al.* 1995	India	fish and shellfish	65	17.2(finfish)		*L. monocytogenes*
					12.1(shellfish)		
17	Jeyasekaran *et al.,* 1996	Tropics	fresh shellfish		12.1%		*L. monocytogenes*
18	Cordano and Rocourt, 2001	Chile	crustaceous shellfish	-	11.6%		*L. monocytogenes*
19	Huss *et al.,* 2000	Denmark	Cold-smoked fish	110	0.9%	<100	*L. monocytogenes*
				70	6%		
				160	41%		
20	Gombas *et al.,* 2003	USA	Smoked seafoods	2644	4% (Smoked)	<0.3-	*L. monocytogenes*
			Seafood salads	2446	5% (salads)	$1.5X10^5$	
21	Inoue *et al.,* 2000	Japan	Smoked salmon	92	5.4%	<10	*L. monocytogenes*
			Raw seafood	213	3.3%	<0.3->100	
22	Uyttendaele *et al.,* 2009	Belgium	Smoked fish	90	27.8%	>100	
23	Vitas *et al.* 2004	Spain	smoked salmon	100	41%		*Listeria* spp
					28%		*L. monocytogenes*
24	Jørgensen *et al.,* 1998	Denmark	Cold-smoked salmon	-	34-43%		*L. monocytogenes*
			Cold-smoked halibut		45-60%		
25	Thimothe *et al.*, 2004	USA	raw fish,	234	16.7%		*Listeria* spp.
			finished products,	233	9.0%		*Listeria* spp.
			environmental samples	553	27.3%		*Listeria* spp.
			raw fish,		3.8%		*L. monocytogenes*
			finished products,		1.3%		*L. monocytogenes*
			environmental samples		12.8%		*L. monocytogenes*
26	Busani *et al.,* 2005	Italy	Fish & fish products	3160	6%		*L. monocytogenes*
27	Markkula *et al.,* 2005	Finland	Rainbow trout	149	4%		*L. monocytogenes*
		(2 sites)		117	4%		
28	Mena *et al.,* 2004	Portugal	Different fish species	25	12%		*L. monocytogenes*
29	Herrera *et al.,* 2006	Spain	Marine fish	50	10%		*L. monocytogenes*
30	Van Coillie *et al.,* 2004	Belgium	fish (halibut) and meat products	252	33.33%	<10;>100	*L. monocytogenes*

Appendix 2: *Samples of live fresh blue mussels from the mussel processing company around Iceland and microbiological test results for the presence of L. monocytogenes / Listeria spp.*

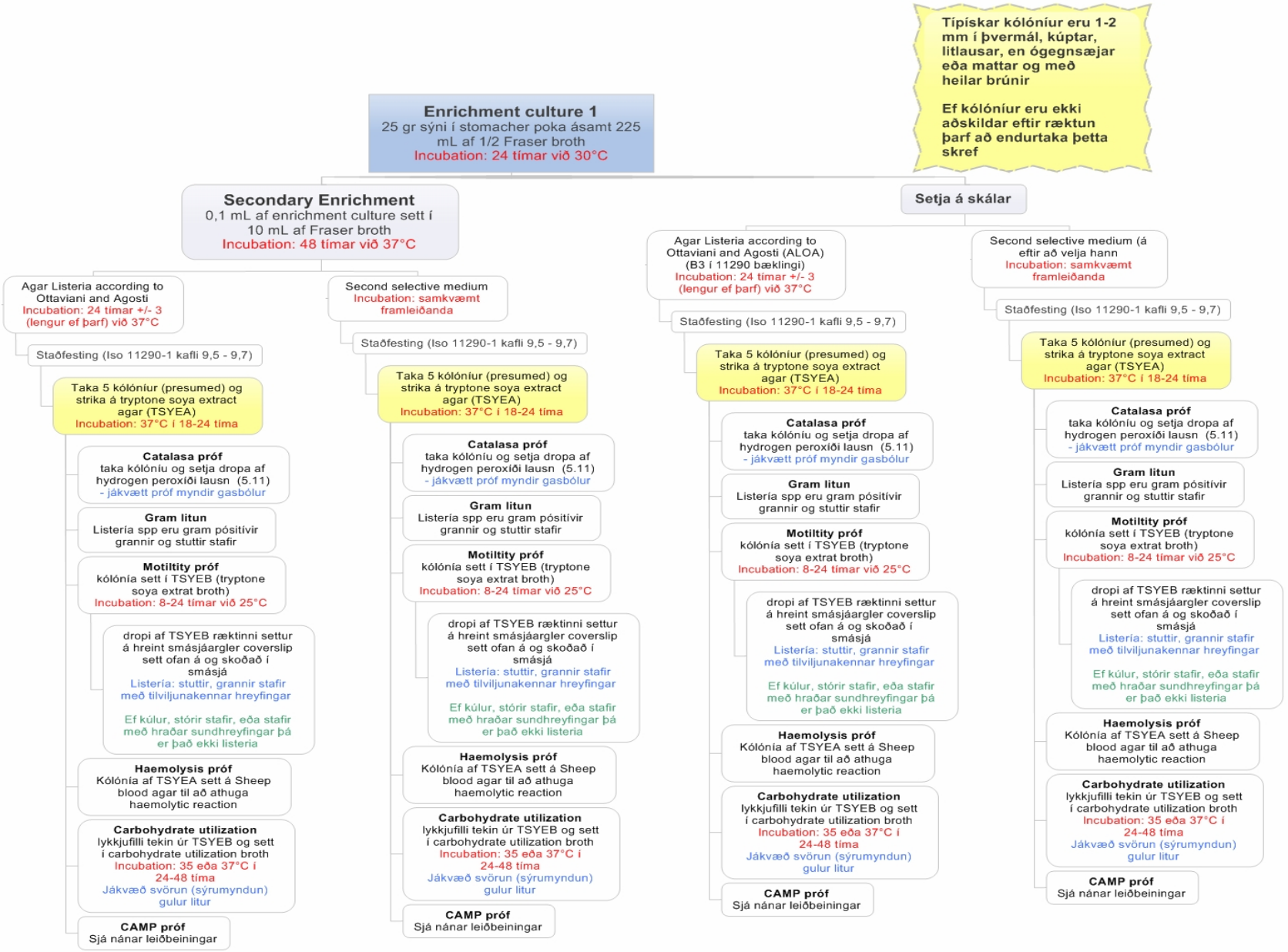
Sl. No.	Producer	Mussel Type	Lot No.	Expire Date	Received Date	Trial begins	Comments
1	**Norðurskel ehf,** Sjávargata, Hrisey	Retail pack	A03211	10.2.2011	2.2.2011	3.2.2011	*Listeria* not detected
2	- Do -	- Do -	A03211	10.2.2011	2.2.2011	3.2.2011	- Do -
3	**St2 ehf,** Drangsnes, Drangsnesvegur, Western Fjords.	Raw Mussel	-	-	17.3.2011	18.3.2011	- Do -
4	- Do -	- Do -	-	-	17.3.2011	18.3.2011	- Do -
5	- Do -	- Do -	-	-	24.3.2011	24.3.2011	- Do -
6	- Do -	- Do -	-	-	24.3.2011	24.3.2011	- Do -
7	- Do -	- Do -	-	-	24.3.2011	24.3.2011	- Do -
8	- Do -	- Do -	-	-	31.3.2011	31.3.2011	- Do -
9	- Do -	- Do -	-	-	31.3.2011	31.3.2011	- Do -
10	- Do -	- Do -	-	-	7.4.2011	7.4.2011	- Do -
11	- Do -	- Do -	-	-	7.4.2011	7.4.2011	- Do -
12	- Do -	- Do -	-	-	14.4.2011	14.4.2011	- Do -
13	- Do -	- Do -	-	-	14.4.2011	14.4.2011	- Do -
14	**Vogaskel ehf,** Vaglasel, Greater Reykjavík Area (Near Kopavogur Airport)	- Do -	-	-	5.5.2011	5. 5.2011	- Do -
15	- Do -	- Do -	-	-	5.5.2011	5. 5.2011	- Do -
16	**Norðurskel ehf,** Sjávargata, Hrisey	- Do -	-	-	11.5. 2011	5. 5.2011	- Do -
17	- Do -	- Do -	-	-	11.5.2011	12. 5.2011	- Do -
18	- Do -	Retail pack	015706	8.6.2011	3. 6.2011	3.6.2011	- Do -
19	- Do -	- Do -	016106	17.6.2011	15. 6.2011	15.6.2011	- Do -
20	**BioPol ehf** Skagaströnd, Norðurland vestra	Raw Mussel	-	-	23. 6.2011	27.6.2011	- Do -
21	- Do -	- Do -	-	-	7.9.2011	8.9.2011	- Do -
22	**Norðurskel ehf,** Sjávargata, Hrisey	- Do -	-	-	7.10.2011	10.10.2011	- Do -
23	- Do -	Retail pack	028010	14.10.2011	11.10.2011	12.10.2011	- Do -
24	- Do -	- Do -	029010	24.10.2011	20.10.2011	21.10.2011	- Do -

25	- Do -	- Do -	029210	26.10.2011	24.10.2011	25.10.2011	- Do -
26	- Do -	- Do -	029310	27.10.2011	24.10.2011	25.10.2011	- Do -
27	- Do -	- Do -	030010	3.11.2011	31.10.2011	31.10.2011	- Do -
28	- Do -	- Do -	030611	9.11.2011	3.11.2011	4.11.2011	- Do -
29	- Do -	- Do -	031911	22.11.2011	17.11.2011	17.11.2011	- Do -
30	- Do -	- Do -	031911	22.11.2011	17.11.2011	17.11.2011	- Do -
31	- Do -	- Do -	032911	2.12.2011	26.11.2011	26.11.111	- Do -
32	- Do -	- Do -	032911	2.12.2011	26.11.2011	26.11.2011	- Do -
33	- Do -	- Do -	33512	07.12.2011	2.12.2011	3.12.2011	- Do -
34	- Do -	- Do -	33512	07.12.2011	2.12.2011	3.12.2011	- Do -
35	- Do -	- Do -	1812	25.01.2012	20.01.2012	22.01.2012	- Do -
36	- Do -	- Do -	2501	01.02.2012	26.01.2012	27.01.2012	- Do -
37	- Do -	- Do -	3202	09.02.2012	03.02.2012	03.02.2012	- Do -
38	- Do -	- Do -	3902	15.02.2012	09.02.2012	10.02.2012	- Do -
39	- Do -	- Do -	4602	22.02.2012	16.02.2012	17.02.2012	- Do -
40	- Do -	- Do -	4602	22.02.2012	16.02.2012	17.02.2012	- Do -
41	- Do -	- Do -	5402	30.02.2012	24.02.2012	26.02.2012	- Do -
42	- Do -	- Do -	6002	07.03.2012	05.03.2012	07.03.2012	- Do -
43	- Do -	Raw Mussel	-	-	14.03.2012	14.03.2012	- Do -

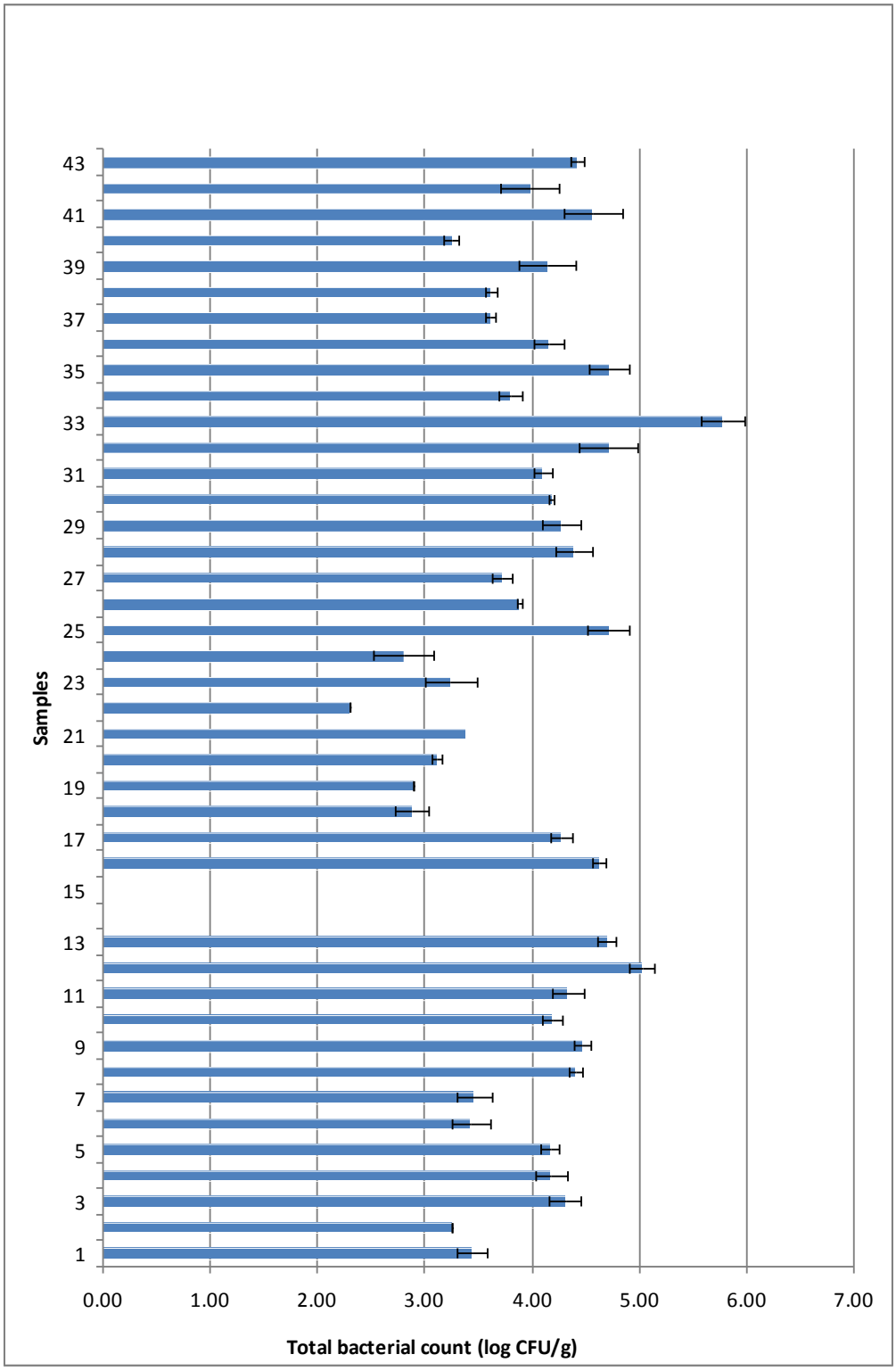
Appendix 3: *Samples of swabs from Norðurskel ehf. and microbiological test results for the presence of L. monocytogenes / Listeria spp.*

Location No.	Sample Location	Sample type	Comments
1.	Water drain in raw material reception	10x5 cm area	*Listeria* not detected
2.	Mussel declumper/Grader machine	- do -	- do -
3.	The tub with the fresh mussel in mussel reception room	- do -	- do -
4.	An empty tub in mussel reception room	- do -	- do -
5.	Grader in mussel reception room	- do -	- do -
6.	Conveyer belt in mussel reception room	- do -	- do -
7.	Swap from the floor under the "grader" in mussel reception room	- do -	- do -
8.	Swap from the drain in mussel processing room	- do -	- do -
9.	Swap form the end of the assembly line in mussel processing room	- do -	- do -
10.	Swap from the "washer" in mussel processing room	- do -	- do -
11.	Swab from under the conveyer belt mussel processing room	- do -	- do -
12.	Swap from tank at the end of the "washer" mussel processing room	- do -	- do -
13.	The drain in mussel packing room	- do -	- do -
14.	The floor at the end of the conveyer belt in mussel packing room	- do -	- do -
15.	Swap from the conveyer belt in mussel packing room	- do -	- do -
16.	Swap from the table with the scale in mussel packing room	- do -	- do -
17.	Swap from the conveyer belt in mussel packing room	- do -	- do -
18.	Swap from the size grader in mussel packing room	- do -	- do -
19.	Swap from the packing machine in mussel packing room	- do -	- do -
20.	Swap from the floor in the middle of mussel packing room	- do -	- do -
21.	Under the Conveyer belt in mussel packing room	- do -	- do -
22.	Swab from "washer"	- do -	- do -
23.	Swab from "washer"	- do -	- do -
24.	Swap from outside the factory (left side of the door)	- do -	- do -
25.	Swap from outside the factory (right side of the door)	- do -	- do -
26.	swap from outside the factory (right side of the door of mussel packing room)	- do -	- do -
27.	swap from outside the factory (left side of the door of mussel packing room)	- do -	- do -
28.	Swab from outside of the factory (from a dirty steel tub)	- do -	- do -
29.	From mussel collection tub in mussel processing room	RODAC	- do -
30.	From floor under the conveyer belt in mussel processing room	- do -	- do -
31.	From floor in mussel processing room	- do -	- do -
32.	From the end of the assembly line in processing room	- do -	- do -
33.	From the sink in changing room	- do -	- do -
34.	From the table next to the scale in packing room	- do -	- do -
35.	From the steel board on the side of the conveyer belt in mussel packing room	- do -	- do -
36.	From the floor next to the drain in the middle of the mussel packing room	- do -	- do -
37.	From the floor next to the drain (by the door) in mussel packing room	- do -	- do -
37.	From the floor next to the door in mussel packing room	- do -	- do -
39.	From "grader" in the mussel reception room	- do -	- do -
40.	From the "collection tray" in the mussel reception room	- do -	- do -
41.	From the steel slide on the "afklasari" in the mussel reception room	- do -	- do -
42.	From the mussel tub in the mussel reception room	- do -	- do -
43.	From the table in the mussel reception room	- do -	- do -
44.	From the "afklasari" in the mussel reception room	- do -	- do -
45.	From a empty tub in the mussel reception room	- do -	- do -
46.	From the plastic that covers the door in the mussel reception room	- do -	- do -

Appendix-4: *Flow Diagram of Microbiological analysis of Listeria monocytogenes (ISO. 11290-1:2004 (E) and ISO. 11290-2:2004 (E).)*



Appendix 5: Total bacteria count (Log CFU/g) in mussel



Appendix 6: *Results for coliforms for mussel samples*

Sample no.	LST-mpn	BGLB-mpn	EC-mpn	Sample no.	LST-mpn	BGLB-mpn	EC-mpn
1	4.9	0.68	1.1	23	0.2	<0.18	<0.18
2	0.78	0.78	0.78	24	0.2	<0,18	<0.18
3	2.2	0.68	<0.18	25	0.78	<0.18	<0.18
4	1.4	0.79	<0.18	26	2.3	0.2	0.2
5	7.8	<0.18	<0.18	27	0.78	<0.18	<0.18
6	17	0.18	<0.18	28	0.45	<0.18	<0.18
7	23	1.4	<0.18	29	<0.18	<0.18	<0.18
8	2.3	<0.18	<0.18	30	<0.18	<0.18	<0.18
9	2.2	<0.18	<0.18	31	<0.18	<0.18	<0.18
10	2.3	<0.18	<0.18	32	0.2	<0.18	<0.18
11	0.2	<0.18	<0.18	33	1.3	<0.18	<0.18
12	2.3	<0.18	<0.18	34	0.45	<0.18	<0.18
13	2.3	<0.18	<0.18	35	<0.18	<0.18	<0.18
14	7.8	<0.18	<0.18	36	0.78	<0,18	<0.18
15	23	<0.18	<0.18	37	1.3	<0.18	<0.18
16	2.3	<0.18	<0.18	38	<0.18	<0,18	<0.18
17	0.45	<0.18	<0.18	39	<0.18	<0.18	<0.18
18	3.3	<0.18	<0.18	40	1.3	<0.18	<0.18
19	2.3	<0.18	<0.18	41	<0.18	<0.18	<0.18
20	4.9	<0.18	<0.18	42	0.45	<0.18	<0.18
21	<0.18	<0.18	<0.18	43	0.78	<0.18	<0.18
22	<0.18	<0.18	<0.18				

[End of Paper I]

Paper II. Consumption pattern of Blue mussel (*Mytilus edulis*) in Iceland

II

Abstract

Aims

There are no consumer surveys done before to find out consumption patterns of blue mussels (*Mytilus edulis*) in Iceland. A consumption survey was conducted for the essential inputs for Quantitative Microbiological Risk Assessment (QMRA) of *L. monocytogenes* in the blue mussel in Iceland.

Methods

The survey was conducted under LisRisk 08196 E.U. SAFEFOODERA-ERA-NET project and conducted by RHA (The Research Centre of the University of Akureyri, Iceland). The survey was included consumption data of shellfish and blue mussel in Iceland- frequency of consumption, vulnerable groups in a population and age distribution of the consumers as well as characterization of home storage practices. The survey was conducted by using a pre-prepared questionnaires, distributed the questionnaires among the people around Iceland through preserved emails.

Results

The total number of respondents for the whole survey was 619. Shrimps and lobster are dominating among consumers, blue mussel taken over than shellfish when the consumption frequency is 1-6 times/year. An average consumption of blue mussel in Icelandic population was found 0.028 kg/capita/year.

Conclusion

A clear output of consumer attitude of shellfish especially blue mussel consumption was found.

Significance and Impact of study

The respondent group was highly urban and may not truly represent regional population distributions of Iceland.

Keywords: Shellfish, Blue mussel, consumer, Iceland.

1. Introduction

1.1. Background

In general seafood consumption is safe. For the general safety of seafood we use GMP, HACCP etc. But for the launching of a new product the assessment of risk is very important and prioritory task at present days.

Aquaculture and harvest of blue mussel in Iceland is new and need risk assessment approach (Hazard identification, Exposure assessment, Hazard characterisation and Risk characterization). The part of the risk assessment tasks is to find out the consumption pattern of blue mussel into Icelandic population, so the consumer survey of blue mussel was done around Iceland.

1.2. Seafood/shellfish consumption survey worldwide

Many of the surveys conducted on consumer attitudes and consumption of seafood, the major issues studied were seafood acceptability, variety, convenience, home preparation, taste, purchase site and product forms i.e. fresh or frozen. But very limited surveys were conducted on shellfish consumption separately. No survey works was found on blue mussel consumption.

A few consumption survey on seafoods were conducted in USA. An internet survey about the consumer perceptions about seafood was conducted in USA by Doris *et al.,* 2008. A Connecticut survey on consumer preferences for ecolabeled seafood was done by Cathy *et al.* (2004) in USA. A survey on Missouri fish and seafood consumption were done by Charles (1998). A preliminary survey was conducted by Liao and Smith (1972) for the marketing opportunity for freshwater shrimp in South Carolina, USA. A consumer survey of the northern United States was conducted to gather market information regarding the decision to purchase fish in 1995 by Nauman *et al.* A survey was conducted to study characterizes seafood consumption patterns of ten Asian and Pacific Islander (API) ethnic groups (Cambodian, Chinese, Filipino, Hmong, Japanese, Korean, Laotian, Mien, Samoan, Vietnamese) within King County, Washington, USA. The primary purpose of this study was to describe Asian and Pacific Islander seafood consumption rates, species, and seafood parts commonly consumed and cooking

methods. The average overall consumption rate for all seafood combined was 1.891 (g/kg/day) body weights; the predominant seafood consumed was shellfish (46% of all seafood). A survey of New York seafood consumers found a preference for Integrated Multitrophic Aquaculture over Monoculture (IMTA), about 34% of the respondents initially expressed a positive attitude toward farmed seafood, 48% were indifferent, and the remainder had a negative attitude towards IMTA (Shuve *et al.*, 2009). A seafood consumption survey was conducted in the Gulf of Mexico after the Exxon Valdez oil spill to justify the FDA (Food and Drug Administration) assumption on seafood consumption among the local residents. An elevated rate of seafood consumption was observed among the Gulf Coast residents compare to FDA assumption (NDRC, 2010).

A survey was performed to discover local seafood consumption habit and preferences in Halifax, Nova Scotia, Canada. The survey participants revealed a strong empathy for locally caught, direct marketed seafood products (EAC, 2010). A representative survey of Canadians' public opinion was conducted to measure attitude and behaviours of the study population about the aquaculture industry and support for a national aquaculture act. The survey assessed Canadian fish and seafood consumption and preference (Coletto *et al.,* 2011).

There are many others research attention of consumer survey had been paid for the seafood products, Greece (Batzios *et al.,* 2003), Hong Kong and Southern Mainland China (Perishable Group, 2010), Australia (ANZMAC, 2010), Norway (Myrland *et al.*, 2000).

Altintzoglou *et al.* (undated) evaluated various new seafood product concepts among young adults in Norway and Iceland. A study on seafood consumption and attitudes among 18-80 years old Icelander was carried out by MATIS (Icelandic Food and Biotech R & D institute) (Sveinsdóttir *et al.,* 2011). But no specific survey on shellfish especially blue mussel consumption was conducted yet in Iceland.

1.3. Blue mussel in Iceland

The blue mussel (*Mytilus edulis*) is a native species and is widely distributed in the west, north and east coasts of Iceland. It is a seashore and shallow water species. It is purely a filter feeder, attached to the bottom by filaments. The blue mussel farming in Iceland is new and it's developing continuously. The blue mussel farming is relatively easy because it requires no artificial feed and hatcheries to produce seed. The annual production of blue mussel has been a few tones per year, mostly sold on domestic market. About nine blue mussel farms (figure 1) are in operation in 2009, mostly experimentally. The blue mussel has been cultured and harvested in limited amounts for local consumption and for bait. The processing of blue mussel has not yet reached industrial scale. Norðurskel is the only biggest farm, grows mussel on an industrial basis (Icelandic Fisheries, 2012).

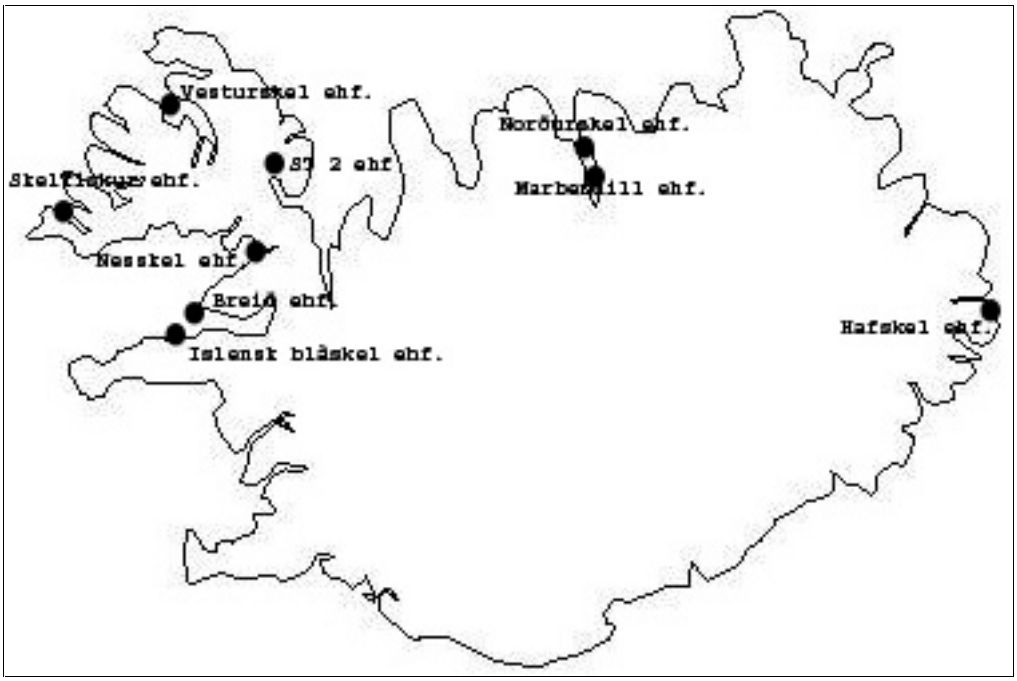


Figure 1: The location of mussel farm sites

The Icelandic fjords are very clean and good condition for mussel farming. There are few health issues associated with the farming water - the level of Cadmium and toxicity formed by DSP (Diarrhetic Shellfish Poisoning) and PSB (Paralytic Shellfish Poisoning). Both the Cadmium and toxicity are formed naturally but not polluted from human activities. The most of the Icelandic people prefer live fresh blue mussel, cooked by light boiling just before consumption, brings risk to be contaminated by certain

pathogens like *Listeria* spp. The quality of the Icelandic blue mussel is good and constant through seasons compared to other regions. The price of fresh live mussel is rising every year due to limited supply than demand. There is an opportunity to export Icelandic blue mussel all over Europe (MFA, Iceland, 2008).

1.4. Global Blue mussel production

Global farmed production of blue mussels increased rapidly in the last half of the 20th century. The total blue mussel production in Europe is around 700.000 tonnes per year and the demand is rising. A closer look at the major producers of blue mussels in the world reveals that Netherlands is by far the largest producer, even though production has decline in recent years. The largest producer in 2007 was France, where the production has been growing steadily since the 1995s. Other large European producers are Ireland, UK and Canada. The most mussel farming countries are - France, Netherlands, Ireland, Canada, United Kingdom and Germany. In 2007, France produced 29% of world blue mussel; Netherlands produced 21%, Ireland produced 18%, both Canada and UK produces 12% and Germany produced 5%. The rest of the countries- Norway, Sweden, Denmark, Argentina, Channel Islands, Iceland and Falkland Is.(Malvinas) produced only 3% of the world production (figure 2). Iceland is being producing a very limited quantity of blue mussel. From 2003-2007, Iceland producing four tones of blue mussel every year which value is 8,000 USD, but in 2008 the production increased to 10 tones (figure 3).

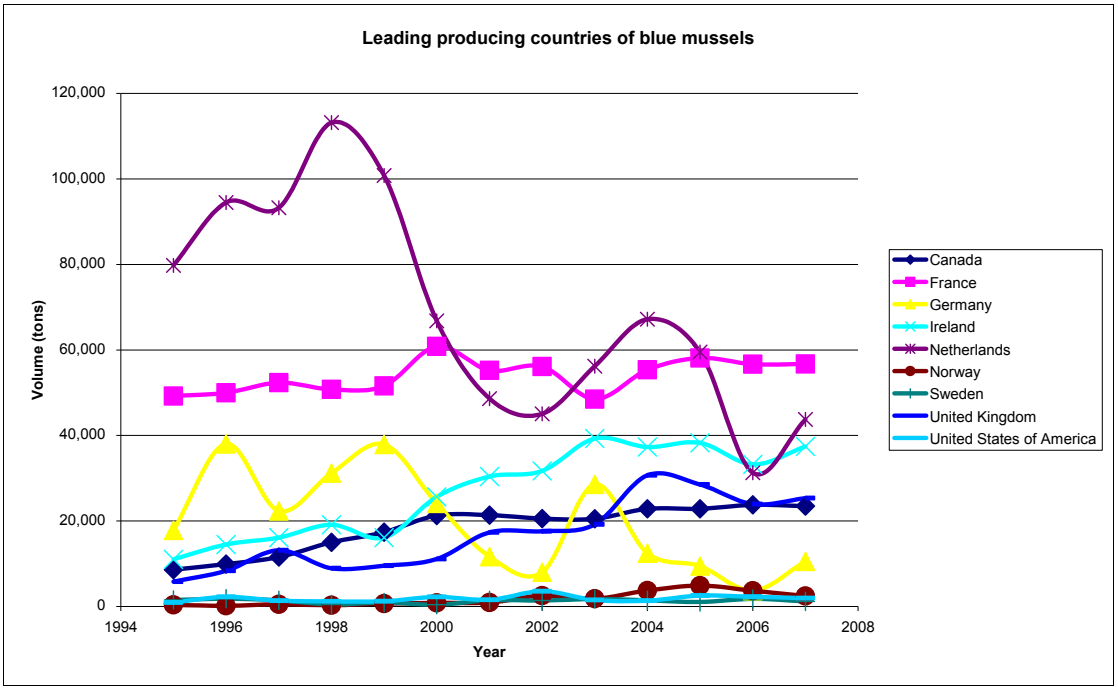


Figure 2: Biggest producers of blue mussels 1995 - 2007 (FAO/Fishstat Plus).

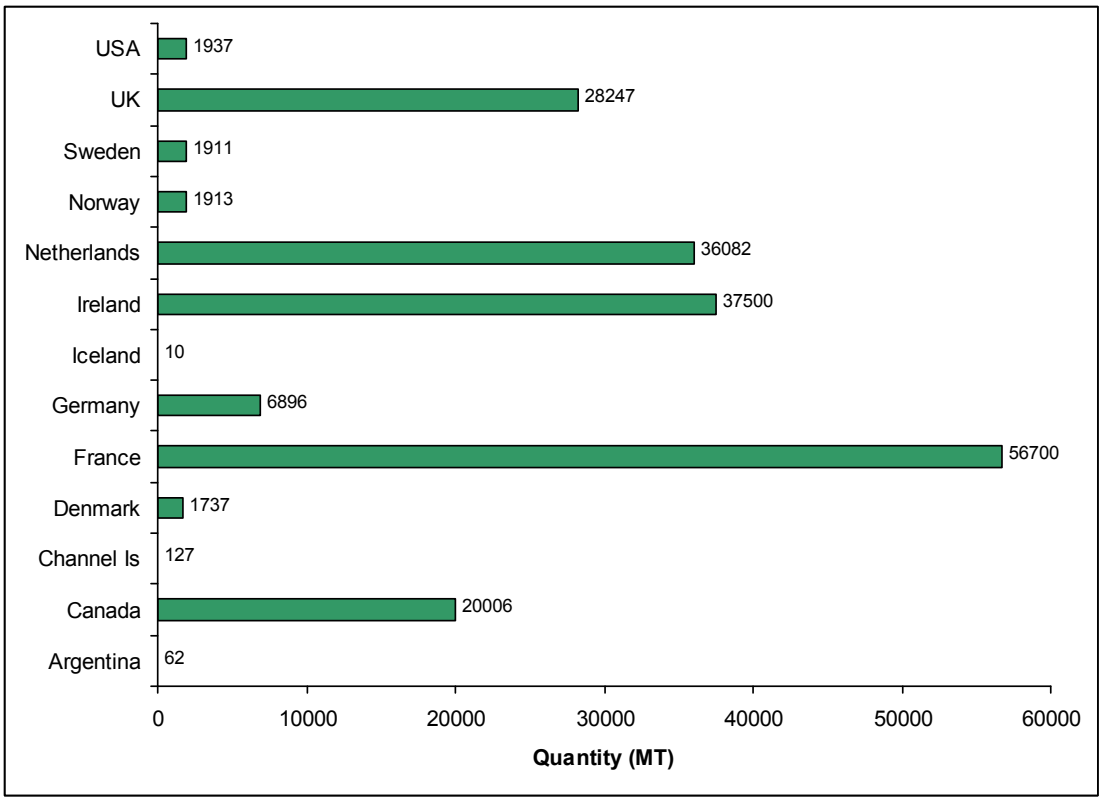


Figure 3: Aquaculture production of blue mussel by major producer countries in the year 2008 (FAO, 2010)

Consumer disbursement information regarding a particular product can contribute to public decisions which will insure a more consistent flow of raw products to the processing sector. The consumer's attitude is changeable, mainly due to socio-economic transformation such as the improvement of the standards of living, the greater expansion of the media, the promotion of biological products, the development of the tourism industry etc. The consumers play a significant role, which linked between supply and demand and the consumer's requirements and choice are necessary to execute new strategies for the product development and value addition and marketing channel development to congregate the consumers' demand on shellfish specially the blue mussel in Iceland.

1.5. Global Blue mussel consumption pattern

The consumption pattern of few European countries (figure 4) shows that the local production might not be proportional to local consumption, but now a day products are globalized beyond the country border, the local consumption for a particular food indicates it demand to create a market, insist to promote export-import to reach its

consumers' fork. Iceland are lacking behind having such a local consumer data for blue mussel consumption. This paper demonstrated a consumer survey of blue mussel consumption into Iceland population to explore the consumers' preferences and attitudes regarding basic marketing aspects of blue mussel consumption. These aspects are reflected in a number of questions posed to Icelandic consumers in order to highlight their attitudes and preferences towards shellfish especially blue mussel consumption. This could establish to be very advantageous for a more realistic organization of the distribution roots, which in turn would promote blue mussel consumption in Iceland.

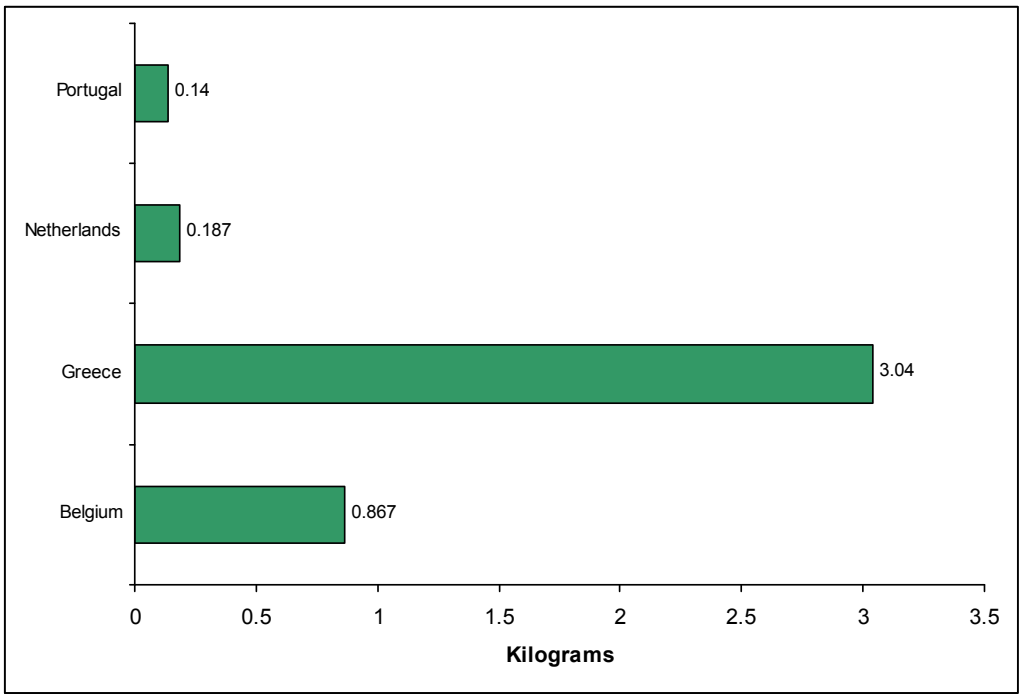


Figure 4: Consumption pattern (kg/capita/year) of mussels among few EU countries (modified from Willemsen, 2003)

2. Objectives

Information about the preferences and behavior of shellfish consumption in Iceland is limited. Because the shellfish industry in Iceland offers the greatest possibility for aquaculture expansion, information is needed to determine specific marketing opportunities and targets. This study was a first step towards gathering necessary information for a risk assessment**.** The objective of the consumer survey on shellfish in Iceland was to quantify consumer practices relating to the purchase, transport, storage, and preparation of fresh produce, with emphasis on practices that affect safety.

It has the following sub objectives-

- To get the demographic information about the shellfish specially the blue mussel consumer in Iceland.

- Figure out the consumption pattern of blue mussel in contrast to other shellfish available in Iceland market.

- Figure out the consumption rate of blue mussel in Iceland.

3. Materials and Methods

To accomplish the objectives of this study, a consumer survey feedback form (Appendix 5) was developed focusing shellfish especially blue mussel consumption in Iceland. Participation in the survey was voluntary. The consumer survey feedback form was undertaken through a set of questionnaires designed and developed to investigate the Icelandic consumer's attitude and preferences towards the shellfish and blue mussel market. 1,000 consumers, randomly selected, filled in a set of questionnaires by online email posting. A sample of 1000 people aged 17 to 65+ years in Iceland was drawn from the RHA register during 19 November, 2010. All members of the sample population held Icelandic citizenship. All the 1000 people were emailed an introductory letter together with a two-page questionnaire (survey feedback form). There were 24 questions into the questionnaire were prepared for the respondents to reply. The questionnaire was developed in Icelandic language. The questionnaire was included both open-ended and close-ended questions. The questions were divided into three sections: questions about respondent's status (demographic profile), questions for the adult respondents (age group 17 to 65+ years) and questions for children (age < 16 years). The demographic section contained questions regarding age, gender, household income, education and household size. Each respondent was asked to return the completed questionnaire. Those unwilling to participate were asked to return the uncompleted questionnaire. After one week a reminder was emailed to those who had not returned the original invitation. Descriptive statistical analysis (frequencies, percentages) and cross tabulations were carried out using Microsoft Excel. We aggregated survey responses according to various demographic groupings and summarized the results using percentages.

4. Results

4.1. Status of the survey respondents

This chapter reviews the demographic profile of the survey respondents obtained from the consumer survey. Comparisons of the consumer survey to Iceland Census survey data of 2010 (Statistics Iceland, 2011) are provided to determine if the sample is reasonably representative of the Iceland population. Since the survey respondents include only seafood consumers, and are thus representative of that particular market segment, variations from census population data are expected. The survey included screening questions designed to elicit responses the primary fresh seafood (shellfish) shopper.

4.1.1. Age distribution

Among the 1000 consumers there were 619 respondents replied the survey questionnaire representing the Icelandic population for age, gender, income, ethnicity and geographic location, the respondents for this age distribution question only 611 people replies. There were five different age groups of the respondents; from 17-21, 22-35, 36-45, 46-55 and more than 65+ years. Figure 5 compares respondents' age distribution to the census age distribution. The comparison indicates that survey responses over-represent census between ages 36 and 65 years. The survey responses also underrepresented census over age 65+ years, as well as those between 17 and 35 years. The highest number of respondents was from the age groups 46-55 (30%) and 36-45 (27%) years. The younger and the older people were found not likely that much steady to fill the survey form.

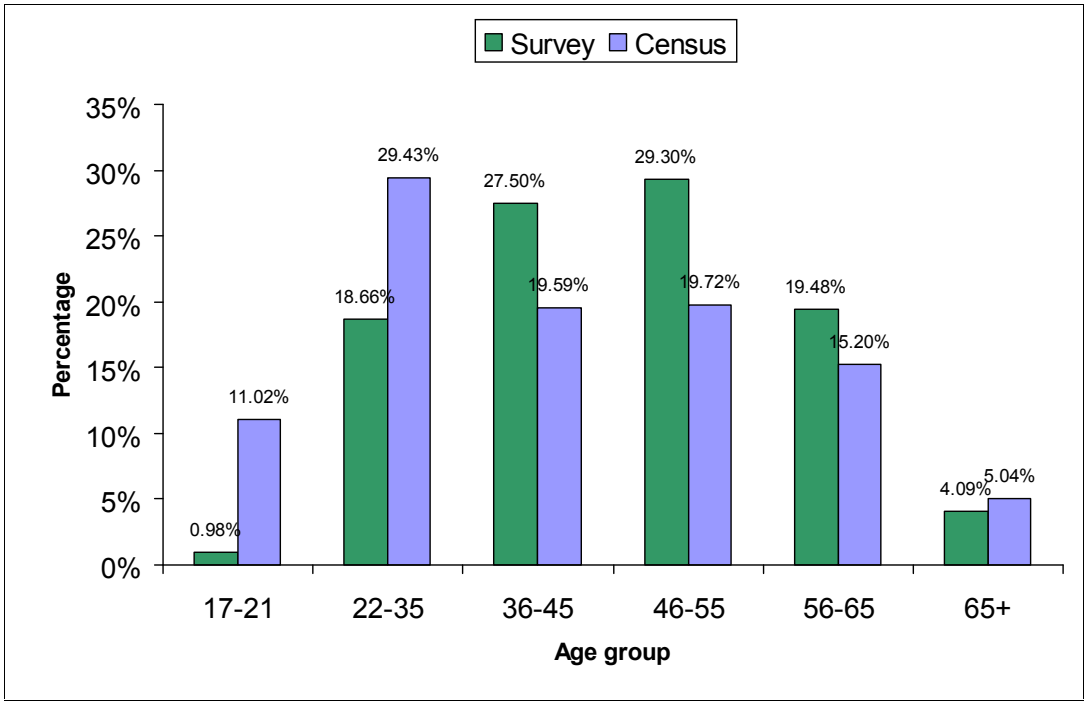


Figure 5: Age distribution (N = 611)

From the surveyed population of 563 families it has been observed that 42.98% has no children under 16 year's age, 23.09% has one child, about 22.56% has two children and only 10.12% has three children less than 16 years age (figure 6).

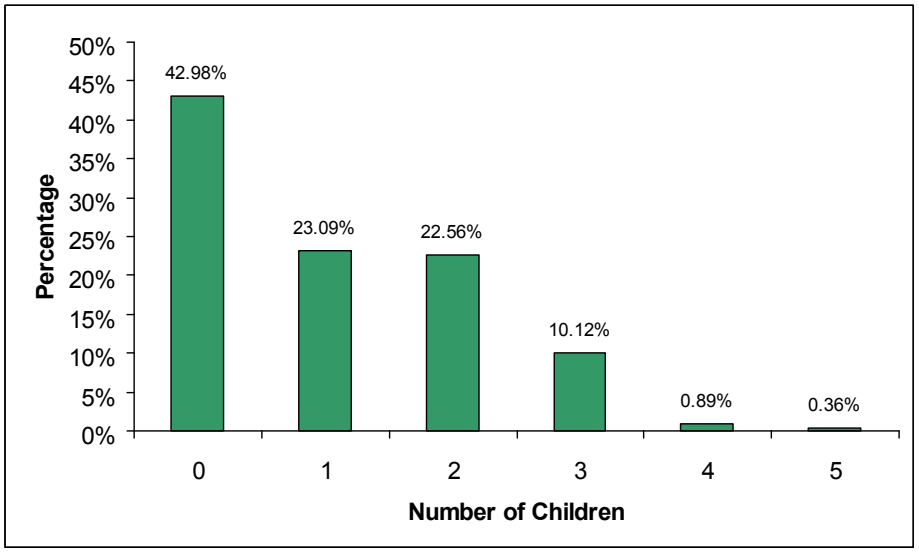


Figure 6: Number of children under age 16 years (N = 563)

4.1.2. Gender

Figure 7 shows the gender of the individual those who attained the survey, a comparative with census survey data in Iceland. The comparison indicates that survey responses over-represent to census for male but underrepresented for female consumers. Here, of the 609 respondents who chose to indicate their gender, 65% were male and rest 35% were female respondents of which only four female respondents (2%) were pregnant.

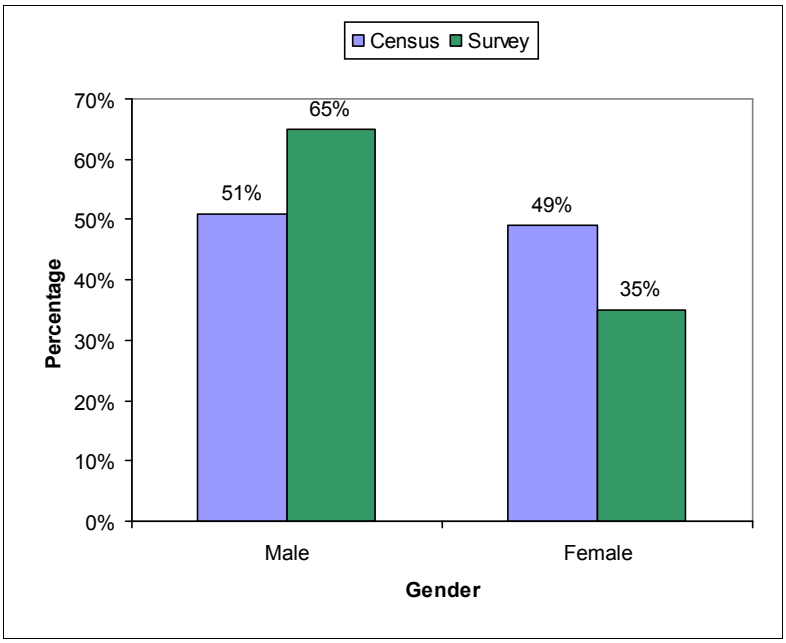


Figure 7: Respondent gender (N = 609)

4.1.3. Family status

About 94% of the respondents are urban dwellers, only 6% living in rural areas (figure 8). In Iceland the number of family members mostly varies from two to five. The survey data shows that 27.72% (168) people having no kids, 23.76% have 2 kids (144), 19.64% have one kid (119) and only 16.83% (102) people have three kids (figure 9).

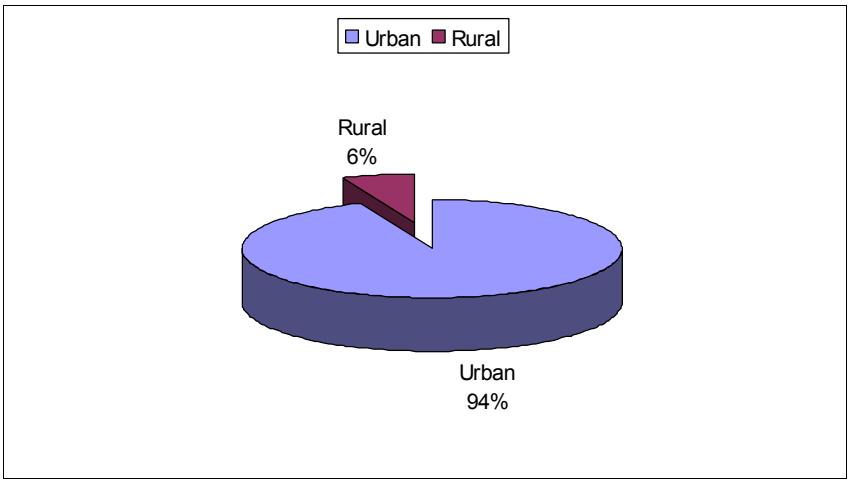


Figure 8: Ratio between Urban and Rural dwellers (N = 609)

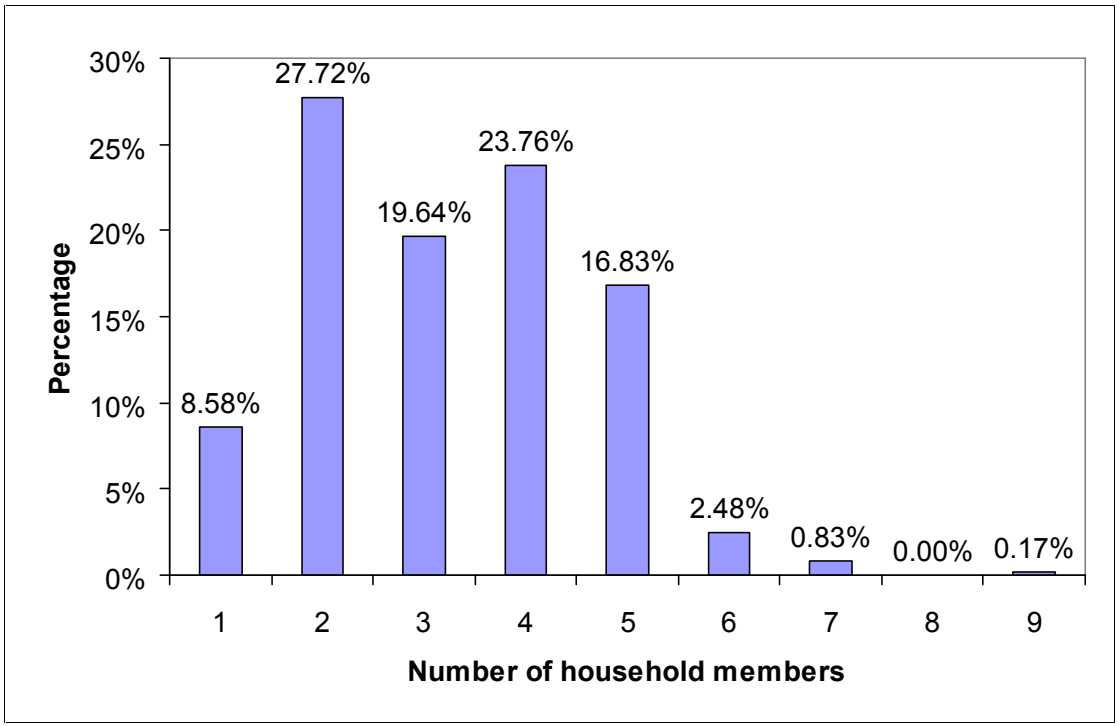


Figure 9: Number of family members in Iceland (N = 606)

From the survey it has been noticed that 80.9% respondents are the head of the household. Most of the respondents (93.80%) working full time and a few (4.90%) working part times (figure 10).

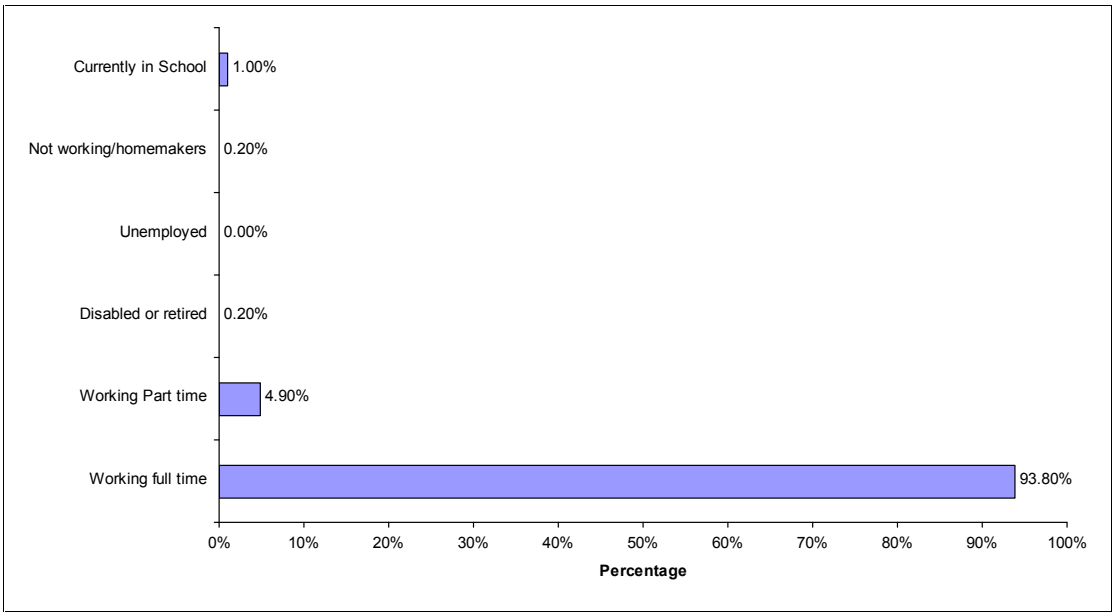


Figure 10: Employment situation (N = 576)

About 72.60% of the respondents have a University or equivalent degree but only 21.00% respondents have a Diploma or Industrial training (figure 11). The family net income varies from one-10 million Icelandic Krona (MISK) per year. About 30.40% people have an average income of six-10 MISK (figure 12).

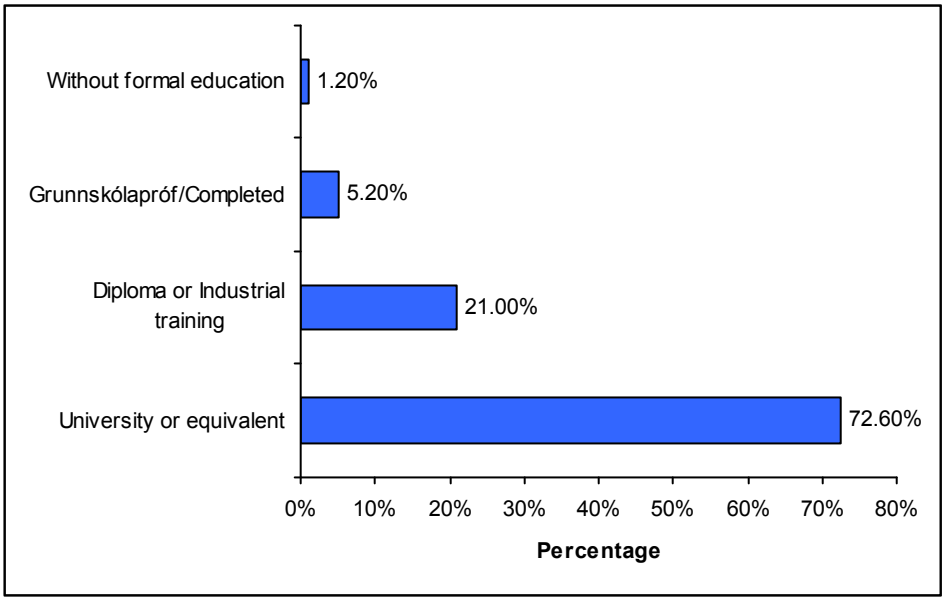


Figure 11: Education status (N = 572)

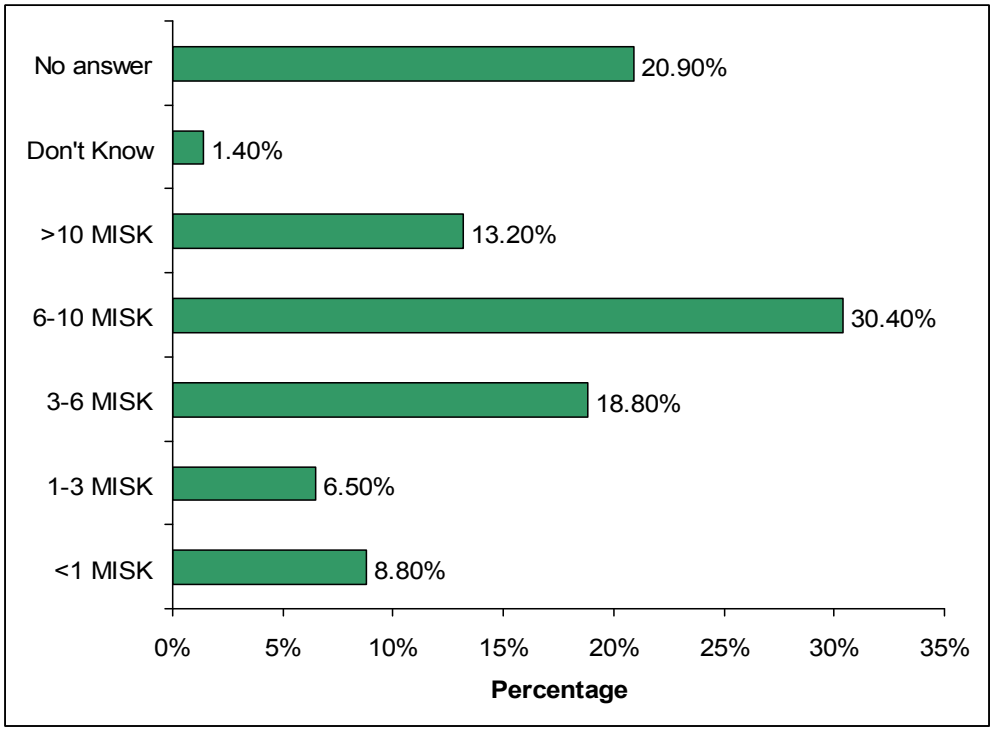


Figure 12: Household income (N = 569)

4.2.Status of the shellfish consumption by respondents

This section reviews the respondents' shellfish consumption patterns including taste preferences, frequency of consumption, purchasing habits and expenditures.

4.2.1. Shellfish types

Shellfishes are common food items for Icelander. The most common shellfish has been eaten in Iceland are shrimps (96.8%) and lobster (90.6%). Scallop is the third choice (55.4%). Mussels are used to eat by 49.2% population in Iceland (Figure 13). There are cross choices in between species, some people like more than one types of shellfish.

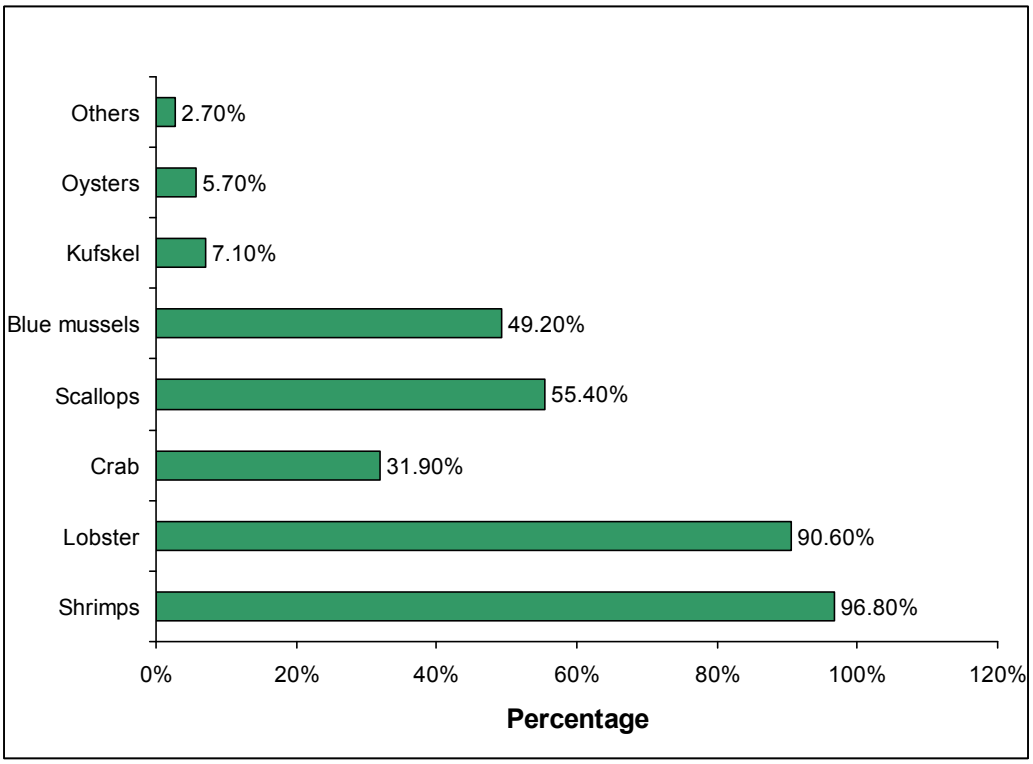


Figure 13: Kinds of shellfish eaten by respondents

4.2.2. Frequency of shellfish and blue mussel consumption

The survey data reveals that only 24.5% people used to eat shellfish one time every month, on the other hand only 2.0% eat blue mussels once in a month. But when the frequency goes down (1-6 times/year), consumption ration increases to 26.4% and 8.20% for blue mussels and shellfishes respectively. The highest time limit consideration for blue mussel consumption is monthly basis, once in a month. Blue mussel is not for everyday or weekly meal (figure 14). About 70.50% Icelandic people used to eat less than 12 mussels in a meal (figure15); they (67.20%) prefer to have it in restaurants than to home (57.40%) (figure 16).

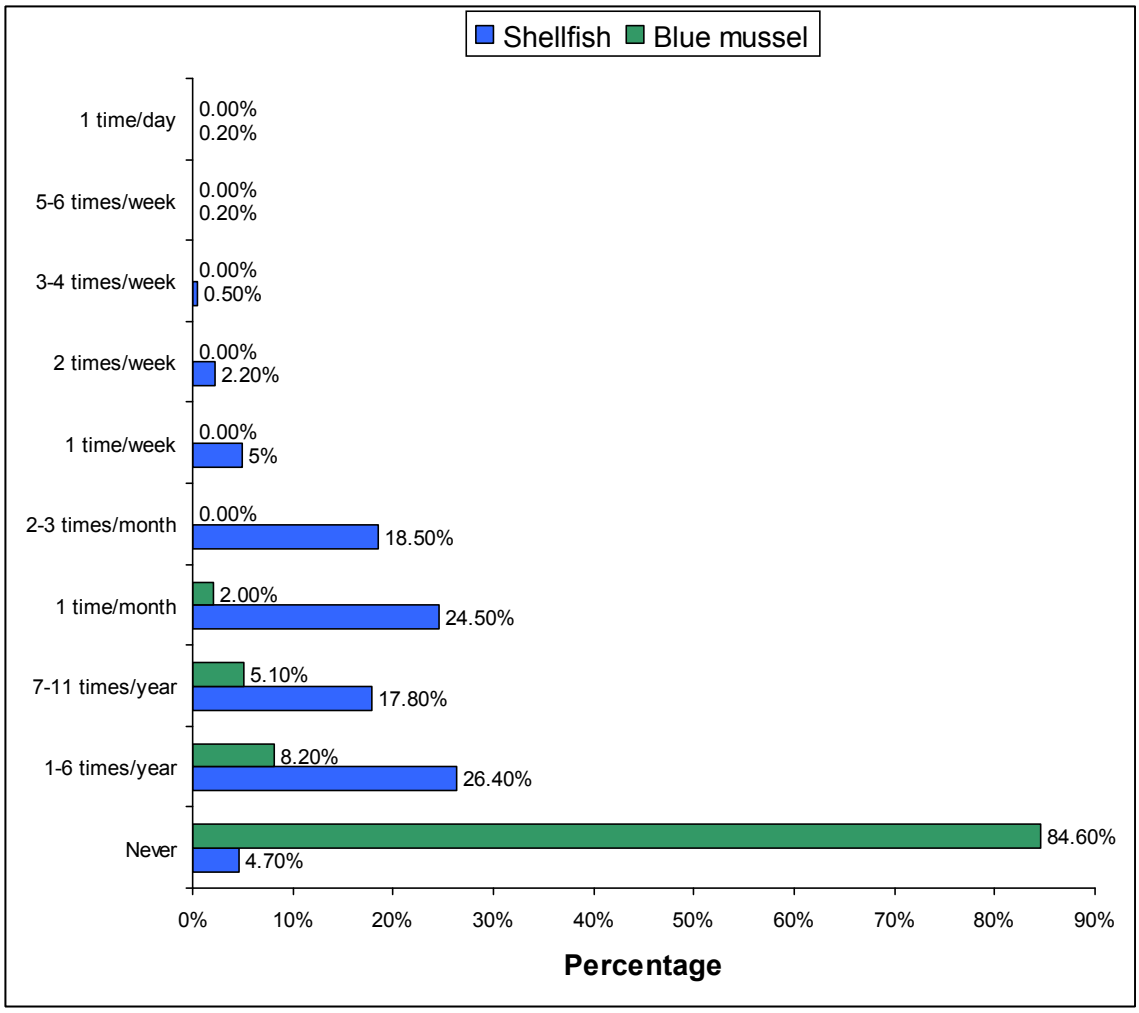


Figure 14: Frequency of eat shellfish (N=595) and blue mussel (N=293).

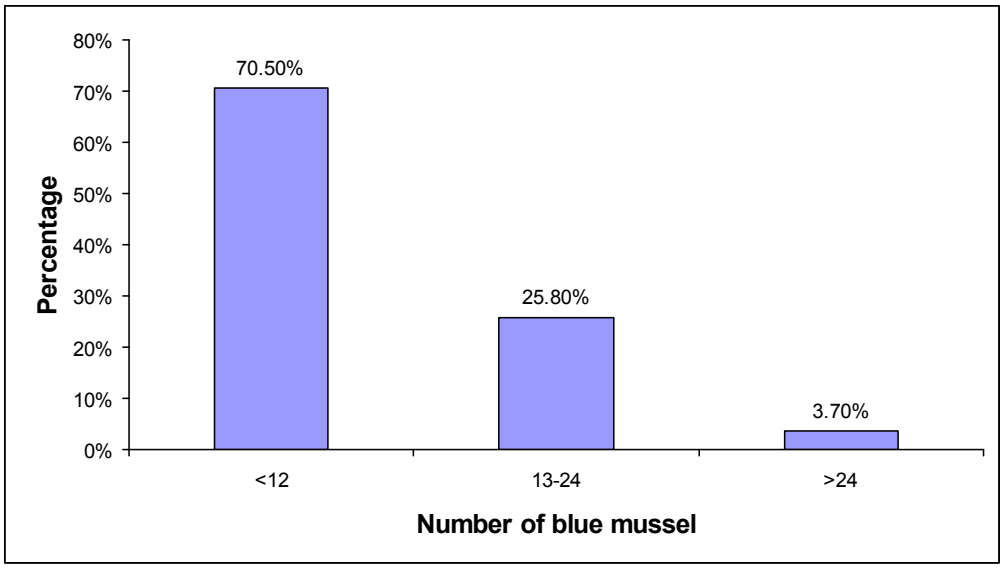


Figure 15: No. of mussels eaten in one meal (N=295).

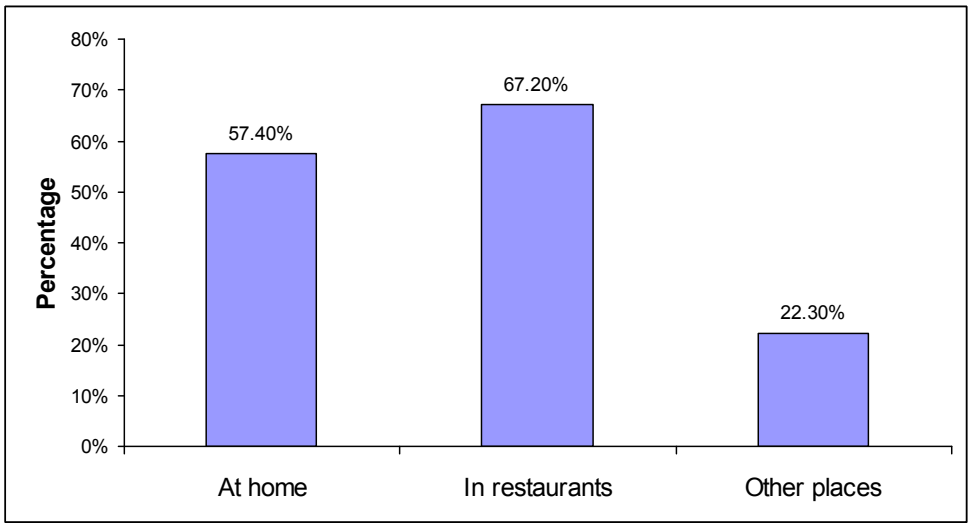


Figure 16: Place of Blue mussel consumption

4.2.3. Types of mussel (preservation and cooking type) eaten by respondents

Mussels are available in market as fresh, frozen and canned. But the local producing blue mussels are mostly selling as live-fresh in retail. Most of the respondents (69.5%) prefer fresh mussels for their meals. But some people used to eat frozen (33.30%) and canned (30.00%) mussels as well (Figure 17).

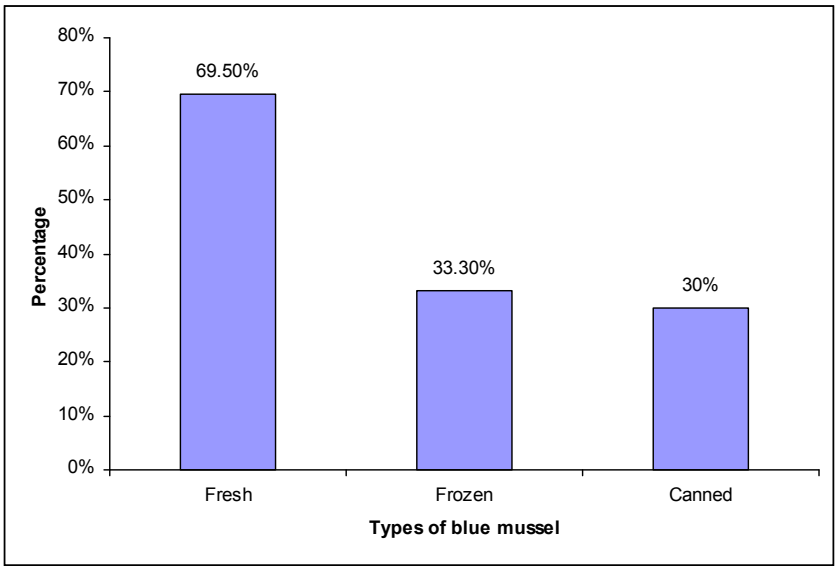


Figure 17: Types of mussels eaten (N = 279).

Most of the Icelander prefers to make boiled before eat blue mussels, either it is fresh or frozen (89% and 82.9% respectively), but they prefer canned mussels to eat readily from the can (69.1%). Sometimes they like to have it chilled after boiling (Figure 18).

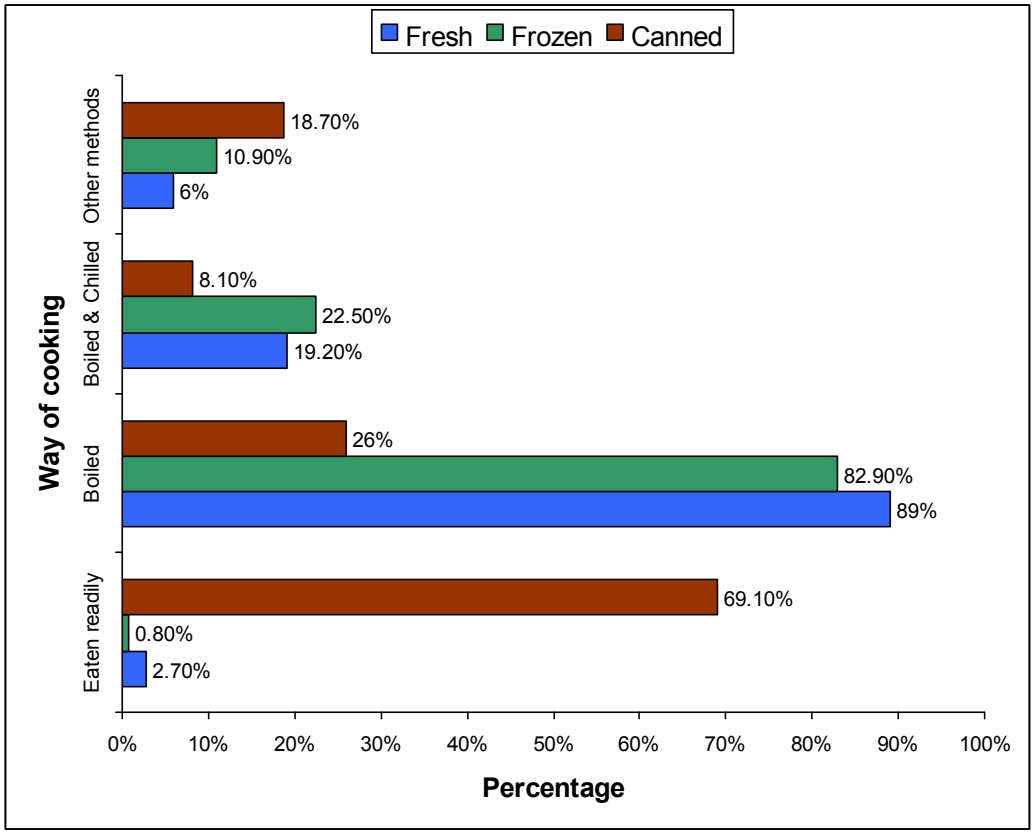


Figure 18: Ways of blue mussel cooking

4.2.4. Delay between purchase and consumption

The majority of the consumer prefers to eat mussels within three days after purchase. About 28% consumer prefer to eat the same day of buying and 28% prefer to eat just after one day of purchasing, about 11.54% consumer like to consume it within two days of purchasing (figure 19).

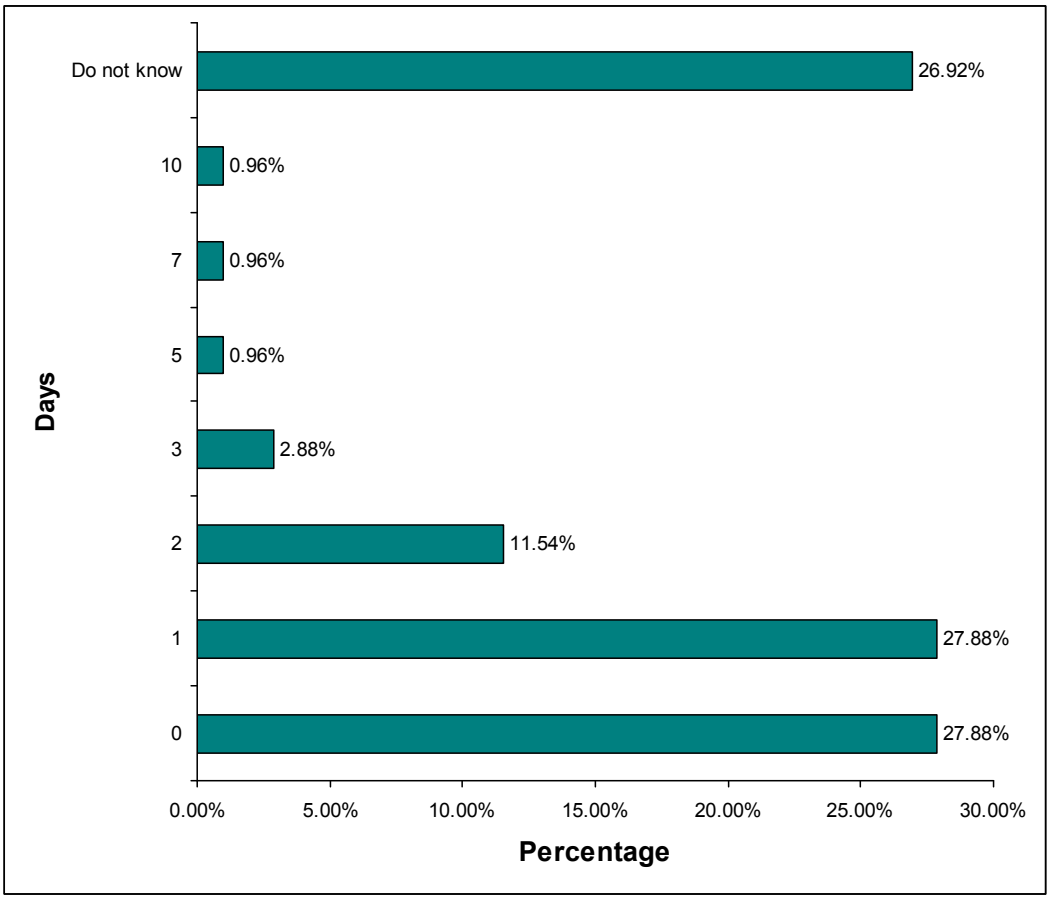


Figure 19: Days between purchase and consumption (N = 104).

4.3. Consumption pattern of blue mussel for children under age 16 years

About 53.4% Icelander has children under the age of 16 years (figure 20) and the age of the eldest one is mostly (58.9%) between 11-16 years (figure 21).

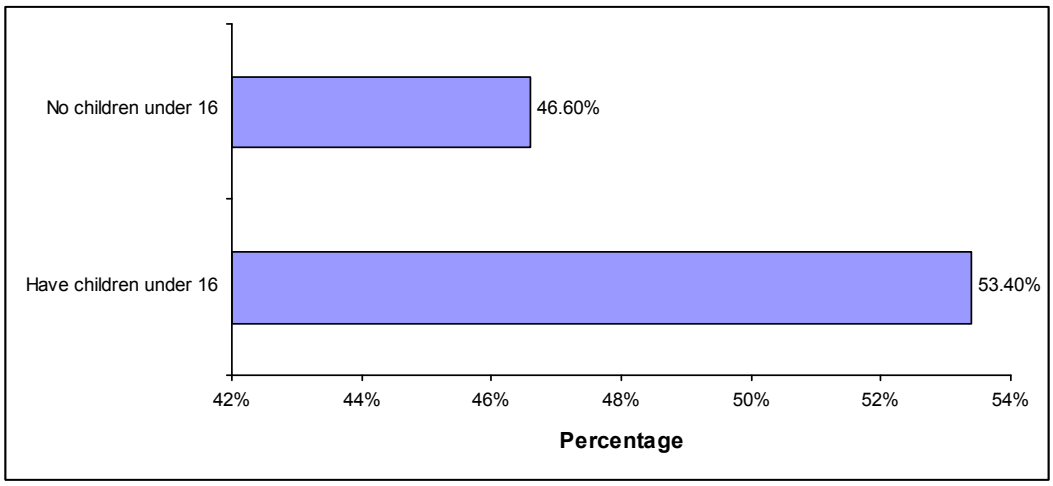


Figure 20: Family members having children under age 16 years (N=580)

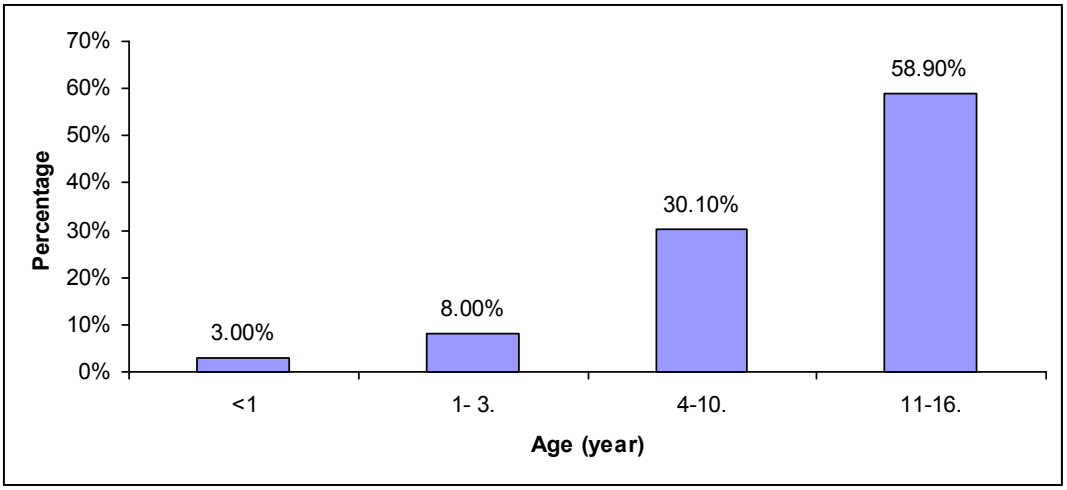


Figure 21: Age limit of the eldest child (N=299)

Shrimp (88.5%) and lobster (84%) was found the common shellfish eaten by the children under age 16 years, scallop (34%) is the third choice, mussel/ blue mussel is the fourth choice for the children, only 20% consumer like to have mussels/ blue mussels for their meal (figure 22).

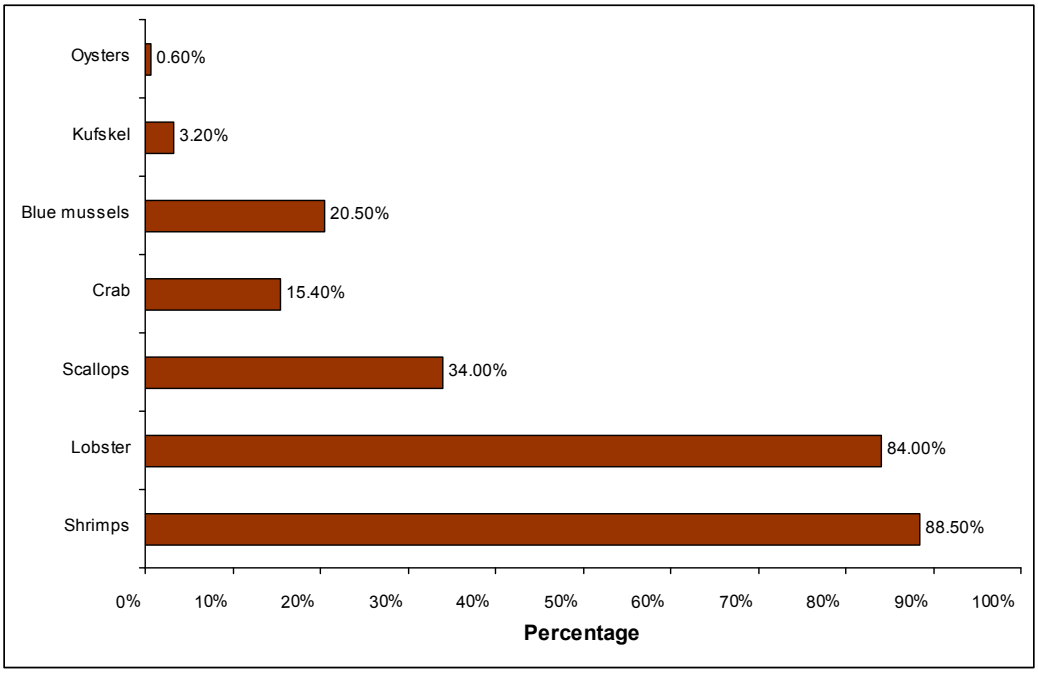


Figure 22: Types of shellfish eaten by children under 16 years age

The children under age of 16 years don't like a bulk amount of shellfish to eat for their meal. Only 32.8% young consumer prefers to consume it 1-6 times per year, whereas 48.2% children never like to eat blue mussel for their meal (figure 23, 24 and 25).

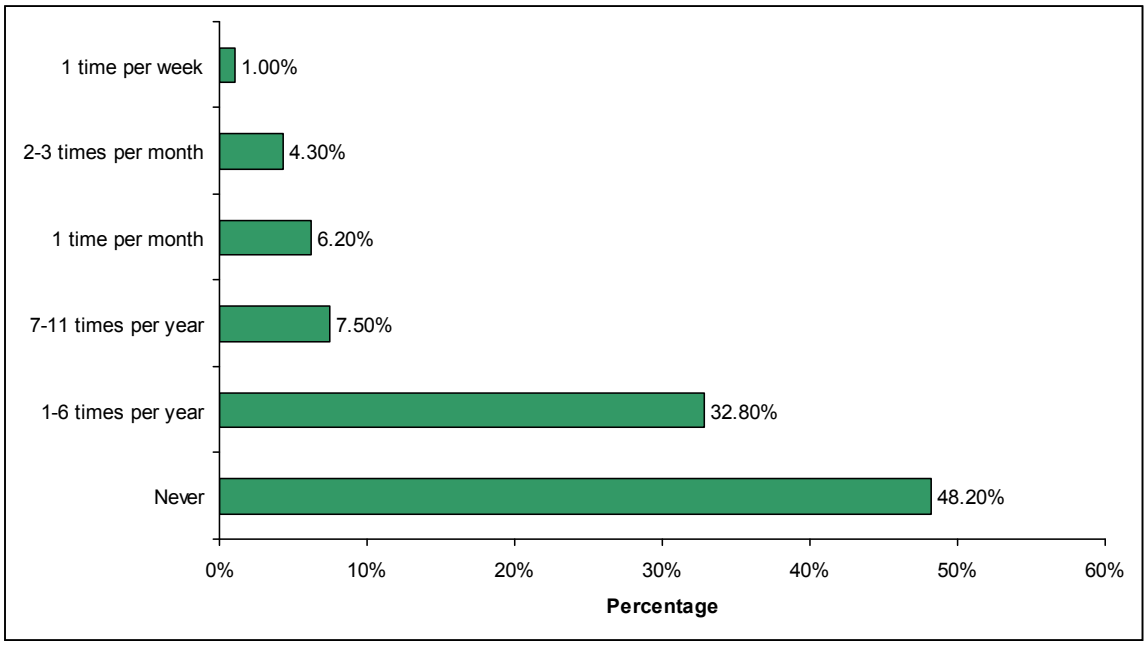


Figure 23: Frequency of eating shellfish by children under 16 years of age

The mussel consumption is not that much frequent among the children, only 21.20% children under age 16 prefer to have mussels 1-6 times per year for their meal (figure 24

and 25). Most of the young people (95.10%) prefer about 12 blue mussels for their meal (figure 26).

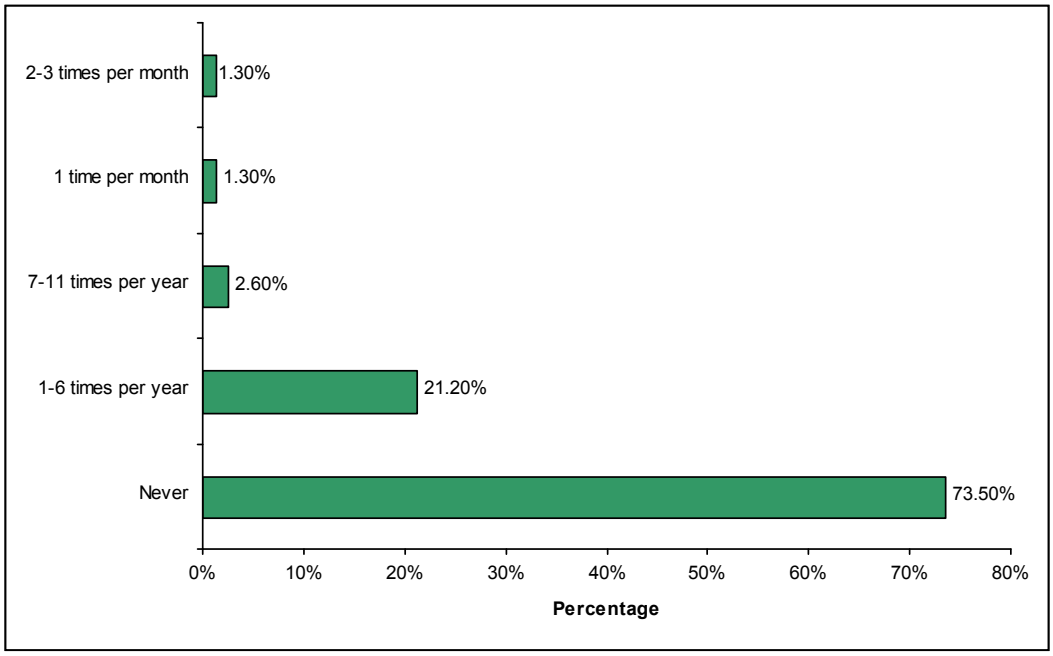


Figure 24: Frequency of eating mussels by children age below 16 years

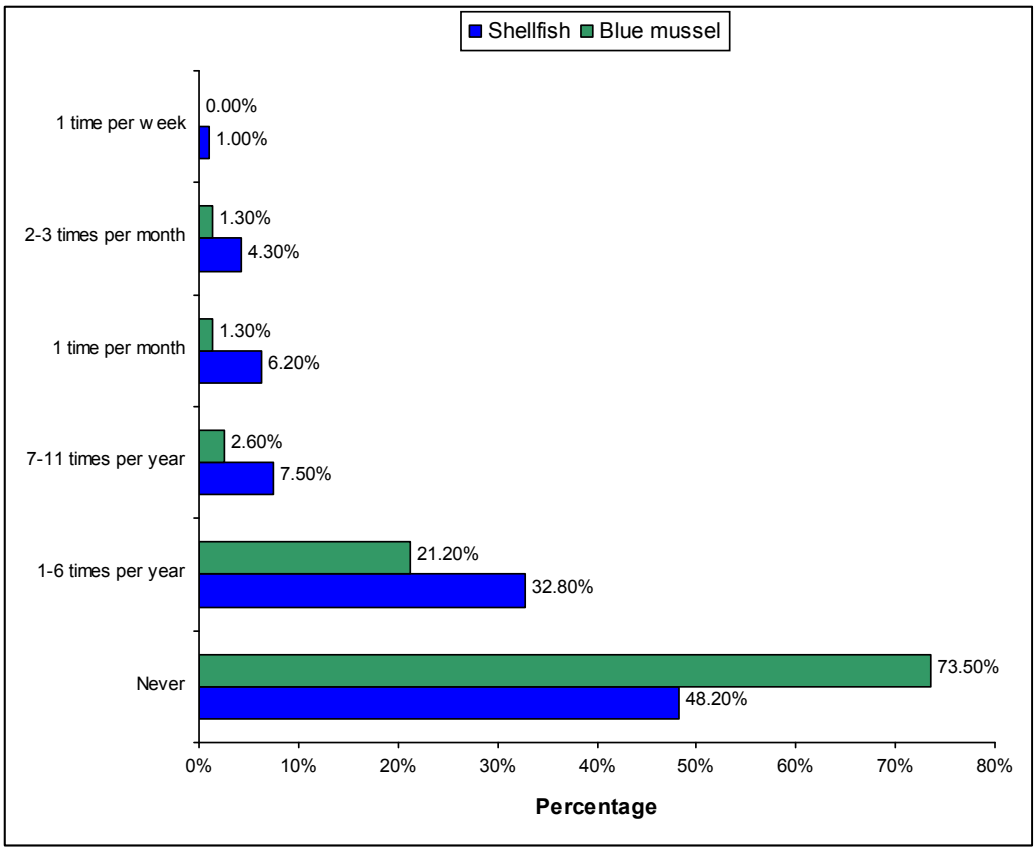


Figure 25: Frequency of eat shellfish (N=305) and blue mussel (N=151) by children under age 16

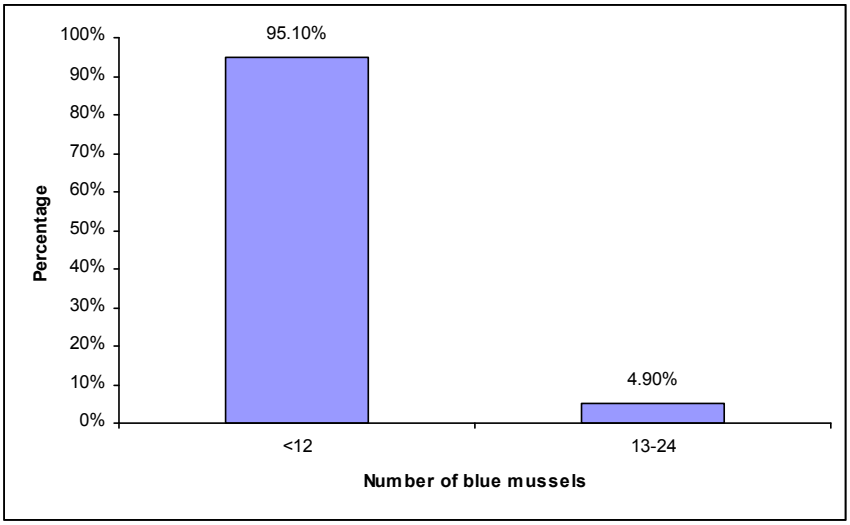


Figure 26: Number of mussel eaten by children under 16 in one meal

4.4. Comparative consumption pattern between mature people and children

4.4.1. Shellfish types

A comparison for types of shellfish consumption between mature people and children shows that there are similarity to choice shellfish types; shrimp and lobster are the best two for both types of consumer, scallops and blue mussel are more liked by mature people than to children (figure 27).

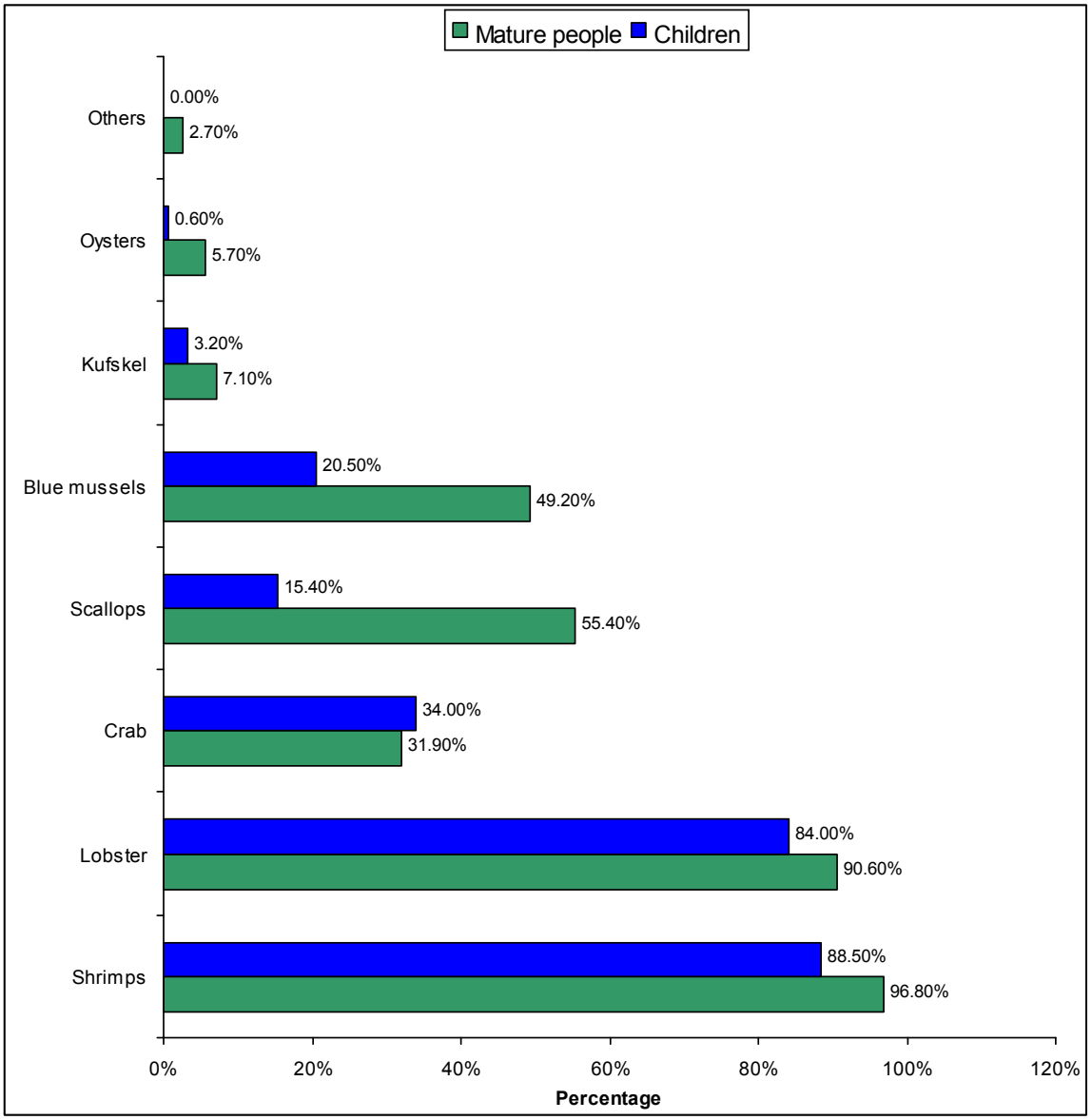


Figure 27: Kinds of shellfish eaten by mature people and children

4.4.2. Frequency of consumption shellfish

Shellfish are more chosen by mature people than children. As weekly or monthly meals the children are not expect shellfish but as an occasional food they prefer shellfish more than to mature people, 32.8% children prefer shellfish as a meal for 1-6 times per year, 26.4% mature people prefer it for the same frequency of shellfish consumption (figure 28).

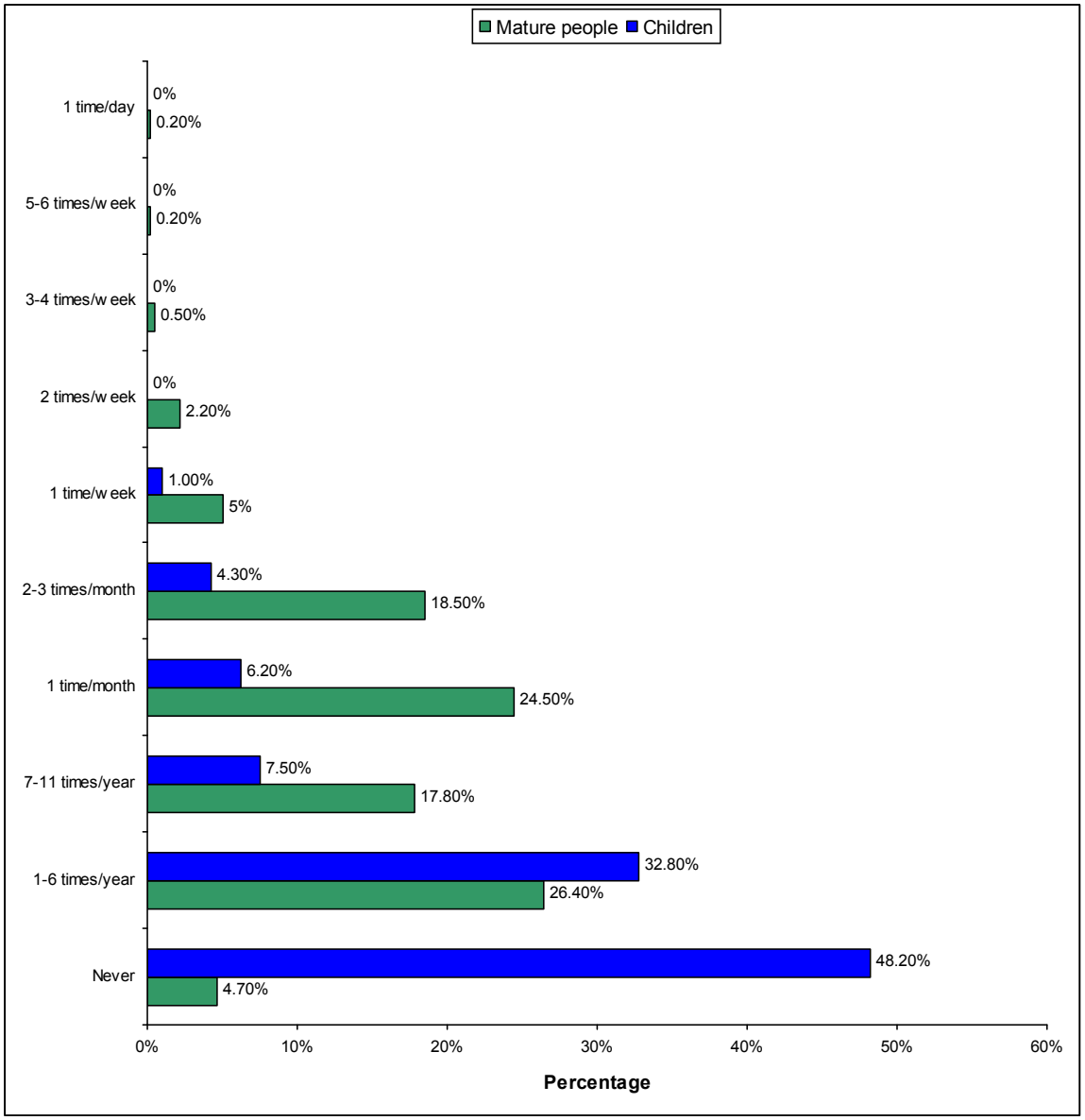


Figure 28: Frequency of eat shellfish by mature people and children (N=595 and 305)

4.4.3. Frequency of consumption blue mussel

Blue mussel are choose as an occasional food, 1-6 times per year by both mature people and children (84.6% and 73.5%) (figure 29) .

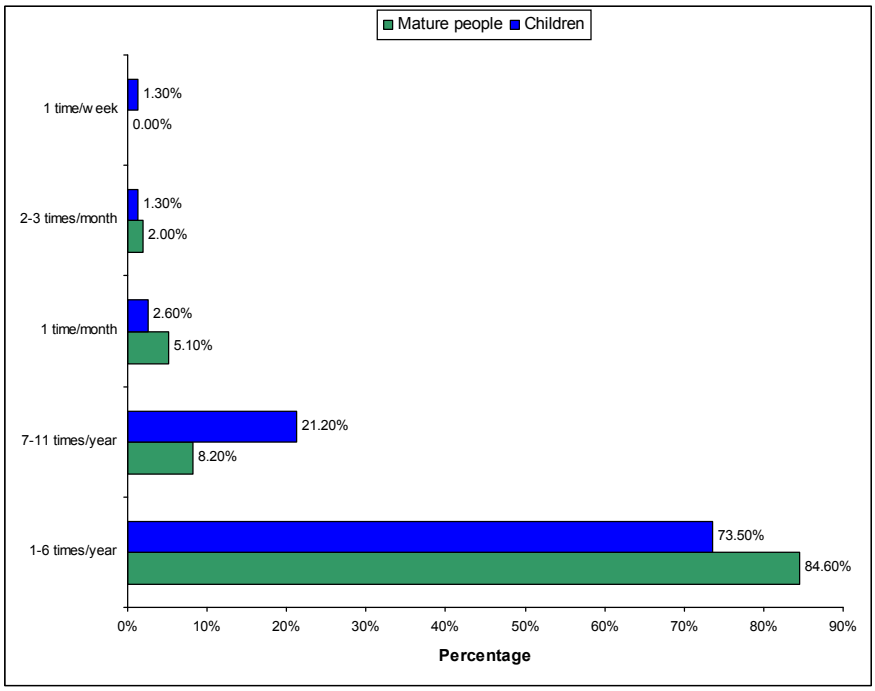


Figure 29: Frequency of eat blue mussel by mature people and children (N=293 and 151)

4.4.4. Calculation of consumption rate from the survey data

On the 1st January, 2011, the total population of Iceland was 319,090, among them 203,159 were from the age group 20-65+ years, those age group was included our survey, belongs to the most blue mussel consumer. Average weight of marketable size blue mussel found 10 g of meat. Among the 293 survey respondents, average number of serving/ year was 54.5, and then the calculated number of serving among the age group 20-65+ years was 37788.96. In an average people consume 15 mussels in a meal, so 150 g meat of mussel consumed in each meal. We retrieved the total quantity of meat served in one year was 5668.34 kilograms. So, the consumption (kg/capita/year) was 0.028 (table 1).

Table 1: Calculation of Consumption of blue mussel in Icelandic population

Total Population of Iceland	319,090
Age group (20-70)	203,159
Quantity of meat in 1 mussel(g)	10
Total No of respondent	293
No of serving/year of 293 respondents	54.5
No of serving/year of age group(20-70)	37789
Average serving (g)	150
Quantity of meat served in 1 year (kg)	5669
Consumption (kg/capita/y)	**0.028**

5. Discussions

The consumer knowledge and attitude toward shellfish especially blue mussel consumption was assessed by means of a nationwide internet (email) survey, the representative respondents are those who has internet connection with computer. The survey respondents are the employed working people, from the capital city Reykjavik and the northern city Akureyri. The survey represents the consumer pattern of blue mussel and shellfish among the Icelandic population. This paper presents the results of a preliminary survey among shellfish consumers conducted in the northern and western part of Iceland, from 19 November to 02 December, 2010. The main objective of this study was to obtain consumer practices of purchase, transport, storage, and preparation of blue mussel as RTE food.

The total population in Iceland was 319,090 at the 1st January, 2011, where 75,631 (23.70%) were under 17 years (Statistics Iceland, 2011). Of 1,000 deliverable surveys, 619 were returned the reply; the data reflects the input of 619 respondents about the consumption pattern of shellfish among their family members. The average response rate was 61.9%.

Among the five age groups responses did varies, mid-aged people are more likely to reply the questionnaire, male are found more responsive than female. The survey respondents dominated by urban dwellers (94%), mostly having University or equivalent degree (72.6%), about 93.8% of the respondents working full time, most average (30.40%) income varies between 6-10 MISK (Million Icelandic Krona), 27.72% respondents family has no children at all and 42.98% has no children under 16 (appendix 1).

Shrimps and lobster are dominating among consumers, blue mussel taken over the shellfish when the consumption frequency is 1-6 times/year (appendix 2). The number of shellfish consumption in each meal is <12 for both adult (75.50%) and children (95.10%) (appendix 2 and 3). Consumer prefers to eat blue mussel in restaurant than

home (Appendix 2). Fresh and boiled (89%) mussel is the best choice (appendix 4), customer prefers to consume within same day or one day after buy (appendix 2).

The young children (age <16) is an important age group of shellfish consumption, shrimp and lobster are their best choice as well, blue mussel is the 4th choice among them, their preference frequency of blue mussel consumption is 1-6 times per year (appendix 3).

Sveinsdóttir *et al.* (2011) found that average fish consumption frequency in Iceland is around two times a week, haddock is the most frequently consumed fish, eaten once in a week, the consumption of fresh fish and ready fish meal increasing now a day among 18-26 years aged people. Our survey revels that 96.8% (age group 17-65+) and 88.5% (age group <16) people prefer shrimp as the 1st choice among the shellfishes, 46.2% (age group 17-65+) and 20.5% (age group <16) people prefer blue mussel as a meal and 69.5% (age group 17-65+) of them prefer fresh mussel. From both surveys a common attitude of fresh seafoods consumption being trendy. We found out from our cross-sectional study that both shellfish and blue mussel consumption is higher for mature people (17-65+) than children (<16) (appendix 2 and 3).

The consumption frequency in Iceland (0.028 kg/capita/year) found too low compare to other European countries (figure 4).

The largest blue mussel producing nations France and Netherlands have stabilized in terms of blue mussel production. Canada and Ireland noticeably increased their farmed blue mussel production between 1993 and 2002. The future mussel industry is getting greater emphasis on value addition products (RTE dishes) rather than fresh live mussel and with the intension of higher contest for raw materials might be the limiting factor. The inability to control algal toxins is a major limiting factor for blue mussel aquaculture. The impact of various biotoxins in Europe and North America is being well documented to protect the shellfish consumer from diarrhetic shellfish poison (DSP) and paralytic shellfish poisoning (PSP). PSP and DSP toxins have been recorded throughout the European continent, as well as in the Gulf of Maine (ASAKUA, 2012).

The increasing trend of blue mussel culture in Iceland is motivating towards monitoring toxic algae along with the blue mussel culture sites. Since 2005 a systematic monitoring of toxin phytoplankton is being observed by Marine Research Institute, Iceland (MRI, 2012). This survey result can use for PSB poisoning analysis and its after effect among the shellfish and blue mussel consumers. The final output of this survey is to figure out the present consumer pattern of shellfish and blue mussel in Iceland to predict upcoming potential of blue mussel culture in Icelandic water body.

Icelandic Fisheries Minister committee suggested increasing knowledge, improving organization and reducing risk in order to make the mussel industry attractive to investors. Mussel farming is an environment friendly industry as the mussel is not fed on introduced food but feeds itself on natural plankton. The mussel industry in Iceland will probably rely on good quality high technology level and high quantity production (MFA, Iceland, 2008).

As blue mussel has an environmental friendly farming with no feed at all, as the Icelandic bay is suitable, blue mussel seed is from the natural source, the Icelandic Government and private entrepreneur should take initiatives to spread this blue mussel culture all over the suitable water areas in Iceland. Blue mussels need more promotion among the consumer in Iceland and in the possible exporting countries.

Now the Icelandic blue mussel processing companies are producing only fresh live blue mussel for the local consumption, they should think about value addition products like frozen and canned mussels.

6. Conclusion

This survey is a base requirement for the risk assessment. The marketing system coordinates the production decisions of producers with the purchase decisions of consumers; consumer survey is an important tool for the marketing systems improvement. The survey focused on consumer purchase behavior as well as preferences and attitudes towards shellfish consumption. Survey using online email contacts of panellists is a quick and less labour-intensive. However, the respondent pool was highly urban and may not truly reflect regional population distributions. More research and studies on household consumption and expenditure behaviour are likely to compensate dividends to the seafood industry specially the blue mussel farming in Iceland.

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8. Appendix

Appendix 1: *Demographic characteristics of survey respondents (N=619)*

Demographic categories	Frequency	Percent	
		Survey	Iceland Census
Age			
17-21	06	0.98	11.02
22-35	114	18.66	29.43
36-45	164	27.50	19.59
46-55	179	29.30	19.72
56-65	119	19.48	15.20
65+	25	4.09	5.04
Gender			
Male	397	65	51
Female	213	35	49
Pregnancy	4	2.88	N/A
Number of household members			
One	52	8.58	N/A
Two	168	27.72	N/A
Three	119	19.64	N/A
Four	144	23.76	N/A
Five	102	16.83	N/A
Six	15	2.48	N/A
Seven	5	0.83	N/A
Eight	0	0	N/A
Nine	1	0.17	N/A
Number of children under 16 years			
Zero	242	42.98	N/A
One	130	23.09	N/A
Two	127	22.56	N/A
Three	57	10.12	N/A
Four	5	0.89	N/A
Five	2	0.36	N/A
Regional population distribution			
Urban	572	94	N/A
Rural	37	6	N/A
Employment situation			
Working full time	540	93.80	N/A
Working part time	28	4.90	N/A
Disabled or retired	1	0.20	N/A
Unemployed	0	0.00	N/A
Not working/homemakers	1	0.20	N/A
Currently in School	6	1.00	N/A
Education status			
University or equivalent	415	72.60	N/A
Diploma or Industrial training	120	21.00	N/A
Grunnskólapró / Completed	30	5.20	N/A
Without formal education	7	1.20	N/A
Family net income (1 year)			
<1 MISK (Million Icelandic Krona)	50	8.80	N/A
1-3 MISK	37	6.50	N/A
3-6 MISK	107	18.80	N/A
6-10 MISK	173	30.40	N/A
>10 MISK	75	13.20	N/A
Do not know	08	1.40	N/A
Choose not to answer	119	20.90	N/A

Appendix-2: *Consumption pattern of shellfish among respondent of age group 17-65+ years*

	Frequency	Percent
Types of shellfish consumed		
Shrimps	547	96.8
Lobster	512	90.6
Crab	180	31.9
Scallop	313	55.4
Blue mussel	278	49.2
Kufskel	40	7.10
Oysters	32	5.70
Others	15	2.70
Frequency of eat shellfish		
Never	28	4.70
1-6 times/year	157	26.4
7-11 times/year	106	17.8
1 time/month	146	24.5
2-3 times/month	110	18.5
1 time/week	30	5.00
2times/week	13	2.20
3-4 times/week	3	0.50
5-6 times/week	1	0.20
1 time/day	1	0.20
2 or more times/day	0	0.00
Frequency of eat blue mussel		
1-6 times/year	248	84.60
7-11 times/year	24	8.20
1 time/month	15	5.10
2-3 times/month	6	2.00
1 time/week	0	0.00
2times/week	0	0.00
3-4 times/week	0	0.00
5-6 times/week	0	0.00
1 time/day	0	0.00
2 or more times/day	0	0.00
Number of blue mussel eaten in a meal		
<12	208	70.50
13-24	76	25.80
>24	11	3.70
Place of blue mussel consumption		
At home	170	57.40
In restaurants	199	67.20
Other places	66	22.30
Types of mussel (preservation style) eaten by respondents		
Fresh	147	69.50
Frozen	70	33.30
Canned	63	30.00
Delay between fresh live blue mussel purchase and consumption		
0 days	29	27.88
1 days	29	27.88
2 days	12	11.54
3 days	3	2.88
5 days	1	0.96
7 days	1	0.96
10 days	1	0.96
Don’t know	28	26.92

Appendix-3: *Consumption pattern of shellfish among respondent of age group <16 years*

	Frequency	Percent
Types of shellfish consumed		
Shrimps	138	88.50
Lobster	131	84.00
Crab	24	15.40
Scallop	53	34.00
Blue mussel	32	20.50
Kufskel	5	3.20
Oysters	1	0.60
Others	0	0.00
Frequency of eat shellfish		
Never	147	48.20
1-6 times/year	100	32.80
7-11 times/year	23	7.50
1 time/month	19	6.20
2-3 times/month	13	4.30
1 time/week	3	1.00
2times/week	0	0.00
3-4 times/week	0	0.00
5-6 times/week	0	0.00
1 time/day	0	0.00
2 or more times/day	0	0.00
Frequency of eat blue mussel		
Never	111	73.50
1-6 times/year	32	21.20
7-11 times/year	4	2.60
1 time/month	2	1.30
2-3 times/month	2	1.30
1 time/week	0	0.00
2times/week	0	0.00
3-4 times/week	0	0.00
5-6 times/week	0	0.00
1 time/day	0	0.00
2 or more times/day	0	
Number of blue mussel eaten in a meal		
<12	39	95.10
13-24	2	4.90
>24	0	0.00

Appendix-4: *Ways of blue mussel cooking*

Ways of cooking	**Preservation type**		
	Fresh (%)	**Frozen (%)**	**Canned (%)**
Eaten readily (%)	2.70	0.80	69.10
Boiled (%)	89.00	82.90	26.00
Boiled and chilled (%)	19.20	22.50	8.10
Other methods (%)	6.00	10.90	18.70

Appendix-5: *Survey questionnaire*

MUSSELS CONSUMPTION QUESTIONNAIRE
LISRISK 08196 E.U – SAFEFOODERA – ERA-NET

INSTRUCTIONS

- Please answer each question as best you can. Estimate if you are not sure.
- ***Put an X*** in the box next to your answer.
- Do not make any other marks on this form.

1. Age

Less than 17	17-21
22-35	36-45
46-55	56-65
More than 65	……

2. Sex

Male Female

⇓

2.a. Are you pregnant?

YES NO

3. Your place of **residence** is considered to be:

Urban Rural

4. How many people are currently living in your **household**, including yourself?

_____

5. How many are **children under 16** years of age? _____

6. Are you the "**head of the household**"

YES NO

7. How **often** do you eat **shellfish**?

NEVER (GO TO QUESTION 9)

1-6 times per year	2 times per week
7-11 times per year	3-4 times per week
1 time per month	5-6 times per week
2-3 times per month	1 time pes day

8.a. What **kind of shellfish** do you eat more often? (Multiple Answers)

Shrimps	Lobster	Crab
Clams	Mussels	Scallops
Oysters	Winkles	

Other (please state): _____

If **MUSSELS ARE** among the shellfish you eat more often **GO TO QUESTION 8b.** If **NOT GO TO QUESTION 9**

8.b. How often did you eat **mussels**, in the last 12 months?

1-6 times per year	2 times per week
7-11 times per year	3-4 times per week
1 time per month	5-6 times per week
2-3 times per month	1 time per day
1 time per week	2 or more times per day

8.c. How many mussels, do you usually eat in one meal?

Less than 6
Less than12
13-24
More than 24

8.d. Where do you consume **Mussels? (Multiple Answers)**

At home
In restaurants
Other (please mention):_____

1 time per week 2 or more times per day

If you **DO** consume **MUSSELS** at home **GO TO QUESTION 8e.** If you **DON'T** consume **MUSSELS** at **HOME GO TO QUESTION 9**

8.e. What **kind of mussels** do you eat the most? (Multiple Answers)

Fresh Frozen Canned

8.f. If you eat **FRESH mussels** how **many days (maximum**) between purchasing and consuming? _____ days

8.g. How are each kind of **mussels prepared**? **(Multiple Answers)**

Fresh mussels
Only seasoned
Cooked and cooled
Other (please state)_____

Frozen mussels
Defrosted + seasoned
Cooked and cooled
Other (please state) _____

Canned mussels
Direct from can
Cooked and cooled
Other (please state) _____

9. Have you got children **under** the age of 17?

NO (GO TO QUESTION 11)
YES
⇓
9.a. How old is the **eldest** of them?
⇓
Age

Less than 1	1-3
4-10	11-17

9.b. Sex:

Male Female

10.a. What kind of shellfish does he/she eat most often? (Multiple Answers)

Shrimps	Lobster	Crab
Clams	Mussels	Scallops
Oysters	Winkles	

Other (please state): _____

If **MUSSELS ARE** among the shellfish you eat more often **GO TO QUESTION 10.b.** If **NOT GO TO QUESTION 11**

10.b. How often did he/she eat **mussels over the past 12 months**?

NEVER (GO TO QUESTION 11)

1-6 times per tear	2 times per week
7-11 times per year	3-4 times per week
1 time per month	5-6 times per week
2-3 times per month	1 time per day
1 time per week	2 or more times per day

10.c. How many mussels, does he/she eat in one meal?
Less than 06
Less than 12
13-24
More than 24

11. Check the box that best corresponds to your current **work situation**.

Working full time	Unemployed
Working part time	Not working
Disabled or retired	Currently in school

12. What is the highest **level of education** you have completed?

University or college or equivalent
Intermediate between secondary level and university (e.g. technical training)
Secondary school
Primary school only (or less)
None

13. What is your total combined **family net income for one year**, from all sources, wages, public assistance/benefits, help from relatives, alimony, and so on?

Less than € 5,000	€100,000-€149,999
€5,000-€19,999	More than €150,000
€20,000-€49,999	Don't know
€50,000-€99,999	Choose not to answer

14. How was the questionnaire completed?

Self administered by the participant
Face-to-face interview
Both self-administered and interview
By phone interview
Postal or electronic mail

15. Country

Iceland
Cyprus
Others ……..

16. Date _____ / _____ / _____

17. Place:

16. Contact Information:
(If you want to write)

Thank you very much for completing this questionnaire. We want to be able to use all the information you have provided, so we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- Have not skipped any pages and
- Completely erased any changes you may have made.

[End of Paper II]

Paper III. Growth of *L. monocytogenes* in Blue Mussel (*Mytilus edulis*) in Iceland

III

Abstract

Aims

The aim of the study was to observe the growth of *Listeria monocytogenes* in Icelandic blue mussel at refrigerated temperature, also to examine the anticipated growth using predictive microbiology; these growth studies are important phase of Quantitative Microbial Risk Assessment (QMRA).

Methods

A particular strain of *L. monocytogenes* (DSM 20600) serovar 1/2a was used to observe its growth into live blue mussel, its depurated water and blue mussel meat in environmental and refrigerated temperature. For the enrichment and enumeration of *L. monocytogenes* in blue mussel ISO 11290-1:2004(E) & ISO 11290-2:2004(E) was followed. The predictive growth of *L. monocytogenes* was observed by 'Growth Predictor'.

Results

There was a reduced growth of *L. monocytogenes* found from two hours to seven days shelf-life at 6.6°C, but it grows moderately until 2 hours. A relative promising growth observed in the depurated water of live mussel within 2 hours. The predictive growth at 15°C found a short lag phase (11 hours).

Conclusion

No stable and exponential growth was observed. Further growth study of *L. monocytogenes* in blue mussel is recommended to sketch a unique growth model.

Significance and Impact of study

The growth from the microbiological study was found uneven due to unknown reason, further growth study is recommended.

Keywords: *Listeria monocytogenes,* growth, *Mytilus edulis,* prediction, Iceland.

1. Introduction

1.1. Background

The increasing consumer demand for fresh foods with minimal cooking and preservation has led to increase the sales for RTE (Ready to Eat) foods globally. Many refrigerated RTE foods are treated with mild heat processes, with maximum temperature typically reaching 70-95^{0}C, packed in a vacuum or with modified atmospheres (usually anaerobic), and then refrigerated (Peck, 2006). The combination of heat treatment and refrigerated anaerobic storage is designed to prevent the growth of non-spore forming pathogens and spoilage organisms. Inadequate killing steps, post-process contamination and characteristics of the product may allow for the survival and growth of pathogens.

The pathogenic bacterium *L. monocytogenes* is of particular concern because of its ability to grow in the absence of oxygen, at refrigerated temperature, and survive in the processing plant environment where it can contaminate foods during pre or post-processing (D'Amico and Donnelly, 2008). An extended shelf-life exacerbates the problem by providing additional time for *L. monocytogenes* to grow to numbers high enough to cause illness. One of the main factors for the increase of the emergence of *L. monocytogenes* is the production of minimally processed foods like fresh blue mussel; Icelandic people prefer to eat fresh blue mussel after three minutes heat at 65^{0}C temperature. This is especially the case for RTE foods in which *L. monocytogenes* can grow and that will not receive a heat treatment during production, and for foods that may be contaminated from the environment, include the production environment, during their manufacture and storage condition until consumption.

1.2. Growth of *L. monocytogenes*

In general *Listeria* strains are able to grow between 1^{0}C and 45^{0}C under aerobic and facultative conditions. Their optimal growth temperature is between 30^{0}C and 37^{0}C. Minimal growth temperatures were determined for 100 strains of *Listeria* (Junttila *et al.,* 1988), the mean minimum temperature for *L. monocytogenes* growth was found 1.7^{0}C,

no differences in growth temperature were observed among strains isolated from different sources.

The major factors which control the growth of *L. monocytogenes* in seafood are temperature, pH, water activity, organic acid and preservatives. Lower pH is correlated to higher lactate concentration; most seafood has higher pH and less lactate than meat products. *L. monocytogenes* is a facultative anaerobic bacterium which can grow with a minimum water activity (a_w) 0.91 to 0.93, pH 4.2 to 4.4, temperature -0.4^{0}C to +4^{0}C, optimum temperature 30-37^{0}C, maximum water activity (a_w) >0.997, pH 9.4 to 9.5, temperature ~45^{0}C and maximum % water phase salt (NaCl) 10 (FAO, 1994; Ross *et al.,* 2000).

Kaysner *et al.* (1990) found no growth of *L. monocytogenes* in raw oyster at 4 °C until 21 days, whereas *L. monocytogenes* grows well in cold-smoked salmon at 4°C to 10°C from 2.0 log cfu/g to 6.5 log cfu/g (Duffes, *et al.*, 1999; Hudson and Mott, 1993; Dillon and Patel, 1993; Nilsson *et al.*, 1997; Peterson *et al.*, 1993; Pelroy, *et al.*, 1994a; Pelroy, *et al.,* 1994b. Susan *et al.* (1988) also observed growth at 4°C and in addition at 7°C, 10°C, 20°C, 30°C with pH values 4.39, 4.39, 4.62, 4.62 and 5.23 respectively. Cole *et al.* (1990) observed that an initial concentration of 10^4 cells of *L. monocytogenes* survived until four weeks with the pH and temperature combinations which are 4.66 at 30^{0}C, 4.36 at 10^{0}C and 4.19 at 5^{0}C. Jørgensen and Huss (998) found that *L. monocytogenes* shown moderate growth in naturally contaminated cold-smoked, and 'gravad' fish while the growth appeared faster in hot smoked fish. Farber *et al.* (1992) observed the growth of *L. monocytogenes* at 30^{0}C in an a_w value of 0.93. Vermeulen *et al.* (2007) found that most of the *L. monocytogenes* strains were not ab le to grow at a_w <0.930, pH <4.3 or a total acetic acid concentration >0.4% (w/w).

1.3. Microbiological Identity of *L. monocytogenes*

Among all species of the genus *Listeria*, only *L. monocytogenes* is typically implicated in human food borne illness. *L. monocytogenes* is a facultatively anaerobic, non-sporulating, catalase-positive, oxidase-negative, short, non-branching, gram-positive rod that grows readily on blood agar, producing incomplete β-hemolysis. The identification of *Listeria* species is based on a limited number of biochemical markers, among which

absence or presence of hemolysis and arylamidase are used to differentiate between *L. monocytogenes* and *L. innocua*. The CAMP (Christie, Atkins, Munch-Petersen) test must be interpreted. Chromogenic media are based on both the specific chromogenic detection of phosphatidylinositol phospholipase C and the xylose fermentation and give specific and direct identification *of L. monocytogenes* and *L. ivanovii*. Isolates of *L. monocytogenes* with atypical properties require tools of molecular biology for final identification, serotyping although not allowing speciation, serves a useful purpose for confirming the genus diagnosis *Listeria* (Allerberger, 2003).

1.4. Predictive microbiology

Predictive microbiology is a powerful tool to aid the exposure assessment phase of QMRA, the predictive microbiology can estimate the changes of microbial population on food product between harvest / production and consumption from product, storage and physical parameters, e.g. storage or cooking temperature, pH, water activity/salt content, storage atmosphere, preservatives etc.(Ross *et al.,* 2000). It is possible to infer exposure to *L.monocytogenes* at the time of consumption from the initial microbiological condition of blue mussel and its history from production to consumption. This study has tried to fit a predictive output for the growth of *L. monocytogenes* in blue mussel having data from prevalence, level of contamination and product, storage and physical parameters.

2. Objectives

There is an immense need to understand the survival and growth of *L. monocytogenes* on fresh blue mussel during refrigeration. Refrigeration is a well known method for preservation of food products until the shelf-life expired. For the home storage of blue mussel refrigeration is common, where temperature varies from 2 to 8^0C. Mostly fresh blue mussels are consumed in Iceland on the day of purchased whereas sometimes remain in the home refrigerator for few days.

The purpose of this study is to monitor the growth of *L. monocytogenes* in fresh blue mussel (*Mytilus edulis*) with contaminated water and also the growth during retail and home storage facilities over the shelf-life with reasonable environmental conditions. The study checked the survival of *L. monocytogenes* at refrigerator temperature during retail and home storage.

The goal of this study is to figure out the survival and growth level of *L. monocytogenes* from purchase of blue mussel until consumption. The objective of this growth study is to measure the growth of *L. monocytogenes* in the fresh live blue mussel and into the meat of the mussel. The study has the following sub-objectives -

- Growth study of *L. monocytogenes* into fresh live blue mussel in refrigerated temperature.

- Growth study of *L. monocytogenes* into blue mussel meat at refrigerated temperature.

- Predictive growth study of *L. monocytogenes* into blue mussel using observed physical parameters.

The data collected from growth study can be used for further growth potential study and to determine the shelf-life and risk assessment study of fresh live blue mussel in Iceland.

3. Materials and Methods

3.1. Experimental design

A particular bacteria strain *L. monocytogenes* serover 1/2a (DSM 20600) was used to contaminate artificially to the fresh blue mussel. Blue mussel was collected as fresh and lives from a producing company and retail stores. The growth was observed into both live mussels and mussel meat in refrigerated temperatures, the growth of the pathogen in contaminated water in ambient temperature also observed. Based on observed physical properties of blue mussel a predictive growth of the contaminated pathogen was sketched and a comparative analysis was drawn for further progress of growth study of the pathogen in blue mussel in Iceland (figure 1).

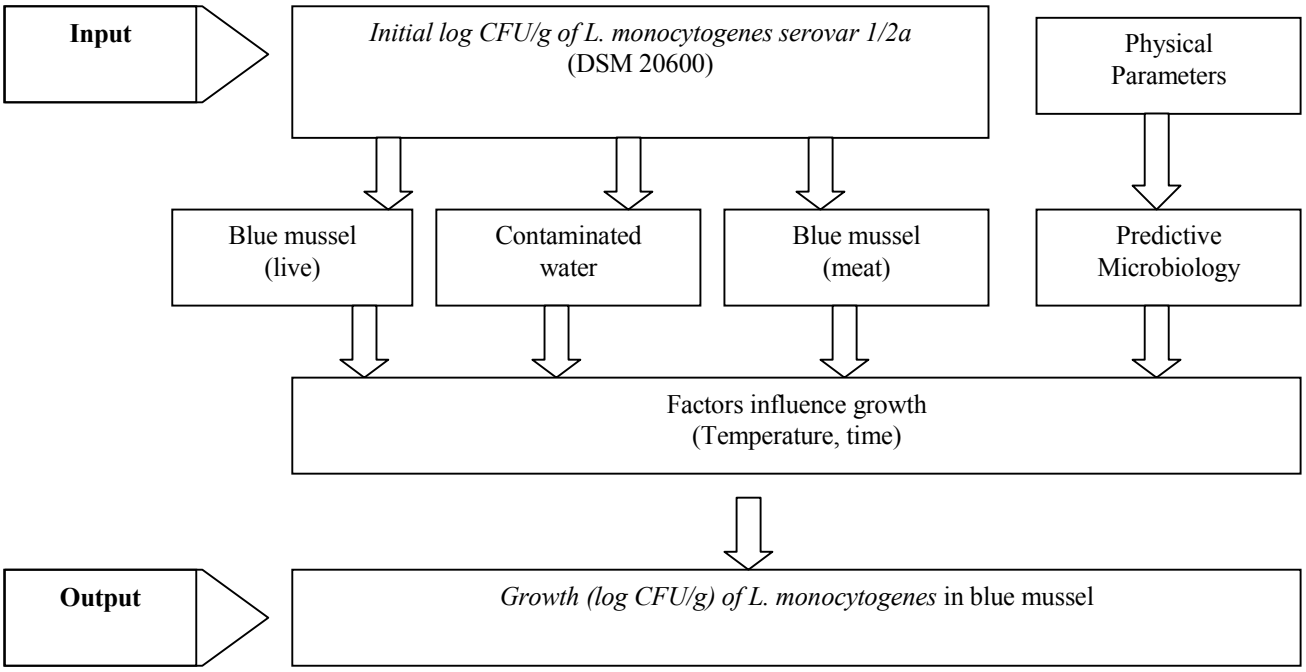

Figure 1: Experimental design of L. monocytogenes growth study in blue mussel.

3.2. *L. monocytogenes* strain

All members of the genus *Listeria* are widely distributed in nature. Epidemiological data from different countries show that the majority of the human outbreaks are associated with three *L. monocytogenes* serotypes (1/2a,1/2b and 4b), despite the fact that there are 13 serotypes potentially capable of infecting humans (Wiedmann, 2002). Due to their

ubiquitous presence, *Listeria* in general and *L. monocytogenes* in particular are also used as hygiene indicators in all stages of the food processing chain. Single *Listeria* strains can spread in manufacturing plants and even establish themselves as endemic organisms (Senczek *et al.,* 2000).

The pathogenic bacteria used for this study was *L. monocytogenes* (DSM 20600) serovar 1/2a, was collected from DSMZ (DSMZ, 2012). The strain was reconstructed according to the manufacturer instructions, incubated in BHI (Brain Heart Infusion) (Difco) broth at 37°C for 24 hours, streaked in BHI (Brain Heart Infusion) (Difco) agar. A selective colony of the strain from BHI agar was mixed carefully into 10 ml BHI broth and incubated at 37°C for 24 hours, 1.0 ml enriched broth from the first enrichment was mixed into a new 10 ml BHI broth and incubated at 15°C for 24 hours, the 2nd enriched culture was diluted up to 10^{-8} and streaked on PCA (Plate Count Agar) (Difco) plates for counting, incubated at 37°C for 24 hours.

3.3. Preparation of blue mussel for growth study of *L.monocytogenes*

Mussel samples were collected from local retail stores in Akureyri, Iceland and directly from the producer, Norðurskel ehf, Sjávargata, Hrisey, Iceland. Every time about one kg of fresh live blue mussel was collected. The samples were brought to the laboratory at the University of Akureyri, Iceland for the growth study of *L. monocytogenes*.

3.3.1. Inoculation into live blue mussels

There were few contamination study in live blue mussel were conducted, among them three were successful. For each contamination study about 600g live fresh blue mussels were put in a container along with 3000 ml of artificial seawater. Enriched culture of *L. monocytogenes* (DSM 20600) from BHI broth were mixed accordingly (table 1) with the seawater. The live mussels were kept in the seawater bath for two hours to be evenly contaminated with the *L. monocytogenes*; contaminated mussels were put in refrigerator (6.58^0C) for until seven days. After seven days mussels' samples were brought out from refrigerator to check the growth rate *L. monocytogenes* at refrigerated temperature. 25 g of mussel meat were separated from the shell, put in a stomacher bag (BagPage,

Interscience, France) along with 225 ml of butterfield buffer water, blended using stomacher lab-blender 400 (Seward Laboratory, England, Sussex). From the stomacher bag 0.1ml of inoculum were spread on oxford agar (Oxoid, England) and OCLA (Oxford Chromogenic *Listeria* Agar) (Oxoid, England) agar plates, incubated for 24 hours at 37°C and checked the presumptive colony for *L. monocytogenes* and counted to verify the growth.

Table 1: Concentrations of L.monocytogenes used for live fresh mussel contamination study

Batch No.	**Concentration of *L.monocytogenes* (cfu/ml)**	**Concentration of *L.monocytogenes* (log cfu/ml)**
1.	$3.02X10^4$	4.48
2.	$7.94X10^3$	3.90
3.	$5.62X10^2$	2.75

3.3.2. Inoculation into mussel meat

There were 11 contamination studies in blue mussel meat completed, among them eight were successful. For each contamination study 200g of mussel meat were separated from the one kg of live mussel, weighted, put in a stomacher bag, placed into the lab-blender and blended for two minutes, after that the sample was ready for contamination with *L. monocytogenes* strain. Enriched culture of *L. monocytogenes* from BHI broth were mixed accordingly (table 2) with the mussel meat. 1.0 ml BHI broth from the 2^{nd} enrichment was homogenized with the blended mussel meat and was put once more in the Lab-blender for one minute, afterward kept in the refrigerator (6.6°C) where the temperature was measured using a StowAway temperature logger (Onset, Cape Cod, Massachusetts), the logger recorded the temperature in every five minutes (figure 2).

Table 2: Concentrations of L.monocytogenes used for mussel meat contamination

Batch No.	**Concentration of *L.monocytogenes* (cfu/gm of meat)**	**Concentration of *L.monocytogenes* (log cfu/gm of meat)**
1.	$8.74X10^3$	3.94
2.	$8.74\ X10^2$	2.94
3.	$3.80\ X10^3$	3.58
4.	$3.80\ X10^2$	2.58
5.	$2.00X10^1$	1.29
6.	$4.14X10^3$	3.62
7.	$16.33\ X10^3$	4.21
8.	$1.63\ X10^3$	3.21

3.3.3. Sampling of contaminated mussel meat

To measure the growth of *L. monocytogenes* into the refrigerated mussel meat, the meat sample was checked frequently, after 2 hours, 4 days and 7 days, 25 g contaminated meat (2 samples fo each batch) was collected from the refrigerated bag and put in a new stomacher bag along with 225 ml of butterfield buffered water, blended and mixed thoroughly; two serial dilutions ($10^{-2,}$ 10^{-3}) were made, 0.1 ml of each dilution were placed on Oxford and OCLA agar plates, incubated at 37°C for 24 hours and counted the positive colonies for *L. monocytogenes*.

3.4. Enrichment and Enumeration Methods

ISO 11290-1:2004(E) & ISO 11290-2:2004(E) was followed for the enrichment and enumeration of *L. monocytogenes* in blue mussel. The positive colony in OCLA and OXFORD agar were checked, typical colonies of *Listeria* spp grown on oxford agar for 24h are small (1mm) greyish colonies surrounded by black halos. After 48h, colonies become darker, with a possible greenish sheen, and are about 2 mm in diameter, with black halos and sunken centres. And the typical colonies of *Listeria* spp grown on OCLA are clearly visible blue/green colonies with *L. monocytogenes* colonies showing a distinctive opaque white halo. Sub-culturing of the colonies of presumptive *L. monocytogenes* in particular agar plates, plated out and confirmation by means of appropriate morphological, physiological and biochemical tests. For the confirmation of the presence of *L. monocytogenes* the confirmatory tests- catalase test, gram staining, motility test, haemolysis test, carbohydrate utilization test and CAMP test were accomplished done according to ISO 11290-1:2004(E).

Enumeration method

Calculate for each of the plates for number of colonies of *L. monocytogenes* present, using the following formula:
a = (b/A)xC, where

b is the number of colonies conforming to the identification criteria.
A is the number of colonies plated out for confirmation.
C is the total number of characteristic colonies enumerated on the petri-dish.

Calculate the number N of L. monocytogenes present in 1 ml or 1 gram of product, using the following formula:
$N= \sum a / \{V(n1+0.1n2)d\}$, where

∑a is the sum of the colonies of L. monocytogenes calculated after confirmation, on all the dishes retained at two consecutive dilutions, one of which at least contains at least 15 identified colonies;

V is the volume of the inoculum applied to each dish in millilitres.
n1 is the number of dishes retained at the first dilution;
n2 is the number of dishes retained at the second dilution;
d is the dilution factor corresponding to the first dilution retained.

3.5. *Listeria* Identification tests

Several tests were made to try to identify *Listeria* in the mussels, catalase reaction test, gram staining, motility test and haemolysis test were performed and finally a API (Analytical Profile Index) test were conducted on the bacteria strains that meet the condition for the test.

3.5.1. Catalase reaction test

The catalase reaction test was performed by removing one of the colonies that had been grown on TSYEA (Tryptone Soy Yeast Extract Agar) and putting them on a slide, one drop of hydrogen peroxide solution (3% m/m i.e. 10 volume solution) was then put on the slide. If the contact of the hydrogen peroxide solution caused a formation of gas bubbles it was considered as a positive reaction.

3.5.2. Gram staining

Gram staining is an empirical method of differentiating bacterial species into two main groups (Gram-positive and Gram-negative); the method is based on reaction due to the chemical and physical properties of their cell walls. The test was performed by taken colonies from TSYEA putting them on a microscope slide and spread around the slide. Then the sample is put over a fire for a short period of time and then covered with crystal violet colour for one minute, then the colour is washed off with water and iodine solution put on it for one minute before it is washed off. After washing the sample a 96% solution of ethanol is put on it for 30 seconds and then the sample is washed once again and then the sample is covered with safranin red and washed and dried before examine in a microscope. If the bacteria are purple they are classified as Gram-positive if they are pink they are classified as Gram-negative.

3.5.3. Motility test

A loopful culture from TSYEB (Tryptone Soy Yeast Extract Broth) was put on a slide and cover slip put on top and examine with a microscope. *Listeria* spp. appear as a short and slim rods with tumbling motility

3.5.4. Haemolyses test

One colony was taken from TSYEA agar and streaked on blood agar. The plate was then incubated for 24 hours at 37°C and then the indication of haemolytic reaction was exanimate.

3.5.5. API- Test

API is a classification of bacteria based experiment allowing fast identification. The API range introduced a standardized, miniaturized version of existing techniques, which up until then were complicated to perform and difficult to read. API *Listeria* is a 24 hours identification of all *Listeria* species (API *Listeria* 2010).

The API Listeria strip consists of 10 microtubes containing dehydrated substrates which enable the performance of enzymatic tests or sugar fermentations. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading table (table 3) and the identification is obtained by consulting the profile list (appendix 1).

Table 3: Reading table of sugar fermentations in API test

Tests	Active Ingredients	Results	
		Negative	Positive
DIM	Enzymatic substrate	ZYM B /< 3 min	
		pale orange	orange
ESC	Esculin Ferric Citrate	pale yellow	black
αMAN	4-nitrophenyl-αD-mannopyranoside	colorless	yellow
DARL	D-ArabitoL	red / orange-red	Yellow / yellow-orange
XYL	D-Xylose		
RHA	L-Rhamnose		
MDG	Methyl-αD-glucopyranoside		
RIB	D-Ribose		
G1P	Glucose-1-Phosphate		
TAG	D-Tagatose		

Inoculation of the strip

The bacteria suspensions are distributed into each tube, avoiding the formation of bubbles, 100 µl suspensions into the cupule DIM and 50 µl into the other cupules. After closing the incubation box incubated for 18-24 hours at $36^0C \pm 2^0C$ in aerobic conditions.

Reading and Interpretation

Reading the strip: A drop of ZYM B reagent added to DIM test. All the reactions are read within 3 minutes by referring to the reading table 3 and recorded the reactions are positive or negative (+/-) on the result sheet, the type of haemolysis also recorded.

Interpretation

On the result sheet, the tests are separated into groups of three and a value 1, 2 or 4 is assigned to each, by adding together the values corresponding to positive reactions within each group, a 4-digit numerical profile is obtained which constitutes the numerical profile. Having the numerical profile the specific species were recognized from the appendix 1.

3.6. Growth of *L. monocytogenes* of blue mussels using predictive microbiology

To get the predictive growth of *L. monocytogenes* from 'Growth Predictor' the product, storage and processing parameters are required. The physical properties of blue mussel (pH, a_w, amount of NaCl content in water phase), storage time and storage temperature are the inputs to obtain the forecast possible growth of *L. monocytogenes.*

3.6.1. Physical properties of blue mussel

3.6.1.1. pH measuring

The pH level was measured by weighing five gram of the mussel flesh in to a stomacher bag, then five gram of distilled water was added and mixed thoroughly using the stomacher lab-blender. The pH electrode was then put in to the solution and the pH level of the solution was measured using the pH meter (ThermoFisher, USA, Waltham MA).

3.6.1.2. Water activity

The water activity measurement was performed with Aqua Lab (Series 3TE, USA, Pulman, Washington).The Mussel flesh was removed from the shell and measured when it had reached room temperature.

3.6.1.3. Calculation the amount of NaCl in water phase

The amount of Sodium Chloride (NaCl) inside the mussel meat were measured by using Kohler theory-

We know from simplified Kohler theory (Kreidenweis *et al.*, 2005)-

$$a_w = Nw/(Nw + \nu Ns),$$

where, a_w =Water activity of the solution droplet

Nw = Number of moles of water in solution(1 Litre) = Molarity of water = 55.56.

ν = Number of ions of solute present in solution.

Ns = Number of moles of solute (NaCl) in solution= 1 Molal

3.6.2. Storage time

The average shelf life of fresh blue mussel in Iceland was found seven days (168 h). From the consumption survey we found that 28% people prefer to eat the blue mussel just after buy from retail and the same number of people (28%) prefer one day after buy. But there are chance to buy few days old blue mussel from retail, because the product may arrive few days ago in the retail. The minimum (2 h), most likely (96) and maximum storage (168 h) times were used to develop the distribution of storage times from retail to home storage until consumption for the growth prediction of *L. monocytogenes* in fresh blue mussels in Icelandic population.

3.6.3. Growth from predictive microbiology

The software that was used to predict the growth of the *Listeria* was 'Growth predictor' (Growth predictor, 2012). The results from product characteristics trials were used as critical factors that are the average a_w, pH level and salt (NaCl) contents in water phase of fresh raw mussels; and the model that was used was *L. monocytogenes/innocua* with CO_2(%). Predictions were made for 1°C, 4°C, 7°C and 15°C temperature for initial log number of bacteria per gram of blue mussel (log 1 cfu/g).

4. Results

4.1. Growth of *L. monocytogenes* in mussels

A temperature logger was used inside the refrigerator, recorded temperature after each 5 minutes interval (figure 2). The gross average temperature was found from all the four observations are 6.58^{0}C, all the growth study of *L. monocytogenes* in blue mussel observed in these refrigerated temperatures.

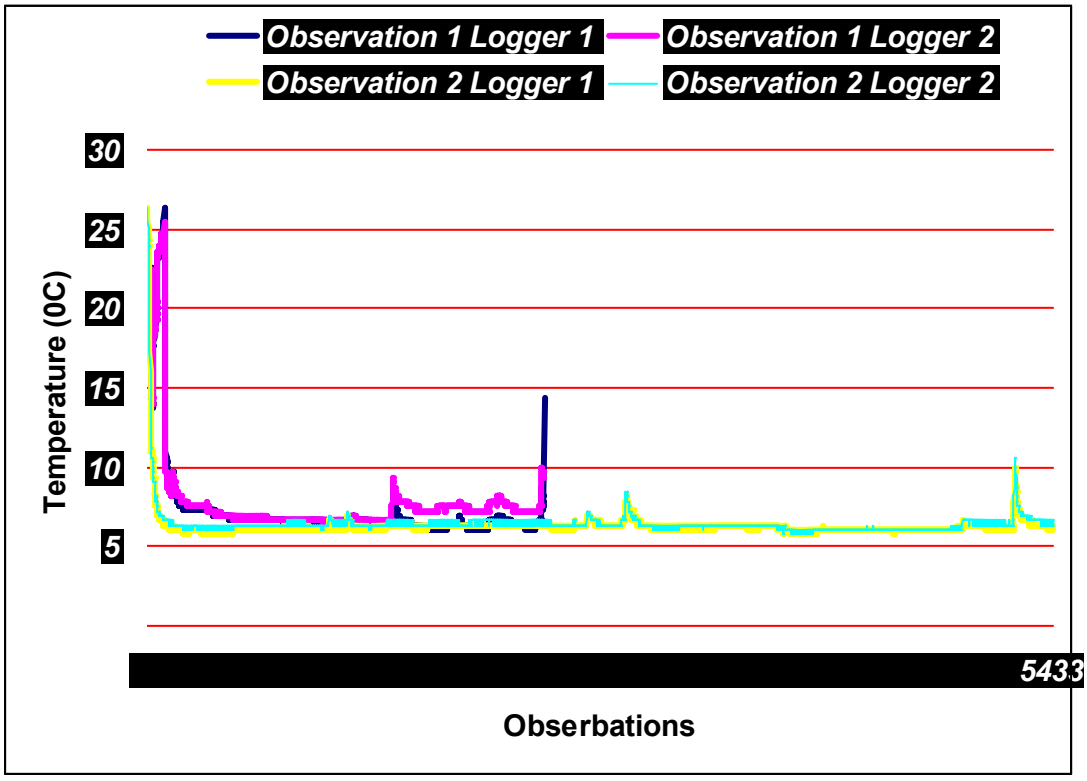

Figure 2: Refrigerator temperature recorded by logger in each 5 minutes interval

4.2. *L. monocytogenes* growth in live blue mussels

The following figure (figure 3) shows the growth of *L. monocytogenes* into live blue mussel in OCLA agar –

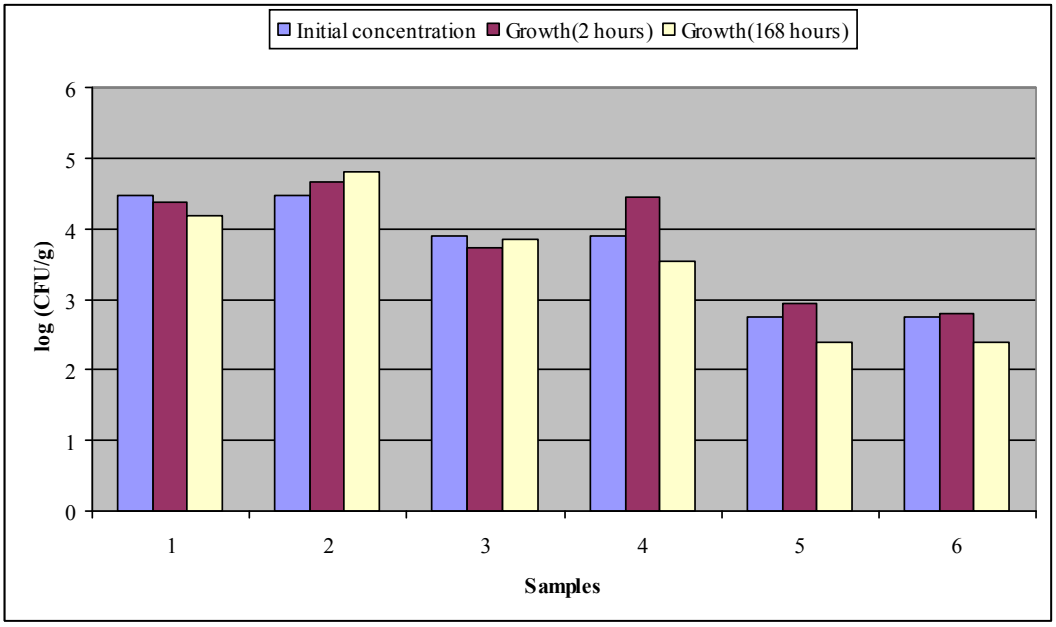

Figure 3: Growth of L.monocytogenes in live blue mussel (grows in OCLA agar)

There were three different concentration level *L. monocytogenes* were inoculated into six batches of live mussel (table 1), each inoculum was used for two samples. There were decreases of growth into the two samples (sample 1 and sample 3, figure 3) of live shell after two hours. But the other four samples (samples 2, 4, 5 and 6) found growth after two hours and sample 4 shows a moderately higher and rapid growth until two hours of depuration. There were decrease of growth after 168 hours (7 days) in all the samples except sample 2, where the inoculum grown exponentially with time (figure 3).

The following figure (figure 4) shows the growth of *L. monocytogenes* into live blue mussel in OXFORD agar-

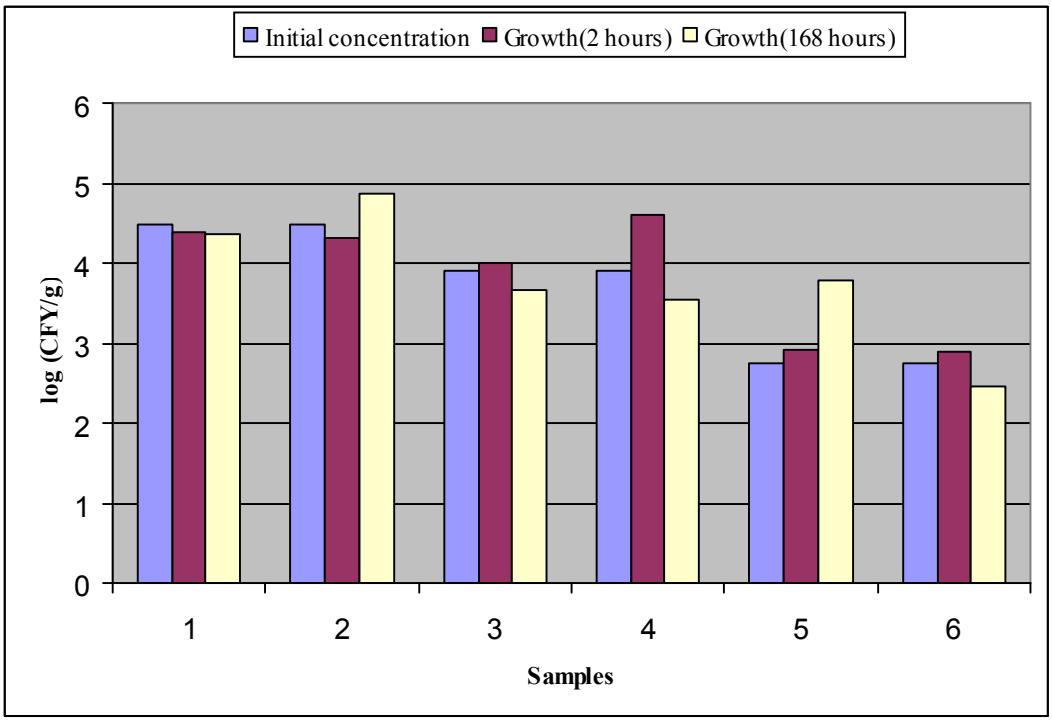

Figure 4: Growth of L.monocytogenes in live blue mussel (grows in OXFORD agar)

The number and concentration of inoculated inoculum and sample numbers were same as OCLA agar in OXFOR agar. In OXFORD agar there were decrease of growth in sample 1 and sample 2 but increase other 4 samples until two hours. In seven days there is increase of growth in sample 2 and sample 5, sample 5 shown an exponential growth from 2 hours to seven days. In sample no 3, 4 and 6 showed a better growth in OXFORD agar than OCLA agar within 2 hours. There was a high growth in oxford agar after 168 hours in sample 5, sample 2 also showed a moderate growth in oxford agar (figure 4).

The following figure (figure 5) shows the growth of *L. monocytogenes* into contaminated water in OCLA agar-

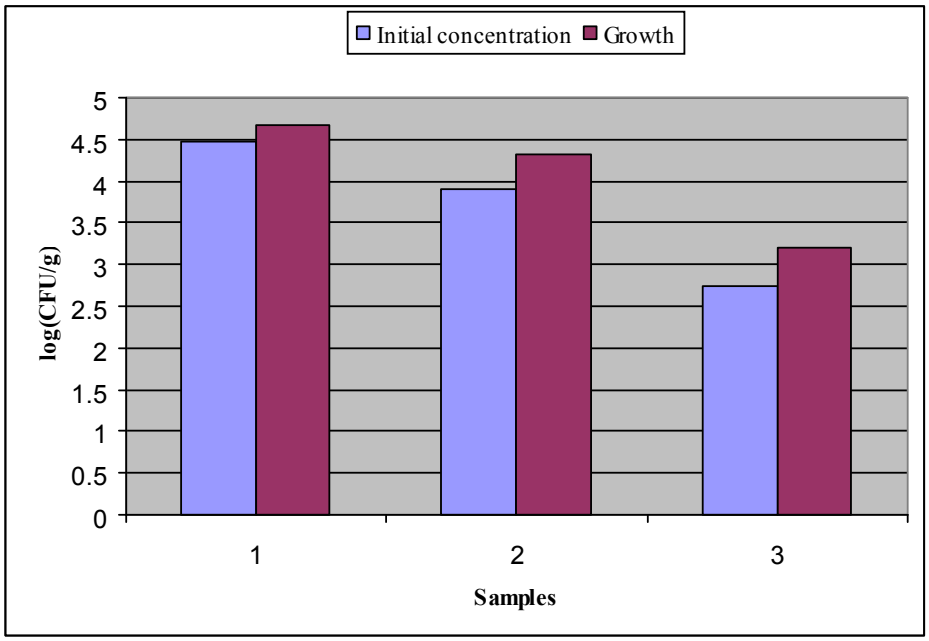

Figure 5: Growth of L.monocytogenes in contaminated water (grows in OCLA agar)

The following figure 6 shows the growth of *L. monocytogenes* into contaminated water in OXFORD agar-

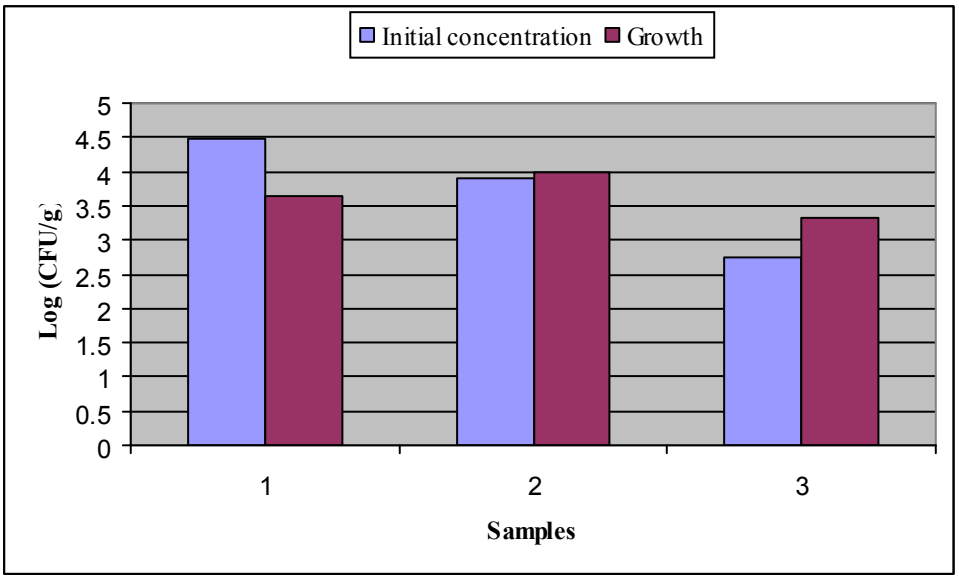

Figure 6: Growth of L.monocytogenes in contaminated water (grows in OXFORD agar)

The growth of the *L. monocytogenes* inoculum observed in depurated water with blue mussel, the growth observed two hours after contamination. Among the three depuration studies all the three shown reasonable growth in OCLA agar (figure 5), sample 2 and sample 3 showed growth in OXFORD agar but a reduction of number of counts in the 1st sample in OXFORD agar (figure 6).

4.3. *L. monocytogenes* growth from mussels meat

The growth of *L. monocytogenes* in eight batches of contaminated mussel meat were given below (figure 7) -

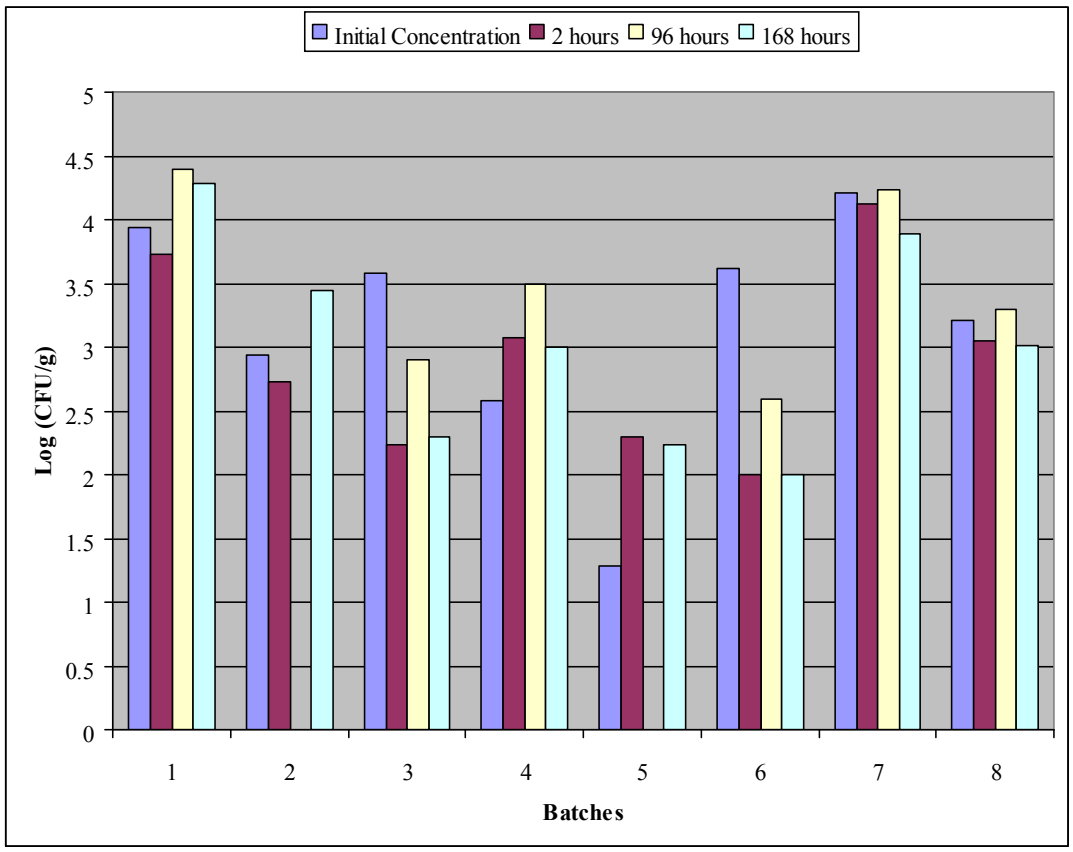

Figure 7: Number of Listeria from contaminated mussel (OCLA agar)

Figure 7 shows the growth of *L. monocytogenes* from the contaminated mussel meat after two hours, 96 hours and 168 hours. Among all eight contamination experiments of *L. monocytogenes* in mussel meat, six samples (sample 1, 2, 3, 6,7 and 8) showed no growth/ reduced growth after two hours, only two samples (sample 4 and 5) showed growth after two hours. After 96 hours samples 1, 4, 7 and 8 showed growth, no growth in samples 2 and 5 and reduced growth in samples 3 and 6. After 168 hours (7 days) samples 1,2,4 and 5 showed growth but the other four samples showed reduced growth after 168 hours. There was an remarkable observation that all samples shown a reduced growth from 2 hours to 168 hours except samples 5 where no growth found might be because of any experimental error (figure 7).

4.4. Growth of *L. monocytogenes* of blue mussels using growth prediction software

4.4.1. Physical properties of blue mussel

Results obtained from measurement of pH level and water activity on mussel meat.

4.4.1.1. pH and a_w

Average pH level was 6.50; the highest pH level found 6.77 and the lowest was 6.08. The highest water activity measurement was 0,999 but lowest one was 0,964 and the average was found 0.989 (Appendix 2).

4.4.1.2. Calculation the amount of Sodium Chloride (NaCl) in water phase

We know from Kohler theory -

$$a_w = Nw/(Nw + \nu\, Ns),$$

$$a_w = 55.56/(55.56 + \nu\, X1\ ),$$

We found the average water activity of blue mussels is 0.989

$$\text{So, } \nu = 55.56/0.989 - 55.56 = 0.618$$

Then NaCl in water phase = (0.618/2) *58.44=18.06 gm/liter, where Molecular weight of water is 58.44. So, NaCl in water phase % = 1.806

4.4.2. Growth from growth predictor

The prediction of growth of *L. monocytogenes* observed in four different temperatures- 1^0C, 4^0C, 7^0C and 15^0C (figure 8).

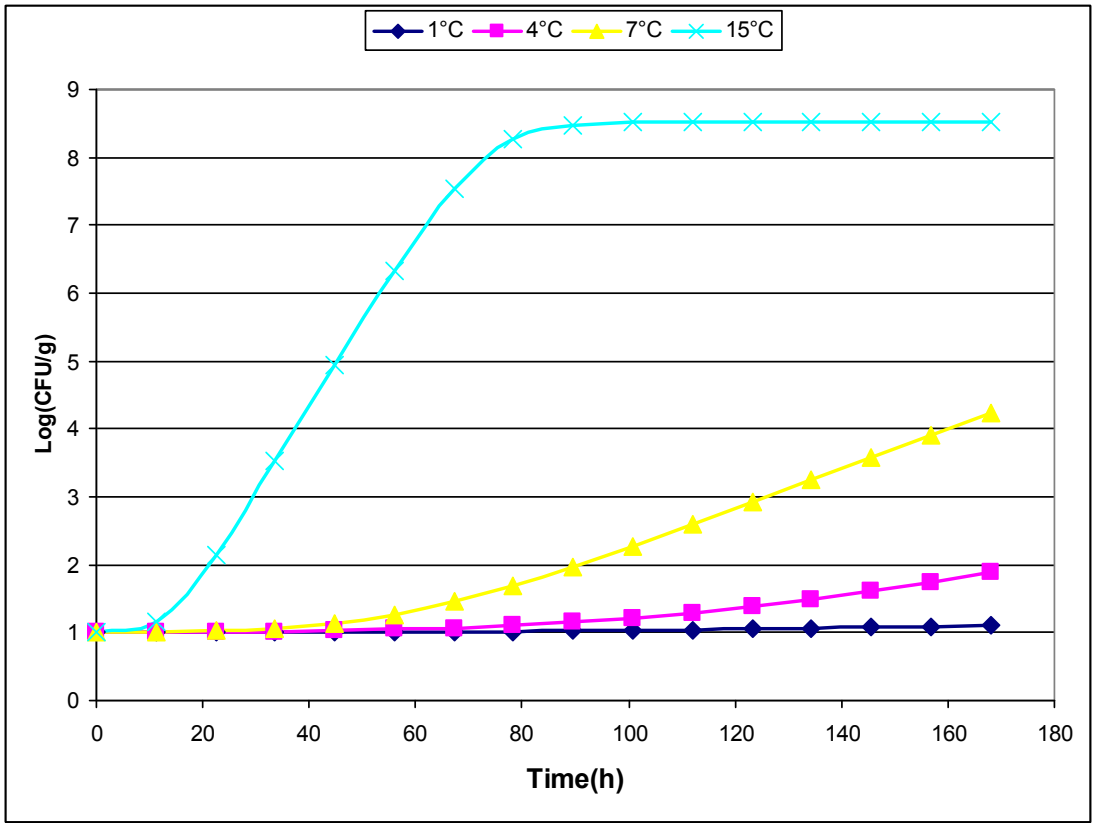

Figure 8: Prediction of growth of L. monocytogenes with time and temperature variations

The 'Growth Predictor' showed an exponential growth of *L. monocytogenes* from zero hour to 168 hours (7 days) in four different temperatures (1°C, 4°C, 7°C and 15°C). For this prediction model the initial numbers of *Listeria* was log 1 units. According to the growth predictor results the influence of the temperature is high on the predictive time period. In all the virtual cultures the number of *Listeria* is rather stable for the first 10 hours, but at 15°C the number of the bacteria starts to grow. For those seven days of storage time the enumeration of *Listeria* would be more than 4 log units if stored in 7°C at given condition but 1.11 log unites at 1°C for same conditions. If the prediction for 15°C is observed for log 1 the growth was very fast and it only taken about 70 hours to reach maximum growth (figure 8). The lag phase continued from zero hour to until 50 hours when the growth temperature was 1°C to 7°C. But when the growth temperature is 15°C, lag phase started after 11 hours, and there was a rapid growth until 67 hours, then until seven days the growth is steady or no growth. The maximum growth observed in 7 days are 1.11 cfu/g in 1°C, 1.89 cfu/g in 4°C, 4.24 cfu/g in 7°C and 8.6 cfu in 15°C (figure 8).

5. Discussion

Contamination of *L. monocytogenes* in seafood varies with product category; the potential extent of its growth also varies among different foods, being dependent of pathogen's growth rate in a specific food, which is a function of the product's composition and storage conditions, and on the shelf-life of the product. Factors affecting the growth of *L.monocytogenes* are product formulation, storage time and temperature, and interaction with other microorganisms present in the product (FAO/WHO, 2004).

The growth study included the initial level of *L. monocytogenes* in the blue mussel at retail where the food is purchased, the exponential growth in home refrigerator until consumption. The growth study also included the variations of storage temperature (1^0C, 4^0C, 7^0C and 15^0C) and time (2h, 96h and 168h). The growth study has two parts - growth study from the microbiological contamination and growth prediction from the 'Growth Predictor'. The inputs of microbiological growth study were initial log (cfu/g) of *L. monocytogenes*. The live fresh blue mussel, depuration water of blue mussel and mussel meat were the three medium of made contaminated by initial concentration of *L. monocytogenes* inoculum. The growth of the *L. monocytogenes* was observed influenced by the two major factors- temperature and time. The input of the 'Growth Predictor' was the physical parameters of blue mussel- pH, a_w and salt contents in water phase of blue mussel meat. The predictive growth of the pathogen observed influenced by the same physical parameters- temperature and time (figure 1).

For the microbiological growth study, all the experiment was observed at 6.58^0C refrigerated temperature (figure 2), the growth of *L. monocytogenes* inoculated into fresh blue mussel (live and meat) and depurated water was measured. The growth of *L. monocytogenes* in live mussels into OCLA (figure3) and OXFORD (figure 4) agar indicated that there is a noticeable growth until the 1st two hours after contamination, out of six samples, four samples shown growth both in OCLA and OXFORD agar until two hours. but a reduction of growth found from two hours growth to seven days growth at 7^0C, five samples in OCLA agar and four samples in OXFOR agar shown reduce growth until 168 hours. It proved that at extended shelf life of blue mussel may

reduce the further growth of *L. monocytogenes* at 7^0C or below. The growth of *L. monocytogenes* into depurated water observed at ambient temperature (20-22^0C). A good exponential growth was observed into water with live mussels both in OCLA and OXFOR agar; it indicated that *L. monocytogenes* can grows well at 20-22^0C (figure 5 and 6). *L. monocytogenes*'s growth in blue mussel's meat shown a different scenario than growth in live mussel, out of eight samples six samples shown reduced growth until 1st two hours into meat; but an increase of growth observed in six samples from two hours to 96 hours and 168 hours. In the observed growth study there was a common phenomenon found for both live mussel and mussel meat - a reduced growth observed at the end of 168 hours (7 days) which is the declared shelf life for blue mussel being sold in Iceland.

Carrasco *et al.* (2006) found growth increases when the temperature approaches the optimal growth temperature of *L. monocytogenes* (30°C), in our growth prediction experiment we found a rapid growth when temperature increases from 7°C to 15°C. Jørgensen and Huss (1998) found that RTE fish products are commonly contaminated with *L. monocytogenes* and that growth occurs during normal storage conditions (no temperature abuse), the prevalence of *L. monocytogenes* was 4% in cured seafood (brined shrimps and surimi, caviar and marinated herring) but no growth was observed during storage at 5°C, indicating that these products are of low-risk with respect to *L. monocytogenes*. Our predictive growth study shown the maximum growth at low refrigeration temperatures (4°C) was often less than growth in the same foods (blue mussel) at higher temperatures (7°C), at 4°C, growth increased from 1 log cfu/g to 1.89 log cfu/g, but at 7°C the growth increases to 4.24 cfu/g at the end of 168 hours (figure 8). It was concluded that refrigeration temperature and storage time are not independent factors. High storage temperatures and long storage times would not be likely to occur because this combination would lead to obvious spoilage and the food would not be consumed.

6. Conclusion

L. monocytogenes is a food borne pathogen that has the ability to survive freezer and refrigerator temperature, so consumers should remember that fresh blue mussel needs to be properly heated to destroy the pathogen.

At the end of the shelf-life (7^{th} day after production) of blue mussel there are possibilities to reduce the number of *L. monocytogenes* present, but this study would not give any guarantee of absence of the bacterium.

Reducing the ranges of refrigerator temperatures by eliminating storage at the high temperatures reduced the predicted cases of Listeriosis by reducing growth *of L. monocytogenes* in the foods that permit growth.

This growth study will help to assess the risk of growth of *L. monocytogenes* in blue mussel is affected by formulation, process and storage conditions. Data collected from this study can be used to determine the shelf-life of blue mussel. To do this successfully there is a need for research to increase our knowledge of the contamination route of *L. monocytogenes* in blue mussel processing sites.

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8. Appendix

Appendix 1: *API test strip numeric profiles of Listeria spp*

Numerical Profiles	*Listeria* spp.	Numerical Profiles	*Listeria* spp.
2150	*Listeria ivanovii*	3750	*Listeria ivanovii*
2170	*Listeria ivanovii*	3770	*Listeria ivanovii*
2250	*Listeria ivanovii*	6010	*Listeria monocytogenes*
2310	*Listeria seeligeri / ivanovii*	6110	*L. monocytogenes / innocua*
2311	*Listeria welshimeri*	6120	*Listeria grayi*
2330	*Listeria ivanovii*	6130	*Listeria grayi*
2340	*Listeria ivanovii*	6150	*Listeria monocytogenes*
2350	*Listeria ivanovii*	6310	*Listeria seeligeri/welshimeri*
2370	*Listeria ivanovii*	6311	*Listeria welshimeri*
2410	*Listeria monocytogenes*	6410	*Listeria monocytogenes*
2510	*Listeria monocytogenes*	6450	*Listeria monocytogenes*
2711	*Listeria welshimeri*	6510	*Listeria monocytogenes*
2750	*Listeria ivanovii*	6520	*Listeria grayi*
2770	*Listeria ivanovii*	6550	*Listeria monocytogenes*
3110	*Listeria seeligeri /inncua/ ivanovii*	6701	*Listeria welshimeri*
3120	*Listeria grayi*	6711	*Listeria welshimeri*
3130	*Listeria grayi / ivanovii*	7110	*Listeria innocua*
3150	*Listeria ivanovii*	7111	*Listeria welshimeri*
3170	*Listeria ivanovii*	7120	*Listeria grayi*
3210	*Listeria seeligeri / ivanovii*	7130	*Listeria grayi*
3250	*Listeria ivanovii*	7301	*Listeria welshimeri*
3270	*Listeria ivanovii*	7310	*Listeria seeligeri / welshimeri / innocua*
3300	*Listeria seeligeri / ivanovii*	7311	*Listeria welshimeri*
3310	*Listeria seeligeri / ivanovii*	7320	*Listeria grayi*
3311	*Listeria welshimer*	7330	*Listeria grayi*
3330	*Listeria ivanovii*	7500	*Listeria innocua*
3340	*Listeria ivanovii*	7510	*Listeria innocua*
3350	*Listeria ivanovii*	7511	*Listeria welshimeri*
3360	*Listeria ivanovii*	7520	*Listeria grayi*
3370	*Listeria ivanovii*	7530	*Listeria grayi*
3510	*Listeria innocua*	7701	*Listeria welshimeri*
3520	*Listeria grayi*	7710	*Listeria welshimeri / innocua*
3711	*Listeria welshimer*	7711	*Listeria welshimeri*
3730	*Listeria ivanovii*	7720	*Listeria grayi*

Appendix 2: *Physical properties (pH and water activity) of mussel samples*

Sl. No.	P^H	Water activity	Sl. No.	P^H	Water activity
1.	6.64	0.987	23.	6.53	0.992
2.	6.63	0.985	24.	6.43	0.995
3.	6.76	0.985	25.	6.30	0.994
4.	6,74	0.985	26.	6.49	0.993
5.	6.53	0.988	27.	6.46	0.991
6.	6.68	0.987	28.	6.57	0.982
7.	6.74	0.992	29.	6.21	0.986
8.	6.65	0.978	30.	6.17	0.987
9.	6.53	0.990	31.	6.35	0.985
10.	6.65	0.994	32.	6.51	0.987
11.	6.54	0.990	33.	6.30	0.999
12.	6.41	0.993	34.	6.08	0.964
13.	6.77	0.995	35.	6.61	0.993
14.	6.44	0.989	36.	6.46	0.992
15.	6.68	0.984	37.	6.47	0.998
16.	6.38	0.989	38.	6.56	0.990
17.	6.50	0.993	39.	6.54	0.995
18.	6.76	0.987	40.	6.58	0.979
19.	6.18	0.990	41.	6.50	0.993
20.	6.56	0.993	42.	6.52	0.993
21.	6.37	0.994	43.	6.38	0.994
22.	6.48	0.977			
	Average			**6.50**	**0.989**

(End of Paper III)

Vita

Md. Murad Mufty was born in Patuakhali, Bangladesh in 1976. He enjoyed academics as well as music and travelling. In 1994, he enrolled as Fisheries and Marine Resource Technology Discipline in Khulna University, Bangladesh. After finishing his bachelors (Honours) degree in 1999, he began his job as "Microbiologist" in a Fish Processing industry in Khulna, Bangladesh. During the Spring of 2005, he met his wife, Bosra Khanam and married in June of 2005. In July 2005 he joined in Marine Fisheries Office, Chittagong, Govt. of Bangladesh, as "Marine Production Officer". Currently, Murad is living in Chittagong and working for the Fish Inspection and Quality Control Office, Department of Fisheries, Govt. of Bangladesh, as a "Microbiologist" from September 2005. In 2009 Murad has received a six months fellowship from the United Nations University-Fishedies Training Program (UNU-FTP), Iceland on 'Quality Management of Fish Handling and processing'. Since September, 2010 he has been studying M.Sc. on 'Fisheries Science' in University of Akureyri, Iceland, supported by UNU-FTP. After completion his M.Sc. degree he will continue his Job as 'Microbiologist' and will try to implement his knowledge for the betterment of fisheries sector of Bangladesh.

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