FINAL REPORT

A review of the published literature and current production and processing practices in smoked fish processing plants with emphasis on contamination by *Listeria monocytogenes*

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EXECUTIVE SUMMARY

A critical review of literature relating to *Listeria monocytogenes* contamination of raw and finished fish during the hot and cold smoking processes was undertaken from a variety of academic and other bibliographical sources. The review was conducted using a systematic approach, with a scored assessment of publication quality a system adapted from the Oxford System originally described by Jadad and Murray (2007). The review purpose was to identify key production and processing practices that could potentially influence *L. monocytogenes* prevalence and numbers associated with ready-to-eat smoked fish throughout the production chain from farming or being caught at sea to final product packing. A series of visits to fish farms and commercial smokers, mainly in Scotland, were also undertaken to gather information on practices that may contribute to, or control, the risk of *Listeria* contamination; and also to gather information on any perceived barriers to the effective management of *L. monocytogenes* food safety risks.

A survey of environmental health officers (EHOs) in Scotland involved with inspections of fish smoking plants was also undertaken to identify how informed EHOs thought the food businesses they inspected were with regard to *Listeria* and smoked fish, and also how informed EHOs themselves were on the same topic. Based on the findings from these studies, recommendations for key areas where there were gaps in knowledge or areas where additional guidance for both EHOs and food business operators (FBOs) would be beneficial were developed. The key findings from each section of the study are summarised below.

LITERATURE REVIEW

From the literature it was identified that *L. monocytogenes* can be isolated from fresh surface waters such as rivers and also from coastal waters, but rarely from deep sea waters. There is evidence that environmental conditions such as rainfall or tidal movements can influence *Listeria* levels in water and as a result fish, both farmed and wild, can have *Listeria* present on their skin surfaces. Raw fish entering smoking plants have been reported to be contaminated with *L. monocytogenes*, with prevelances ranging from 0% to 75%, and 15-20% being most typical.

It was also identified from the literature that some *L. monocytogenes* biotypes can persistently colonise plant processing environments for extended periods of several years and these plant resident biotypes are more likely to be isolated from the final products than biotypes present on batches of raw fish entering the plants. It is possible that raw fish could be the original source of the plant resident biotypes, although this cannot be conclusively ascertained from the literature available. Specific process stages and regions within smoked fish processing plants appear to be more susceptible to persistent colonisation by *L. monocytogenes.* Such *r*eservoirs include drains, skinning machines, brine injection units and fish slicers. Plant workers may also act as fomites, spreading *L. monocytogenes* around processing environments via their hands and equipment.

INDUSTRY VISITS

Overall, fish farmers considered it important to maintain strict cleaning regimes underpinned by microbiological (or other) monitoring to ensure cleaning effectiveness throughout the entire farming process. In Scotland, there is routine and widespread monitoring for *Listeria* on the fish and on the equipment used to handle the fish (e.g. fish pumps, well-boats and killing plant). It was observed during farm visits that sea- caught fish can often be transported using plastic crates. These crates can often be damaged and scratched, which may present a surface for growth of *L. monocytogenes*, although there was no firm evidence available to confirm any microbiological implications from damaged crate surfaces.

Visits to commercial fish processing and smoking premises identified that larger throughput plants have well-informed technical staff and make considerable efforts to attempt to prevent *L. monocytogenes* contamination of products. A number of medium-sized FBOs used external consultants to provide microbiological support. These consultants tended to service more than one smoker and we note they could effectively communicate best practices or guidance to a range of businesses. Most smaller throughput plants did not have ready access to sound microbiological advice.

The majority of the smoking plants visited were observed to have issues with condensation and ceiling drippage, particularly in their chillers. An absence of drain disinfection measures was observed in a number of premises. Some of the businesses visited had never undertaken shelf life determinations – an observation that was particularly true of the smaller enterprises. Numbers of *Listeria* at the end of shelf life tests were perceived to be expensive and test results

from lab-cultured *L. monocytogenes* inoculations were not viewed as being typical of naturally-contaminated product by the industry in general.

EHO SURVEY

It was identified that the main areas of concern for EHOs related to plant cleaning and sanitation, the control of key hazard microorganisms (not just *Listeria*), salt monitoring, post process handling and shelf life determinations; both in terms of FBO practices and their own knowledge gaps. A minority of EHOs (12%) indicated they believed that effective salting and brining represented a critical control point for *Listeria* (i.e. they believed that salting was listericidal) during the pre-smoking preparation processes, which is not reliably the case. The survey also identified that EHOs felt there is a lack of understanding regarding HACCP in general in FBOs (particularly in smaller businesses). It was also apparent that some EHOs felt they did not fully understand the smoking process; the nuances associated with it and how these influenced the risk of *Listeria* contamination of final product.

The study also determined the preferred format that any aides or guidance produced by the agency to fill the identified knowledge gaps should take. There was a strong preference for interactive web-based systems (online audio/video and decision support systems) as well as training sessions or workshops. There was a relative dislike of paper-based booklets and other printed material.

STUDY CONCLUSIONS AND RECOMMENDATIONS

The study highlighted that guidance in the following areas would be of benefit to FBOs and/or EHOs involved in the smoked fish industry:

- The importance and purpose of testing the processing environment, how samples should be collected, where samples should be collected; and at what frequency.
- Improving knowledge of how the principles of HACCP should be used to minimise food safety risks from *Listeria* should be aimed primarily at smaller FBOs. Such information should include general good microbiological practices and basic guidance on the fundamental principles of microbiology.
- The sourcing of high quality raw ingredients and information covering the auditing of raw fish suppliers was felt to be worthwhile for smaller FBOs. In

addition, the provision of information describing risk factors that may increase the chances of contamination of raw fish was felt to be important. Furthermore, there was a widely acknowledged gap in the most effective way to sample raw fish and how to choose a laboratory test method for *Listeria*; again, aimed primarily towards small FBOs and EHOs

- The cleaning and sanitation of food contact surfaces and the plant environment generally; along with further information describing the importance of verifying cleaning effectiveness was identified as an area where some FBOs would value additional information.
- A number of smaller FBOs had not heard of a 'multiple hurdles approach' to product safety and would value further information regarding strategies to minimise the chances of final product contamination.
- The prevention of post processing contamination during storage, including effective freezing and the operation of condensation-free chillers was felt to be beneficial by all sizes of FBO.
- Information describing the numbers of *L. monocytogenes* on products at the end of shelf life using FBO products inoculated with laboratory cultures or naturally contaminated products was valued by small FBOs and EHOs.
- Monitoring various stages of smoking process, particularly salting and brining, and the operation of these premises using HACCP principles aimed specifically at EHOs.

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1 INTRODUCTION

Listeriosis is caused by invasive infection with the bacterium *Listeria monocytogenes* (*L. monocytogenes*). Listeriosis is a rare condition in healthy people. However listerial infections can cause serious illness in vulnerable groups, such as people with impaired immunity, the elderly and expectant mothers (Lyytikainen, 2006). In individuals that are immunocompromised or elderly, it is normally the blood stream or central nervous system that is infected and Listeriosis usually presents as septicaemia or meningitis. Amongst these vulnerable populations, the illness is severe with a terminal prognosis for 20-25% of patients (Farber, 2000; Gillespie, 2010). In pregnant women the mother is rarely affected, but the condition can result in spontaneous abortion, stillbirth of the foetus or delivery of a severely ill baby due to infection. Non-invasive infection with *L. monocytogenes* can occur, but in these cases the illness caused is febrile gastroenteritis (Miettinen *et al*, 1999). *L. monocytogenes* is generally classified into 13 different bacteria subspecies, or serovars, on the basis of reactions with a range of standardised antibodies. Although each serovar has the potential to infect humans; historically it has been serovars 1/2a, 1/2b and 4b that have caused most human illness (Farber, 2000).

Listeria monocytogenes is a ubiquitous Gram-positive bacterium which occurs naturally in the terrestrial environment, fresh and salt water, livestock manures, decaying plant materials and also in many raw foods associated with these environments (Gram, 2001). The organism has an optimum growth temperature of 30-37^{o}C, but can survive between 1 and 45^{o}C, and is known to be able to multiply at refrigeration temperatures (Hutchison *et al*. 2005). It is also halotolerant up to solutions of 12% w/v NaCl (Niedziela *et al*. 1998). *L. monocytogenes* was first recognised as a foodborne human pathogen in the early 1980s when a disease outbreak, caused by the consumption of contaminated cabbage in coleslaw, caused 18 deaths in Eastern Canada (Schlech *et al*, 1983).

Between 2000 and 2007 in the UK, a number of vulnerable groups have presented an apparent doubling in (apparent) foodborne *Listeria* infections (Adak, 2005; Gillespie, 2010). Whilst actual numbers of cases of Listeriosis within the UK and Scotland are low, the condition is severe with almost all cases requiring hospitalisation and up to a third resulting in death. While *Listeria* is frequently detected in a number of ready-to-eat foods, the primary source(s) of the Listeriosis are infrequently identified. Epidemiological investigation of the increased incidence of Listeriosis has provided some valuable information and, most importantly, demonstrated that

the observed increased incidence is not the consequence of a single outbreak (i.e. the increase is not the result of a single point-source event; HPA, 2009).

The Food Standards Agency commissioned a survey of *Listeria monocytogenes* in hot and cold smoked over a period of 4 months between July and November 2006. Overall, just over 10% of samples were positive for *Listeria* spp.; with *L. monocytogenes* detected in 8% of tests. Of the cold smoked fish tested, 20.5% contained *Listeria* spp., of which 17.4% were *L. monocytogenes.* All cold smoked fish in which *L. monocytogenes* was present were found to be below the 100 cfu/g legal limit. Of the hot smoked fish tested, 5.2% contained *Listeria* spp. and 3.4% of these were *L. monocytogenes*. Three of the hot smoked fish samples (0.06%) were in breach of the limits (>100 cfu/g) for *L. monocytogenes* as laid down in the Microbiological Criteria Regulations (Regulation (EC) 2073/2005, as amended).

Although the survey concluded that *L. monocytogenes* was rarely found to exceed the statutory criteria in UK smoked fish, it was apparent that smoked fish supports the growth of *L. monocytogenes*; even under vacuum packaging conditions at refrigeration temperatures (Farber, 1991; Rørvik *et al*, 1991; Jemmi and Keusch, 1992; Cortesi *et al,* 1997). Thus there is the potential for growth and human illness if low numbers of the pathogen contaminate smoked fish products; even under normally-adequate refrigeration. Therefore there is a need to develop tools to help the ready-to-eat smoked fish industry minimise the risks and prevelance of contamination with *Listeria* of this potentially high risk food.

Study aims

The scope of this commission by the Food Standards Agency in Scotland, was to review current practices in the management of *L. monocytogenes* by smoked fish manufacturers. A key focus was the identification of key risk areas in the whole processing chain (from farm to end-product) and possible gaps in the management of any identified risks. This study sought to summarise current knowledge for Food Business Operators (in particular SMEs) and Local Food Authorities on the key risk areas *for L. monocytogenes* contamination and the monitoring and management of these risks.

This work involved the critical consideration of peer-reviewed and other published literature relating to *L. monocytogenes* and the contamination of smoked fish to produce a literature review. The review focus extended across the whole smoked fish production chain from

farming and fishing, through processing to the extended refrigerated storage of the final smoked product. The aims of the review were to define practices and pathways that allow *L. monocytogenes* contamination to occur, to identify any known factors influencing the introduction of *L. monocytogenes* to any smoking process and to identify any known factors influencing *L. monocytogenes* survival in the processing environment.

In addition, attempts were made to review and summarise any literature regarding reports of human illness outbreaks and sporadic cases. The results of anonymised summaries of industry-held data for out of specification product and the effect of different smoking processes and process stages on *L. monocytogenes* contamination of smoked fish in the UK with emphasis on Scotland were also included in the review.

A series of visits to fish farms and food business operators (FBOs) carrying out smoking, mainly in Scotland, were also undertaken to gather information on practices that may contribute to or control the risk of *Listeria* contamination and also to gather information on perceived barriers to control.

A survey of environmental health officers (EHOs) in Scotland involved with inspections of fish smoking plants was also undertaken to identify how informed EHOs thought the food businesses they inspected were in regard to *Listeria* and smoked fish, and also how informed they themselves were on the same topic. Based on the findings from the study recommendations for key areas where there were gaps in knowledge or areas where additional guidance (for EHOS and FBOs) would be beneficial were developed.

2 LITERATURE REVIEW

A critical review of literature relating to the smoking processes and their various stages was undertaken from a variety of academic and other bibliographical sources (e.g. the Thompson ISI databases, PubMedNet and Dialog Select) to identify relevant information from experimental, survey and other work in Europe and elsewhere relating to fish smoking and *L. monocytogenes*. As outlined previously, the review focussed on all stages of the ready-to-eat smoked fish production chain from farming and fishing, through processing to the extended refrigerated storage of the final smoked product. The aims of the review were to define practices and pathways that allow *L. monocytogenes* contamination to occur, to identify any known factors influencing the introduction of *L. monocytogenes* to any smoking process and to identify any known factors influencing *L. monocytogenes* survival in the processing environment.

2.1 METHODOLOGY

The review of the production and smoking processes and stages review was undertaken using a systematic approach with a scored assessment of each paper's quality using the Oxford System (Jadad and Murray, 2007).

Briefly, to allow the literature to be shaped, a set of key questions that the report aimed to answer were formulated for two key search areas

For searches relating to *Listeria* and raw fish the questions formulated were:

- "What is the contamination or infection prevalence (i.e. established infection/contamination) of wild or farmed fish by *L. monocytogenes*"
- "What factors affect incidence (i.e. the rate of new infections)?",

For searches relating to *Listeria* and smoked fish, the questions formulated were:

- "What are the risks of human Listeriosis from smoked fish?" and
- "What are the processing risk factors and interventions for human Listeriosis from smoked fish?"

These questions were used to generate an initial set of key words which were used to search three electronic databases from their inception dates until the end of March 2011. The databases included were: Thompson ISI Web of Science from 1899- March 2011; Thompson ISI MEDLINE from 1950- March 2011 and PubMed.Net from 1950– March 2011. The bibliographic databases used included food safety and processing, public health and agriculture or aquaculture subject areas. The initial set of key words used was the same for all three databases with minor variations in syntax and are presented in Appendix A

For both searches, citations and abstracts were uploaded from each of the three electronic databases into Reference Manager version 12.0.3 (Thompson Reuters, Paris, France). The references were processed using the 'find duplicates' automated functionality of the program and the duplicates were removed. To ensure that all of the pertinent papers were identified, the search strategy was verified by checking the generated list of references against the cited reference lists of a random selection of five publications for all searches. To ensure completely random selection of articles, the papers were sorted by author name and each assigned a sequential number. The formula =n * rand() was used in Excel (version 2010; Microsoft Corp. Redmond, WA, USA) to generate a list of random numbers corresponding to the papers. The 'cited by' functionality of the Thompson ISI Web of Science was furthermore used to identify that articles published after the five randomly-selected references, which cited these papers, were similarly included in the search-generated reference list.

A set of criteria were used to appraise each publication's abstracts for relevance. Publications that were determined as not relevant were discarded. Abstracts were assessed as relevant if they:

- Reported the *L. monocytogenes* incidence or prevalence for either live wild fish or raw fish at retail or on arrival at a processing plant
- Reported factors which influenced the contamination of aquatic or farm environments by *L. monocytogenes* and made attempts to correlate between fish contamination and their environment
- Undertook evidence-based investigations to identify sources of *L. monocytogenes* contamination for raw or fresh fish
- Described primary research on the fate of *Listeria* contaminating smoked fish or the smoked fish processing environment

- Described the assessment of risk factors for Listeriosis in relation to the consumption of smoked fish
- Described interventions which reduced contamination of smoked fish or human illness associated with the consumption of smoked fish
- Reported the prevalence or the numbers of *Listeria* associated with smoked fish or smoked fish processing environments
- Isolated *Listeria* spp. during a study which included a reference to smoked fish
- Described an outbreak of foodborne illness caused by *Listeria* which was associated with, or proven to be, caused by the consumption smoked fish

For both searches, the criteria were independently applied to the abstract of each paper by at least two members of the research team. For each citation, a consensus was reached that the citation was relevant for inclusion. Arbitration by a third researcher was used to settle conflicting appraisals. Full articles were obtained for all abstracts which passed the inclusion criteria.

For each publication identified, two researchers read the abstract of the publication and a single collaborator read the entire paper. Each paper was appraised using a standardised form whenever possible and a basic scoring system used to objectively quantify the robustness of the work. The range for scoring (0-5) and the areas focused on for quality was as outlined by Jadad and Murray (2007). More specifically, the questions on the standardised form included:

- How robust were any reported results based on:
    - The source(s) of the samples (e.g. multiple farms, fish species or processing plants)
    - How many samples were tested overall
    - If appropriate, whether the study included control samples
    - An assessment of the appropriateness of any microbiological test methods
    - The choice of sampling method/ carcass region sampled/ sample format
    - Consideration of cross contamination between samples
    - Whether the study used naturally-contaminated or artificially-inoculated samples
        - If artificially inoculated; whether multiple strains were used

- Whether the study was lab-based or undertaken under commercial processing conditions
- If applicable, the practicality of adopting any proposed intervention based on:
  - Effectiveness of the treatment
  - Legal barriers to implemention
  - Consideration of the impact to commercial processes

For disease outbreak publications, the robustness of the report was assessed primarily as the strength of the association between the human isolate and the likely seafood source. Studies where molecular genomic typing methods were used were favoured compared with studies which used antibody-based or other biochemical typing methods. The appraisal score for each of the papers identified by the systematic search is provided in reference list.

A brief review of each paper was written and used to produce the formal review of the literature relating to the effect of different processes/processing stages to *L. monocytogenes* prevalence/numbers on smoked fish.

2.2 OUTCOMES OF THE LITERATURE IDENTIFICATION

In combination, the three search engines chosen covered more than 700 million published articles as summarised by the following peer reviewed literature databases: The Biosis Citation Index, CAB Abstracts, Conference Proceedings Citation Index, Current Chemical Reactions, Current Contents Connect, Derwent Innovations Index, Essential Science Indicators Index, Food Science and Technology Abstracts, Global Health, Index Chemicus, Inspec, Index Medicus, Medline, Science Citation Index, Web of Science and Zoological records (Thomson Reuters, 2008).

For the search relating to raw fish, the number of papers returned by the Thompson ISI Web of Science database was 158. Of these, 103 papers were discarded on the grounds they did not meet some or all of the assessment criteria. The search keywords returned 75 papers from PubMed.Net, of which 32 were not relevant and therefore discarded. From the search using MEDLINE, 34 results were returned with 18 being discarded as not relevant. After the removal of duplicates, there were 76 unique papers identified for further appraisal. The five papers randomly selected from the raw fish literature search for verification purposes were Bourdin

(2009); Colburn *et al*. (1990); Dominguez *et al*. (2001); Markkula *et al*. (2005) and Notermans and Hoornstra (2000). For the searches relating to smoked fish products the randomly-selected publications were Lappi, (2004); Vaz-Velho, (2006); Tome, (2008); Hwang, (2009a) and Klaeboe *et al*. (2010).

Searching the reference lists in the randomly selected papers identified no additional publications for the raw fish search. A further seven additional papers including one paper in press were identified for the smoked fish search. One of these papers was published in Japanese (Jin *et al*, 1994) and two were Italian language papers (Leoni and Moriggi, 1996; Quaglio and Messi, 2000). No previously-unidentified papers were tagged by the 'cited by' search for either literature list. In total, there were 76 papers identified for the raw fish search and 165 papers relating to smoked fish and listeria. The two searches were merged and a second round of duplicate removal was undertaken. The final number of unique and relevant papers was 235. A summary and a critical appraisal of these publications formed the backbone of this review. Additional papers were cited to support general or related assertions as required. The total number of references cited by the review was 311.

2.3 A REVIEW OF THE PEER-REVIEWED LITERATURE.

2.3.1 FOODBORNE DISEASE OUTBREAKS ASSOCIATED WITH SMOKED SEAFOODS

Identifying outbreaks of Listeriosis can be difficult since cases tend to be sporadic and low in number (Gillespie *et al*, 2010). Consequently, it is difficult to relate outbreaks to a particular food. However, using molecular techniques and in particular pulsed field gel electrophoresis (PFGE), a number of studies have determined indistinguishable strains isolated from smoked fish and patients ill with Listeriosis (Garrido *et al* 2008). This section reviews available literature relating to cases of foodborne Listeriosis that have been attributed or linked to smoked seafood products, with the details of each summarised in Table 1.

Table 1 A summary of smoked fish/shellfish that have been confirmed as the causes of foodborne Listeriosis outbreaks

Smoked seafood implicated in Listeriosis outbreak	Smoking method	Packing method	Outbreak country	Reference
Mussels	Not reported	Not reported	New Zealand	(Brett *et al*, 1998)
Rainbow trout	Cold	Vacuum	Sweden	(Ericsson *et al*, 1997)
Rainbow trout	Cold	Vacuum	Finland	(Miettinen *et al*, 1999)

The first microbiologically-confirmed link between cases of human Listeriosis, contaminated smoked seafood and a contaminated smoked food processing environment. occurred in Auckland, New Zealand, in the early 1990s (Brett *et al*, 1998). Two patients became ill after consuming smoked mussels. Investigation by NZ healthcare officials resulted in the isolation of *Listeria monocytogenes* from an unopened packet of mussels collected from the refrigerator of one of the cases. The culture from the unopened pack and those from the two patients were indistinguishable when macro-restricted DNA fingerprints were compared using PFGE. Furthermore, PFGE analysis of isolates from additional cases of Listeriosis of unknown origin in

New Zealand in 1991 and 1992, revealed two more isolates with indistinguishable PFGE patterns. Further investigation of the outbreak resulted in additional *L. monocytogenes* isolates obtained from a variety of sources including 15 packs of mussel products sampled at retail sale, the refrigerator of one of the patients, environmental swabs taken in the processing factory; and an isolate from a mussel product imported from NZ into the United Kingdom (Brett *et al*, 1998). No information other than 'factory environmental swab' was provided for the areas of processing factory that were sampled.

Further evidence of the potential health risk of *L. monocytogenes* in fish products was highlighted a complex incident described by Ericsson *et al* (1997). Over an extended period of more than one year, nine cases of Listeriosis in Sweden affecting two immuno-compromised cancer patients, three pregnant women (and their three unborn children) and four elderly patients of >70 years old was investigated. There was a single fatality over the course of the investigation as a direct consequence of Listeriosis. Molecular typing isolated several different, but closely related strains of *L. monocytogenes* from the patients and suspected foodstuffs identified by case control-style interviews with those infected. One of the strains was isolated from six of the patients, from "gravad" (dill-flavoured, salt/sugar cured rainbow trout) and from a refrigerator in one of the patients' homes as well as from the grocery store chiller where the fish were purchased. A second strain was also isolated from a different patient's fridge and the fridge of the (different) store where the cold smoked rainbow trout was purchased. However this second strain was different to the one that caused the patient's illness. Although all of the stains implicated by the investigation were closely related, the cold smoked trout and the gravad were processed by different manufacturers. Traceback of the outbreak was undertaken by the testing unopened packages of the suspected contaminated produce obtained directly from a processor referred to as Processor Y. Fish in these packs were also found to contain a *Listeria* strain indistinguishable from the one of those which caused disease in six patients. A single sample of fish residue taken from a packing machine in processing factory Y also tested positive for the same strain. Following extensive testing it was not possible link the sources of all of the *Listeria* isolated from patients, fish and the refrigerators as a consequence of the time between the commencement of the outbreak and the start of the investigation.

The authors were however able to conclude that six of the nine cases were caused by the consumption of gravad or cold-smoked rainbow trout made by Processor Y and that the products from this fish processor were contaminated by multiple strains of *Listeria*; one of

which had caused the fatality. The same Swedish outbreak was further discussed by a later publication which reiterated that a single fish processing plant can spread multiple clonal varieties of *L. monocytogenes* (Tham *et al*, 2000). There is little new information regarding the outbreak in the later paper although it states that a Swedish local health authority had isolated the *Listeria* strain which caused the fatality during a routine inspection some six months before the outbreak, indicating that particular *L. monocytogenes* strains can colonise processing environments for extended periods of at least 18 months. Although not mentioned in the 1997 report, the later study revealed that the cold smoked trout and the gravad were vacuum packed.

An outbreak of febrile (fever-causing) non-invasive gastroenteritis following the consumption of *L. monocytogenes*-contaminated of smoked fish was described by Miettinen and colleagues (1999) in five otherwise healthy people. Stool samples were taken from all five patients and were tested for a range of standard gastrointestinal human pathogens, which initially excluded *L. monocytogenes*. However, when these initial tests revealed no obvious infections, the stool samples were examined for *L. monocytogenes* in a second round of testing which commenced one week after the onset of symptoms. The re-test swabs were found to contain *L. monocytogenes*. Interviews with all five of the patients revealed that they had shared a meal which included cold smoked rainbow trout purchased from a retail store. The store was investigated. The temperature measured in the retail chiller containing the smoked fish showed a 7^{o}C differential between the top and bottom of the unit. The fish were stored at the top of the fridge where the temperature was 11.6^{o}C. A sample from the same production batch of fish was tested for a wide range of human pathogens which included *L. monocytogenes* and was found to contain 1.9 x 10^{5} cfu *Listeria* /g fish. Serotyping revealed both the fish and human isolates were serotype 1/2a and further typing studies revealed that PFGE profiles of all strains were indistinguishable. The authors concluded that they had confirmed a foodborne case of febrile gastroenteritis caused by *L. monocytogenes*.

2.3.2 PREVALENCE OF *LISTERIA* IN WATER, ON RAW FISH AND READY TO EAT SMOKED FISH

2.3.2.1 WATER

Listeria is a ubiquitous environmental organism. It is commonly found in soil and vegetation, but has also been isolated from fresh and marine waters (Ben Embarek, 1994; Gram, 2001). Since it can be isolated from water it is possible that this can result in a contamination of fish as a raw ingredient and ultimately the processing environment and smoked fish. This section reviews literature relating to the prevalence of *Listeria* in water, raw fish and the final product. Run-off water represents the greatest influx of *Listeria* to surface waters such as lakes, streams and rivers. Catchments with rivers that have passed agricultural units have been shown to contain *L. monocytogenes* (Lyautey *et al*, 2007). Around 30% of ruminants in Britain shed *L. monocytogenes* in their faeces (Hutchison *et al*, 2004), which can potentially enter the water and river systems. Lyautey *et al*, 2007 observed that there was a correlation between the numbers of dairy farms in a catchment, which increased prevalence of *Listeria* isolations from surface waters. Furthermore, Lyautey and colleagues also observed that the presence of crops upstream of watercourse was also a risk factor for the detection of *Listeria* in surface water. The researchers hypothesised that the application of livestock wastes containing *Listeria* as soil and crop fertilisers, along with soil disturbances during the manure application, may have played a role in explaining their findings (Lyautey *et al*, 2007).

In contrast, several studies have shown not all surface water from agricultural areas is contaminated with *L. monocytogenes*. A Danish study did not find any *Listeria* in freshwater streams feeding into a number of Danish fish farms (Hansen, 2006). In Spain, Gonzalez *et al* (1999) did not detect *L. monocytogenes* in water samples collected from nine rivers. Furthermore, Jemmi and Keusch (1994) investigated *L. monocytogenes* contamination associated with three Swiss rainbow trout farms and water inflow from agricultural land. One farm in particular recorded a high level of *Listeria*-contamination. Results showed however that none of the water samples tested from each of the three farms contained *L. monocytogenes* (Jemmi and Keusch, 1994). Overall these observations suggest agricultural activity is a risk factor for *L. monocytogenes* contamination of surface waters, but that the correlation is not absolute as it is possible to farm without contaminating surface waters (Jemmi and Keusch, 1994).

The linkage between farming activity and surface water contamination by *L. monocytogenes* may be a dynamic one. Miettinen and Wirtanen (2005) examined 510 rainbow trout originating from fish farms in lakes and sea areas around Finland for the presence of *Listeria monocytogenes* and concluded that *Listeria* contamination of aqueous environments may be a phenomenon that occurs and disappears rapidly and sporadically. Therefore an accurate profile of *Listeria* contamination of surface water can only be undertaken over a period of time, during different seasons and a range of weather conditions. To a large extent, Miettinen and Wirtanen's findings highlight methodological flaws in a number of previous studies relating to water contamination either because the conclusions were based on small numbers of samples or samples were taken over short time intervals (Gonzalez *et al*, 1999; El-Shenawy and El-Shenaway, 1996; Watkins and Sleath, 1981; Colburn *et al* 1990).

Climatic conditions may have a strong influence on the probability of finding *Listeria* spp. in surface waters. The numbers of samples contaminated with *Listeria* spp. increase after periods of sustained, elevated rainfall or when sampling was performed immediately after a few days of intermittent rain during an otherwise dry period. In addition, during dry periods with clear (i.e. not muddy) water there were usually decreased numbers of contaminated fish. The primary contamination source for a fish farm may be a brook, river and other surface waters entering the farm following increased rainfall (Miettinen and Wirtanen, 2005).

There is a wealth of evidence that supports the theory that increased bacterial contamination of surface waters occurs after rainfall events (Mallin *et al*, 2009; Reifel *et al*, 2009; Sinclair *et al*, 2009; Stumpf *et al*, 2010). Studies have shown that even light rain falling on fresh faecal material can transport *E. coli* significant distances overland (Collins *et al*., 2005). The steeper the slopes of any hills above a watercourse, the greater distances that bacteria from animal manure can be transported (Collins *et al*., 2005). There appears to be no reports of similar transport mechanisms operating for *L. monocytogenes.* However, it is plausible that both soil-borne listeria and *L. monocytogenes* commonly contained in British livestock manures are simply washed into rivers and surface waters upstream of the fish farms.

In addition to contamination as a consequence of rainfall or agriculture, El-Shenawy and El-Shenawy (2006) found that in the Agba and Suez Gulf and the red sea, contamination of water with *L. monocytogenes* occurred mostly where there were cities or industrial/tourism activities. The finding led to speculation as to the impact of discharging of untreated sewage into seawater

and the practice having a possible role for the contamination of fish with *L. monocytogenes*. Consequently, water contamination may not be an issue only associated with fresh water fish farms. Indistinguishable *L. monocytogenes* biotypes have been isolated from river water and coastal sea water demonstrating that bacteria have the potential to be present on both fish farmed in freshwater and those that spend part , or all, of their life cycle in seawater (Miettinen and Wirtanen, 2005).

A recent report has shown that tidal water movements can influence the microbiology of estuaries (Solo Gabriele *et al*. 2000). In addition to observing *E. coli* increases in surface waters as a consequence of heavy rainfall, Solo Gabriele *et al* observed that *E. coli* numbers in estuaries varied in a cyclical pattern that correlated with the tidal cycles. The highest bacterial concentrations were observed during high tide, and the lowest were observed at low tide. Extensive sampling by the authors revealed that the cyclical pattern of increased and decreased *E. coli* numbers was caused by the rapid growth of *E. coli* within riverbank soils. The soils were subsequently washed by the water during high tide which released *E. coli* from the riverbank soils into the water. *E. coli* growth in soils of up to three orders of magnitude was found to be a function of increasing soil water content. It is not currently known if numbers of *L. monocytogenes*, a commonly isolated soil inhabitant, cycle in the same manner. However, *L. monocytogenes* can multiply in tidally-washed, coastal soils and river mouth waters are known to be highly contaminated by *Listeria* spp. (Sidorenko and Buzoleva 2007, Bou-m'handi and El Marrakchi 2002).

Although fish may be farmed/caught in water contaminated with listeria, the risk of fish developing an infection is relatively low. Temporary colonisation of the farm environment by *Listeria* has been observed (Miettinen and Wirtanen, 2005). However, the authors report that farmed rainbow trout were unlikely become infected with *L. monocytogenes*. The researchers isolated 12 strains of *L. monocytogenes* from farm water during their study; but only two out of these 12 strains were able to infect fish. Furthermore, even if the fish were colonised, the infections were transient and short-lived. Miettinen and Wirtanen (2005) considered it unlikely that shedding of *L. monocytogenes* into the faeces of infected fish significantly further increased environmental contamination on-farm.

2.3.2.2 RAW FISH

As discussed in section 2.3.2.1, it is clear that *Listeria* is often found in surface waters and other waters containing fish. Thus it is not unreasonable to assume that the external surfaces of fish swimming in contaminated water have the potential to become contaminated with *L. monocytogenes* and that fish therefore are not required to be infected in order to act as fomites (objects which spread contamination) for the transfer *L. monocytogenes*. This section reviews the literature relating to the prevalence of *Listeria* on raw fish surfaces prior to any smoking process.

A comprehensive study reported that 8.8% out of a total of 510 rainbow trout originating from fish farms in lakes and sea areas around Finland were contaminated with *L. monocytogenes* (Miettinen and Wirtanen 2005). The study tested pooled samples of five fish and determined that prevalence varied greatly between different fish farms. The measured range was from zero to 100% in pooled samples and from zero to 75% when a smaller number of fish were tested individually (Miettinen and Wirtanen 2005).

A Swiss study investigated two rainbow trout farms that used ground or spring water and different management practices and farm layouts. There were no *L. monocytogenes* found in 30 fish skin or 30 faecal content samples on one of the farms which used concrete-walled ponds and hygienic management regimes (Jemmi and Keusch 1994). However, when samples were tested from the other farm that used river water and natural (earth) walled ponds, five out of 15 skin samples and six out of 15 samples of faecal content were contaminated with *L. monocytogenes* (Jemmi and Keusch 1994).

However, no *L. monocytogenes* were isolated from any samples collected for a study that reported the prevalence in salmon in a Norwegian coastal fish farm (Ben Embarek, 1994). Although only ten fish were collected for testing, the fish gills, skin and viscera were individually tested. Furthermore, a previous Finnish study that sampled 55 fish from six farms by cutting out 25 g of the neck region including the gills found them to be all negative for *L. monocytogenes* (Johansson *et al*. 1999). Similarly, another Finnish study only found one out of 60 rainbow trout samples was contaminated with *L. monocytogenes* (Autio *et al*. 1999). Medrala *et al* (2003) found that only six out of 72 samples of Norwegian coastal cage bred salmon and sea trout entering a Polish processing plant contained *L. monocytogenes*.

A number of wild fish species (e.g. salmon, whitefish sablefish) from a variety of sources (e.g. Norwegian salmon, West coast USA salmon, Chilean salmon) were collected and their skin surfaces tested for *L. monocytogenes* by Hoffman and colleagues (2003). Differences were noted when the raw fish test results were scrutinised. Although only 14.6% of the raw fish harboured *L. monocytogenes* on their external skin, there were species- and source- specific differences between the isolations. Wild west USA coast salmon had a prevalence of 29.5%, whereas only 3.6% of wild sablefish samples were contaminated. Hoffman and colleagues advised that careful selection of raw fish from sources associated with low *L. monocytogenes* counts could help prevent reduce *L. monocytogenes* of raw fish entering smoking plants.

A study carried out in Portugal that examined trout and salmon samples from the production lines of three cold smoking plants detected *L. monocytogenes* in 25 out of 183 samples (13%) (Duarte *et al*. 1999). However, this study collected samples during processing, rather than before the commencement of processing, so there is a possibility the fish were contaminated from the plant environment and that the real prevalence may have been lower. Miettinen *et al*, (2001), determined, as part of a large study involving 28 fish processing factories in Finland, a prevalence of 18.8% (2/11) for *L. monocytogenes* detections in raw fish of undesignated species.

It is apparent from the literature that, the prevalence of *L. monocytogenes* on raw fish tends to be low, but can be variable. Table 2 shows a summary of reported prevalences of *L. monocytogenes* from raw fish. A study by Gram (2001) found that *L. monocytogenes* contamination on raw fish entering fish processing plants varied greatly from 2% of all trout batches examined entering processing plants in Switzerland up to 79% of salmon batches entering a single USA plant. This variation could be due to a number of factors, such as farm practices, surface waters, weather conditions at harvesting, hygienic practice. Miettinen and Wirtanen (2005) suggested during their study that the section of the fish carcass that is sampled could also be important when determining prevalence. Out of 510 fish, 43 gills tested positive for *L. monocytogenes*, compared with only 1 positive test for skin samples and 1 positive visceral test. Thus, since the majority of papers reporting prevalence used fish surface samples, the historically-reported prevalences may have been under-estimated.

In addition to different prevalences, the numbers of *L. monocytogenes* associated with different areas of farmed fish carcasses also show statistically significant differences. In rainbow trout (Miettinen and Wirtanen 2005), gill samples have significantly higher numbers when compared

with skin samples. The findings may be consequences of the large volumes of sporadically-contaminated water that are filtered through fish gills (Miettinen and Wirtanen 2005). In Turkey, *Listeria monocytogenes* has been frequently isolated from both gill (25%) and skin (52%) samples of raw freshwater and marine fish (n=30; Yucel *et al* 2010), which included brown trout and horse mackerel. A survey of raw crawfish in the USA (Thimothe *et al*. 2002) found that 29.5% contained *Listeria* spp. and 1% *L. monocytogenes*. When fish are contaminated with low numbers of *L. monocytogenes* or when contamination is sporadic, the literature appears to suggest that there may be merit in selective sampling of the gills. There is evidence that gill-filtered water containing small quantities of *L. monocytogenes* will concentrate the bacterium on the gill gas-exchange surfaces (Miettinen and Wirtanen, 2005).

The sporadic nature of reports of *L. monocytogenes* isolations from fish in waters containing *L. monocytogenes* could be due to physiological differences in the fish. Lie *et al* (1989) undertook biochemical profiling of the fish organs and surface mucus of 13 species including trout and salmon and observed lysozyme activity in almost all of the samples and species examined. However, there was species-specific variation in the form of lysozyme isozymes as well as in the assayed antibacterial activity. Most strikingly, there were differences between rainbow trout and Atlantic salmon with "lysozyme activity in the former at least 20 times greater than in the latter" (Lie *et al*, 1989). Furthermore, in rainbow trout, the kidneys appeared to have the highest lysozyme levels, followed in descending order by alimentary tract, spleen, skin mucus, serum, gills, liver and muscle. Lie and colleagues concluded that lysozymes play an important role in infection control in fish. The relatively low concentrations of lysozyme in gills, coupled with the potential concentration of pathogens during filtration of the water, may be a contributory factor to reports of higher isolations from these organs. However, to date there is no published data confirming or investigating this potential theory. Also it should be remembered that, due to reporting of differences in lysozyme type and activity mean that observations made for one fish species regarding *L. monocytogenes* contamination of farm water poorly correlating with fish infections, do not necessarily apply across different fish species.

Another USA study (Pao *et al*. 2008) sampled fresh fish fillets for *Listeria spp.* (including *L. monocytogenes*) from local markets (Virginia) and nationwide (internet purchased). Overall, 9.3% of local samples and 15.9% of the internet samples were positive for *L. monocytogenes*. In

salmon, the prevalence of *L. monocytogenes* was similar for both markets (9.4% local market vs. 11.8% internet market).

Table 2 A summary of the reported prevalences of *L. monocytogenes* contamination of raw fish

Fish species	Origin	Sampling site	Prevalence	Reference
Rainbow trout	Switzerland	Skin	5/45 (11.1%)	Jemmi and Keusch 1994
Rainbow trout	Finland	Skin	1/60 (1.7%)	Autio *et al*. 1999
Brown trout and horse mackerel	Turkey	Skin Gill	52% 25%	Yucel *et al* 2010
Salmon and salmon trout	Portugal	Skin, belly cavity	25/183 (13.7%)	Duarte *et al*. 1999
Salmon	Norway	Gills, skin, viscera	0/10 (0%)	Ben Embarek *et al*. (1994)
Rainbow trout	Spain	Gills, skin, viscera	0/30 (0%)	Gonzalez *et al*. (1999)
Brown trout	Spain	Gills, skin, viscera	0/12 (0%)	Gonzalez *et al* (1999)
Salmon and sea trout	Norway	Tissues	6/72 (8.3%)	Medrala *et al*. (2003)
Rainbow trout	Finland	Gills, skin, viscera	45/510 (8.8%)	Miettinen and Wirtanen 2005
Salmon, whitefish, sablefish	USA	Skin	46/315 (14.3%)	Hoffman *et al*. 2003
Raw fish (species not given)	Finland	Not given	2/11(18,2%)	Miettinen *et. al.* (2001)
Raw fish filet from local market (Virginia) **-salmon** **-trout** **-tilapia** **-catfish**	USA	25 g of whole fillet	 9.4% 2.8% 2.8% 22.2%	Pao *et al*. 2008
Raw fish fillet from internet market (Nationwide) **-salmon** **-trout** **-tilapia** **-catfish**	USA	25 g of whole fillet	 11.8% 8.8% 18.8% 25%	
Crawfish	USA	25g whole fish	0%	Thimothe *et al*, 2002

2.3.2.3 READY-TO-EAT SMOKED FISH

Listeria is commonly associated with smoked fish products (Gram, 2001) and there have been numerous studies which have determined the prevalence of *Listeria monocytogenes* in smoked seafoods. A summary of the reported prevalences of *Listeria* in smoked fish is discussed in brief here and the publications cited in this section are summarised in Table 3.

An early survey of the prevalence of *L. monocytogenes* in fish was undertaken by Dillon *et al* (1994) who purchased 258 random samples of smoked fish (cod, mackerel, caplin, eels, herring, salmon, charr, trout and turbot) from retail outlets in Newfoundland, Canada. Of these, 12 samples contained *L. monocytogenes* as determined by enrichment (4.65% of samples were positive). Samples of both hot smoked (cod) and cold smoked (herring) were found to be contaminated with *L. monocytogenes*. Dillon and colleagues were interested in the observation that hot smoked fish was contaminated because they believed that the hot smoking process should have been sufficient to kill the *L. monocytogenes* and, consequently, investigated further. Although not fully conclusive, the final discussions of the authors were that the *L. monocytogenes* contamination in the final product was due to local producers not adhering strictly to their hot smoking protocols (e.g. not moving fish partway through smoking to ensure even heat and smoke penetration) or that there was another unidentified source of contamination in the handling chain downstream of the hot smoking process.

Around the same time, Ecklund *et al.* (1995) enumerated *L. monocytogenes* from 48 finished products collected from six different processing plants in the NW of the USA. The samples collected were considered likely to be contaminated with *L. monocytogenes*. *L. monocytogenes* populations ranged from 0.3 to 34.3 cells /g, with a mean of 6.2 per g and a median of 3.2/ g. Since the Ecklund work selected samples from plants which were likely to have been contaminated by *L. monocytogenes*, it is questionable whether the reported numbers and incidence are typical of those which would be determined from a random selection of samples.

At the same time as the Ecklund and Dillon studies were being undertaken, Heinitz *et al* (1998) examined 1,080 smoked finfish and shellfish between 1991 and 1995 from processors and distributors, again in the United States. In contrast to the Ecklund work, the samples were selected without making consideration of the likely status of the product and so the Heinitz survey results should be considered to be less biased with respects to the prevalence of *L. monocytogenes* contamination. The Heinitz results showed that, for the samples where a

smoking method was identified, 51 out of 240 (17.5%) cold smoked samples contained *L. monocytogenes* compared with 19 out of 215 (8.1%) hot smoked samples. From samples imported into the US from the UK, Heinitz reported that of 20 out of 124 (16.1%) contained *L. monocytogenes*. A slightly increased number of positive samples were detected in smoked fish imported into the USA from Norway; 31 out of 131 (23.7%; Heinitz *et al*, 1998).

Survey work to determine the *L. monocytogenes* prevalence for smoked fish products has also been undertaken outside of the USA. Samples of smoked and cold-salted fish products (n=110) were initially collected at retail in Finland in 1996 and tested for *L. monocytogenes* (Johanssen *et al*, 1999). *L. monocytogenes* was isolated in 20% (22/110) of the samples from the retail market, with ten of these positive samples containing *L. monocytogenes* at >100 cfu/g, 17% (5/30) of cold-smoked fish and a single hot-smoked fish product (2%) were found to be positive by enrichment. Later work, using far less sample collections, was undertaken by Miettinen *et al* 2001 as part of a larger study that included 28 fish processing factories. Miettinen *et al* 2001 reported that 2/17 (11.8%) cold smoked fish and 1/8 (12.5%) hot smoked fish were positive for *L. monocytogenes*. Jin *et al* (1994) detected *L. monocytogenes* in 12 samples of cold smoked salmon out of 76 (15.7%) in Japan. A later Japanese retail survey of however, recorded a significantly lower prevalence, with 5 out of 92 samples of smoked salmon (not specified whether hot or cold smoked) bought from retail outlets in Japan containing *L. monocytogenes*; all at levels of below 10 cfu/g (Inoue *et al*, 2000). A third Japanese study (Nakamura *et al*. 2004) found that 12/66 (18%) of samples of cold smoked salmon and trout purchased at retail contained *L. monocytogenes*. Recently, also in Japan, Miya *et al*. (2010), found that 1/33 (3%) samples of cold smoked salmon contained *L. monocytogenes*. Thus, there has been a range of prevalences reported from Japan with two surveys reporting low prevalences (<4%) and two reporting prevalences of at least 15%. None of the later surveys makes any attempt to explain the differences between the reported prevalences of *L. monocytogenes* in smoked fish. Overall, the Finnish and Japanese prevalences are broadly similar to those reported in the USA

In broad agreement with the findings of Inoue *et al* (2000) report, low numbers of *L. monocytogenes* were recorded in positive smoked fish samples collected by Ecklund *et al*. (1995). Ecklund and colleagues reported that *L. monocytogenes* populations in positive samples ranged from 0.3 to 34.3 cells per g, with a mean of 6.2 per g and a median of 3.2 per g. The recurring theme of contamination typically comprising low numbers of *L. monocytogenes* was reinforced by Cabedo *et al* (2008) who collected 89 retail and food industry samples of smoked

salmon (not specified whether hot or cold smoked) and found that seven of them contained *L. monocytogenes*. For each of the seven positive samples, *L. monocytogenes* was present at a concentration of between 10 and 100 cfu/g.

Jorgensen and Huss (1998) sampled both cold smoked and hot smoked fish from producers in Denmark. A total of 64 out of 190 samples (34%) of cold smoked salmon tested positive for *L. monocytogenes* and nine out of 20 samples (45%) of cold smoked halibut samples were also positive. In contrast, only four out of 74 samples (5%) of heat-treated seafood(which included hot smoked fish, fish patés and fish cakes) contained *L. monocytogenes*. Although the Danish prevalences appear to be much higher than those reported in Finland and the USA, a comparatively small number of samples were collected Denmark.

For the 64 cold smoked salmon samples contaminated with *L. monocytogenes,* Jorgensen and Huss (1998) determined that 53 contained less than 10 cfu/g of *L. monocytogenes*. Of the remaining positive samples, nine contained between 10 and a 100 cfu/g and two contained between a 100 and a 1000 cfu/g (Jorgenesen and Huss, 1998). The fish tested by Jorgensen and Huss (1998) were processed by one of ten participating plants. Three of these plants produced smoked fish where no contamination was detected whereas two producers were found to have all of their tested lots contaminated with *L. monocytogenes* (Jorgensen and Huss, 1998). Detections in the remaining plants were sporadic between different batches of fish. A major conclusion of the Jorgensen and Huss (1998) study was that it was possible to produce cold smoked salmon without detectable *L. monocytogenes* contamination. However there was great variation between the abilities of different producers to do so; even when using the same raw materials.

Loncarcevic *et al* (2006) isolated *L. monocytogenes* from 12 of 58 'gravad' rainbow trout fish samples, three of 26 cold-smoked rainbow trout and one of the 66 hot-smoked rainbow trout sample that were surveyed in Sweden. Although the prevalences for the cold smoked fish were significantly lower than those reported for other countries, levels of *L. monocytogenes* of greater than 100 cfu/g of fish was found in 10 of the 16 positive samples. Furthermore, the highest numbers of *L. monocytogenes* (132,000/g fish) was found in the sample of hot-smoked rainbow trout. The *L. monocytogenes* serogroup 1/2 was most frequently found, followed by 4 and 3. The authors did not extensively discuss the likely sources of *L. monocytogenes* on the fish, although they considered that the high numbers of *L. monocytogenes* isolated from the hot

smoked trout were most likely the result of post smoking contamination. The temperatures achieved during hot smoking were generally considered to be high enough to control *L. monocytogenes*, providing further speculation that post smoking contamination of the product may have been an issue.

Couvert *et al* (2010) tested a total of 551 batches of vacuum-packed cold smoked salmon processed by ten French manufacturers between June 2006 and June 2007 by enrichment. Couvert and colleagues observed 42 batches contained *L. monocytogenes* (7.6% positive). Latorre *et al* (2007) and Di Pinto *et al* (2010) reported the results of various foods sampled at retail in Southern Italy over a 12 year period between 1993 and 2004. The involved study of Latorre and colleagues determined that smoked salmon had the highest prevalence of *L. monocytogenes* (10.6%) over the period in the region sampled. However the prevalences reported by the second Italian study by Di Pinto *et al* (2010) undertook detection to determine that 45 out of 132 (34.1%) smoked salmon samples collected from supermarkets in Southern Italy from February 2007 to January 2009 contained *L. monocytogenes*. The Di Pinto *et al* (2010) study did not discuss possible reasons for reporting a threefold increase in prevalence compared with the earlier work of Latorre *et al* (2007). Both studies were based in the same general region of Italy.

Gonzalez-Rodriguez *et al* (2002) purchased and examined 30 samples of vacuum-packed cold-smoked salmon and 24 rainbow trout fillets from two large chain supermarkets in Leon (NE Spain). The salmon had been imported from Norway and Scotland and the Rainbow trout had been reared in farms located on Spanish river systems. The samples were homogenised and subjected to a two-step enrichment process to detect *L. monocytogenes*. None of the samples were found to contain *L. monocytogenes* (Gonzalez-Rodriguez *et al* 2002). A later survey, again retail based in Northern Spain, collected 102 samples of smoked salmon and 40 of smoked trout from supermarkets from 2003 to 2005 (Garrido *et al* 2009). This survey determined that 11 and 25 % were contaminated with *L. monocytogenes*, for smoked salmon and smoked trout respectively. Of the 11 contaminated samples of vacuum packed smoked salmon four contained *L. monocytogenes* at a level greater than 1000 cfu/g. Likewise of the ten positive trout samples, four contained *L. monocytogenes* at a level greater than 1000 cfu/g. The findings of Garrido *et al* (2009) were broadly supported by a third Spanish study (Vitas *et al* 2004) which reported 28/100 (28%) of cold smoked salmon samples contained *L. monocytogenes*. Taken together, the findings of the three Spanish studies are similar to that reported for the Italian

studies since for both countries the earlier studies show very low or no prevalence, but later ones report a significantly increased prevalence, typically with no reason offered by the later authors for the increases to the *L. monocytogenes* isolations. A potential criticism of the earliest Spanish survey is that very few samples were collected.

A survey of *Listeria* contamination in RTE foods in Austria (Wagner *et al* 2007) found 20% of 88 smoked fish samples (mixture of salmon, trout, mackerel and other species) contained *L. monocytogenes*. Furthermore, of these positives, 6% contained more than the 100 cfu/g EU statutory limit for ready to eat foods (Regulation (EC) 2073/2005).

In summary, there are a range of *L. monocytogenes* prevalences which have been reported from a wide selection of countries. Frequently, there is no general agreement between prevalences; even when surveys have been undertaken in geographically-close regions during the same time interval. A general theme is that earlier surveys tend to show lower prevalences that more recent ones. None of the survey authors make comment on or attempt to explain why there are sometimes marked the differences between their prevalences and those reported by previous studies. One commonly-encountered reason for increasing detections of bacteria in microbiology is improvements in the laboratory methodologies. There is however, no evidence that is the reason why there is a trend towards increasing prevalences for the more recent surveys.

Table 3 A summary of reported prevalences of *L. monocytogenes* isolated from smoked fish.

Region	Product Type	Prevalence (%)	Mean *L. mono* count (cfu /g fish)	Range (cfu /g fish)	Reference
New Zealand	Cold smoked salmon	9/12 (75)	ND	ND	Hudson *et al* 1992
Canada	Cold smoked salmon	10/32 (31.2)	ND	ND	Farber 1991
Italy	Cold smoked salmon	0/37 (0)	ND	ND	Valenti *et al* 1991
Iceland	Smoked salmon	3/13 (23)	ND	ND	Hartemink and Georgsson 1991
NW USA	Cold smoked salmon		6.2 (n=48)	0.3 -34	Ecklund *et al* 1995
Norway	Cold smoked salmon	3/33 (9)	ND	ND	Rørvik and Yndestad 1991
Switzerland	Cold smoked salmon	4/64 (6.3)	ND	ND	Guyer and Jemmi 1990
Switzerland	Cold smoked salmon	24/100 (24)	ND	ND	Jemmi 1990a
Spain	Cold smoked salmon	28/100 (28)	ND	ND	Vitas *et al* 2004
Japan	Cold smoked fish (salmon and trout)	12/66 (18)	ND	ND	Nakamura *et al* 2004
Switzerland	Cold smoked fish	44/324 (13.6)	ND	ND	Jemmi 1990b
Canada	Cold smoked fish	31/71 (4.4)	ND	ND	Dillon and Patel 1992
Switzerland	Cold smoked fish	49/434 (11.3)	ND	ND	Jemmi 1993
Finland	Cold smoked fish	2/17 (11.8)	ND	ND	Miettinen *et al* 2001
Finland	Cold smoked fish	5/30 (17)	ND	<100 - 13700	Johanssen *et al* 1999
Switzerland	Hot smoked fish	44/496 (8.9)	ND	ND	Jemmi 1990b
Finland	Hot smoked fish	1/50 (2)	ND	<100 - 13700	Johanssen *et al* 1999
Peru	Smoked marinated fish	3/32 (9)	ND	ND	Fuchs and Sirvas 1991

	(ceviche)				
New Zealand	Smoked mussels	5/14 (35.7)	ND	ND	Hudson *et al* 1992
Finland	Hot smoked fish	1/8 (12.5)	ND	ND	Miettinen *et al* 2001
Austria	Smoked fish (salmon, trout and mackerel)	18/88 (20)	ND	5/88 had counts >100 cfu/g	Wagner *et al* 2007
Italy (Samples from the EU including Scotland)	Smoked fish (salmon and swordfish)	6/50 (12)	ND	<10 to 11,660	Meloni *et al*, 2009
Japan	Cold smoked salmon	3%	ND	ND	Miya *et al* 2010

ND denotes that a result was not determined.

2.3.3 THE FISH SMOKING PROCESS IN THE UNITED KINGDOM

Fish smoking in the United Kingdom is undertaken in either one of three different ways: cold, hot or chemically smoked. Traditionally, fish are either hot or cold smoked using the smoke from burning or smouldering hardwood (commonly oak) logs or chips. The amount of exposure that the fish get to the heat generated by the burning wood determines whether the fish protein denatures (cooks). The temperature and the exposure time to smoke are the primary differences between hot and cold smoking (Arvanitoyannis *et al*, 2009; Table 4).

More recently, chemical smoking using wood smoke condensate has become available. Referred to anecdotally as mechanical smoking, fish are loaded into a sealed vessel (which can be heated or not). Smoky air is blown across the fish to flavour the flesh. The smoky air is generated either by using condensate-derived smoke (solid or liquid) or by burning hardwood in a separate compartment which is external to the kiln holding the fish. Mechanical smoking attempts to mimic the temperature ranges found inside traditional hot and cold smokers. The primary advantage of mechanical smoking is that it can provide a more consistently reproducible product as a consequence of electronic control over kiln temperature and air flow/smoke application (if smoke condensate is used). For mechanical smoking, the key factor for reproducibility is not to vary the smoke condensate source and composition, since condensate change influences the sensory attributes and shelf-life of the finished product (Martinez *et al*, 2007).

A summary of typical temperatures and processing times for each of the three smoking methodologies, along with their relative general strengths and weaknesses is shown below as Table 4. The parameters should be considered indicative because different fish species and masses of fish influence the temperatures and intervals required for their processing. While smoking practices in each establishment will vary, due to fish species, volumes smoked, business size and type and smoking method, most do tend to follow similar production stages. A rough outline of the process stages is shown in Figure 1, although it should be remembered that not all businesses will use all stages and there may be some slight differences in the order.

Briefly, raw fish enters the processing plant in either a chilled or frozen state. The fish is kept refrigerated until it is processed. If the fish is frozen, it will be allowed to thaw during the refrigerated storage. If not already done so, the fish will be gutted, filleted and rinsed. Depending on the type of final product, the fish may or may not be skinned at this stage. The

fish are then prepared for salting which can be done using brine or dry salt at a time/concentration combination to achieve the desired salt content for that particular product. The fish are then smoked (cold, hot or mechanically) before being prepared for packaging and final sale.

Table 4 A summary of common fish smoking methods.

Smoking method	Typical characteristics	Relative advantages	Relative disadvantages
Hot smoked	Drying 20°C - 30°C for 0.5 - 1h Smoking at 60°C-90°C for 1-1.5h	Rapid process, can be done in 2hrs Generates a ready to eat product Significant microbial kill	Denatured protein causes undesirable flesh colour Short maintenance intervals for equipment
Cold smoked	Drying (or optional weak smoke) at 25°C-35°C for 1-2h Smoking at 21°C-30°C for 6-48h	Little change to the flesh colour Improved product odour Less nutritional changes Long maintenance intervals for equipment	Diminished microbiological kill Does not kill parasites Can take two days to make the finished product
Mechanical smoked	A sealed oven where either smoke condensate or burning wood in an external chamber is blown over the heated or unheated product. Mechanical smoking strives to mimic either the hot or cold smoke exposure times and temperatures provided above.	If heat is generated by the oven, it enhances microbial kill. Exceptionally rapid smoking time	Variable microbiocidal activity possibly dependent on phenol concentration of smoke condensate

Adapted and updated from previously published information (Arvanitoyannis *et al*, 2009; Sunen, 1998; Milly *et al*, 2008)

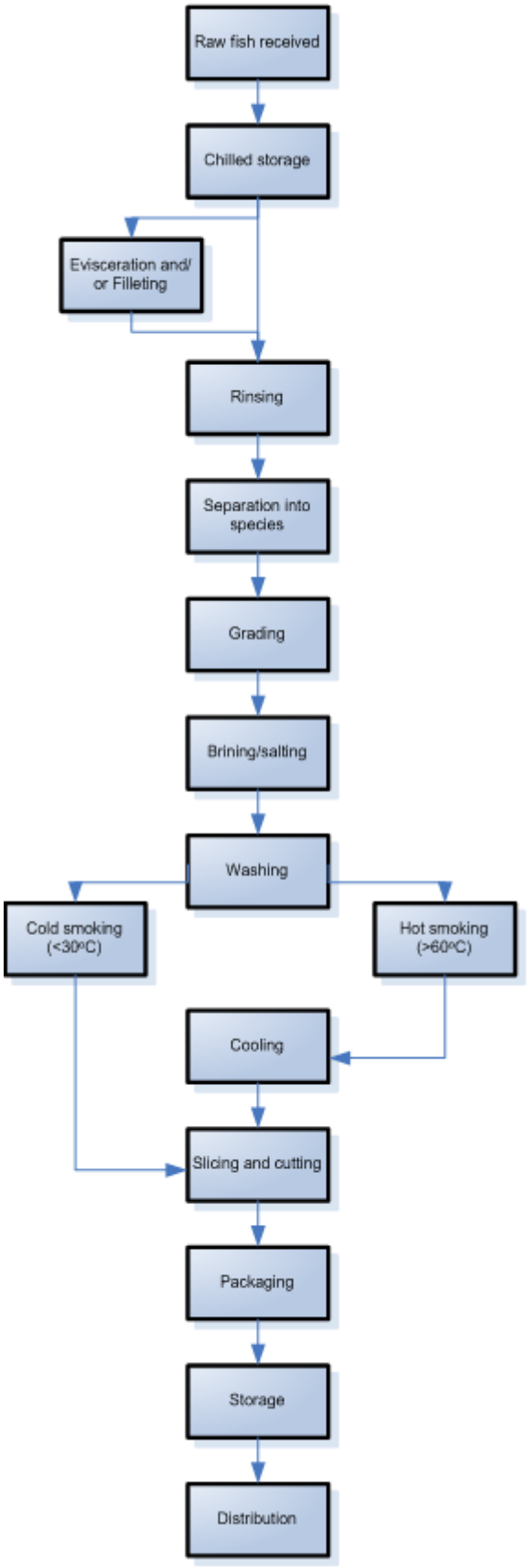

Figure 1 A general flow diagram depicting the main process stages involved in the production of smoked fish

2.3.3.1 A DETAILED OVERVIEW OF THE TRADITIONAL HOT AND COLD FISH SMOKING PROCESSES IN SCOTLAND

A more detailed overview of the smoking process as typically carried out in Scotland is presented in this section. A case study of a small throughput traditional smoking establishment visited as part of the study is also given to show the steps in practice.

The first stage of processing is evisceration. On the NE coast of Scotland, most wild fish sourced from marine fisheries are landed at Aberdeen or Peterhead with very small amounts sourced from smaller ports such as Arbroath, Crail and Anstruther. The majority of the fish are typically eviscerated on the fishing boat soon after they are caught. Even allowing for the reduced amount of discarded, inedible material; eviscerated fish cost more per kilo than those that have not been gutted. Thus on-boat evisceration is popular because it is a way to increase the value of a catch. In addition, chilling and freezing costs on the boat are reduced if the viscera are removed because the mass to be chilled is lowered. For those reasons, evisceration of sea-caught fish does not commonly take place in smoking plants. For farmed fish such as salmon, the raw fish may arrive at the plant either pre-eviscerated or with the viscera intact. For fish that are destined for smoking, there are differences in how the fish are filleted depending on their evisceration status. Fish with the viscera intact are filleted using the block filleting method. In brief, block filleting involves slicing the meat away from the bone with the bone and viscera then discarded. If the fish arrive pre-eviscerated, then angel-style filleting is used to remove the bone out of the meat. Block filleting results in two individual fillets, angel filleting results in one fillet with the two halves still joined. Filleting can be done mechanically or by hand. Fish generally arrive with their heads intact (Figure 2). The head is removed by cutting through just behind the gill slit.

After evisceration and filleting, the fish are optionally skinned. Automated skinning involves passing the fillet through a skinning machine that slices a thin section of skin from the surface of the meat (Figure 3). Skinning machines have a variety of designs but it is common for them to consist of a spinning blade inside a stainless steel housing. There is a very narrow gap between the underside of the blade and the surface of the housing which is the thickness of the skin to be removed. The small gap can become clogged with detritus during routine use and skinning machines typically require frequent rinsing. It is not uncommon for skinning machines to be completely disassembled and the blade removed for effective cleaning and sanitation.

After the optional skinning, the fish are briefly washed in potable water before brining or salting (Figure 4 and Figure 5). The primary purpose of salting or brining is to preserve the fish and increase its shelf life. A further benefit of brining is that it promotes the formation of a pellicle on the fish flesh which helped prevent lipid loss during hot smoking. Brining also improves the aesthetic appearance of fish, causing the surface of the meat to become shiny and glazed.

Whether fish are treated with finely ground salt crystals or liquid brine is decided by the smoking method and how the fish are supported during smoking. Fish that are tied in pairs for smoking, are tied to a smoking frame or that have their skin intact will normally be processed using salt crystals. It is common for salt crystals, equivalent to roughly 10% of the mass of the fish to be cured, to be evenly layered between layers of fish. Salt crystals cause the surface of the fish to become less slippery and make it less likely the fish will slip out of the tied string or off the smoking frame. Curing using salt crystals is undertaken for 30 minutes to an hour. The use of dry salt curing reportedly produces a sensorially-improved cold smoked fish product when compared with liquid brine (Muratore *et al* 2007). Generally, lowering the amount of salt added increases the required curing time. For liquid brining, it is common for salt concentration of the brine to be near the point of saturation (around 13% w/v at 5^{o}C). At such concentrations, the fish typically will be cured in a period of less than one hour.

Fish that are cured using salt crystals are normally rinsed by immersion into a volume of water. Fish cured in brine are generally not rinsed before being hung for smoking. In both cases, the fish are allowed to drip dry before the commencement of smoking (Figure 6 and Figure 7) hot smoking, the crystal salt cured and water rinsed fish are either tied together at the tail end and laid over batons of oak with tapered (triangular) sides (Figure 8) or otherwise fixed to a smoking frame (e.g. by the use of tenterhooks). The oak batons are called smokie sticks. Wood is normally used because it does not heat significantly during hot smoking. In contrast to metal, wood does not burn fish. The position of the fish are changed halfway through the smoking to ensure they are all evenly flavoured and cooked.

Figure 2 Haddock delivered to a smoking plant with their heads intact and pre-eviscerated (arrow)

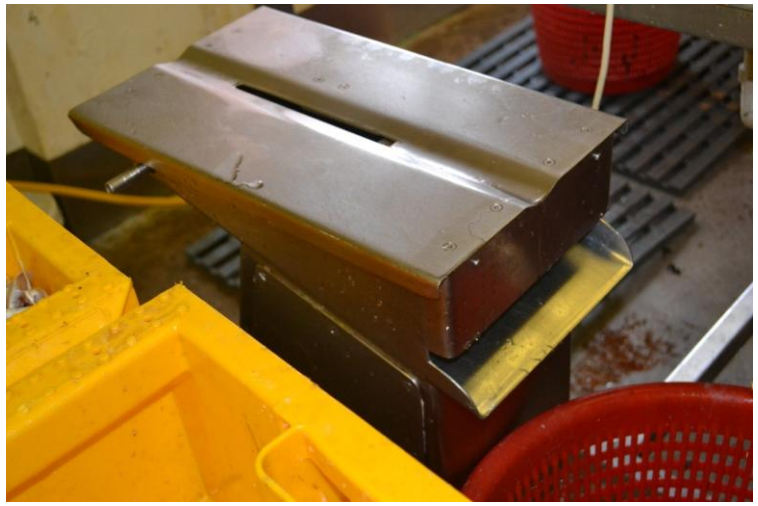

Figure 3 A fish skinning machine

Figure 4 Skinned fish soaking in 13% (w/v) brine

Figure 5 Fish curing using salt crystals

Figure 6 Skin on haddock being dried prior to hot smoking.

Figure 7 Skinned, dyed (colouring E102; front) and not dyed (rear) haddock being dried on metal Finnan sticks prior to cold smoking

Figure 8 Hot smoking of tied haddock pairs over a fire pit

For cold smoking, fish are laid across stainless steel supports called Finnan sticks to dry (Figure 7). Metal supports are used for cold smoking because they last longer and are more easily cleaned. As is the case for hot smoking, the positions of the fish are changed halfway through smoking to ensure the product attains an even flavour. Cold smoking takes 4-5 hours routinely or overnight for a very strong flavour. After smoking, cold smoked product are immediately refrigerated. Hot smoked product requires a cooling stage of 30 minutes to 1 hour before it is packed and refrigerated.

CASE STUDY OF A SCOTTISH FISH SMOKER

A small throughput establishment that carried out hot and cold smoking was visited as part of this review. There were no discernible differences between the generic description of fish preparation (i.e. evisceration, filleting and salting/brining as described above) and the practices undertaken at the plant visited. For both hot and cold smoking, fish were flavoured by burning or smouldering oak or apple hardwood. The wood was chipped for cold smoking and either chipped or cut into small (20cm x 5cm x 5cm) logs for hot smoking. Kerosene firelighters were used to start the logs burning for hot smoking and sufficient time for the kerosene to burn off was allowed before exposing the fish to the flames. Skin-on fish were hot smoked for 1-1.5 hours. At the plant visited, a small tray (50cm x 30cm x 5 cm) of wood chips (300-400g) was placed at the base of the cold smoking kiln. A shovelful of embers from the hot smoke kiln was placed into the tray to initiate smouldering of the chips. Cold smoking kilns at the plant had doors which are closed and had adjustable vents to prevent excessive oxygenation of the chips (which could cause the wood to ignite and result in hot smoking).

2.3.4 THE EFFECT OF SELECTED PROCESSING STAGES ON *L. MONOCYTOGENES* GROWTH AND SURVIVAL

A number of studies have attempted to determine the microbiological consequences of raw fish as it progresses through the various stage of processing to become the final smoked product. This section reviews the effects of that the steps in the smoking process can have on *Listeria monocytogenes* survival and growth.

Generally, when reviewing literature, a greater value is attached to those studies that are undertaken under commercial processing conditions, as compared with lab-based work. However, it has been shown that plant environmental *L. monocytogenes* can contaminate fish during processing (as described in this report, section 2.3.4). Therefore, plant based studies are

likely to represent the effects of each processing stage to the microbiota of the fish as well as an unpredictable and plant-specific effect related to plant environmental contamination. The unpredictable environmental contribution in combination with the comparatively few numbers of publications describing the effects of each processing stage means that laboratory-based studies are considered here to be more reliable indicators of the microbiological consequences of each processing stage for this section of the review.

2.3.4.1 CHILLED STORAGE AND FREEZING

Large quantities of seafood are harvested from cold water, therefore the microflora associated with fish tends to predisposed to low temperature survival and growth may not be inhibited as effectively by refrigeration as the microflora associated with other foods (Nickelson *et al*., 1980). Adequate refrigeration for seafood is consequently more important for controlling both spoilage organisms and those of public health concern compared with other animal-derived foodstuffs.

There is little evidence that prolonged frozen storage, either of the raw fish or packed product results in significant reductions to *L. monocytogenes* numbers. Furthermore, sub-zero temperatures do not cause sufficient injury to *L. monocytogenes* cells to affect their subsequent regrowth at chill temperatures after thawing fish (Gram,2001, El-Kest et al1991). Gram (2001) hypothesised that the high lipid concentrations found in fish flesh may protect bacteria against sub-zero temperatures and ice formation damage. As might be expected, given the observation that long-term frozen storage does not significantly affect *L. monocytogenes* viability, unpublished preliminary work undertaken at the Danish Institute for Fisheries Research (cited by Gram, 2001) determined that the growth of *L. monocytogenes* in cold-smoked salmon was not significantly affected by the ice formation at the point of freezing.

There appears to be little in the published literature relating to the common practice of freezing raw fish prior to smoking, and any consequences to *L. monocytogenes* numbers after smoking. However, there is limited information in related areas. For example, 250 samples of frozen sushi from supermarkets and fresh sushi from sushi bars in Northern Germany were surveyed for *L. monocytogenes* contamination. Only three samples were found to contain *L. monocytogenes* (Atanassova *et al*. 2008) and all three isolates were from fresh sushi. Generally, when other hygiene indicators such as *Escherichia coli* were determined; the fresh sushi was of a poorer microbiological quality than frozen sushi. Evidence of this type however is at best considered anecdotal because although sushi is raw fish, the foodstuff is minimally processed

before consumption. Thus, it may not be appropriate to interpret differences between fresh and frozen fish used for sushi as a relevant indication of the status of fresh or frozen raw fish destined for smoking. Atanassova and colleagues concluded that the sushi processing stages may influence the microbiological quality of the sushi and that the final product status was dependant on the skills and habits of the cooks preparing it.

In conclusion there is quite a strong body of evidence that shows *L. monocytogenes* on fish flesh is largely unaffected by freezing, even for extended periods of time.

2.3.4.2 SALTING AND BRINING

Vogel *et al*. (2010) recently reported that exposure of *L. monocytogenes* to sodium chloride helps the bacterium endure long-term desiccation. The authors speculate that stress-response genes required for *L. monocytogenes* to survive desiccation are up-regulated/activated upon exposure to salt. Thus brining may pre-dispose *L. monocytogenes* to extended survival in the plant environment. This finding is quite important because the authors believe that one of the reasons why *L. monocytogenes* persistently survives in smoked fish plants is because it is resistant to desiccation.

There are earlier reports which support a theory of resistance to desiccation as an important survival factor for *L. monocytogenes*. Jensen et al. (2007) reported that there was an unknown protective mechanism and enhanced adherence and aggregation for *L. monocytogenes* when growing as a biofilm exposed to sodium chloride. In combination, the Vogel and Jensen studies support a supposition that *L. monocytogenes* strains exposed to salt during brining become predisposed to biofilm formation as a consequence of the exposure. *L. monocytogenes* growing as biofilms have enhanced resistance to stresses such as drying results in improved survival. Further evidence to support such a supposition is provided by Tomkin (2002) and Klaeboe *et al*, (2010) who report that persistent *L. monocytogenes* in food processing plants are due to sessile (attached to surfaces) rather than planktonic (free swimming and motile) cells.

2.3.4.3 SMOKING

As indicated previously, there are three main smoking methods used in the production of ready-to-eat smoked fish and the effect of each of these on *L. monocytogenes* growth and survival are considered in turn.

COLD SMOKING

L. monocytogenes numbers artificially inoculated onto the surface of brined salmon portions showed insignificant change during a cold-smoking process that used an air temperature of 22.2^{o}C to 30.6^{o}C for 20 h duration (Ecklund *et al* 1995). At those temperatures, there was no significant difference to *L. monocytogenes* numbers whether the smoke was applied or not. In contrast, lower ambient temperatures (17.2- 21.1^{o}C) were more effective in reducing *L. monocytogenes* for a smoke treatment compared with a non-smoked control. At lower temperatures, *L. monocytogenes* populations decreased 10- to 25-fold when smoke was applied. When *L. monocytogenes* was injected into the interior of the fish fillets rather than surface-applied, the numbers of *L. monocytogenes* increased 2- to 6-fold at 17.2^{o}C to 21.1^{o}C and 100-fold -fold at 22.2^{o}C to 30.6^{o}C, irrespective of whether smoke was present. Conversely, *L. monocytogenes* and other isolates were found in fish samples after all processing stages except those taken immediately after cold smoking, and before further handling of the product raising the possibility that cold smoking could be potentially be optimised to reduce numbers of *L. monocytogenes* (Rørvik *et al*., 1995). Further investigations have tended to support a hypothesis that cold smoking can initially reduce *L. monocytogenes* numbers. However the reduction is not permanent, *L. monocytogenes* apparently recover during chilled storage and the initial reduction can depend upon other factors within the production process such as salting (Aase and Rørvik, 1997, Porsby *et al*. (2008), Rørvik (2000). For instance, exposure of naturally contaminated salmon fillets to temperatures ranging from 19 to 22^{o}C and smoke exposure times from 3 to 10h, sampled immediately after smoking and again tested for *L. monocytogenes* the next day and after 1 week of storage in sealed plastic bags at 4^{o}C generated the following observations: Of 200 samples taken before smoking, 108 (54%) were *L. monocytogenes* positive, the corresponding figure immediately after smoking was 19 (9.5%); Eleven pre-smoking samples contained 10–300 cfu/g fish, while all post-smoked samples contained <10 cfu/g fish.

Similarly, a single log reduction in *L. monocytogenes* numbers from 10^3 to 10^2 cfu/cm^2 immediately after cold-smoking salmon was observed by Porsby *et al*. (2008). The greatest reductions in *L. monocytogenes* numbers were observed when smoking dry-salted or brine-injected fillets. The cold smoking of unsalted fresh fillets did not result in significant reductions to the numbers of *L. monocytogenes* associated with the fish (Porsby *et al*. 2008).

Rørvik (2000) summarises the findings from a number of cold smoking studies as: "cold-smoking has been shown to eliminate *L. monocytogenes* in **challenge** tests at temperatures from 17.1 to 21.1°C while from 22.2 to 30°C the bacteria survived [the cold smoking process]". Rørvik (2000) makes a clear there should be distinctions made between inoculated and naturally contaminated fish and also notes that for natural contamination "cold-smoking (19 to 22°C) [caused] the frequency and level of *L. monocytogenes* to decrease."

HOT SMOKING

Although the fat composition of fish can influence how it reacts to temperature (Ben Embrek, 1994), fish protein starts to rapidly denature (i.e. starts to effectively cook) at temperatures above 60°C (Table 4). Thus by definition, hot smoking temperatures typically exceed 60°C (Table 4) in order to cook fish as it smokes. Consequently, Jemmi and Keusch (1992) inoculated trout with *L. monocytogenes* at a concentration of 10^6 cfu g^{-1} and determined the fate of the bacteria after exposure to hot smoking conditions at 65°C. Complete kill of the *L. monocytogenes* was observed after 20 minutes. Similar findings were reported by Ben Embrek (1994) for fish inoculated with *L. monocytogenes* at 100 cfu g^{-1} who reported that no *L. monocytogenes* was recovered after hot-smoking at 65°C for 15 minutes.

Although Poysky (1997) summarises a number of reports of thermal inactivation of *L. monocytogenes* as 'temperatures approaching 70°C are required for effective bacterial kill', mild hot smoking of Atlantic mackerel at 60°C resulted in an approximate a two log unit reduction in total mesophilic aerobe numbers (Kolodziejska *et al*, 2002).

Taken in combination, these publications provide strong evidence that the temperatures and exposure duration typically experienced by fish during hot smoking are sufficient to completely kill any *L. monocytogenes* present on the surface of the fish. Consequently, hot smoking is a critical control point for *L. monocytogenes* and any contamination issues with hot smoked product are highly likely to be caused by recontamination of the fish after the smoking stage. Common contamination routes are discussed in section 2.3.5.4.

CHEMICAL SMOKING

Chemical smoking uses wood smoke which has been cooled to condense it into a liquid. The smoke condensate is used to flavour fish either by direct application to the fish flesh (e.g. by

dipping the fish in a solution of condensate), or by heating the condensate so that it returns to a gaseous state and using that in a more traditional manner.

The literature has some conflicting reports regarding role of chemical smoking in controlling *L. monocytogenes*. For example, a minimum temperature of 67.2^{o}C was required during hot (mechanical) smoking to completely inactivate *L. monocytogenes* on salmon fillets if the temperature was applied in combination with smoke (Poysky *et al*. 1997). When an increased quantity of liquid smoke was applied by dipping fillets in a solution of smoke condensate, inactivation was achieved at the lower temperature of 58.9^{o}C (Poysky *et al*. 1997) suggesting that the smoke condensate was anti listerial. If no smoke was applied, a temperature of 82.8^{o}C for at least three hours was required for complete inactivation of *L. monocytogenes*. 82.8^{o}C is towards the high end of the temperatures achieved by a traditional wood-burning hot smoke process and thus there exists the possibility the fish that are on the periphery of a wood burning process may still harbour *L. monocytogenes* after smoking. To ensure even heating and smoking it is recommend to reposition of fish during hot smoking.

A French study (Thurette *et al*. 1998) used predictive modelling validated with experimental data to determine growth rates of *Listeria* during the smoking of salmon. The study determined, using liquid smoke, that phenol concentrations as high as 20 ppm and temperatures as low as 4^{o}C could limit the growth rate of *L. monocytogenes* during smoking. Thus the French study also provides evidence that chemical smoke can be anti-listerial and at much lower temperatures than those reported by Poysky *et al.* (1997).

A study, which was similar in design to the Thurette study, was undertaken by Sabanadesan *et al.* (2000). Looking at the inactivation of *Listeria innocua* on salmon fillets during mechanical cold smoking, it was found that under industrial conditions, temperatures of 18-30^{o}C did not affect the inactivation of *L. innocua*, but that smoking time had a significant effect, with a 3 log reduction observed for the longest time assessed (12 h; Sabanadesan *et al.* (2000)).

Vitt *et al* 2001 investigated the inhibitory effect of 5 liquid smokes. The two that had greatest effect were the 'Charsol' and 'Supreme' brands with optimum inhibition on *L. monocytogenes* growth shown to be after a five minute marinade. However, sensory acceptability of the smoked product reduced rapidly after 2 minutes immersion in the smoke condensate.

In contrast to the four previous studies showing a role for smoke in reducing *L. monocytogenes* numbers, Neunlist *et al*. (2005) observed no differences in the numbers of *L. monocytogenes* before and after exposure to smoke condensate at cold smoking temperatures under laboratory conditions. Furthermore, an earlier study by Sunen *et al*. 2001 evaluated the antimicrobial activity of one dried and three liquid smoke extracts. In contrast to the indications initially suggested by Thurette *et al*. (1998), Sunen and colleagues found there was no relation between the antimicrobial activity and the concentration of phenols in the smoked products. Confoundingly, two of the liquid smoked products Sunen *et al.* (2006) tested reduced the populations of *L. monocytogenes*, whereas the other products studied did not.

More recently, in the most comprehensive study identified, Sunen *et al*. (2003) have revisited the antimicrobial consequences of exposing *L. monocytogenes* inoculated onto the surface of rainbow trout before exposure to one of four commercial smoke wood condensate preparations. The fish were smoked under cold smoking conditions before vacuum packing and stored at 4^{o}C for 21 days (Sunen *et al*. 2003). In agreement with their previous findings, only two of the four smoke extracts effectively controlled the numbers of *L. monocytogenes* keeping them below the detection limit for the quantitative enumeration test method used. However, *L. monocytogenes* was still detectable by enrichment after treatment with any of the four condensates examined. In contrast to their earlier findings (Sunen *et al*. 2001), the two smoke wood condensates that had significant anti-*Listeria*l activity were the two containing the highest quantities of phenolic compounds (Sunen *et al*. 2003).

It is apparent that some smoke condensates appear to be able to cause reductions to some *L. monocytogenes* strains inoculated onto fish. There is also evidence that these anti-listeria effects are influenced by exposure time and smoking temperature. The later findings of Sunen and colleagues broadly support the initial speculations of Vitt *et al* (2001) who were the first to suggested a linkage between phenolic compound concentrations and antimicrobial activity. However later work, which modelled the cold fish smoking process using smoke condensates. indicated that the antimicrobial effect cannot be determined solely on the level of phenolic compounds (Cornu *et al*. 2006). Furthermore, based on the output from a model built from laboratory-generated data, Hwang (2009) believes that the concentrations of phenol required to *reliably* prevent the growth of *L. monocytogenes* may be too high to be attained practically in smoke condensates (Hwang *et al*, 2009). Although of little practical use for cold smoking, the conclusions of Hwang *et al* (2009) broadly agree with those of Poysky *et al*. (1997) and both

suggest that smoking at the highest temperature possible is the best strategy for the inactivation of *L. monocytogenes.*

Although it is somewhat removed from commercial processing conditions, Guilbaud *et al* (2008) observed decreases in *L. monocytogenes* numbers when liquid smoke was added to laboratory broth cultures at 37^{o}C. However, there was no attempt to match the experimental conditions to those found in commercial smoking plants. The Guilbaud work is mentioned in this report only because the authors report that smoke condensate addition triggered changes in *L. monocytogenes* gene expression, suggesting there are potential adaptation mechanisms in *L. monocytogenes* to smoke. If that is the case, then any adaptations may well be strain specific because Porsby *et al*. 2008 found that plant environment persistent strains of *L. monocytogenes* were no more resistant to the salmon smoking process than other strains. They also found that combining a drying step after brining and liquid smoking reduced significantly the levels of *L. monocytogenes* over a 24 h period. In contrast to the findings of Guilbaud *et al* (2008) a second lab-based study by Neunlist *et al*. (2005) observed no differences in the numbers of *L. monocytogenes* before and after exposure to smoke condensate at cold smoking temperatures under laboratory conditions.

In summary, there are a number of papers which have investigated the effects of chemical smoke on *L. monocytogenes*. The majority of papers report that some condensates can reduce *L. monocytogenes* on fish and it is apparent that the phenolic concentration of smoke or smoke condensate can impact negatively on *L. monocytogenes* growth. However phenolic concentration is not a reliable indicator of the *L. monocytogenes* contamination status of smoked fish although a number of authors have further reported smoke with high phenolic concentrations can cause *L. monocytogenes* numbers to decline. Whilst the inhibitory effect of smoke towards *L. monocytogenes* appears to be enhanced by higher smoking temperatures, it is important to note that smokes with high phenolic content do not reliably decontaminate fish at the low (<30^{o}C) temperatures experienced during cold smoking. For hot smoked fish, it is likely that the elevated (>60^{o}C) temperatures rather than the smoke that causes reductions in *L. monocytogenes* numbers.

2.3.4.4 CUTTING AND SLICING

The cutting and slicing of fish fillets have been shown to influence the shelf-life of cold smoked salmon. Whole cold-smoked fillets had a significantly longer shelf-life compared with sliced cold

smoked salmon (Hansen *et al* (1998). Sliced salmon may become unsatisfactory for eating principally because of spoilage as a consequence of the excessive growth of background bacteria. In contrast, whole fillets became unfit to eat due to changes in texture, with the fish becoming soft although there were much lower numbers of bacteria present (Hansen *et al* 1998). This observation may be explained as, compared with sliced salmon the whole fillets have far less processing and there was less opportunity for contamination. In addition, there was a lower surface area to volume ratio with the whole fillets with less damaged fish cells providing potential nutrients for microbial growth on the flesh surfaces (Hansen *et al* 1998).

2.3.5 POTENTIAL SOURCES OF *LISTERIA MONOCYTOGENES* CONTAMINATION FROM FARM TO FINAL PRODUCT

A number of studies have acknowledged smoked fish contamination by *L. monocytogenes* and have sought to determine the source(s) of the bacteria (Dauphin *et al*, 2001; Autio *et al*, 1999; Eklund *et al*, 1995; Hoffman *et al*, 2003). In the early 1990s, investigative studies of this type relied upon traditional microbiological testing. Having the advantage of nucleic acid-based typing methods, recent studies have been better able to accurately track *L. monocytogenes* through plants as well as determine which *L. monocytogenes* sources contribute to the contamination status of the final product. Furthermore, modern typing methods have allowed researchers to determine that isolates of *L. monocytogenes* obtained from retail smoked fish have a tendency to be closely-related and are genetically distinct from strains emanating from other foodstuffs (Corcoran *et al* 2006).

Common sites for *Listeria* isolation in the preparation of ready-to-eat foods have been identified. Possible reservoirs in the plant included drains, floors, equipment framework, walls (especially if there were cracks that retained moisture), condensate, trolleys, forklifts, cleaning equipment (sponges, brushes, floor scrubbers) and maintenance tools, packaging equipment, conveyors, slicers, dicers and blenders, racks for transporting finished product, spiral and blast freezers as well as containers such as tubs and bins (Tompkin et al.1999).

2.3.5.1 FEED FOR FARMED FISH

The most likely source of contamination of the raw fish at the farm level is the presence of *Listeria* in the growing and surface waters (as discussed earlier in this report). This section considers the potential of feed as a contamination source at the farm level. To date few studies

report the incidence of *L. monocytogenes* contamination of fish feed. Furthermore, those few studies that do report feed test results have sampled small numbers of feeds. From the limited evidence available, there does not appear to be a widespread problem of fish feed being a source of fish contamination. A Danish study failed to detect *L. monocytogenes* within eight fish feed samples of unspecified type collected from both fresh and sea water farms where rainbow trout were grown (Hansen *et al*, 2006). Similarly, no *L. monocytogenes* were found when two feed samples were taken from a Turkish trout farm and tested using two different testing protocols (Kýsla *et al*, 2007). *Salmonella* was considered the only significant concern for catfish feed in the USA (McCoy *et al*. 2011). Many of the safeguards applied to animal and fish feed for *Salmonella* control (e.g. high pressure and temperature extrusion of the feed through a dye) would also be expected to provide adequate controls for *Listeria* spp. The Norwegian government routinely monitors the farmed fish feed used in the country. However, the routine tests undertaken measure presence of *Salmonella*, and numbers of *Enterobacteriaceae* and moulds. The Norwegians do not test for *L. monocytogenes* because they do not consider there is a significant issue with it contaminating fish feed (Amund Måge, Norwegian National Institute of Nutrition and Seafood Research; Personal communications, 29/03/11).

2.3.5.2 CRATES USED FOR FISH TRANSPORT

There is limited literature available relating to the contamination of fish crates, but there is some evidence that if they are not lined, transport crates can act as fomites. For instance, boxes used to transport shrimp to a processing plant were found to be contaminated with *L. monocytogenes;* suggesting that the boxes could be the source of contamination of steps further down the processing chain (Destro *et al* 1996). In addition, two different salmon suppliers had the same biotype *L. monocytogenes* isolated from their fish, although it was not known if original source of fish (i.e. the fishing boat) was the same (Thimone *et al* 2002). A study of fish processing factories in Greece also detected *Listeria monocytogenes* on wooden boxes (1/2) used to transport fish (Soultos *et al*. 2007). A French paper showed that there were genetically indistinguishable *L. monocytogenes* biotypes on fish sourced from Scotland and Norway (Dauphin *et al*., 2001). Whilst it is possible both vessels were fishing in the same region of the North Sea, *L. monocytogenes* is rarely isolated from oceanic waters (Ben Embarek *et al* 1997). Furthermore, although *L. monocytogenes* is halotolerant, its numbers do decrease in seawater (Hansen *et al* 2006). A number of authors have speculated that predation and competition for the niche may be the mechanisms operating (Hansen *et al* 2006; Bremer, 1998). We consider it unlikely that an *L. monocytogenes* strain crossed the North Sea given the

volumes of water, the dilution effects of these volumes to *L. monocytogenes* numbers and distances involved. However if the crates weren't cleaned and sanitised properly such an explanation could plausibly explain the observation.

In Scotland, interviews with fish smokers revealed that for the bulk transport of sea fish, ice filled transport crates are used for convenience. In brief, the crate system operates as follows: When new batches of fish are delivered (or bought at a market), an empty crate is exchanged for the full one. The empty crate then goes to a "box pool" where it is cleaned and turned around for reuse. The largest Scottish crate pool is located in Peterhead, which seems to be a major hub.

For farmed fish such as trout and salmon, the fish are transported from the farm into fish wholesalers (who will fillet/eviscerate as necessary before selling it on) packed in polystyrene (Styrofoam) boxes full of fish and ice. It is a standard practice throughout Scotland to reuse these Styrofoam boxes without any cleaning. Generally, the boxes that a batch of farmed fish came in on will be the one they go out in, but not always – boxes from different batches can be mixed up. However, it is also standard practice throughout Scotland to line the recycled polystyrene boxes with blue polythene liners before they are recycled. Typical 25kg Styrofoam box polythene liners are a fraction of a penny each (£40 for 10,000) and so there is a physical barrier to reduce the likelihood of cross contamination on those occasions that the boxes are re-used for different batches.

Recognising that there may be a microbiological issue with crate recycling generally rather than specifically for *L. monocytogenes*, there has been unpublished industry-funded research aimed at determining whether this represents an issue. The work was funded in the 1990s in the UK by the crate rental operator CHEP (originally the acronym stood for the Commonwealth Handling Equipment Pool; an organisation set up after the Second World War to dispose of the transport infrastructure left behind by the US armed forces in Australia. CHEP has grown to become a fully commercial global organisation with a US headquarters) and concentrated on the likelihood that recontamination would occur after effective cleaning and disinfection.

In summary, the studies involved washing and disinfecting crates, checking the effectiveness of these procedures and then storing the crates under a series of different conditions. The CHEP-funded work suggested that the procedures and storage conditions used by manufacturers at

the time of the study may require review to ensure that crates and trays remain in a good hygienic condition before they are commissioned for use.

In more detail, the microbiological findings of industry work included:

- There was no difference between shrouded (covered) and non-shrouded trays for yeasts and moulds
- The post-clean crate storage time had no significant impact on total aerobic mesophilic counts (TAMC)
- Trays retained moisture under shrouds and TAMC increased to unacceptable levels if shrouds were used
- Only the top trays in a stack were particularly vulnerable to airborne bacteria

The results from these studies suggest that shrouding of top trays of stacked crates may be worthwhile, but there would appear to be little value in shrouding an entire stack of crates. The following recommendations were made in the report as potential good practice elements as a result of these findings:

- Trays should be thoroughly dry before being stacked and stored
- Shrouding of the top tray in the stack should take place no more than 15 minutes after washing
- Shrouds should be micro-perforated to ensure that moisture does not accumulate on crate stacks
- Monthly microbiological checks should be carried out by the tray washing business to verify washing efficacy
- It may be necessary for manufacturers to shroud product in trays (e.g. in despatch areas) to reduce the potential for gross contamination.

CURRENT INDUSTRY PRACTICES RELATING TO CRATE USE AND CLEANING

A large crate pool in Scotland was visited to gather industry practices on crate cleaning and sanitation practices. The visit revealed that a range of crate capacities are used in the UK. Typical crate loads range from 15kg to 50kg. In the white fish sector, crates are commonly called boxes by the industry. Most of the plastic crates used by fishing vessels to store and transport fish to processors are owned by a crate rental company. CHEP is the largest crate rental company in the UK. Crates are typically delivered to the fishing boats by a rental

company before they leave harbour. The boat's catch is stored, marketed and sold in the crates. After the crates have been used to deliver the fish to a processor, they are typically retrieved for cleaning and reuse by the rental company. The crate rental company that was visited classified crates as dirty (A), rinsed (B) or cleaned (C). At the crate pool visited, there were separate areas for storage of each category of crate. Crates which could handle loads of 15kg to 50kg were washed by an automated machine. The crate pool visited also contained a small selection of very large capacity plastic storage bins which could be used to transport 500kg-1000kg of fish and ice. The larger capacity crates were washed manually using pressure hoses.

The machine washing was undertaken using a fork lift truck to remove crates from the delivery lorries. The crates were hand lifted onto a conveyor belt which fed the crates into the washer. The washer had a capacity of washing 20 boxes per minute and used water at a temperature of 50^{o}C. The mechanism of action for the automated washer was to apply water under high pressure from hoses housed inside a stainless steel carcase. A range of different cleaning and disinfection chemicals manufactured by Biotrace were routinely used at the crate pool visited. After washing, the boxes were inspected for visible cleanliness. Boxes which were not visibly clean were rewashed. Microbiological or other checks on the effectiveness of cleaning were not undertaken by the staff who were interviewed.

After washing, the boxes were manually stacked onto pallets and a fork-lift truck was used to transport the crates from the washing area into the dispatch area. The crates were stored indoors at all times. Figure 9 shows general views of the washing hall (A), including the washing machine (B) and the storage area for the washed crates (C) at the pool that was visited.

The customer renting the crate stipulated what chemicals were used in the washer at the crate pool visited. Depending on the chemicals used, the cost of washing varied between 18 pence and 60 pence per crate. The company visited had a robust infrastructure for keeping track of its boxes. Staff at the plant informed the research team that it was common for crates to travel around the UK as well as continental Europe. The plant visited washed crates for both fishing companies and the processors who bought the caught fish. However, the pool visited was not involved with farmed fish such as salmon or trout. The reason for the lack of activity in the farmed sector was that there were specific biosecurity requirements for farms which were fundamentally incompatible with the crate systems used for sea-caught fish.

A

B

C

Figure 9 A crate pool washing hall (A), crate washer (B) and crate storage area (C).

As was mentioned previously, the insides of many crates typically were scraped and scratched. Since there was no information on the effectiveness of crate washing, it was unclear whether there were any microbiological implications for the damaged surfaces. On the one hand, we speculate that the roughened surfaces may have provided a bacterial niche into which sanitisers would not effectively penetrate. However, we also note that a number of authors have already determined that plant-resident *L. monocytogenes* rather than the *L. monocytogenes* contaminating raw fish are the major source of contaminated final product (Dauphin *et al*, 2001; Autio *et al*, 1999; Eklund *et al*, 1995; Hoffman *et al*, 2003). Thus the role of imperfectly washed and disinfected crates is likely not to be significant.

2.3.5.3 ICE

Information relating to *L. monocytogenes* contamination of ice is limited. Briefly, at a single Finnish processing plant, a total of one out of seven test ice packs taken from fillet transporting boxes tested positive for *L. monocytogenes* (Autio *et al* 1999). However, the paper authors were of the opinion that the fish themselves had contaminated the ice rather than the converse (Autio *et al* 1999).

2.3.5.4 THE PROCESSING ENVIRONMENT

Over the last 20 years there have been at least a dozen studies which have attempted to determine how cold smoked fish may become contaminated with *L. monocytogenes* whilst in the processing environment.

The literature describes several areas in the processing environment that can be potential reservoirs for *Listeria monocytogenes*. These include racks for transporting finished product, drains, floors, equipment framework, walls, condensate, trolleys, forklifts, cleaning equipment and maintenance tools (Tompkin *et al*, 1999). In addition to establishment of listeria in one of the niches above, Tompkin *et al*, (1999), also identified a number of situations that could cause a contamination problem. These included: significant alteration of a processing line, used equipment being brought into the factory, equipment breakdown, the employment of a new worker unfamiliar with hygienic practices, heavy over production and the clogging of drains.

Rørvik *et al*. (1995) were one of the first research teams to investigate *L. monocytogenes* in a smoked salmon processing plant in Norway using biochemical classification technologies. The

plant environment was examined for the presence of *L. monocytogenes* and other *Listeria* spp. In all, 475 samples were collected and *L. monocytogenes* was detected in 16% of samples. Other *Listeria* species were isolated from 22% of the samples. The smoking plant was part of a slaughterhouse, but *L. monocytogenes* was more frequently detected in smokehouse-derived samples. The isolation prevalence for *L. monocytogenes* was 29% for the smokehouse environment samples but only 17% of the raw fish from the smokehouse were contaminated. Eleven percent of the vacuum-packed smoked salmon product sampled contained *L. monocytogenes*.

The authors report that the slaughterhouse was sporadically contaminated, with *L. monocytogenes* as was seawater outside the slaughterhouse. Multilocus enzyme polyacrylamide gel electrophoresis was used to divide *L. monocytogenes* strains into 11 electrophoretic types (ETs). The authors commented that one ET, ET-6, which was the most common ET in Norway, seemed to have colonised the smokehouse environment. ET6 was the only *L. monocytogenes* strain isolated from vacuum-packed final product. While this study demonstrated that the smoke house was more likely to be contaminated, it did not provide any suggestions as to the source of this contamination or whether particular stages of processing were responsible for the contamination seen in the final product.

Rørvik and colleagues (1997) expanded their initial investigations of the prevalence of *L. monocytogenes* and other *Listeria* to a further 40 Norwegian salmon smoking plants, where potential sources of contamination were identified. Samples were taken from the final product and the plant drains. *L. monocytogenes* was detected in smoked salmon in 13/40 plants (33%) and in the drains of 25/40 (63%) of the plants. Other *Listeria* species were found in smoked salmon samples at 16/40 (40%) of plants and in the drains of 30/40 (75%) of the plants. Multivariate analyses of relationships between microbiological test results, operating hygiene, management practices and the state of repair of the production facilities showed that job rotation (employees periodically changing their processing task to prevent boredom) was the strongest expressed risk factor for isolation of *L. monocytogenes* from the smoked salmon. Well-maintained facilities and use of vats for salting of the fillets, showed a preventive effect. *L. monocytogenes* in the drains was found to be a sensitive predictor for the presence of *L. monocytogenes* in the smoked salmon (Rørvik *et al*, 1997). In general, there was no correlation between the presence of other *Listeria* species in a plant and the presence of *L. monocytogenes*.

The Rørvik *et al*. (1997) study was the first piece of surveillance to show a relationship between plant environmental strains of *L. monocytogenes* and contamination of the final product.

Another comprehensive early study was undertaken by Ecklund and colleagues (1995). The Ecklund team surveyed the environments of six cold-smoked salmon processing plants in the Pacific Northwest (WA) of the USA to determine the sources of *L. monocytogenes* contamination for cold smoked fish. In addition, the researchers assessed the cleaning and sanitation procedures in some of the plants. The cleaning studies concluded that the standard practices adequately eliminated *L. monocytogenes* from the processing line and associated equipment surfaces. However, Ecklund *et al*. (1995) observed that recontamination of food contact surfaces occurred to cleaned and sanitised surfaces almost immediately after the resumption of processing. The Ecklund publication concluded that the primary source of contamination of the product and the processing environment was the surface areas of frozen or fresh raw fish coming into the plant.

Ecklund and colleagues did not isolate *Listeria* from the flesh of fish except when there was cross contamination during filleting. The issue with cross contamination further strengthened their assertion that *L. monocytogenes* naturally occurring on the fish surfaces were a major contributor to the contamination problem. The Ecklund study also noted that contamination could also occur as a consequence of injecting recirculated brine into fish. Small amounts of contamination were also noted at bruised regions of the fish flesh.

Routine surveillance of smoked fish in Finland in the mid-1990s showed a higher than expected prevalence of *L. monocytogenes* (Johansson *et al*, 1999). Subsequent investigation by Johansson and colleagues revealed that the majority of the fish had been smoked at a single plant. The researchers investigated further by taking 200 samples from the plant environment and final products of the processor over two extended periods of August–September 1996 and May–September 1997. In addition, the six fish farms providing raw material fish to the plant were sampled (n=55) during September 1997–January 1998. The *L. monocytogenes* isolates were all typed by serotyping and PFGE. The production environment was found to be contaminated with at least four of the pulsotypes isolated from the smoked fish. The areas that were contaminated with the same *L. monocytogenes* that was isolated from the final product were the plant drains, the skinning and salting equipment, conveyor belts and the packing machinery. The farm samples and raw material fish all tested negative for *L. monocytogenes*. In

contrast to Ecklund *et al* (1995), Johansson and colleagues reported that the plant environment was the major source of *L. monocytogenes* contamination, rather than the raw fish entering the plant (Johansson *et al*, 1999). The authors hypothesised that difficulties cleaning and santising effectively without disassembling the salting and slicing equipment was the main reason for the contamination.

Autio and colleagues (1999) investigated the sources of *L. monocytogenes* in a Finnish processing plant that manufactured cold smoked rainbow trout. The general approach was to sample two lots of fish being processed through the plant at various different process stages; and to gather corresponding samples from various locations in the plant environment. Since *L. monocytogenes* contamination of the raw fish at the beginning of the process was low, the researchers were able to determine the processing stages at which the product became contaminated with *L. monocytogenes*. In the plant used for the study, the brining stages of processing was shown to significantly contaminate the fish. Correspondingly, the brining and post-brining areas, and the brine injectors were the environmental samples that had the heaviest concentrations of *L. monocytogenes*. To a lesser extent, there was also increased *L. monocytogenes* contamination of the fish associated with positive isolations from the machinery used to remove the skin from the raw fish and also the post-smoke slicing equipment. Employees, and their equipment (especially ineffectively-cleaned aprons and gloves), were also considered to be minor vectors of contamination.

Over 300 *L. monocytogenes* isolates were further characterised as part of the Autio study using PFGE; the results revealed that the plant-environment strains of *L. monocytogenes* were the ones predominantly isolated from the final product, indicating that contamination of fish with *L. monocytogenes* occurred during processing. One pulsotype (type I) was found on raw fish and skinning, brining and smoking areas. Pulsotype II was detected in brining equipment, fish during processing and final product. Fish sampled after brining had only pulsotypes I and II, suggesting these fish were contaminated during brining. Pulsotypes I and III were found in the slicing and packing areas. It was thought that the fish had spread pulsotype I into these areas. Pulsotype III was associated with the slicing machine. The *L. monocytogenes* present on the raw fish entering the plant were either infrequently or not isolated from the final product. The findings suggest raw fish seemed not to be the most important factor in the contamination of cold-smoked rainbow trout, but that the processing environment was the key risk. However the pulsotyping suggests that the raw fish may initially contaminate some of the equipment

early in the process so the authors conclude that raw fish as a primary source of *L. monocytogenes* contamination could not be ruled out. Autio and colleagues state that more research is needed to establish the role of raw fish in cold-smoked fish product contamination by *L. monocytogenes*.

Consequently, the research team devised a deep clean programme for the plant in an attempt to eradicate the resident *L. monocytogenes*. In brief, hot (80^{o}C) water, steam and hot (80^{o}C) air ovens were used to sanitise the affected, disassembled equipment components and plant areas. Five months after the deep clean, the researchers returned to the plant and were unable to isolate *L. monocytogenes* from the previously-contaminated areas. It is unclear from the paper whether the deep clean was undertaken once and the effects lasted 5 months, or if the deep clean methods were adopted into the routine cleaning procedures of the plant. The researchers concluded that inadequate cleaning and sanitation in the plant had allowed *L. monocytogenes* to become established as a long term plant environment coloniser. In any event, the study was important because it was one of the first to show that the plant environment can be a major cause of *L. monocytogenes* contamination of cold smoked fish. The study re-iterated the advice provided by Johansson *et al*. (1999) regarding the importance and benefits of effective cleaning and sanitation.

The contamination routes of *L. monocytogenes* in cold-smoked salmon processing plants were investigated by analysing almost 4000 samples from the products and environments of two Danish smokehouses (Vogel 2001a). The product samples related to smoked fish manufactured between 1995 and 1998 and processing environment swabs were taken only between 1998 and 1999. Final product contamination in one plant varied from 31 to 85%, with no *L. monocytogenes* isolated from raw fish at the start of the smoking process. At the second plant, the prevalence of *L. monocytogenes* for both raw fish and product contamination varied from 0 to 25% (16 of 185 raw fish samples and 59 of 1,000 product samples). A total of 429 isolates of *L. monocytogenes* were profiled by random amplified polymorphic DNA (RAPD) profiling, and 55 different RAPD types were found. The RAPD types detected on the products and processing equipment were indistinguishable, suggesting that contamination of the final product in both plants was due to contamination during processing rather than to contamination from raw fish.

However, the authors do not exclude the possibility that raw fish could have been an important source of contamination of the processing equipment and the plant environment.

Contamination of the product occurred predominantly in the brining and slicing areas of both plants. In the first plant, the same RAPD type was found over a 4-year period in the final product and over a two year period in the environmental samples. Therefore, the strain was exceptionally persistent and apparently unaffected by routine hygienic procedures. In the second plant, the prevalence of *L. monocytogenes* was lower, several different *L. monocytogenes* RAPD types were isolated and there were no apparent long term plant environment colonisers. The authors conclude the contrasting conditions found to exist between the two plants show that the establishment of persistent strains may be avoided by effective cleaning and sanitation.

Dauphin and collaborators (2001) also investigated the sites of *L. monocytogenes* contamination in three cold smoked salmon and herring plants in France. All three plants imported their raw fish from Scotland and Norway. Samples were taken from the fish during various processing stages as well as from the plant environment. The samples were tested for *L. monocytogenes* to determine the processing stages that increased contamination of the fish. This approach was similar to a study conducted in Finland by Autio *et al*. (1999). An important difference between the two studies is that the French plants all used salt crystals to affect curing of the fish rather than the saline injection reported by Autio *et al*. (1999). As before PFGE was used to determine contamination routes and the sources of the *L. monocytogenes* on the final products. Briefly, Dauphin reported that 42% of the 141 samples taken from the three processing plants tested positive for *L. monocytogenes*. In contrast, more than 80% of samples taken from a variety of raw seafood entering the plants contained *L. monocytogenes*. As was reported previously by Autio *et al*. (1999), the pulse-types of *L. monocytogenes* on the raw product and the final product were not the same.

Each of the three French plants had different characteristics; no general conclusions were made regarding likely contamination hotspots that were present in all of the plants. In Plant I, 70% of the environmental isolates belonged to a single *L. monocytogenes* pulse type (pulse type 1). At Plant 1, the final product exclusively contained pulse type 1. None of the *L. monocytogenes* types present on the raw fish at the commencement of processing were isolated from the final product. Furthermore, pulse type 1 was never isolated from any of the raw fish entering Plant 1. Pulse type 1 was also persistent in Plant 1; the researchers were able to recover it for more than two months. As was reported by Autio *et al*. (1999) the French researchers highlighted

issues with ineffective cleaning and sanitation in the plants which contributed to the persistence of pulse type 1 at plant 1.

In Plant 2 87% of the surfaces of the raw fish entering the plant harboured *L. monocytogenes*. The researchers noted that the same pulse type was isolated from batches of farmed salmon sourced from Scotland and Norway. Although not investigated further, we speculate that the finding may be a consequence of ineffective cleaning of recycled fish crates or contamination of the ice used to pack the raw material. At Plant 2, one of the pulse types present on the raw material was isolated from the final product. However the researchers considered, in keeping with the opinion of Autio *et al*. (1999) that it was rare for the pulse types present on the raw material to survive through to the smoked product. Plant 2 was assessed by the Dauphin team to be exceptionally efficient at producing cold smoked fish flesh that was free of *L. monocytogenes* (i.e. the fish skin was contaminated but the edible portions of the smoked fillets were *L. monocytogenes*-free). The authors attributed the high quality product to the good manufacturing practices at plant 2, and the particular efforts made to prevent contact between the skin of one fish and the flesh of another during processing. At Plant 3, 100% of the final products were contaminated with a unique clone of *L. monocytogenes*. The authors considered that the contamination occurred during the slicing of the final product, although no substantial evidence was provided to support slicing as the contamination source.

In summary, the French study largely reaffirms the findings of the Finnish one. In all of the three plants investigated, contamination of final products did not appear to significantly originate from the *L. monocytogenes* present on raw salmon entering the processing environment. Both sets of authors also agreed that majority of the *L. monocytogenes* isolated from the final product was sourced from the processing environment. In addition, there was a suggestion that plant persistent clones may originate from raw fish, employees or the environment external to the plant. Ineffective cleaning and sanitation was again considered to be a risk factor for the contamination of cold-smoked fish by *L. monocytogenes* (Dauphin *et al*, 2001).

A Polish study investigated *Listeria monocytogenes* contamination in a smoked fish processing plant (Medrala *et al*, 2003). Seventy-one presumptive strains were isolated over a year from 152 samples of raw fish (salmon and sea trout) and the final product of vacuum-packed cold-smoked sliced salmon. Contamination of raw materials ranged between 4.3–15.4%, whereas

final products were more significantly contaminated with a prevalence of up to 77.8%. The significantly higher prevalence in the finished product suggested that there was a persistent *L. monocytogenes* resident in the plant. Although no environmental samples were taken from the plant by Medrala and colleagues, PFGE was used to determine that the *L. monocytogenes* on the smoked product were different from those entering the plant on raw fish. The authors speculated that the product became heavily contaminated towards the end stages of processing (i.e. smoking, slicing, and/or packaging) by plant-resident strains of *L. monocytogenes*. There was a dominant *L. monocytogenes* clone identified by PFGE. This dominant clone was further classified by additional restrictions into several closely-related strains. The authors further speculate the minor changes in the PFGE bands correspond to a clone selection process and that DNA of the original strain has changed as the organism became more adapted to each of its colonised environmental niches. In keeping with the conclusions of other studies (Dauphin *et al*, 2001; Johansson *et al*, 1999), the Polish group believe that *L. monocytogenes* on raw material were a minor contributor to final product contamination at the plant they investigated. Furthermore, the authors speculate that *L. monocytogenes* became established in the plant environment as a consequence of ineffective cleaning and sanitation procedures (Medrala *et al*, 2003).

In 2004, Lappi and colleagues reported the effect of applying interventions of improved employee training and targeted sanitation procedures to four smoked fish processing plants in the USA which had isolated either *Listeria* or *L. monocytogenes* from the plant environment or final smoked product. The consequences of the interventions were followed for two years. Prior to the application of the interventions, samples were collected from the processing plant environment and from raw and finished product at monthly intervals. The samples were tested for *Listeria* spp. and *L. monocytogenes*. Before the interventions were applied, 19.2% of raw product samples (n = 276), 8.7% of finished product samples (n = 275), and 26.1% of environmental samples (n = 617) tested positive for *Listeria* spp. During and after implementation of *Listeria* control strategies, 19.0% of raw product samples (n = 242), 7.0% of finished product samples (n = 244), and 19.5% of environmental samples (n = 527) were positive for *Listeria* spp. In one of the four plants studied, no environmental samples tested positive for *L. monocytogenes*. The plant was excluded from en masse statistical analyses. Based on the combined results from the other plants, environmental *Listeria* spp. prevalence was significantly lower ($P < 0.05$) after the implementation of control strategies. However, *Listeria* prevalence for floor drains was similar before and after implementation of controls (49.6 and 54.2%,

respectively). Regression analysis revealed a significant positive relationship ($P < 0.05$) between *L. monocytogenes* prevalence in the plant environment and in finished products before implementation of control strategies; however, this relationship was absolved by implementation of the interventions. Molecular subtyping (EcoRI ribotyping) revealed that specific *L. monocytogenes* ribotypes had persisted in three processing plants over the entire two years of the study's duration. The persistent ribotypes were responsible for all six finished product contamination events detected at one of the plants. Ribotype data also indicated that incoming raw material is only rarely a direct source of finished product contamination (Lappi *et al*, 2004). The Lappi study is another example of researchers who believe *Listeria* that effective cleaning and sanitation can reduce cross-contamination and prevalence of *Listeria* spp. and *L. monocytogenes* in smoking plant environments. However, the authors also acknowledged that the effective and sustained removal of persistent *L. monocytogenes* strains from smoking environments is problematic.

Pulsed field gel electrophoresis was used to type *L. monocytogenes* and trace contamination routes and in raw fish and the environments of four cold smoke salmon plants in Iceland (Gudmundsdottir *et al*, 2005). Intermittently, 125 samples were taken from raw fish and 522 from the processing environments during an interval between 1997 and 2001. *L. monocytogenes* was isolated from 11.3% of all the samples taken, although the cold-smoked salmon final product incidence was only 4%. *L. monocytogenes* was commonly isolated from raw fish, floors and drains, the staff in the processing environment and processing equipment. Environmental isolations were made both before and immediately after cleaning. The study typed more than 200 *L. monocytogenes* isolates by PFGE in order to determine contamination sources.

In Plant A, 93% of the isolates belonged to a single pulsotype (Type 1). When Type 1 was restricted further for more precise typing, it was sub-classified into three closely-related types (type 1A, 1B and 1C). Type 1A was commonly isolated for at least two years from the drains, air, personnel and processing equipment in Plant A. Type 1A was also isolated from fish sourced from a single supply fish farm. Pulsotype 1B was never isolated from the raw material, but originated mainly from drains/floors during processing at Plant A. Type 1B was isolated from the final product 32 times during the course of the study. Nine isolations of 1B were from the clothes and hands of plant personnel.

With samples from Plant B, 10 different pulsotypes were identified. The sources of these pulsotypes were cleaned forklifts (immediately after cleaning), brine, personnel and raw material. For Plant B, the authors concluded that contamination of final product could be linked to both ineffective cleaning allowing the establishment of resident *L. monocytogenes* and also contaminated raw material (Gudmundsdottir *et al*, 2005)

The seven isolates from Plant C were classified into one of four different pulsotypes. Three types were all isolated from the plant environment rather than raw fish. The plant environments that were contaminated with *L. monocytogenes* were hatches and newly-cleaned transporter vehicles. The authors noted that despite detections of *L. monocytogenes* in environmental samples after plant clean up and during processing, none of the final product samples were found to be contaminated. There were five *L. monocytogenes* isolations in Plant D. All of the isolations were environmental; from the floors, drains or processing equipment sampled during processing.

The comprehensive Icelandic study (Gudmundsdottir *et al*, 2005) concluded that raw fish, floors and drains and staff were the major sources of *L. monocytogenes* found on cold-smoked salmon products in the plants that were studied. The authors believed that the hygienic design and cleaning of processing plants and equipment, and staff behaviour (hygienic processing practices) were the important factors in controlling the spread of *L. monocytogenes* through processing environments.

Further US-based studies were undertaken by Hoffman and colleagues that focussed on *L. monocytogenes* contamination of two cold smoke fish processing plants on the Eastern coast (NY) of the United States (Hoffman *et al*, 2003). More than 800 samples were collected from the processing environment and the raw fish entering the plants over an 8 week period in spring and summer. The test results showed that there were significant differences between the isolations from each of the plant environments. Plant A in the study had an environmental prevalence of 43.8% (112 of 256 samples). Furthermore, greater than 60% of samples collected from the drains at plant A tested positive for *L. monocytogenes*. The non-drain Plant A environmental samples showed a prevalence of 32.3% overall, with only 3.1% of samples collected from food contact surfaces testing positive (Hoffman *et al*, 2003). Plant B had a prevalence of 1.2% (3 of 256 samples).

Positive isolates from the Hoffman study were typed using a PCR-based fingerprinting method that targeted rDNA. Sixteen separate *L. monocytogenes* subtypes were present on raw fish entering the plant. Nine of these raw fish ribotypes were not found in the plant environment. The authors believed their results indicated a disparity between the subtypes found on raw fish and those found in the processing environment. In keeping with the conclusions of Autio *et al*. (1995) and Dauphin *et al*. (2001), Hoffman *et al*. (2003) concluded that environmental contamination in the cold fish plants they investigated was distinct from the contamination on new batches of incoming raw fish. Furthermore, the Hoffman study provided more molecular evidence that the persistent, *L. monocytogenes* in one of the plants, was a major source of contamination and was likely to be a consequence of imperfect cleaning and sanitation. Unfortunately, the Hoffman study did not test final product and so no conclusions could be drawn regarding the contribution to final product contamination by persistent *L. monocytogenes*.

One final major finding of the Hoffman study was that drains in smoked fish processing plants can be a source of persistent *L. monocytogenes*. The finding is a recurring theme since similar conclusions for drains as problem areas for *L. monocytogenes* have been reported for other processed food sectors; particularly meat slaughterhouses where it is an acknowledged problem (Gudbjornsdottir *et al*, 2004; Kushwaha *et al*, 2009).

Hansen *et al*. (2006) note that cold-smoked fish is often contaminated by *L. monocytogenes* that are persistent in smokehouse environments. A number of previous publications (Autio, 1999; Dauphin *et al*, 2001; Hoffman *et al*, 2003) had assumed that the original source of plant persistent *L. monocytogenes* was the raw fish brought into the plant for smoking. Hansen and his colleagues report their efforts to confirm whether the assumption was correct by determining the original sources of plant-persistent *L. monocytogenes* in Denmark. The study undertook surveillance of internal and external environments that were connected to fish processing. A total of 400 samples were collected from diverse environmental sources, which included fish slaughterhouses, fish farms, and smokehouses. In general, Hansen and colleagues reported that *L. monocytogenes* prevalence increased with the degree of human activity. The isolations from various sources related to fish smoking were 2% of seawater samples from fish farms were *L. monocytogenes*-positive, 10% of freshwater fish farms, 16% of environmental samples from fish slaughterhouses and 68% of samples from a single fish smokehouse. No seasonality was observed for *L. monocytogenes* isolations.

Discussing their findings, Hanson et al. 2006 noted that the for the smokehouse isolates, the pattern of RAPD types was fairly homogeneous (i.e. the majority of the isolates were the same type). There was a much greater diversity for the *L. monocytogenes* isolated from outside environments. The RAPD type dominating the inside of the fish smokehouse was sporadically-isolated from a water sample taken on a freshwater fish farm. The authors concluded that "*L. monocytogenes* in the outer environment associated with Danish fish processing is probably of minor importance to the environment inside a fish production plant" (Hansen *et al*, 2006). However, the isolation of a smoke plant resident *L. monocytogenes* from water taken from a fish farm is an important finding because it provides the first good evidence that the environment used to farm the fish could be the original source of plant-resident *L. monocytogenes*. Furthermore, it is likely that the source of *L. monocytogenes* responsible for persistent plant contamination come into the plant on the skin of raw fish.

A joint Portuguese-English study (Vaz-Velho *et al* 2001) aimed to identify the sources of *Listeria* isolated from smoked salmon. Samples were taken for *L. monocytogenes* testing from fresh fish suppliers, raw materials, factory sites and finished product. Sero- and phage-typing were carried out on the isolates and it was determined that the same strains isolated from fish supplier samples were not found on the processing lines. Furthermore, the strains isolated from fresh salmon differed between location, and that the isolates from farm water were different to those isolated from fish farmed in the water. It was not possible to identify the source of the contamination in the final product.

More recently, Dass and colleagues (2010) surveyed an Irish cold smoke salmon plant for a period of one year monitoring for *L. monocytogenes* in the processing line, processing environment, personnel, raw fish brought into the plant and the final smoked product. The purpose of the study was to determine whether genetically similar strains were able to colonise different environmental niches in the plant as well as gathering information on contamination sources and vectors. The overall prevalence of *L monocytogenes* over the course of the project was 24.54% (n = 444). Molecular methods (Multiple Locus Variable number tandem repeats Analysis; MLVA) were used to type the isolates. Eight unique MLVA types were isolated over the course of the study. The final product was most commonly contaminated by two types of *L. monocytogenes*. In contrast to the other molecular studies, one predominant type originated from the raw material (type a) although the other predominant type (type c) was a persistent

coloniser of the initial part of the production line environment. The authors concluded that *L. monocytogenes* can progress through the entire production chain and result in contamination of the final product. This finding is at odds with the conclusions from other studies where plant environment *L. monocytogenes* are more likely to be isolated from the final product than *L. monocytogenes* from raw fish.

The Dass study organised the processing plant into four different zones. Their observations showed that each zone had one dominating strain type, a result that caused the authors to hypothesise that some *L. monocytogenes* strains present on raw fish may be better adapted to establishing specific environmental niches in a processing environment. The Dass study clearly showed that there were a number of areas in the plant that routinely harboured *L. monocytogenes* and which were difficult to decontaminate. The samples with the highest *L. monocytogenes* isolations were raw fish surfaces, filleting boards, drains, floors, conveyer belts and slicer/skinning equipment. The authors noted that each of these areas would be rigorously cleaned before the start of the production but that the cleaning procedures were largely ineffective against the established *L monocytogenes* populations. The authors recommended that new cleaning and disinfection protocols should be considered to more effectively control *L. monocytogenes* in the plant (Dass *et al*, 2010).

In an effort to understand *Listeria* transmission and contamination patterns in fish processing environments, Hu *et al*. (2006) undertook surveillance of a cold-smoked fish processing plant for five consecutive days. The study was again based in NY on the east cost of the USA. Intensive sampling of finished products (60 per day) and the plant environment at the processing stages of smoking, skinning, trimming, slicing and packing (66-108 per day) was undertaken during the beginning, middle, and end of each processing period. Overall, a total of 782 samples comprising 300 finished products and 482 environmental samples were collected and tested for *Listeria* spp. and *L. monocytogenes*. A total of 28 finished product and 57 environmental samples (9.3 and 11.8%, respectively) were positive for *Listeria* spp. Only one sample of finished product and five environment samples were positive for *L. monocytogenes.* No meaningful analyses were possible for the *L. monocytogenes* detections. There were no significant differences in *Listeria* prevalence amongst the samples collected from the beginning, middle, and end of the production day. Furthermore, patterns and prevalences were highly variable between days and within a given day. The authors concluded that their "findings indicated that chance events played an important role in the contamination of finished products".

It is important to note that the plant studied by Hu *et al*. (2006) was the same plant investigated by Hoffman *et al*. (2003). Furthermore, this same plant has been extensively studied, either individually or as one of a group of plants, continuously by various members of the same research team (Lappi *et al*, 2004; Thimothe *et al*, 2004; Norton *et al*, 2001a and 2001b). After more than seven years of near-continuous on-going study, extensive testing and academic intervention, it is questionable whether the plant should be considered as a typical smoked fish processor. From such a viewpoint, the authors' conclusions relating to random events being the main factor which governs contamination of the final product could be considered valid only for those plants which have exceptionally good control of environmental *L. monocytogenes*. If contamination events are truly chance occurrences (and in no way related to *L. monocytogenes* on fish coming into the plant), it would seem there is little more that could be done to further improve *L. monocytogenes* contamination of smoked fish after any resident environmental *L. monocytogenes* are effectively controlled.

From the literature reviewed above, key areas where *Listeria* have been detected or where *Listeria* contamination has been found to be the highest in the processing environment can be identified. Several processing stages where *Listeria* has been detected is repeatedly reported by many authors. These process stages could potentially be sources of contamination of the final product during ready-to-eat smoked fish production. The riskiest production stages and practices are summarised as Table 5. Along with key risk areas being identified in the literature, it also appears that the a number of general conclusions and common themes can be identified. In brief, these are:

- Raw fish entering smoking plants are can be contaminated with *L. monocytogenes,* to varying degrees (Table 2).
- *L. monocytogenes* isolated from the final smoked product can be the same biotype as was present on the raw fish, but that is a fairly infrequent occurrence.
- It is possible that *L. monocytogenes* biotypes enter the plant on raw fish and that some strains are able to persistently colonise the plant processing environment. In extreme cases, such colonisations can be for extended periods of several years. However, the literature relating to original sources of persistent *L. monocytogenes* is, at times, contradictory and so more research is needed to confirm the original sources.

- Plant environment *L. monocytogenes* are more likely to be isolated from final product than those present on raw fish.
- Drains and difficult-to-clean skinning, brine injection and slicing equipment have been highlighted as frequent reservoirs of persistent *L. monocytogenes* colonisation in smoked fish plants.
- Plant workers can spread *L. monocytogenes* around processing environments via their hands and surfaces which contact their hands (e.g. knives, other tools, machine controls and door handles)
- The key to preventing persistent *L. monocytogenes* colonisation and decontamination of the plant environment is through effective cleaning and sanitation which can be difficult to accomplish.

Table 5 A summary of key areas where *Listeria monocytogenes* has been identified in fish processing environments

Key risk area	Reference
Incoming raw fish	Ecklund *et al* 1995; Gudmundsdottir *et al* 2005; Hoffman *et al* (2003); Dass *et al* 2010
Workers	Rørvik *et al*. 1997; Autio *et al* (1999); Gudmundsdottir *et al* 2005;
Drains/floors	Rørvik *et al*. 1997; Johansson *et al*, 1999; Gudmundsdottir *et al* 2005; Hoffman *et al* (2003); Dass *et al* 2010
Filleting and/or evisceration	Ecklund *et al* 1995
Skinning	Johansson *et al*, 1999; Autio *et al* (1999); Dass *et al* 2010
Salting/brining	Johansson *et al*, 1999; Autio *et al* (1999); Vogel 2001a;
Slicing	Vogel 2001a; Dauphin *et al* 2001; Medrala *et al* 2003; Dass *et al* 2010
Conveyor belts	Ecklund *et al* 1995; Dass *et al* 2010
Packing	Ecklund *et al* 1995; Autio *et al* (1999)

2.3.6 CONTROL OF *L. MONOCYTOGENES* DURING PROCESSING

Once estab*lished, L. monocytogenes* has been shown to be difficult to eradicate from fish smokeries and other food processing environments (Tomkin, 1999; Gram, 2001; Møretrø and Langsrud, 2004 and summarised here in chapter 2.3.5.4). Furthermore, if complete eradication of *L. monocytogenes* can be achieved, there are no guarantees that re-colonisation will not occur unless effective cleaning and sanitation procedures are rigorously and meticulously adhered to.

Various studies have examined the effects of specific intervention treatments on *Listeria* and its contamination of smoked fish products. Regulation (EC) 853/2004 lays down specific hygiene rules for products of animal origin stating that food business operators shall not use any substance other than potable water to remove surface contamination from products of animal origin, unless the substance has been approved by the Commission. It is recognised that other countries can and do use antimicrobial treatments. Additives, such as antioxidants and preservatives, which are permitted for use in food, can also occasionally exert an anti-microbial effect, even though that is not their intended purpose or primary function. Various treatments and additives that have been shown to have an effect on *Listeria* have been considered and reviewed in the following section.

Please note that the use of additives are restricted to certain foodstuffs within the EU and their discussion in this report does not imply that these products are permitted for use in the production of smoked fish products within the UK. Food business operators should satisfy themselves that any additive they are using, or wish to use, conforms to EU legislation and any applicable domestic regulations.

2.3.6.1 CLEANING

Recent studies have assessed the various cleaning and sanitation procedures used in smoked fish plants for sanitation effectiveness in both laboratory and plant-based studies have supported this theory (Bagge-Ravin *et al*. 2003; Robbins *et al*, 2005). These studies report a range of apparent effectiveness of cleaning strategies. This was highlighted in a study that took environmental surface samples from a factory in England after cleaning and before the commencement of processing and found these to be positive for *Listeria*. *Listeria* was isolated

from 15 of the 23 factories specifically sampled (65%). In those processing facilities that were *Listeria* positive, 7.2% of all the surfaces sampled tested positive (Miettinen *et al*, 2001). *L. monocytogenes* is known to exist in two forms: as planktonic cells (i.e. as free-swimming, motile single cells) or as a sessile biofilm (i.e. as part of a group of individual cells living in close proximity enmeshed within a web of protective polymers). Planktonic forms of *L. monocytogenes* are susceptible to most mainstream cleaning and sanitization agents and so can be effectively controlled (Frank *et al*., 2003). However, as a biofilm, *L. monocytogenes* can be very resistant to the action of cleaners and sanitisers (Frank *et al*., 2003; Sommers and Lee Wong, 2004). Compared with planktonic forms, biofilm *L. monocytogenes* requires 10-20 times more chemical to achieve the same degree of kill under laboratory conditions (Robbins *et al*, 2005). When *L. monocytogenes* persists in food processing environments, it is predominantly in the form of a biofilm (Tomkin 2002; Klaeboe *et al*, 2010). Biofilm formation prior to desiccation was found to increase the survival of *L. monocytogenes* cells on stainless steel coupons (Truelstup *et al* 2011). Furthermore, bacteria could be transferred from the biofilms to smoked or fresh salmon on contact.

One sanitiser formulation that has been reported as fairly effective against *L. monocytogenes* derived from a biofilm is peroxyacetic acid (PAA; an active oxygen-based sanitiser composed of hydrogen peroxide, peracetic acid, and acetic acid in combination; Stopforth *et al*., 2002). Stopforth created an artificial biofilm by inoculating cattle carcass washings onto stainless steel tiles under laboratory conditions. The Stopforth study concluded that PAA, in contrast to a number of other sanitisers that were assessed, was more effective in killing sessile (attached) cells compared with cells treated in suspension. Somers *et al*. (2000) compared the effectiveness of two sanitizers at controlling *L. monocytogenes* in a meat (i.e. not a fish) plant. Combination A used a chlorinated-alkaline, low-phosphate detergent, and dual peracid sanitizer. Combination B used a solvated-alkaline environmental sanitation product and hypochlorite sanitizer. Both detergents significantly removed or inactivated biofilm bacteria. The sanitizers also reduced biofilm numbers but this was not significant in most cases for the dual peracid (Combination A).

A later study by Bagge-Ravn and colleagues (2003) attempted to build on the findings of Stopforth et al. (2002) by determining the effectiveness of PAA in a commercial fish smoking environment. As part of their studies, the Bagge-Ravn team applied a fog of PAA to the slicing area at a salmon smokehouse and compared its effectiveness with that of a foam sanitizer that

used sodium hypochlorite as the active agent (the established sanitation process routinely performed at the smokehouse). The effect of each procedure on *L. monocytogenes* populations was assessed. Two hundred twenty-three environmental samples were collected with sponges and swabs after each of the sanitization procedures, and 68 samples were collected post clean during production. Using a selective isolation method, strains of *L. monocytogenes* were isolated and subsequently genetically characterized by RAPD. Following chlorine foam sanitisation, 14 to 42% of the samples contained <10 cfu *L. monocytogenes* per site, whereas 29 to 78% of the samples collected after fog sanitization contained the same proportion of undetected *L. monocytogenes*. Although a higher proportion of samples had lowered numbers of *L. monocytogenes* for PAA, the overall prevalence of *L. monocytogenes* was unchanged. For both treatments, *L. monocytogenes* was found only in poorly cleaned areas such as drains. The authors make specific note that, in keeping with established dogma, effective cleaning is a pre-requisite for effective sanitation (Bagge-Ravn *et al*, 2003). The RAPD types from every single positive drain sample were identical to the type that had persisted in the smokehouse over a seven year period, emphasising the importance of drains as a persistent *L. monocytogenes* niche. The Bagge-Ravn study is further notable because it demonstrates that the method of sanitiser application can influence the effectiveness of sanitation. The original Stopforth *et al* (2002) study applied PAA directly to *L. monocytogenes*-contaminated films and demonstrated effective kill. Although more convenient for a commercial premises, when the PAA was applied as a fog by the Bagge-Ravn study and kill effectiveness was significantly reduced.

The resistance of *L. monocytogenes* biofilms to various sanitizing agents and disinfection procedures has been evaluated (Belessi *et al.*, 2011). Biofilms were formed under laboratory conditions aimed at mimicking processing conditions where stainless steel surfaces were contaminated with liquid rich in food residues. The first sanitation assessment involved biofilm formation on stainless steel coupons (SS) using 0.5%, 7.5% and 9.5% NaCl and at one of two different temperatures (5^{o}C and 20^{o}C). The biofilms formed were exposed to water at 60^{o}C for 20 min, or to 2% PAA for either 1, 2, 3 or 6 min. The warm water treatment, designed to mimic water-based cleaning caused no significant reductions in the attached *L. monocytogenes* populations when compared with unwashed controls. In contrast, *L. monocytogenes* numbers on SS coupons decreased as the exposure time to 2% PAA increased and no cells were detected by culture after 6 minutes exposure. Biofilms formed at 20^{o}C were more resistant to PAA than biofilms formed at 5^{o}C. Salt concentration in the growth medium had no marked impact on the resistance to PAA. A second sanitation procedure included biofilm formation of non-adapted

(NA) and acid-adapted (AA) at 4°C. Coupons coated with biofilm were periodically exposed to chlorine (0.465%), quaternary ammonium compound (1% QAC) or 2% PAA. The most effective sanitizer was QAC followed by PAA and chlorine. However the results from this study must be interpreted with caution as no evidence was provided to support the experimental salt concentrations, temperatures and chemicals chosen. Furthermore, a lack of valid temperature and nutrient component controls make comparison between the first and second sample groups weak.

2.3.6.2 WORKER HYGIENE

A mathematical model of cross contamination within a fish processing plant was developed and its predictions validated based on real world observations (Ivanek *et al,* 2004). The model showed that a simple intervention such as changing gloves with greater frequency could significantly reduce the within lot prevalence of contaminated fish (Ivanek *et al*, 2004).

Electrolysed oxidising water (EOW) is generated by passing an electrical current through a weak solution of sodium chloride dissolved in tap water. The electrolysis generates hydrogen gas and hydroxide radicals at the cathode. At the anode, the chlorine ions from the salt are neutralised and form chlorine gas. If the chlorine at the anode is reacted with hydroxide at the cathode, hypochlorite (the active agent in bleach) is formed. If the pH of the solution is lowered, the hypochlorite equilibrates to its acidic form, hypochloric acid (Fabrizio *et al* 2002). EOW is used to describe solutions of hypochlorite, hypochloric acid and mixtures of these two antibacterial agents. EO water is becoming increasingly popular in the food industry as a method for reducing microbial numbers on food contact surfaces (Loretz *et al* 2010).

The effectiveness of acidic EOW to in reducing *L. monocytogenes* contamination on the gloves of seafood processing workers(Liu and Su (2006). A variety of latex and nitrile disposable and reusable gloves were assessed by inoculation with a five strain cocktail of *L. monocytogenes* at a high concentration of 5 x 10^7 cfu/cm^2 of glove material. Gloves were inoculated with and without organic residues derived from shrimps. *L. monocytogenes* survival was poor on clean (i.e. no shrimp residue) reusable gloves and its populations decreased rapidly to non-detectable levels within 30 min at room temperature (which was not specified). High numbers of *L. monocytogenes* cells were recovered from clean disposable gloves after 30 min of inoculation. The presence of shrimp meat residue on gloves enhanced the survival of *L. monocytogenes*. The bacteria were detected on soiled reusable and disposal gloves for at least two hours after

inoculation at room temperature. Immersing inoculated gloves in EO water at room temperature for 5 min completely inactivated *L. monocytogenes* on clean gloves and significantly reduced the contamination on soiled gloves when compared with a tap water treatment EO water was shown to be suitable for use as a sanitizer for reducing *L. monocytogenes* contamination on gloves and the risks of transferring *L. monocytogenes* from gloves to RTE seafood (Lui and Su, 2006).

2.3.6.3 METALS

The antimicrobial properties of copper were investigated as a potential intervention to help control *Listeria* spp. in the factory environment (Rogovskyy 2006). Rogovskyy found that drains fabricated from copper reduced the counts of *Listeria* spp. by more than one log. However, copper coated surfaces and copper-impregnated concrete did not exhibit any antimicrobial activity.

2.3.6.4 WASH WATER AND CHLORINE

Under current EU legislation disinfection of meat with chlorine is not permitted. Chlorine is a typical component of treated potable water. When rendering water potable, 5ppm is a normal upper limit used by water companies initially to decontaminate the pipe work and the water flowing through it for existing established water networks. After the initial decontamination, the concentration of chlorine is typically lowered to 0.5 -2.0 ppm for an on-going routine disinfection of mains water (Drinking Water Inspectorate, 2009). No peer reviewed papers were found to describe the effect of chlorine at the concentrations typically used for water purification on *L. monocytogenes* associated with smoked fish. However, Thiessen *et al* (1984) assessed 1.33 ppm chlorine as a control for *Salmonella* spp. in poultry chiller water and found practically no reduction (<0.5 log cycles) in bacteriological counts on the skin and meat of chicken carcasses. In comparison, Bautista *et al* (1997) observed almost complete eradication from poultry carcasses by treatment with high levels (300-400ppm) of chlorine. Based on the bacteriological reductions observed at potable concentrations of chlorine for chicken, it is considered unlikely that chlorine at 0.5-2.5 ppm would cause a significant reduction to the numbers of *L. monocytogenes* associated with smoked fish flesh.

There is evidence to suggest that high levels of chlorine may demonstrate a negative effect on *Listeria* growth in fishery products. Bremer and Osborne 1998 report that 200ppm chlorine

caused significant reductions in *L. monocytogenes* numbers on the surfaces of raw king salmon. However the 200ppm concentration of chlorine was exceptionally high. One acknowledged problem with chlorine is that it can react with organic materials such as fish flesh and skin to form carcinogenic (cancer-causing) side products such as trihalomethanes (Shikongo-Nambabi et al, 2012).

2.3.6.5 STEAMING

Currently, there is a lack of evidence on the effectiveness of smoky steam on the numbers of *L. monocytogenes* associated with smoked fish. However it is proposed that the process of cold smoking could be altered to include a steaming step (Dimitradou *et al* ,2007). In fish steamed in a mixture of liquid smoke and water, the numbers of naturally present total aerobic mesophiles (TAMC) reduced from an initial 5.9 x 10^5cfu/g to 25 cfu/g. After prolonged storage for 91 days at 4^{o}C, if the fish had not been previously dried, the TAMC was still 25 cfu/g. However, there was no *L. monocytogenes* contamination of the fish by either artificial or environmental routes therefore there is a lack of evidence as to the effectiveness of steaming in reducing *L. monocytogenes* numbers.

2.3.6.6 FREEZING AND SUPER-CHILLING

The effect of freezing stress on *L. monocytogenes* has been studied (Yoon *et al* (2004). Cold smoked salmon fillets inoculated with *L. monocytogenes* were frozen at 20^{o}C for 5 days and subsequently stored at 4 and 10^{o}C for up to 60 days. The freezing treatment increased the lag phase before *L. monocytogenes* growth by 10 -15 days when stored at 4^{o}C, and 4 days when stored at 10^{o}C. The numbers of freeze-stressed *L .monocytogenes* stored at 4^{o}C never reached those of the not-frozen controls, even after 60 days. However, when refrigeration was at 10^{o}C and after as little as 16 days, *L. monocytogenes* numbers increased to more than 7 Log cfu/g fish which was similar to numbers achieved by the unstressed controls.

Guyer and Jemmi (1991) undertook three separate trials during which they observed the growth of inoculated *L. monocytogenes* on salmon fillets during their processing and storage. There were no significant differences between the growth of a reference strain and a salmon fillet derived-strain of *L. monocytogenes* used for the studies. A general conclusion of the work was that freezing of the finished product, followed by thawing and refrigerated storage had no

significant effect on the numbers of inoculated *L. monocytogenes* compared with an unfrozen inoculated control.

Super-chilling involves reducing the temperature of fish uniformly to a point slightly below that obtained in melting ice and has been used to extend the shelf life of the fish. Midelet-Bourdin *et al*. (2008) found that super chilling of smoked salmon to -2^{o}C for 14 days reduced the prevalence of *L. monocytogenes* to 9.0% compared with 39.0% for storage above 0^{o}C.
In summary, the treatment of smoked fish by temperature reduction to below 0^{o}C appears to have a fairly limited impact on *L. monocytogenes* proliferation during subsequent storage at higher temperatures: a below zero cold treatment followed by refrigerated storage does not eradicate *L. monocytogenes* from smoked fish. The best case scenario is that if the subsequent storage temperatures are low enough, the below zero temperature treatment can extend the amount of time before *L. monocytogenes* commences exponential growth. Freezing and super cooling as treatments followed by refrigerated storage are not reliable interventions for *L. monocytogenes* control. Keeping fish frozen with thawing only at the point of use of course prevents *L. monocytogenes* growth for the frozen stored period.

2.3.6.7 IRRADIATION

Food irradiation is a processing technique which exposes food to electron beams, X-rays or gamma rays. The radiation exposure causes reductions in numbers of microorganisms, with high exposures effectively sterilising food. In contrast to pasteurisation, cooking or other forms of heat treatment, irradiated food tends not to change colour or have an altered texture, although there can be minor chemical changes to foods subjected to the process. A great deal of research spanning several decades funded by the WHO, UN-FAO and the USDA has shown that irradiation of food is safe and an effective way to kill bacteria and preserve food.

Irradiated foods in the EU are subject to some strict controls applied at the individual member state level. In the UK, the Food Standards Agency licences premises to use irradiation subject to approval by the European Commission. At the time of writing, there is only one food processing premises in the UK with a licence to irradiate food and its authorisation is restricted solely to herbs and spices. The Food Irradiation Regulations (2009) oblige food processors to clearly label food exposed to ionising radiation and restrict irradiation only to a number of food classes.

Fish and shellfish are an allowed food group which can be exposed to up to 3 kGy provided:

- There is a reasonable technological need
- The food presents no health hazard and the radiation treatment is not used as a substitute for hygiene and health practices or for good manufacturing or agricultural practices
- The irradiation benefits consumers

In addition, other restrictions apply to ensure that food irradiation may only be used to:

- reduce the incidence of food-borne disease by destroying pathogenic organisms,
- reduce spoilage of foodstuffs by retarding or arresting decay processes and destroying spoilage organisms,
- reduce loss of foodstuffs by premature ripening, germination or sprouting,
- rid foodstuffs of organisms harmful to plant or plant products

E-BEAM IRRADIATION

Electron beam (e-beam) irradiation uses electrons to irradiate food and reduce the numbers of microorganisms associated with it. The process targets microorganisms' nucleic acids and is becoming popular in the USA because it is a cold process which does not significantly alter the structure or flavour of a number of foods. Informally in the USA, the process is referred to as 'cold pasteurisation' (Anon, 2011). E-beam irradiation can be used to inactivate *L. monocytogenes* in cold smoked fish (Medina *et al*, 2009). An initial *L. monocytogenes* inoculum of 9 Log cfu/g on cold smoked salmon was decreased by 7 log units after exposure to a dose 4 kGy of e-beam radiation. A dose of 1 kGy produced a 2 log inactivation (Medina *et al* 2009). However, in contrast to many foods, E-beam doses of 2 to 4 kGy produced marked off odours for cold smoked salmon.

X-RAY IRRADIATION

The effectiveness of X-ray irradiation on reducing *L. monocytogenes* numbers in ready-to-eat vacuum packed smoked mullet has been assessed (Robertson *et al*. 2006). Robertson found that a dose of 2 kGy was required to eliminate an initial level of 10^4 cfu/g. No change of sensory quality was detected in the product.

Although there are limited data, a brief summary of Robertson's findings are that exposure of cold smoked salmon to 1 kGy of x-rays would decrease the natural bacterial population and increase the shelf-life by about 20 days with only slight off-odours detectable after 35 days storage at 5^{o}C. Also X-ray appeared to be better suited for smoked fish because it did not leave any significant organoleptic evidence of the treatment. Whether smoked fish could be irradiated is questionable because potentially, *L. monocytogenes* could be a health hazard to vulnerable groups and the irradiated food regulations (2009) state that the food presents no health hazard prior to irradiation.

2.3.6.8 HIGH PRESSURE

High pressure processing has been shown to be effective in reducing numbers and delaying the regrowth of *L. monocytogenes* associated with smoked fish. Using a four strain cocktail of *L. monocytogenes*, an initial study showed there was a correlation between growth lag for *L. monocytogenes* and pressure applied (Lakshman and Dalgaard 2004). Samples treated with 250 MPa had a 17 day lag period before *L. monocytogenes* growth commenced compared with one day for untreated controls. However, and texture and colour differences were seen at applications of 200 MPa of pressure or higher.

However, it became clear after subsequent work that pressure, salt and phenol act synergistically to inhibit *L. monocytogenes*. No bactericidal effect was achieved when dolphinfish which had been smoked under mild conditions (1.97% salt and 42 ppm phenol) was exposed to a high pressure treatment of 300 MPa at 20^{o}C for 15 min. However, under more severe salting and smoking conditions (2.93% salt and 82 ppm phenol), pressurization kept *L. monocytogenes* counts under the detection limit throughout 100 days of storage. Both high pressure (Lakshman and Dalgaard, 2004) and increased phenolic compound concentrations (Vitt *et al* 2001) have previously been reported as causing unacceptable organoleptic changes to the product.

Temperature and pH also influence the effectiveness of pressure. A French study investigated the effects of high pressure processing at 100, 150, and 200 MPa combined with sub-zero temperatures of -10^{o}C, -14^{o}C, and -18^{o}C at pH 7.0 and pH 4.5 on *L. monocytogenes* present on salmon fillets (Ritz *et al*. 2008). Perhaps not surprisingly, the study showed that the most effective high-pressure treatment for *L. monocytogenes* inactivation was when extremes for high pressure and low pH and temperature were used. Under these conditions,

L. monocytogenes numbers were lowered more than a six logs rather than extending the lag phase before growth (Ritz *et al*. 2008). However, modifications of the physical properties of the fish flesh were a consequence of the treatment. In particular, the fish needed to be heated before the pressure was released to prevent freezing. Consequently, the researchers observed a pronounced lightening of the pinkish colour of the flesh as well as an increased toughness of the meat which they believed may be acceptable to consumers on the grounds that they were indicators of improved food safety.

High pressure alone (450 MPa) over a period of 10 minutes at 12^{o}C was shown by Medina *et al* (2009) to produce an initial three log decrease in *L. monocytogenes* artificially inoculated onto cold smoked salmon by dipping into a solution containing 3 x10^{6} cfu *L. monocytogenes* per ml. However, the *L. monocytogenes* recovered and after 35 days at 5^{o}C growth of at least a log was observed during storage at 5^{o}C. If the storage temperature was 5^{o}C - 8^{o}C only 21 days were required for a one log growth. However, Medina *et al* (2009) did note that there was no odour given off as a result of the treatment although slight colour changes were observed. A potential criticism of the study was that only a single strain of *L. monocytogenes* isolated from chicken was used.

More recently, the potential of exceptionally high pressure was examined by Gudbjornsdottir *et al* (2010) who found that high pressure treatment (700-900 MPa) was of potential value for the control of *Listeria* associated with fish. In the Gudbjornsdottir study, *L. innocua* was reduced from 4.5 x 10^{3} cfu/g to undetectable numbers. Although no substantial change in the colour of the fish flesh was observed except a minor lightening of the product, the microstructure of the cold smoked salmon was most detrimentally affected at the highest pressure 900 MPa with a treatment time of 60s (Gudbjornsdottir *et al*. 2010). In keeping with the other studies (Ritz *et al*. 2008; Medina *et al*. 2009) the high pressure treatment caused changes in the fish flesh structure resulting in hardening of the fish flesh severe enough to be perceived by consumers (Gudbjornsdottir *et al*. 2010).

Overall, although the microbiological benefits for pressure treatment are beneficial, the principal drawback of high pressure is that the fish flesh colour is changed by the pressure treatment and the fish flesh tends to become tougher. For these reasons, before high pressure processing could be properly assessed as an practical intervention, formal taste testing would

need to be undertaken to determine if the reported physical changes to the product were acceptable to consumers.

2.3.6.9 PULSED LIGHT

Pulsed light involves the use of very high intensity and short duration pulses of broad-spectrum 'white light' that last only a few hundred millionths of a second. In the EU, use of pulsed light would likely be subject to compliance with the consumer safeguards defined in regulation EC 258/97. The key issue is whether the treatment would be classed as irradiation by the regulators.

The wavelengths applied include a section of the electromagnetic spectrum normally filtered out by the Earth's atmosphere. Since the 'light' contains radiation that is not naturally found on the planet's surface, there are few bacterial defence mechanisms that protect against it. A specific subset of wavelengths casually referred to as pulsed UV light has been specifically approved for use with food in the United States by the Food and Drug Administration.

Ozer and Demirci (2006) exposed raw (not smoked) salmon fillets inoculated with *L. monocytogenes* to pulses of high intensity light. The fillets were exposed at different distances from the strobe and also for different lengths of time. The authors found no differences in *L. monocytogenes* population reductions between the skin and muscle of salmon fillets. The reductions were however quite modest and of the order of a single log unit at a distance of 8 cm from the light strobe and a 60s exposure (Ozer and Demirci, 2006). At shorter distances (3 to 5 cm) from the strobe, high enough temperatures were recorded in the fillets to partly cook the flesh. The authors concluded a 60s treatment that was 8cm from the strobe could achieve a single log reduction without quality issues. At a distance of 8cm, a high intensity pulse is however required. Although there is modest microbiological benefit for the use of pulsed light at that distance, the authors note that the generation of high intensity pulsed light is energy intensive and that a significant barrier to uptake is the cost of the required electricity.

2.3.6.10 UV LIGHT

Short wavelength UV-C (100-250nm) has been explored as a decontamination treatment for fish (Bernbom *et al*, 2011). In the EU, use of UV would likely be subject to compliance with the consumer safeguards defined in regulation 258/97. Use of a ceiling-mounted, light source in a

cold smoked salmon production plant reduced *L. monocytogenes* numbers on fish which were close to the light source. The reduction was time-dependant with a three log decrease in total bacterial counts after 48 hours of exposure. The numbers of samples which tested positive for *L. monocytogenes* were not significantly lowered after 7 hours exposure to a UV-C lamp. In contrast, significant reductions in positive test results from fillets in close proximity to UV source were noted after 48 hours exposure. Furthermore, after the extended exposure of the fillets for 48 hours, areas greater than 5 metres away from the source or which did not have any apparent direct UV-C illumination showed a significantly reduced incidence of *L. monocytogenes*. The effectiveness of UV-C light was decreased by the presence of organic materials (Bernbom *et al*, 2011). The study acknowledged the human health implications of having a UV-C source close to workers in a production facility. This hazard could be minimized by placing plant conveyor belts inside a UV-C tunnel.

2.3.6.11 PH AND A_W

There is little published literature relating to the influence of natural pH (i.e. pH not manipulated by acetate, lactate or other preservative) and water activity (a_W) on the survival and propagation of *L. monocytogenes* associated with smoked seafoods. However, a set of key papers from a Belgian research group has attempted to model the behaviour of *L. monocytogenes* as a function of a_W, and pH based on laboratory test results from 26 clinical isolates suspected of causing foodborne illness or food isolates of *L. monocytogenes* (Gysemans *et al* ,2007; Vermeulen *et al* ,2007, and Vermeulen *et al,* 2009). The growth boundary of *L. monocytogenes* was observed during these studies to not be restricted to a narrow transition zone. Indeed "in the studied region, a_w did not have a pronounced influence on the position of the growth/no growth boundary while a low concentration of acetic acid (0.2% (w/w)) and a pH decrease to 5.8 was sufficient to significantly reduce the possibility of growth". The results from these studies are summarised in Table 6. The findings that *L. monocytogenes* is tolerant to high salt concentrations and low water availability supported the evidence previously presented (section 2.3.4.2; Peterson *et al*, 1993; Truelstrup Hansen *et al*, 1998; Jørgensen *et al*, 2000; Gram, 2001).

Although sparse, there is a small amount of data describing typical pH and water activity values for smoked seafood sold in the UK (Table 7). Salt concentrations were observed to vary by product type, given shelf life and country of origin. In the UK, the salt contents of cold smoked salmon sampled at retail were recently found to range from 2.2-3.5%, and had shelf lives from

10-16 days. An earlier MAFF (1991) study of 'The microbiological status of some mail order foods' reported salt concentrations ranging from 3.29-8.11% and shelf lives from 11-20 days. Based on the work of the Belgian researchers, the reported salt concentrations and a_w typical of smoked fish in the UK, it is considered unlikely that the growth of *L. monocytogenes* on fish would be impacted significantly (Table 7).

During challenge studies it has been observed that, while pH and a_w influence the probabilities of growth, the initial inoculum is a major determining factor for *L. monocytogenes* survival or growth in food (Vermeulen *et al* 2009). Vermeulen *et a*l (2009) showed an increasing probability of growth as the initial cell count increased. The authors reported that it was unlikely that a larger *L. monocytogenes* population had a greater chance of growth as a consequence of a higher probability in a larger population than a single stress-resistant cell multiplying. This Belgian study concluded that it "seemed the bacteria influenced each other's growth"; which in is agreement with other observations regarding the importance of sessile *L. monocytogenes* (Tomkin 2002; Klaeboe *et al*, 2010).

Table 6 The results from screening of 26 *L. monocytogenes* strains to determine the a_w, and pH values where growth did not occur

Strain[a]	C/F[b]	a_w	pH
33	F	0.935	4.4
34	F	0.93	4.3
35	F	> 0.950	4.1
182	F	> 0.950	–[c]
207	F	0.93	4.5
212	F	0.93	–
233	F	0.94	–
234	F	0.93	–
235	F	0.93	4.1
236	F	0.93	4.1
349	C	0.935	4.4
350	C	0.935	–
351	C	0.92	4.4
352	C	0.94	4.4
416	C	0.93	4.6
417	C	0.94	4.3
418	C	0.92	4.6
419	C	0.94	4.3
420	C	> 0.950	4.1
421	C	0.94	4.1
422	C	0.935	4.4
423	C	0.94	4.4
424	C	0.935	4.4
425	C	0.95	4.4
680	F	0.92	–
733	F	0.915	–

Table is reproduced from Vermeulen *et al* 2007. Key: [a] Culture collection identifier; [b] Clinical (C) or food (F) isolate; [c] No data was available.

Table 7 Details of cold smoked fish products sold in the UK

Product	VP/MAP	NaCl	Shelf life (chilled)	Process	Notes
Cold smoked salmon	VP	Aqueous >3.5% from top to bottom of salmon side	16 days	22-30°C, 12-24h	UK major multiple
		Unknown	1-6 weeks		International (range)
	VP or MAP	3%	10 days		
Cold smoked salmon side	VP	2.2%	>14 days	22-30°C, 12-24h	UK: Sold on eBay. 'Despatch overnight by express carrier'
Cold smoked trout	MAP (10% O_2, 50% N_2, 40% CO_2)	Aqueous >3.5% from top to bottom of salmon side	16 days	22-30°C, 12-24h	UK. Shelf life limited in practice by organoleptic quality

Source: Industry data (published in Peck, Goodburn, Betts, Stringer, 2006). VP is vacuum packed; MAP is modified atmosphere packaging.

2.3.6.12 MODFIED ATMOSPHERE PACKAGING (MAP) AND VACUUM PACKING

VACUUM PACKING

L. monocytogenes numbers were observed to increase by approximately one log when unsmoked fish was inoculated with *L. monocytogenes*, vacuum packed and stored at 4^{o}C for one week (Rørvik, 1991). Under similar storage conditions after five weeks, populations of *L. monocytogenes* were shown to increase by as much as four log units (Rørvik, 1991). In combination, these findings strongly suggest that vacuum packing does not significantly inhibit the growth of *L. monocytogenes* in either the short or longer terms when the bacterium is using fish as a nutrient source. A potential criticism of the study was the use of an artificial inoculation of *L. monocytogenes*.

Conflicting observations have been reported on the implications of smoking fish prior to vacuum packing Cortesi *et al*, 1997; Nilsson, 1997; Beaufort *et al*, 2007;). Nilsson *et al*. (1997) found that numbers of *L. monocytogenes* increased from three logs to eight logs cfu/g in vacuum packed cold-smoked salmon over refrigerated storage of eight days. In contrast, Porsby *et al* (2008) found that after brining and cold smoking, the numbers of *L. monocytogenes* decreased when the fish were vacuum-packed and stored at 5^{o}C. There appear to be a number of common issues with reports of the fate of *L. monocytogenes* on fish which is vacuum-packed and stored under refrigeration. A large number of the studies (Rørvik 1991; Nilsson, 1997; Guilbaud *et al* 2008; Yilmaz, 2009) use laboratory-grown *L. monocytogenes* strains grown in liquid broth whereas natural contamination is more likely to be from plant environment persistent sessile *L. monocytogenes*. In addition, artificially contaminated fish tend to be inoculated with much larger numbers of *L. monocytogenes* than naturally contaminated fish (Beaufort *et al*, 2007). Lappi *et al* (2004) summarises the other issues as: a unified overview of *L. monocytogenes* growth during storage in naturally contaminated smoked fish has been difficult to interpret due to the heterogeneity of *L. monocytogenes* distribution (Cortesi *et al*, 1997) within samples (Lappi *et al*, 2004) and the variable composition of smoke and smoke condensates (Sunen *et al*, 2003; Stołyhwo and Sikorsky, 2005).

In order to address these confounding issues, Lappi *et al*, (2004) sought to closely mimic a production scenario by observing the fate of *L. monocytogenes* that had naturally contaminated smoked salmon along with refrigerated storage for 28 days under vacuum. Lappi and colleagues

reported that numbers of *L. monocytogenes* did not exceed the EU (2075/2003) statutory limit for ready to eat foods of 100 cfu/g fish flesh (Lappi *et al*, 2004a).

A later, larger French study also determined numbers of *L. monocytogenes* likely to be present on cold smoked salmon after vacuum packing and extended refrigerated storage (Beaufort *et al*, 2007). This study determined initial *L. monocytogenes* presence and numbers for more than one thousand naturally-contaminated samples which were sourced from nine French smoking plants sporadically over a four year period. Samples were initially tested between three and eight days after smoking and the packs were resealed. A second test was undertaken for initially positive packs after refrigerated storage for eight to 15 days at 4^{o}C to mimic cold chain transport and the packs were again resealed. A final retest was undertaken after 8^{o}C storage for 7 days to mimic domestic refrigeration conditions. Although initial detection prevalences in the smoked salmon ranged from 0% to 41%, more than 92% of samples contained *L. monocytogenes* numbers that were below 1 cfu/g fish. After the 4^{o}C storage, there were no significant changes in *L. monocytogenes* prevalence or numbers. The highest numbers of *L. monocytogenes* observed were 7 cfu/g fish. After the 8^{o}C storage, 17% of the contaminated products exceeded 100 cfu/g fish with the highest number observed being 2800 cfu *L. monocytogenes* /g fish. The key findings of the Beaufort study are that if *L. monocytogenes* are present on fish that are vacuum packed, even in numbers less than 1 cfu/g fish, there is the potential for growth during low temperature storage of the fish.

Porsby *et al*. 2008 determined that the numbers of *L. monocytogenes* decreased, but were not eliminated in a liquid smoked salmon product when vacuum packed and stored at 5^{o}C over a period of 20 days. However, although the study comprehensively assessed a number of stages of the cold smoking process, the fillets were all artificially inoculated.

In summary, it is apparent that naturally-present *L. monocytogenes* can multiply under vacuum packing conditions on smoked fish. Although heavy salting in combination with some smoke residues can significantly delay growth and possibly even cause partial *L. monocytogenes* death, vacuum packing in itself is not an effective way of checking *L. monocytogenes* growth or eliminating it entirely during cold storage prior to consumption.

CARBON DIOXIDE MODIFIED ATMOSPHERE PACKING

In the EU, modified atmosphere packaging (MAP) for foods are considered to be food additives and are approved under Annex 1 of 92/2/EC. MAP are permitted for use subject to the conditions and labelling requirements stipulated in directive EC 92/2. In addition, Regulation 33 of the Food Labelling Regulations 1996 sets out that: *A food, the durability of which has been extended by means of its being packaged in any packaging gas authorised pursuant to Council Directive 89/107/EEC, concerning food additives for use in foodstuffs intended for human consumption, shall be marked or labelled with the indication packaged in a protective atmosphere*.

Nilsson et al (1997) report that carbon dioxide modified atmosphere (70% CO_2 [E290] and 30% N_2 [E941]) packed cold-smoked salmon artificially inoculated with a seven-strain cocktail of *L. monocytogenes* had an eight day lag before *L. monocytogenes* growth. Control samples packed under vacuum showed a five log increase in vacuum packaging under the same conditions. However, the CO_2-mediated prevention of growth was a temporary effect because after 27 and 44 days post inoculation, there were three log and four log increases in *L. monocytogenes* numbers respectively.

Increasing the CO_2 concentration to 100%, in combination with either of the bacteriocins nisin or ALTA 2341, was shown to completely suppress growth of *L. monocytogenes* artificially inoculated onto salmon at both refrigeration and abuse temperatures (Szabo and Cahill 1999). Based solely on subjective sensory determinations, Muratore and Licciardello (2005) reported that cold smoked sliced swordfish had a much shorter half-life of 12 days when packed in a modified atmosphere (5% O_2, 45% CO_2, 50% N_2) compared with 42 days when vacuum packed. The study showed no correlation between the shorter sensory shelf-life of the modified atmosphere packed smoked fish and increases to the total aerobic mesophilic counts. Bacterial numbers for both treatments did not significantly increase until at least 15 days storage which was 3 days past the sensory shelf life (Muratore and Licciardello, 2005).

Changes in *L. monocytogenes* numbers during refrigerated storage of artificially contaminated rainbow trout fillets packaged in air (control), vacuum and various modified atmospheres (MAP) has been studied (Yilmaz *et al* ,2009). The MAPs used were 50% CO_2 and 50% N_2 (MAP A); 80% O_2 and 20% CO_2 (MAP B) and 2.5% O_2 in combination with 7.5% N_2 and 90% CO_2(MAP C). Over a

storage interval of 18 days, the *L. monocytogenes* populations multiplied from an initial concentration of 10^4 cfu/g to 10^7 cfu/g in air and to 10^6 cfu/g in vacuum packaging. Yilmax and colleagues observed that modified atmosphere packaging did not eliminate *L. monocytogenes* from rainbow trout fillets. However, all three MAPs retarded the growth of *L. monocytogenes* at 4°C to some extent. There was little difference between the antibacterial effects of each MAP with each having the lowest count at different points along the experimental time course. Although the authors undertook *L. monocytogenes* number determinations in duplicate in two different determinations (n=4), error bars were not reported for the counts. Furthermore, the methods used in this study described that ANOVA was used to determine if differences between the controls and treatments were significant, however there was no mention of significant differences between *L. monocytogenes* numbers for either each treatment or the controls. After 18 days MAP B had the lowest count (5.5 Log cfu/g fish), but MAP C was only slightly higher (6.7 Log cfu/g fish). The air control contained 7.5 Log cfu/g fish. Whether the differences in the treatments were significant is not stated and it is therefore difficult to draw firm conclusions from the interesting, but ineffectively-reported, study (Yilmaz *et al*, 2009).

Although treatment of smoked fish with a CO_2-based MAP can likely delay *L. monocytogenes* exponential growth, there is a significant problem with CO_2 packing which also applies for a number of other MAPs. MAP require an increased volume of packaging to maintain, for example, an effective CO_2 to product ratio. The extra volume of packaging produces a downstream reduction in sustainability through the supply chain. Some analysts have excluded this intervention as not feasible based on the increase in packaging volume (Hansen *et al* 2009). However, there is potential to circumvent this problem by utilizing a CO_2 emitter to maintain an adequate level of CO_2 inside a low volume package during storage (Hansen *et al* 2009). To date no studies have investigated the use of CO_2 emitters as an intervention for *L. monocytogenes* and smoked fish. However, using the CO_2 emitter approach, Hansen and colleagues found that the total bacterial numbers on raw fish remained below 50 cfu/g over the first 14 days of storage at 0.1°C. After two weeks however, the numbers of total aerobes had begun to increase slowly. The average numbers of total aerobic mesophiles had increased by (?) around two log units by 28 days. By comparison, controls without the CO_2 emitters and an air atmosphere had bacterial numbers of around seven logs.

Modified atmosphere packing with elevated concentrations of CO_2 have some issues to be addressed before they could be used as an intervention for the control of *L. monocytogenes* on

smoked fish. It is likely that CO_2 emitters would need to be used to prevent the logistical cost issues associated with increased packaging volumes. In addition, CO_2 does not eradicate *L. monocytogenes* or prevent its growth on smoked fish. Under conditions of perfect refrigeration, high concentration CO_2 MAP at best delays the proliferation of *L. monocytogenes* by up to seven days. In order to be used as a reliable intervention, CO_2 MAP would need to be used in combination with another treatment such as a bacteriocin (e.g. nisin).

2.3.6.13 SODIUM NITRITE

The UK Miscellaneous Food Additives Regulations (1995) apply to enforce EC regulation 95/2/EC. Part C of schedule 2 of 95/2/EC only currently permits the use of sodium nitrite (E250) as a preservation agent if sold in combination with sodium chloride (NaCl) or a salt substitute and only for use preserving cured or canned meat products and liver paté.

Sodium nitrite primarily added to cold smoked fish as an intervention has been shown to inhibit the growth of *Clostridium botulinum* (Pelroy *et al* 1994). Over a period of 40 days at 5^{o}C *L. monocytogenes* growth could be inhibited by a combination of 3% or 5 % NaCl along with 190-200 ppm sodium nitrite when packaged in gas-permeable film or under vacuum. However, when the experiments were performed at 10^{o}C, growth was detected after as little as 5 days of the 40 day study. A combination of nitrite and NaCl was effective at low inoculums (10 cfu/g) of *L. monocytogenes*, but when greater numbers (327 cfu/g) were used, the inhibition was not so pronounced. Even with a low inoculum, growth was detected when fillets were incubated at 10^{o}C with *L. monocytogenes* numbers of 10^5-10^6 cfu/g detected after 30 days (Pelroy *et al* 1994). These results reinforce the importance of having uncontaminated starting material followed by adequate refrigeration for effective *L. monocytogenes* control.

Nitrite used without other inhibitory agents, or in combination with high salt concentrations, has limited use as an intervention *L. monocytogenes* proliferation on smoked fish. Nitrite is effective at postponing, but not eliminating, the commencement of exponential growth by *L. monocytogenes* only when the initial starting numbers of the bacteria are very low (~10 cfu/g fish).

2.3.6.14 SORBATES

In the USA, sorbates can be used as food additives since they generally recognized as safe (GRAS). In the EU, sorbic acid and potassium and calcium sorbates (E200, E202, E203) are approved as set out in Annex III of 95/2/EC, subject to limits on their final concentrations, as **preservatives** for some foods including semi-preserved fish and salted fish products (Miscellaneous Food Additives Regulations, 1995).

The effect of pre-treatment with potassium sorbate on *L. monocytogenes* populations during the storage of freshly caught red mullet and carp in Greece by Tassou *et al* (2004). Gutted fish were dipped for 60 seconds in sterilized water containing *L. monocytogenes* at a concentration of 3 x 10^5 cfu/ml, then placed the fish in a solution of 5% (w/v) potassium sorbate with or without hot water at 60^{o}C (Tassou *et al*, 2004). The fish were subsequently packed in an aerobic or in a modified atmosphere (40% CO_2, 30% O_2, and 30% N_2) and stored at 0-1^{o}C. In the control (untreated) fish, the numbers of *L. monocytogenes* increased from 4.8 to 6.5 log cfu/g. By comparison, *L. monocytogenes* numbers on the potassium sorbate-treated fish stored aerobically remained at 4.0 log cfu/g over a 15 day period. Similar results were seen with red mullet and when using sorbate in combination with hot water (Tassou *et al* ,2004). However, when the fish were stored in a modified atmosphere, the difference between controls and potassium sorbate treated samples were not so marked. The authors speculate that an inhibitory effect to *L. monocytogenes* growth was conferred by the MAP although previous studies have shown little practical benefit for MAP as a control measure for *L. monocytogenes* (section 2.3.6.12). No indications were given on what effect, if any, there was on the sensory quality of the fish.

There are a number of issues relating to the studies of Tassou *et al* (2004) which make potassium sorbate treatment unsuitable for use as an intervention for *L. monocytogenes*. Firstly it is likely that both the MAP and the sorbate caused the observed bacteriostatic effect on raw fish; but the inhibitory contribution of each treatment was not clear. Secondly, the hot water treatment of 60^{o}C used by the researchers was likely to have denatured at least some of the protein in the fish flesh potentially causing organoleptic changes to the finally-smoked product. For these reasons, potassium sorbate treatment was not assessed as a suitable intervention for *L. monocytogenes*-contaminated raw fish.

Potassium sorbate in a combination with other antimicrobials has also been found to be inhibitory to *L. monocytogenes* (Ye *et al.*, 2008). Ye *et al*. (2008), tested a range of GRAS preservatives which included nisin, sodium lactate, sodium diacetate, potassium sorbate and sodium benzoate. Effectiveness testing was for the chemicals individually and also in combination. The study concluded that when used in conjunction with chitosan films, a combination of sodium lactate and potassium sorbate was the most effective of the antimicrobials assessed. The combination prevented the growth of *L. monocytogenes* for over eight weeks at 4˚C (Ye *et al*. 2008).

In a study undertaken in the United States by Neetoo and colleagues (2008), potassium sorbate (PS) used on its own was shown not to significantly inhibit growth of a 12-strain cocktail of *L. monocytogenes* in broth culture at its (EU and US) legal concentration limit of 0.3% (w/v; Neetoo *et al*. 2008). However, when PS was used in combination with nisin (0.00125% and 0.0025% w/v) and sodium diacetate (0.125 and 0.25%w/v) no growth in broth was detected. Although results from broth-based studies can sometimes bear little relation to real-world observations in commercial processing facilities, Neettoo *et al* (2008) were also able to show that a combination of 0.00125% Nisin/0.15% Potassium Sorbate could suppress the growth of *L. monocytogenes* in smoked salmon fillets over three weeks storage at 4^{o}C.

In summary, sorbate is not able to effectively eliminate or prevent the growth of *L. monocytogenes* on smoked fish during storage of the product. Sorbate has been shown to be effective when used in combination with lactate or nisin. However both nisin (Nilsson *et al*, 1997) and lactate (Pelroy *et al*, 1994) on their own showed significant efficacy at preventing the proliferation of *L. monocytogenes*. Thus the observed inhibitory effects for the combination sorbate treatments are probably more due to the lactate and nisin than the sorbate. Consequently sorbate is not assessed as being a particularly useful intervention for *L. monocytogenes* control on smoked fish.

2.3.6.15 NISIN AND OTHER BACTERIOCINS

Bacteriocins are antimicrobial proteins secreted by some bacterial species which tend to inhibit only closely-related bacterial species (Cotter *et al*. 2005). Bacteriocins are secreted by bacteria primarily as part of their niche protection strategy. Nisin is a polycyclic peptide bacteriocin from *Lactococcus lactis* subsp. *lactis* widely used as a food preservative (Delves-Broughton *et al*, 1996). The use of nisin as a food preservative has been widespread in the UK since the 1960s

(Delves-Broughton *et al*, 1996). Nisin (E234) is authorised for food preservation in the European Union by Directive 95/2/EC on food additives other than colours and sweeteners. Nisin is the only bacteriocin currently permitted for use in the EU. Its use is restricted only for ripened and processed cheeses, dairy-based puddings, clotted cream and mascarpone. Specifications for nisin are laid down in Directive 96/77/EC. Nisin may be present in food as a consequence of the direct addition of purified bacteriocin, or it can be secreted from naturally-present or inoculated lactic acid bacteria.

Several bacteriocins have been identified as efficiently reducing *L. monocytogenes* numbers and are discussed here with the caveat that different *L. monocytogenes* strains vary in their susceptibility to nisin (Rasch and Knochel, 1998). Approximately 50 units/g nisin resulted in the survival and growth of *L. monocytogenes* on smoked salmon (Nilsson *et al,* 1997). Low concentrations of nisin remained ineffective even when used in combination with other preservatives such as salting and CO_2 packing. However, raising the nisin concentration to 500 or 1000 U/g of cold-smoked salmon inoculated with *L. monocytogenes* delayed, but did not prevent growth in vacuum-packs stored at 5^{o}C. At higher concentrations of nisin to CO_2 packaged cold-smoked salmon resulted in an initial one to two log reduction of *L. monocytogenes* numbers followed by a lag phase of 8 and 20 days in salmon with 500 and 1000 U nisin/g, respectively (Nilsson *et al*. 1997). An elongated lag were also reported by Szabo and Cahill (1999) who also investigated growth of *L. monocytogenes* in nisin-treated smoked salmon packed under vacuum.

The effects of Nisin, sodium lactate or their combination (1:1) injected into rainbow trout at an industrial scale before the smoking process as well as into the finished smoked product has been reported (Nykänen *et al* (2000). Both nisin and lactate were observed to inhibit the growth of *L. monocytogenes* in refrigerated smoked fish. However, in combination the two compounds acted synergistically (Nykanen *et al*. 2000) and when injected into finished product, decreased the numbers of *L. monocytogenes* from 3.26 to 1.8 log CFU/g over 16 days of storage at 8^{o}C. The numbers of *L. monocytogenes* remained almost constant (4.66-4.92 log CFU/g) for 29 days at 3^{o}C in those samples injected before smoking with nisin and sodium lactate. However any sensory implications of the intervention were not recorded and so it is currently unknown if taint or texture changes are a consequence of the treatment.

Neetoo *et al*. (2008b) examined how *L. monocytogenes* growth changed when nisin-coated plastic films were used to vacuum-pack cold smoked salmon. In control (non-coated) samples, the numbers of *L. monocytogenes* artificially inoculated onto pressed disks of salmon pâté or cold smoked salmon fillets grew from 500 cfu/cm^2 to approximately 1 x 10^7 cfu/cm^2 during storage for 58 days at 4^oC. Pâté samples wrapped in plastic coated with 500 IU of nisin/cm^2 displayed a decreased rate of *L. monocytogenes* growth but eventually reached the same level as the control. When 2000 IU of nisin/cm^2 of film was used, the growth of *L. monocytogenes* on the pâté was inhibited over the 58 days of 4^oC storage. In addition, when the increased concentration of nisin was used, growth on pâté was inhibited for more than 35 days at a higher storage temperature of 10^oC. Inhibition was also found when the cold smoked salmon covered with a plastic film coated with 2000 IU of nisin/cm^2 was inoculated with *L. monocytogenes* at a concentration of 3 logs cfu/cm^2 and stored at 4^oC for 43 days. However, in contrast to the pâté, no inhibition was noted when the salmon inoculated with the higher level of *L. monocytogenes* was stored at a higher temperature (10^oC) which is routinely used to mimic temperature abuse during storage (Neetoo *et al*, 2008b).

Bacteriocins have been isolated from a range of *Lactobacilli* (Ghalfi *et al* 2006). A bacteriocin secreted by *Lactobacillus curvatus* CWBI-B28 was shown to be inhibitory to *L. monocytogenes* growth in broth and on cold smoked salmon fillets(Ghalfi *et al* 2006). A range of different application methods including direct addition of the bacterial strains to fillets, spraying fish with partially-purified bacteriocin and packaging in a bacteriocin-coated film have been explored. Packing films were optimally coated by heat inactivation of a culture containing *Lactobacillus* producer cells and manipulation of cell solution to an acidic pH. The coated film treatment showed promise as an effective control measure. Numbers of *L. monocytogenes* on film-packaged cold smoked salmon declined from 2 log cfu/cm^2 fish to a level of less than the detection limit of 5 cells per cm^2 after three days at 4˚C (Ghalfi *et al* 2006). Furthermore, the film prevented any subsequent increase in the *L. monocytogenes* numbers throughout 22 days of storage at 4^oC.

A bacteriocin-producing *Carnobacterium divergens* strain is known to secrete an intervention potential. The *C. divergens* strain used for the study secreted the M35 bacteriocin (Tahiri *et al*. (2009). Both the culture and the purified M35 bacteriocin were assessed as inactivators of *L. monocytogenes* in cold smoked salmon. A 2.6 log cfu/g reduction in the numbers of *L. monocytogenes* was observed for up to 10 days of storage in samples treated with the

C. divergens culture. Purified divergicin M35 (50 mg/g), or crude culture supernatant showed reductions of a single log cfu/g at the beginning of storage. However, the anti-listerial activity of the supernatants lasted for 15 days compared to only three days for purified bacteriocin. Colour and texture were not significantly affected by any of the treatments.

When evaluating any antimicrobial, the likelihood of the development of resistance by the target organism must be considered. Studies determining high levels of resistance to carnobacteriocin B2 for fish isolates of *L. monocytogenes* (Nilsson *et al*. 2006) suggest that if lactic acid bacteria are used as an intervention for *L. monocytogenes* associated with smoked fish; it may be a better strategy to use lactic acid strains which do not secrete bacteriocins to prevent the development of resistance. It has been argued that cocktails of different bacteriocin-producing strains would reduce the likelihood of the emergence of resistant strains (Galvez *et al* 2010).

In summary, bacteriocins such as nisin show promise as interventions for *L. monocytogenes* associated with smoked fish. The principle drawbacks for bacteriocin use are that large quantities of the antimicrobial (500-1000 U/cm^2 fish) are required for effective *L. monocytogenes* control. Furthermore, it has already been shown that *L. monocytogenes* can develop a resistance to individual bacteriocins. Thus in order to ensure prolonged effectiveness either a cocktail of strains or bacteriocins or an alternative strategy such as bacteriocin in combination with lactate would be required for the prevention of resistance in the longer term.

2.3.6.16 LACTIC ACID BACTERIA

Preservatives such as nitrates, sulphates and sorbate may not be approved in all countries likely to import cold smoked fish and are specifically forbidden in some EU member states where they are banned from being used to enhance the shelf-life of smoked fish (Matamoros *et al* 2009). Therefore there are drivers that have caused some authors to investigate novel methods for reducing *L. monocytogenes* contamination of fish as alternatives to traditionally-used food preservatives. Lactic acid bacteria (LAB) are promising candidates because some members of the group secrete multi-factorial antimicrobial compounds such as lactic acid, hydrogen peroxide and bacteriocins.

Although LAB are part of the natural biota associated with meat, fish, milk and cheese, if they are cultured and added to foods then there may be regulations which apply. A summary of uses

and whether regulations apply are shown as Table 8. The EC regulations which could potentially prevent or restrict the use of LAB on fish are Regulation 258/97/EC on novel foods and novel food ingredients, Directive 89/107/EEC on food additives, Directive 88/388/EEC on flavourings for use in foods and Directive 2002/46/EC on food supplements, Directive 95/2/EC on food additives other than colours and sweeteners (Wessels *et al* 2004).

Table 8 Potential LAB functions in food and regulatory categories (compiled from Wessels *et al* 2004)

Function of LAB	**Regulatory category**			
	Additive	Ingredient	Process aid	Probiotic
Fermentation starter cultures or preservatives. Foods prepared with and containing live LAB.	✓	✓	✓	-
Probiotic (i.e. health-promoting) function for the consumer of the food. Foods containing living or dead LAB	✓	-	-	✓
Function carried out by particular compound produced by LAB other than lactic acid. Such compounds might be aroma compounds, exopolysaccharides, or bacteriocins	✓	-	✓	-

Although LAB have a widely acknowledged ability of inhibiting the growth and multiplication of a variety of food spoilage organisms (Wessels *et al*, 2004), Nilson *et al* (1999) assessed the ability of LAB in inhibiting *L. monocytogenes* growth. The Nilson study used non-identical strains of *Carnobacterium piscicola* that had been found to dominate the biota of refrigerated vacuum packed stored cold smoked salmon along with a strain of *Lactobacillus sake.* Two strains of *Carnobacterium piscicola* were inoculated at a concentration of ~2×10^6 cfu/g onto salmon

slices which were also inoculated with *L. monocytogenes* (~2 × 10^2 cfu/g) before storage at 5°C. On salmon slices without the lactic acid bacteria, *L. monocytogenes* grew to 3 × 10^8 cfu/g (Nilsson *et al* 1999). When co-inoculated, both strains of *Carnobacterium piscicola* were able to inhibit the growth of *L. monocytogenes* on refrigerated salmon slices for over 40 days (Nilsson *et al* 1999). The very large inoculum numbers used by the study meant that the anti-listerial effect may have been due to competition for nutrients. In order to determine if that was the case, external nutrients were applied to the salmon slices. No significant differences between the results for the additional nutrient samples and the standard nutrient samples were observed strongly suggesting that the basis of the inhibition was not nutritional. The sensory profile for *Carnobacterium piscicola* inoculated salmon was reported to be the same as for untreated cold smoked salmon samples. However the strain of *Lactobacillus sake* produced undesirable changes in flavour (Nilsson *et al* 1999).

The Nilsson study (1999) established that *C. piscicola* was antagonistic to *L. monocytogenes* at high numbers. However, later studies have also reported that *C. piscicola* is a promising antagonist of *L. monocytogenes* on smoked salmon even when inoculated at lower numbers. Yamazaki *et al* (2003) found a strain of *C. piscicola* (~10^4 cfu/g) was able to effectively control the growth of *L. monocytogenes* (~10^3 cfu/g) when co-inoculated on to salmon aerobically at 4°C and 12°C. The lactic acid-producing strain was able to completely inhibit the growth of *L. monocytogenes* for over 20 days. At 20°C, the growth of *L. monocytogenes* was still significantly reduced compared with the control (not inoculated with *C. piscicola*) samples. Similarly, Vescovo *et al* (2006) found that a combination of two LAB strains (*Lactobacillus casei* and *Lactobacillus plantarum*) inoculated at 6 logs cfu/g onto cold smoked salmon achieved a reduction in the counts of *L. innocua* of 3.2 logs compared to the control during storage of the product under vacuum for 30 days at refrigerated temperatures.

As discussed by the pioneering studies of Nilsson *et al* (1999), the antagonistic nature of competing bacteria may be multi-factorial and therefore not as easily broken as can be the case with the addition of a single controlling substance. When 57 strains of *L. monocytogenes* were checked for their resistance to three strains of antagonistic Carnobacteria, none were found to be resistant (Brillet *et al*, 2004). Some grouping of more and less sensitive *L. monocytogenes* strains could be made, but generally, the antagonistic abilities of the Carnobacteria were maintained *in situ* on vacuum-packed, cold-smoked salmon refrigerated for 4 weeks (Brillet *et al*, 2004). The most effective strain of the three Carnobacteria was *C. divergens* V41, a strain

previously identified by Duffes *et al* (1999) as being able to antagonise *L. monocytogenes* when added in co-culture on sterile homogenized cold smoked salmon. Further, later, studies were performed to determine the sensory characteristics of cold smoked salmon inoculated with the V41 strain. A trained panel could notice a slight taste difference, but it was felt to be too small for untrained consumers to notice (Brillet *et al* 2005).

Tome *et al* (2006) found that only 41% of LAB strains exhibited an antibacterial effect on *L. innocua* in a plate assay. However, the authors believed that their cultures were very competitive and that they may provide additional protection against the growth of *L. monocytogenes*. An interesting observation of the Tome study was that for cold smoked salmon, storage at refrigeration temperatures (5^{o}C) in vacuum packaging allowed the LAB strains to outcompete other bacteria thereby potentially putting *L. monocytogenes* under competitive as well as selective pressure. A later study (Tome *et al*. 2007) identified the most effective conditions (6h dry salting with sugar, 6 h of drying and 2 h of smoking) for growth of lactic acid bacteria in vacuum packaged cold smoked salmon. The research concluded that the growth of lactic acid bacteria that are anti-listerial can be enhanced by the appropriate selection of processing parameters.

In summary, LAB are able to prevent the growth of *L. monocytogenes* for around three weeks. Increasing the number of strains used tends to extent the amount of time that *L. monocytogenes* growth is restricted. LAB can restrict *L. monocytogenes* even if the storage temperature is imperfect. Although flavour alterations in the product will be influenced by the LAB strains in use, there are some strains which do no cause significant changes to texture or flavour. Subject to compliance with the regulations specified at the start of this section, the use of multiple strains of LAB rather than purified bacteriocin, would be expected to help reduce the likelihood of resistance in susceptible populations of *L. monocytogenes*.

2.3.6.17 TRISODIUM PHOSPHATE

Trisodium phosphate (TSP; E339) has been approved for use as a food additive within the EU. Part C of schedule 2 of 95/2/EC only currently permits the use of TSP as a preservative for creams, vegetable fats and unripened cheeses.

In the United States, TSP is generally regarded as safe for raw food (Mu *et al* 1997). In the US, TSP is routinely used for the decontamination of raw poultry meat by a patented process which

involves the immersion of post-chill whole poultry in a 10% (w/v) solution for 15 minutes (Mu *et al* 1997). However, TSP is a less effective decontaminant for fish. After storage for 9 days at 4°C, pond-reared rainbow trout immersed in either a 10% or a 20% solution of TSP did not show significant reductions in total psychrotrophic counts or numbers of artificially inoculated *L. monocytogenes* when compared with control samples which had been immersed only in tap water (Mu *et al* 1997).

2.3.6.18 SODIUM LACTATE

Sodium Lactate (E325) is approved for food use in the European Union. However, schedules 1-3 of 95/2/EC only currently permits E325 to be used for the adjustment of the pH of baby food, as a preservative for canned and bottled fruit and vegetables, and bread.

The growth of *L. monocytogenes* in cold smoked fish in comminute (minced) raw salmon was mixed with a range of concentrations and combinations of sodium lactate, sodium chloride, and sodium nitrite. Samples of fish were inoculated with 150 *L. monocytogenes* cells, vacuum-packaged in oxygen-impermeable film and stored at either 5°C or 10°C. Periodically, the samples were tested to determine the numbers of *L. monocytogenes* until the end of the product's shelf life (50 days). Pelroy and colleagues (1994) determined that sodium lactate exhibited a concentration-dependent ability to prevent the growth of listeria, but that it did not inactivate *L. monocytogenes*. Furthermore, the inhibition of growth was enhanced by the presence of nitrite and/or increased concentrations of NaCl. The prevention of *L. monocytogenes* growth was more pronounced at 5°C where total inhibition of *L. monocytogenes* growth was achieved for up to 50 days in the presence of 2% sodium lactate and 3% (water-phase) NaCl. At 10°C, total inhibition was achieved for up to 35 days by 3% sodium lactate and 3% (water-phase) NaCl, or by 2% sodium lactate in combination with 125 ppm sodium nitrite and 3% water-phase NaCl.

The antimicrobial effects of different concentrations of potassium lactate (in combination with sodium diacetate) were also evaluated by Yoon *et al*. (2004) for control of *L. monocytogenes*. The use of potassium lactate plus sodium diacetate mixture at all tested dilutions completely inhibited the growth of *L. monocytogenes* on smoked salmon stored at 4°C during 32 days of storage (Yoon *et al*. 2004).

Researchers (Vogel *et al*. 2006) found that a combination of potassium lactate (2.1%) and sodium diacetate (0.12%) delayed the growth of *L. monocytogenes* for up to 42 days in vacuum packed cold smoked salmon stored at 10^{o}C. This procedure did not affect the quality of the product and the authors suggest that it is a suitable technology to prevent the growth of *L. monocytogenes.*

Mejlholm and Dalgaard (2007a) reported that MAP gravad cold-smoked salmon with the addition of 0.15% (wt/wt) diacetate prevented the growth of *L. monocytogenes* for more than 40 days at 8^{o}C, whereas the addition of 0.15% (wt/wt) diacetate reduced the growth rate of the pathogen in MAP cold-smoked Greenland halibut. This difference between the two types of products was explained by a higher content of naturally occurring lactate in cold-smoked salmon (0.77 to 0.98%, wt/wt) than in cold-smoked Greenland halibut (0.10 to 0.15%, wt/wt).

Ye *et al* (2008) assessed the effectiveness of sodium lactate incorporated into a chitosan-coated plastic film. During an initial evaluation, chitosan-coated plastic film containing sodium lactate at 4.5 mg/cm^2 was assessed as effective at inhibiting the growth of a cocktail of 5 x 10^5 cfu *L. monocytogenes* /cm^2 fish at 20^{o}C for ten days. When the lactate-infused film was tested at refrigeration temperature, it completely inhibited the growth of *L. monocytogenes* on smoked salmon for at least 6 weeks. The authors concluded that chitosan-coated plastic films containing 4.5 mg/cm^2 lactate can potentially assist the smoked-salmon processing industry in their efforts to control *L. monocytogenes*.

Edible alginate coatings containing lactate and diacetate have been appraised as possible suppressors of *L. monocytogenes* growth on cold smoked salmon fillets (Neetoo *et al*, 2010). The study incorporated a range of concentrations of sodium lactate and sodium diacetate into five edible (approved in the EU food-grade) coatings (alginate, κ-carrageenan, pectin, gelatin or starch). A range of concentrations of sodium lactate were used, either alone or in combination with sodium diacetate. The researchers applied the coatings onto the surface of cold smoked salmon slices inoculated with *L. monocytogenes* at 500 cfu/cm^2 before storage at room temperature (~22^{o}C) for six days. Despite the high ambient temperatures, the alginate coatings were able to contain *L. monocytogenes* growth at approximately 500 cfu/cm^2 and were the most effective carriers for delivering *L. monocytogenes* growth-inhibiting antimicrobial compounds. Neetoo *et al*, (2010) concluded that alginate-based coatings containing lactate and diacetate could make a meaningful food safety impact for fish consumed without cooking. In

combination, lactate and diacetate delivered in an alginate coating are bacteriostatic, checking the growth of *L. monocytogenes* and thereby enhancing the microbiological safety of filleted and sliced smoked salmon.

In summary, lactate in combination with chitosan, diacetate, high salt and/or nitrite appears able to delay or slow the growth of *L. monocytogenes* for extended periods of at least 30-40 days. If high salt concentration is used, the temperature needs to be meticulously maintained at 5^{o}C or lower to constrain *L. monocytogenes* growth. Lactate in combination with diacetate does not appear to alter the flavour or texture of smoked fish and thus shows real potential as a workable intervention measure. Lactate is not allowed for use in the EU as a bactericidal (decontamination) wash on foods of animal origin.

2.3.6.19 ACIDIFIED SODIUM CHLORITE

Acidified sodium chlorite (ASC) is an antimicrobial treatment and it has been evaluated as a possible control intervention for the growth of *L. monocytogenes* artificially inoculated onto raw whole salmon and salmon fillets (Su and Morrissey 2003). Article 3(2) of Regulation (EC) No 853/2004 of the European Parliament and of the Council lays down specific hygiene rules for food of animal origin and provides a legal basis to permit the use of a substance other than water to remove surface contamination from products of animal origin. However approval of the use of any substance in compliance with 853/2004 requires an evaluation of the decontamination chemical to be undertaken by an Expert Panel of the EU and for the chemical to be assessed as safe. We are unable to find any evidence that a specific evaluation for ASC has been undertaken by an EU Expert Panel for use with fish. However, a number of antimicrobial washes including ASC were evaluated by an EFSA expert panel to determine whether the use of such washes as chicken carcass decontamination treatments would result in the emergence of resistant bacterial strains (EFSA, 2008). The panel found no evidence that resistance would occur. However, the opinion for chicken is only a small amount of the considerations required for a complete safety assessment.

Su and Morrissey (2003) spray washed fish in 50ppm ASC solution for one minute before storage on ice (fillets) for one week or frozen storage (whole fish) for one month. Changes in *L. monocytogenes* numbers associated with the salmon were followed every two days for the fillets and at the end of storage for the whole salmon (Su and Morrissey, 2003). The ASC treatment did not reduce *L. monocytogenes* numbers on the salmon skin for the frozen whole

fish. On the fillets, the initial ASC wash reduced populations of *L. monocytogenes* by roughly 0.5 logs. However, when the fillets were stored in frozen water ice, the *L. monocytogenes* numbers increased slowly. If the fillets were stored in frozen ASC ice, *L. monocytogenes* growth still occurred although it was slower compared with the standard water ice (Su and Morrissey, 2003).

In summary, ASC treatment of raw fish has a small benefit in reducing the growth of *L. monocytogenes* but it only slows and does not completely stop *L. monocytogenes* multiplication. Although Su and Morrissey report that there was no difference in colour of ASC treated salmon, they also say that further studies would be required on sensory characteristics such as taste, before ASC could be adopted as a modest control measure.

2.3.6.20 BACTERIOPHAGES

Bacteriophages are specific viruses which infect and damage bacterial cells. Eukaryotic cells such as human cells are unaffected by bacteriophages. The use and mode of action of bacteriophages in food production is contentious and was the subject of a review by an EU biological hazards expert panel. The opinion summarised the complex regulations and consumer hazards which may apply to foodborne bacteriophages (EFSA, 2009), but really did not offer an opinion as to their legality. A key issue was whether bacteriophages should be considered as antimicrobials, food additives (i.e. preservatives) or as processing aids. The EFSA expert panel reported that, based on data currently available in peer-reviewed literature, it could not be concluded whether bacteriophages are able or unable to protect against recontamination of food, which is the key to identifying if they are processing aids or additives. It also highlighted that if bacteriophage treatments were to be used for removal of surface contamination of foods of animal origin then they would need to be evaluated for safety (as required for all antimicrobial treatments to be used on products of animal origin) and recommended that the Commission produce guidance on what would be required for this. As of yet there has been no further public comment from the Commission on the use of bacteriophages in food production. In essence, the current situation is that the use of bacteriophage as decontaminant on products of animal origin is currently not permitted in the EU and neither has the use of bacteriophage as food additives been authorised.

The use of bacteriophages for the control of *L. monocytogenes* have been evaluated (Soni and Nannapaneni 2010) and it was determined that the commercially-available bacteriophage

LISTEX P100 (EBI Food Safety, Wageningen, Netherlands) could inhibit the growth of *L. monocytogenes* on raw salmon fillet tissue over a 10 day storage at 4°C. The phage was able to lyse *L. monocytogenes* numbers to as low as 0.3 log cfu/g smoked fish, while levels in untreated control samples were as high as 2.6 log cfu/g. In addition to providing a two log reduction to *L. monocytogenes* numbers, Soni and Nannapaneni (2010) believe that, in contrast to most anti-Listerial treatments, application of the phage preparation in a saline wash helps prevent any deteriorations in the quality or aesthetic appearance of the smoked product.

Phage-based control of *L. monocytogenes* has been shown to cause beneficial reductions to *L. monocytogenes* numbers in some foods (Soni *et al*., 2012; Holck and Berg, 2009; Guenther *et al*., 2009). However, the surface of cold smoked salmon has been shown to contain widely-diverse stains of *L. monocytogenes* (Guenther *et al.,* 2009). In studies designed to assess the importance of strain variation, the ability of bacteriophage to control two smoked fish isolates of *L. monocytogenes* was assessed. In brief, phage was added to the surface of cold-smoked salmon previously inoculated with a mixture of *L. monocytogenes* strains each at a concentration of 10^3 cells per fillet. The food was refrigerated for six days at 6°C before examination. The results were that one strain had been reduced by over two log units but that the other *L. monocytogenes* population was not significantly affected by the phage treatment.

2.3.6.21 LYSOZYME

Lysozyme treatment is a recognised preservative under Annex III, Part C, 95/2/EC for cheese and wine. Directive 2000/13/EC (amended by 2001/101/EC) includes a list of food ingredients or substances known as likely to trigger allergic reactions in sensitive individuals; food grade lysozyme tends to be sourced from chicken egg albumen and so products containing lysozyme are required to be specifically labelled.

Datta *et al* (2008) studied the potential of lysozyme treatment in combination with alginate (polysaccharides extracted from brown seaweed) to prevent the growth of *L. monocytogenes* inoculated onto smoked salmon. The effects of exposure to nisin, two forms of lysozyme isolated from oysters and egg white were assessed both individually or in combination with calcium alginate applied to the surface of the fish. None of the treatments caused a statistically significant reduction to the growth of *L. monocytogenes* over a 35 day period at 4°C compared with the untreated controls. The result of using nisin alone was in broad agreement with the other studies dealt with earlier in this review (Nilsson *et al*. 1997; Szabo and Cahill 1999;

Nykanen *et al*. 2000; Neetoo *et al*, 2008b) in that there was a weak and not significant inhibition of growth. A slight reduction in the numbers of *L. monocytogenes* was observed by combining nisin or either of the two lysozymes with calcium alginate, however, the best reduction was achieved by adding both nisin and lysozyme to calcium alginate where there was over a two log reduction compared with the untreated controls. The study of Datta *et al* (2008) also found broadly comparable reductions in numbers of *Salmonella anatum* using the combination of calcium alginate, nisin and lysozyme.

2.3.6.22 ESSENTIAL OILS

Essential oils are complex mixtures of hydrophobic compounds extracted from fruits and vegetables. Essential oils are produced mostly as flavourings for food manufacture, however some essential oils, such as allicin extracted from fresh garlic are also potent antimicrobials (Ankiri and Mirelman, 1999).

In the EU, essential oils in foods are subject to Regulation 1334/2008/EC as a consequence of being flavouring agents. Regulation 1334/2008/EC restricts the use of biologically active agents in flavourings absolutely on the basis of food type and also by capping the maximum permissible concentration of each active compound in those foods which are approved. Lin *et al* (2004) have investigated a combination of oregano and cranberry essential oil extracts, both of which contain phenolic compounds. In summary the essential oils were very good at reducing numbers of *L. monocytogenes*. Numbers of *L. monocytogenes* were measured after the addition of both oregano and cranberry extracts alone and in combination. No decreases in *L. monocytogenes* populations were observed on raw cod slices kept at 4^{o}C and individually treated with either cranberry or oregano extract. However, when a mixture (75% oregano:25% cranberry; v/v) was assessed there was a significant three log unit decrease observed over an 8 day refrigeration period (Lin *et al* 2004). The decrease was observed earlier if lactic acid was used to acidify the extracts to pH 6.0. Under the acidified conditions, a comparable three log decrease was observed at as early as four days under refrigeration. No information was provided on what sensory effects the addition of such extracts might have or the potential increases to manufacturing costs.

2.3.7 COSTS OF INTERVENTIONS TO REDUCE *L. MONOCYTOGENES* NUMBERS ON FINAL SMOKED FISH PRODUCT

Although there are a number of possible interventions that have been summarised in the paragraphs above; frequently the costs of implementation and other practical barriers for widespread implementation are not commonly considered by researchers. In one study however an economic model was developed (Tauer *et al*. 2007) to estimate the minimum costs associated with interventions in fish processing factories aimed at reducing the numbers and prevalence of *L. monocytogenes* contamination of finished cold smoked salmon. Three input factors were considered by the model: non-contamination of raw fillets, non-contamination of plant environment and rate of glove changes on workers. These costs were compared against the potential marginal benefits from reductions in *L. monocytogenes* contamination. In brief, a summary of the publication's main finding was that the model outputs showed that producers may not be able to secure a high enough product price to sustain the costs of any of the modelled interventions.

2.3.8 PREDICTIVE MODELLING OF *L. MONOCYTOGENES* IN SMOKED FISH FINAL PRODUCTS FOR QUALITY ASSURANCE PURPOSES

Predictive microbiology is a quantitative tool that employs mathematical models together with key parameters (e.g. temperature, water activity, salt concentration, pH etc) to predict growth responses in food products of pathogens of concern. Predictive modelling for quality assurance (QA) purposes proceeds from an assumption that a pathogen has contaminated a food. For models which accurately predict the behaviour of foodborne human pathogens in specific foods, the approach provides a rapid determination of likely fate of a pathogen under a series of defined conditions (e.g. imperfect refrigeration or curing of smoked fish). Data used for predictive models are typically assembled from challenge tests of inoculated products under a variety of conditions. The models can be used to identify any likely safety issues with a batch of product created using a set of monitored manufacturing conditions and help assure a food batch's compliance with regulations.

The effects of salt and phenolic smoke compounds on the growth rate of *L. monocytogenes* in cold-smoked salmon have been modelled (Cornu *et al*. (2006). The estimated growth rates, fitted from experimental growth curves, were compared to predictions of existing secondary models. These models considered the effects of temperature, water phase salt content,

phenolic content, and additional factors (e.g. pH, lactate, dissolved CO2) on the rate of growth of both *L. monocytogenes* and naturally-present microbial flora. Although, the phenolic content seemed to have an inhibitory effect on the growth of *L. monocytogenes* in cold-smoked salmon, it was obvious that the studied factors were not enough to describe the uncertainty and variability of the results. Therefore, additional sources of uncertainty and variability affecting the growth rates should be considered in future, such as the between-strain variability and a between-product variability, which was not typically defined by the physicochemical factors measured in experiments.

Mejlholm et al. (2010) determined the performance of six predictive models for *L. monocytogenes* growth and survival in seafoods and other food products. None of the models assessed by Mejlholm took account of smoke components, but several could model the effects of acetic acid, diacetate and lactic acid. One of the models also included the effect of CO_2 packaging and nitrite on *L. monocytogenes* growth and survival. The most complex model had the closest prediction to experimentally observed test results and was able to predict whether *L. monocytogenes* growth or no growth would occur for 89% of the test conditions. Three other models were also assessed by Mejlholm and colleagues as satisfactory. The authors consider that the four successfully validated models are useful for the assessment of risk and the management foods contaminated with *L. monocytogenes* in processed and ready-to-eat (RTE) foods; however a successful prediction rate of 89% may be too low to have practical or reliable benefit in the real world.

A Saltelli global, variance-based sensitivity analysis method was applied to a contamination assessment model, involving quantitative and qualitative factors which had been shown previously to influence the growth of *L. monocytogenes* in cold smoked vacuum packed salmon (Ellouse *et al*. (2010). The model evaluated the numbers of *L. monocytogenes* contaminating the fillets at the end of the food shelf life. Following a chain process in which the food had an initial contamination level of less than 20 cells per portion a subset of ten factors (out of 26) were identified as important for growth of *L. monocytogenes*. These factors were: duration of storage in the refrigerator, physiological state of *L. monocytogenes*, temperature of storage in the refrigerator, optimum specific growth rate, duration of storage at retail, temperature of storage at retail, cold smoked salmon water activity, *L. monocytogenes* minimum temperature for growth, minimum water activity for growth and initial contamination level.

If undertaken under suitable and defined conditions, both the challenge tests studies and the process based models regarding smoked fish and *L. monocytogenes*, are important for establishing food safety regulations with respect the production and marketing activities. Put simply, the outputs from both define how *L. monocytogenes* behaves in defined circumstances and consequently whether those conditions will allow *L. monocytogenes* number to increase to the point when they can cause illness in susceptible groups.

2.3.9 RISK ASSESSMENT IN SMOKED FISH

Risk assessment (RA) is different from modelling undertaken for QA purposes because RA is the scientific method of determining the relationship between exposure to a given hazard and the likelihood of an adverse health effect. Predictive modelling for QA purposes determines the microbiological condition of food at the end of its shelf life from the basis of an assumption of initial contamination. RA attempts to predict whether a food is contaminated, and if so, the consequences of human consumption of the food. The process is well developed for microbiological food safety, and in the last decade a lot of effort has been put into the application of this type of analysis to human food-borne Listeriosis caused by smoked fish.

Several quantitative assessment models have been developed. The first was used to predict the exposure to *Listeria monocytogenes* from cold-smoked salmon (CSS) consumption in France (Puillot *et al*. 2007). The Monte Carlo model took into account the competitive bacterial growth between *L. monocytogenes* and the background competitive flora from the end of the production line to the consumer phase. The purpose of the model was to serve as inputs for further risk assessment and to assess the variability of the exposure to *L. monocytogenes* from CSS consumption in France. A sensitivity analysis ranked the most important factors which affect the *L. monocytogenes* growth as the total duration and the average temperature at the consumer phase, the initial contamination level, the average temperature at the retail phase and the total duration at the retail phase. Risk management strategies were also suggested by model outputs. A reduction in the temperature of household refrigerators to a target temperature (4^{o}C) would be the best strategy to reduce the mean, the median, and the highest percentiles of exposure.

A quantitative risk assessment model to estimate the risk of acquiring Listeriosis from consumption of packaged smoked gravad salmon and rainbow trout was undertaken by Monte Carlo simulation to calculate the probability of illness per serving (Garrido *et al* 2009). The

estimated mean risk per serving varied between 2.8 x 10^{-5} and 1.6 x 10^{-2} in high risk populations. Both of the models used by the researchers overestimated the number of predicted illnesses, although the model using an exponential fit was most accurate (Garrido *et al* 2009).

More recently, Garrido *et al*. (2010) developed a one dimensional risk assessment model which estimated the probability of developing Listeriosis by consumption of ready-to-eat (RTE) smoked fish and sliced-cooked meats. The model used primary data obtained in an exposure assessment step for each type of ready-to-eat products. Hence, the occurrence of pathogen at the point of sale, the percentage of consumers, the size and frequency of ingested food and the growth of pathogen from the point of sale to consumption, according to storage and temperature reported by consumers, were considered in simulation. In addition, the model tested the likely impact of different risk management options, by introducing several mitigations called ‘‘what if” scenarios. Four RTE food categories were considered in simulation: smoked salmon; smoked trout; vacuum sliced cooked ham and non-vacuum-sliced cooked ham. The predicted annual cases of Listeriosis by consumption of each type of product in the Navarra region of Spain were as follows: 2.9 x 10^{-3} in salmon and 2.4 x 10^{-1} in trout, 9.4 x 10^{-1} cases by consumption of vacuum-packed ham and 0.1 x 10^{1} in the case of retail ham.

In terms of mitigation strategies for all products the storage at low temperature has been responsible for the greater decrease of the predicted cases with respect to a baseline model (60 to 80% reduction). Reduction of storage time had a moderate decrease in the number of cases (20 to 40% reduction). However, when storage time was combined with the recommended temperature decrease, the estimated risk had the largest decrease (75 to 85% reduction). With respect to the effect on risk due to reduction of initial pathogen concentration, it has been established as a very effective measure in the trout model (the number of cases estimated was the lowest (1.2 x 10^{-3}).

The risk assessment models mentioned above provide a foundation to assist the potential effectiveness of new strategies for controlling Listeriosis in RTE fish. However, the interpretation of these risk assessments requires a careful evaluation in relation to the scientific knowledge of the production, marketing, and consumption of these products, the degree of variability and uncertainty inherent in the predicted risks and the availability of epidemiological data to validate the models.

2.3.10 POST-MANUFACTURE CONSIDERATIONS

The safety of smoked fish post-manufacture is primarily dependent on the shelf life being established on scientific principles and taking account of realistic storage temperatures, together with storage being in line with labelled instructions and the adherence to the durability date.

European law[1] requires that instructions to enable appropriate usage of the product be given. Since cold-smoked fish is a RTE food, instructions focus on the need for appropriate refrigeration and instructions once the pack is opened, e.g. eat within x days of opening.

A single US study (McCarthy, 1996) investigated the effect of post-process storage temperature on *L. monocytogenes* growth using inoculated (2 log cfu/g) cold smoked salmon. Storage of up to 7 days at -20^{o}C, 6^{o}C and 22^{o}C had no significant effect on *L. monocytogenes* numbers.

2.3.11 REVIEW OF INDUSTRY BEST PRACTICE GUIDES AND GUIDANCE

A number of best practice guides and guidance documents are available and it was felt appropriate to include these in the literature review. These are considered under separate headings below, and are broadly ordered by the production or processing stage(s) covered by the guidance.

2.3.11.1 MANUFACTURE

The '**Recommended International Code of Practice for Smoked Fish**' (CODEX, 1979) applies to a range of smoked fish and fishery products. It contains the technological guidelines and the essential requirements of hygiene for processing, handling, storage and distribution of smoked fish and smoked fish products. It does not provide specific information on particular smoking procedures with regard to a specific species of fish, commenting that "The variations in this field of food technology are too numerous to justify such an attempt". The CODEX guidance is general and not specifically for the control of *L. monocytogenes*.

[1] Article 3.1 (0) of DIRECTIVE 2000/13/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs (OJ L 109, 6.5.2000, p. 29): http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000L0013:20070112:EN:PDF (accessed 5/8/11)

The CODEX code covers, in straightforward language raw material requirements, plant facilities and operation, operating practices and production requirements, step-by-step processing considerations and requirements (brining, pickling and dry-salting), a basic hygiene control programme, laboratory, end-product specifications and a useful table to assist in the preparation of brine of required strength (the amount of salt to be dissolved in water to obtain required brine strengths). The document concludes with guidance on cleaning and disinfection.

At the 31st Session of the Codex Committee on Fish and Fishery Products (April 2011) the Proposed Draft Code of Practice for Fish and Fishery Products (section on smoked fish and relevant definitions) and the Proposed Draft Amendment to Section 3.4.5.1 Water of the Code of Practice for Fish and Fishery Products were advanced to Step 8 and 5/8. However, the Proposed Draft Standard for Smoked Fish, Smoke-Flavoured Fish and Smoke-Dried Fish was agreed to be held at Step 7 pending the development of the section on food additives, comments and consideration at the next session and to further consider food additive provisions in standards for fish and fishery products at its next session. The 32nd Session will be held in October 2012 and is thus beyond the life of this study.

The Scottish Salmon Producers Organisation's (SSPO) and Salmon Processors and Smokers Group's (SPSG) private 'Good Hygienic Practice for the Effective Control of *Listeria* and, In Particular, *Listeria monocytogenes* in the Production of Cold Smoked Salmon' (2008) sets out growing, harvesting, handling and processing standards particularly in relation to cold-smoked salmon that is ready to eat (RTE), i.e. to be eaten without being cooked or reheated. It is therefore also applicable for uncooked products such as sushi and sashimi.

The guidance is written in a straightforward manner and follows standard CODEX hygiene code headings, providing information on:

- Good Manufacturing and Hygienic Practice covering people, water (including ice), hygienic management of fish contact surfaces, equipment and machinery, pest control and transport as well as traceability and incident management
- Microbiological testing, covering sampling, test methods, targets and tolerances
- Specialist requirements for each process step, i.e. salmon farming, harvesting and harvest transport, primary processing, filleting and cold smoked salmon production.

The French language '**Guide de Bonnes Pratiques Hygiéniques Poissons Fumés et/ou Salé et/ou Marinés**' (CITTPM & STF, 2007) was notified to be officially validated in France in 2009 as a Guide under EU hygiene legislation (852/2004). The document does not apply to growing, harvesting or handling of fish, covering only the processing of smoked salmon and trout as well as pickled and other herrings. As with the CODEX guidance, the advice offered is general and not specifically aimed at the control of *L. monocytogenes*. Using technical language and presentation, the production stages are outlined as are regulatory requirements (including labelling), the principal hazards and their control, GMP and GHP, HACCP, validation, traceability, product conformity, the manufacturing environment, hygiene management, product design and shelf life.

The Library Guide on Fish and Shellfish Smoking Business Issues has been started by the Seafood Training Academy[2] (part of Seafish) to provide information on how to set up and manage a small seafood smoking business. It is an evolving and not yet completed resource, intended to be populated gradually with relevant information arising from the Academy's work with a group trying to establish themselves as a seafood smoking business.

2.3.11.2 SHELF LIFE

Shelf life is the period of time for which a product remains safe and meets its quality specifications under expected storage and use conditions. The shelf life determines the use by date. The manufacturer is responsible for setting the shelf life under defined conditions. The maximum permissible shelf life is determined on the basis of microbiological safety, physical condition and organoleptic quality, whichever is the shorter. It is important to note that safety is not determined by testing, but by the design of the product manufacturing process. Human pathogens must be accounted for by the use of safe raw materials and process design.

APPLICATION OF SHELF LIFE BY MANUFACTURERS

The Microbiological Criteria for Foodstuffs Regulation (EC) No. 2073/2005 (as amended) includes limits for the number of *L. monocytogenes* in ready-to-eat (RTE) food able to support the growth of this pathogen, and requires FBOs to be able to demonstrate these will not be exceeded during the shelf life. If FBOs cannot demonstrate this to the satisfaction of the competent authority then a criterion of absence in 25g at the end of manufacture applies.

[2] http://www.seafoodacademy.org/TheLibraryGuidesSeafoodSmokingBusiness.htm

Ready to Eat foods that do not support the growth of *L. monocytogenes* must comply with the limit of 100 cfu/g throughout the shelf life and *L. monocytogenes* must be absent throughout the shelf life in RTE food intended for specific consumption by infants or for special medical purposes.

The Scottish Salmon Producers Organisation (SSPO) and Salmon Processors and Smokers Group (SPSG) (2008) guidance advises that as a minimum, shelf life must be validated using storage and microbiological risk assessment. The number of shelf life trial studies to be carried out must be decided by the Food Business Operator based on HACCP validation requirements.

The CFA, BRC, FSA (2010) 'Shelf life of ready to eat food in relation to *L. monocytogenes* - **Guidance for food business operators**' is aimed at providing guidance for FBOs and enforcement officers. The guidance covers the practical implementation of the European Commission staff working document '***Listeria monocytogenes* shelf life studies for ready to eat foods, under Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs**' by providing clear instructions and draft protocols for the evaluations of *L. monocytogenes* numbers at the end of shelf life.

The CFA, BRC, FSA Guidance is currently being promoted to enforcers and FBOs by FSA throughout the UK. Its principles are at the time of writing are also being incorporated into Food Safety Authority Ireland guidance. However, the general approach for the determination of *L. monocytogenes* numbers is not widely used outside of the UK; despite a statutory requirement for RTE manufacturers to determine this information across the whole of the EU. UK retailers have a tendency to encourage product manufacturers to base their chill shelf life study determinations (thermal storage profiles in particular) on the transit and shelf conditions experienced by retailer distribution chains.

Given the lack of evidence for widespread adoption of the CFA, BRC, FSA guidance or of an established universal protocol for the establishment of shelf life internationally and commercial pressure for extended shelf lives, allocated shelf lives of finished products can vary widely internationally and in the UK (Table 9 and Table 10).

Based on *L. monocytogenes* growth modelling, Finnish Government guidance (Elintarvikevirasto, 2000) regarding *L. monocytogenes* and the shelf life of raw vacuum-packed fish requires that:

- Vacuum packed fish products must be stored as cold as possible, at most at +3°C; and
- Vacuum packed fish products must be on sale for at most 10-14 days. However if self-assessment of the whole of the retail (trade) chain is documented to show that the fish product was no warmer than 3°C, the period of sale can be extended to three weeks.

Table 9 Typical cold smoked salmon shelf lives

Region of product sale	Shelf life (days)
United Kingdom	21-24
Other European Union member states	<120
United States of America	<120

Source: Chilled Food Association/Industry

Table 10 Swedish smoked/gravad salmon shelf lives

Shelf life	Percentage of salmon with indicated shelf life (%)
<1 week	6
2 weeks	4
3 weeks	48
4 weeks	11
5 weeks	29
6 weeks	1

Source: Rosengren and Lindblad (2003)

CONSUMER USE BY DATE UNDERSTANDING AND COMPLIANCE

UK consumer understanding of the "use by date" and "best before date" is poor. Recent FSA data indicates that 27-34% of consumers believe that food past the "use by date" or "best before date" should be thrown away, 24-31% of consumers believe that such food might be past its best but not necessarily unsafe to eat, and 36-37% believe that the action would depend on the food.

Kosa *et al* (2007) reported that in a study of US consumers 63.5% of 289 respondents stated that before purchase they checked smoked seafood product dates 'all or most of the time', with

9.3% 'some of the time'. Roughly half (51.4%) of the same sample stated that they checked product dates 'all or most of the time' and 8.3% 'some of the time' before serving smoked seafood.

2.3.11.3 TEMPERATURE CONTROL

COMMERCIAL

The Scottish Salmon Producers Organisation (SSPO) and Salmon Processors and Smokers Group (SPSG) (2008) recommends the core temperature of the product should be held at a target of 3°C, but no more than 5°C, the temperature and storage time of each batch being monitored and recorded through a documented stock control procedure.

RETAIL AND FOODSERVICE

The maximum temperature specified in legislation for retail of chilled food is 8°C in England, Wales and Northern Ireland. The temperature control requirements for Scotland are set out in Schedule 4 of the Food Hygiene (Scotland) Regulations 2006 (amended). Although the Scottish Regulations do not specify a particular temperature at which food should be refrigerated, the guidance states "As there is no specific temperature mentioned for the chilling of foods that are likely to support bacterial growth, it is recommended that if the food storage place chosen exceeds 8°C then the shelf life of the foodstuff may need to be reduced". In addition, the Scottish regulations also state that "Food should be kept at ambient temperature for the shortest time possible". More generally, the FSA also advises that food refrigerators should generally be kept at a temperature between 0°C and 5°C. Within the EU, there are recommendations for temperatures of 0°C to 8°C specified by different member states.

Within the UK, when held and distributed by the manufacturer, it is likely that chilled food is maintained at not more 5°C. Commonly-agreed retailer 'own label' chilled prepared food temperatures on delivery to retailers' Regional Distribution Centres is routinely set at 5°C maximum through commercial supply agreements. In practice, surveys of all chilled food outlets (including major multiples, farmers markets, small stores and other outlets) indicated that in the UK, the average temperature at retail was 4°C-6°C, with 6% of samples at >8°C. The position appears similar in many other European countries.

MAIL ORDER

Chilled food purchased through mail order is exempt from temperature control legislation[3] in England, Wales and Northern Ireland, although the temperature should be maintained at a "safe level". A MAFF study in 1991 reported that mail order chilled foods spent 70% of their time at 8°C or higher, and that the average temperature on receipt was 15°C. Since 15 years have elapsed, there would be merit in repeating the survey of the temperature control of chilled mail order foods. The Mail Order Fine Foods Association (MOFFA) state that "if it is likely to rise in transit above 8°C, the mail order operator should be confident that this is safe by reference to supporting technical or other data. Long established practices that have proved safe over many years are relevant in this context."

A UK (MAFF, 1991) survey of mail order foods found that the temperature of a simulated food product (sterile agar in water gel) was above 8°C for 70% of the distribution time (Table 11). In this study, the average temperature of mail order foods (smoked salmon and smoked salmon trout) recorded on receipt was 15°C, with a minimum of 11°C, and a maximum of 19°C (Table 12). The time taken for packages to arrive at their destinations was usually between two and three days, with the maximum recorded as 10 days. It was not known whether the delivery temperature data were a valid indicator of the entire temperature profiles during postal delivery of chilled foodstuffs. Furthermore, given the information is now 20 years old, any application of the information to the current operations of the UK postal system is open to criticism.

Table 11 Temperatures of simulated food products - UK mail order (MAFF, 1991)

Temperature range (°C)	Percentage of time at temperature (%)
<5	10
5-8	20
>8	70

[3] The Food Hygiene (England) Regulations 2006 (SI 2006/14); The Food Hygiene (Wales) Regulations 2006 (SI 2006/31 (W.5)); and; The Food Hygiene Regulations (Northern Ireland) 2006 (SR 2006 No 3)

Table 12 Distribution of temperatures of mail order products on receipt (MAFF, 1991)

Temperature (°C)	Number of packs at each temperature on receipt (Percentage of total; %)
11	1 (2)
12	4 (9)
13	7 (16)
14	4 (9)
15	7 (16)
16	5 (12)
17	11 (26)
18	2 (5)
19	1 (2)
Total	43 (100)

The Food Standards Agency (2006) states on its website that foods sent through the post (Royal Mail) requiring refrigeration, including vacuum-packed products such as smoked fish, should be kept cool while they are being transported. Consumers are advised to "check with the supplier what they do to keep it cool until delivery".

In the USA, perishable foods must not be held between 40°-140°F (4.4°-60°C), including those distributed by the mail order industry and foods prepared and mailed from home (FSIS, 2003). However, data demonstrating the level of compliance to the temperature ranges specified could not be found. The USDA (FSIS, 2003) advises consumers as follows regarding the receipt of perishable mail order foods:

- Make sure the company sends perishable items, like meat or poultry, cold or frozen and packed with a cold source. It should be packed in foam or heavy corrugated cardboard.
- The food should be delivered as quickly as possible – ideally overnight. Make sure perishable items and the outer packaging are labelled "Keep refrigerated" to alert the recipient.
- When you receive a food item marked "Keep Refrigerated" open it immediately and check its temperature. The food should arrive frozen or partially frozen with ice crystals still visible. Even if a product is smoked, cured and/or fully cooked, it is still a perishable

product and must be kept cold. If perishable food arrives warm, notify the company. Do not consume the food. Do not even taste suspect food.

- Tell the recipient that the company has promised a delivery date. Or alert the recipient that "the gift is in the mail" so someone can be there to receive it. Don't have perishable items delivered to an office unless you know it will arrive on a work day and there is refrigerator space available for keeping it cold.

FARMERS' MARKETS

National rules apply but little specific guidance is available to operators. FSA Scotland (2005) states that:

- Chilled food should be kept at a temperature of 5°C or less (range 0-8°C)
- Chilled food should be transported by temperature controlled vehicle to and from the market and stored on site under temperature controlled conditions. However, small traders may use icepacks in insulated containers, provided the temperature is kept at 5°C or below.
- In the case of fish, ice should be provided for keeping the temperature at 5°C or below.

No significant data are available to demonstrate the level of compliance with these recommendations. Recent PHLS/HPA surveys (Elson et al., 2004; Sagoo et al., 2006) included sampling from "market stalls" but data are not separated in the reports.

DOMESTIC

It was recommended by Richmond (1991) that the maximum temperature of domestic fridges in the UK should not exceed 5°C. A survey of consumer behaviour in France (Cemagref/ANIA 2004 – see Table 6) established that for short shelf life chilled products, approximately 60% of the shelf life was spent in commercial refrigeration, and 40% in domestic refrigeration. The general applicability of this to other countries is not known, given different practices in various countries.

In 2010, the Waste Action Resources Programme (WRAP) reviewed previous surveys of domestic refrigerator performance, finding that:

- A survey of 75 households recorded that mean operating temperatures were <5°C
- A MAFF survey of 252 households recorded mean operating temperatures of 6°C, with 22% of fridges at temperatures >8°C
- A survey of 150 domestic fridges recorded average operating temperatures of 6.5°C. It was also noted that 26% of larder fridges and 29% of freezer-box fridges operated at average temperatures above 8°C
- Temperature distributions were measured in empty and loaded fridges. When loaded with foods at 5°C, mean temperatures ranged from 0.3-8°C on the top shelf and 2-3.7°C in the fridge door. Average temperatures of two fridges loaded with food at 20°C for 4 hours showed that mean temperatures ranged from 10.8-12°C.

The in-home temperature survey for WRAP (2010) showed that the majority of domestic refrigerators operated at a mean temperature of around 7°C. It was apparent that a proportion of the fridges tested (14 fridges, 29% of the sample) were operating at mean fridge temperatures of 9°C or above. Only 14 of the 48 fridges (29% of the sample) were found to be at mean temperatures of 5°C or less. With 34 fridges (70%) operating below 8°C. The average temperature reading across the whole survey population, as recorded by the interviewers, using the thermometers provided was 5.9°C.

A review of all European studies showed that overall the average air temperature in European refrigerators would appear to be 6.64°C (Nauta *et al*., 2003). Marklinder *et al*. (2004) also found that mean food temperatures were not related to the age or type of refrigerator in Sweden. A figure of 60-70% of domestic refrigerators operating at an average temperature >5°C appears to be relatively common to many studies throughout the world (Table 13).

Table 13 The temperatures measured in surveys of domestic refrigerators

Country	No. samples	Measurement	Tmin	Tmean	Tmax	% >x°C	Reference
UK	75 (air)	Unknown		<5	15	6%>5°C	Rose *et al*., 1990
UK	252 (air)	Data logger	0.9	6.0	11.4	70%>5°C	Evans *et al*., 1991
N. Ireland	150 (air)	Thermometer	0.8	6.5	12.6	71%>5°C	Flynn *et al*., 1992
France	102 (air)	Thermometer			14	70%>6°C	Victoria, 1993
Netherlands	125 (air)	Thermometer				70%>5°C	Notermans *et al*., 1997
New Zealand	50 (air)	Thermometer	0	4.9	11	60%>4°C	O'Brien, 1997
Greece	136 (air)	Thermometer				50%>9°C	Sergelidis *et al*., 1997
USA	106 (air)	Unknown				69%>5°C	Daniels, 1998
UK	645 (air)	Thermometer	-2	7	13	70%>5°C	Johnson *et al*., 1998
USA	939	Unknown				73% <5°C; 4% >8.3°C	CFSAN/FSIS, 2001
France	119 (air)	Data logger	0.9	6.6	11.4	80%>5°C	Laguerre *et al*., 2002
N. Ireland	30	Data logger	-5	4.5	13.0	53%>5°C	Jackson, 2003
Sweden	102 households; 705 food samples	Data logger	0.2 (VP salmon)	7.1	12.3	88%>4°C; 38%>8°C; 11%>10°C	Marklinder *et al*., 2004

			0.2 (fresh herring fillets)	6.5	12.8	83%>4°C; 24%>8°C; 7%>10°C	
			0.6 (milk)	6.9	13.2	92%>4°C; 31%>8°C; 11%>10°C	
			0.8 (minced meat)	6.2	11.3	85%>4°C; 22%>8°C; 6%>10°C	
			1.1 (sliced cooked ham)	7.2	12.3	90%>4°C; 44%>8°C; 10%>10°C	
			1.8 (RTE green salad)	7.4	18.2	94%>4°C; 39%>8°C; 19%>10°C	
			2.4 (soft cheese)	6.8	13.6	93%>4°C; 27%>8°C; 5%>10°C	
France	314 product samples/ fridges	Data logger	yoghurt			47%>6°C; 5%>10°C	Cemagref/ANIA, 2004

			meat			75%>4°C; 5%>10°C	
Ireland	100	Data logger	-7.9	5.4	20.7	59%>5°C	Kennedy *et al*., 2005
Portugal	86	Digital thermometer				70%>6°C	Azevedo *et al*., 2005
Greece	250	Data logger	-2.5			85%>5°C	Koutsoumanis & Taoukis, 2005
Netherlands	31	Glass thermometer	3.8		11.5	21%>7°C	Terpstra *et al*., 2005

Source: (Peck *et al*., 2006)

3 INDUSTRY AND EHO APPRAISAL QUESTIONNAIRES: PERCEPTIONS AND PRACTICES CURRENTLY IN PLACE IN THE SMOKED FISH PROCESS

In order to obtain information describing the production and processing practices currently employed by fish farmers and FBOs in managing the hazard of *Listeria* contaminating their products, a survey of industry and fish producers was undertaken. Opinions were canvassed through a series of smoking business and fish farm visits where interviews based on a questionnaire were undertaken. EHO opinions were also sought regarding *L. monocytogenes* contamination of smoked fish products along with the more general microbiological hazards associated with fish curing and smoking. Questionnaires focussing on areas identified as problematic or potentially problematic during the literature review were developed for each of the three sectors. The questionnaires were reviewed by a steering group that consisted of members of the fish smoking industry, a representative from the relevant trade body (SPSG), environmental health officers and the Food Standards Agency in Scotland before use. The questionnaires were used to form the basis of 'interviews' held during the farm (Appendix D) and industry visits (Appendix C), and an online questionnaire for EHOs (Appendix E).

3.1 METHODOLOGY

3.1.1 QUESTIONNAIRE DEVELOPMENT

PRIMARY PRODUCER/FISH FARM

The fish farm questionnaire was drawn up by reference to the historically problematic areas identified by the literature review. The farm questionnaire sought to cover each of the different stages of the process from farm to slaughter. In some cases, dependant on how the processor organised their operations, the initial stages of primary processing were included on the farm questionnaire rather than the processing plant questions. The specific areas covered in the farm questionnaire were:

- Farm and infrastructure
- Harvesting
- Staff

A copy of the questionnaire is included as Appendix D.

PROCESSORS/SMOKERS

With the key risk areas identified during the literature review in mind, Codex Alimentarius Commission (CAC) guidance was used as the starting basis for the construction of a questionnaire that was to be used for industry to appraise the smoked fish processing risks of final product contamination by *L. monocytogenes*. In brief, the process was commenced by the project team reproducing all of the CAC guidance as a set of bulleted summary points and then eliminating those points that did not have any bearing on smoked fish contamination by *L. monocytogenes*. The key determinant for elimination was whether the previously-undertaken literature review had identified an issue covered by a CAC summary point as important for *L. monocytogenes*. The key sets of relevant points from the CAC guidance with the reasons why they were included underlined, are shown in Appendix B.

The Codex Alimentarius Commission (CAC) has developed a code of practice that describes a basic framework for the manufacture of smoked fish and fishery products intended for human consumption (Anon, 1979). Although published and adopted in 1979, there have been no further revisions made to the guidance. The CAC code concentrates on microbial hazards but also addresses some physical and chemical hazards where these relate to good manufacturing practices (GMP). In terms of specific microbiological hazards such as *L. monocytogenes*, the codex guidance is considered quite broad because all of the offered interventions, controls or good practices relate solely to general cases. Like most CAC advisory notes, the smoked fish guidance rarely mentions phrases such as 'risk assessment'. Neither does it provide specific descriptions of how to control acknowledged hazards. More often than not, CAC stipulates the expected outcome of any decision making process e.g. 'Utensils and food-contact surfaces of equipment should be free from contamination.' without providing practical instruction as to how to achieve the outcome. However, the codex guidance is useful because it does list a set of essential requirements describing the "hygiene for processing, handling, storage and distribution of smoked fish and smoked fish products".

In addition to the basic (edited) CAC framework, whilst reviewing available literature it became apparent that there were gaps in available knowledge regarding current practices in *L. monocytogenes* management that were felt should be addressed by the appraisal questionnaire. A list of issues that were felt to be important by the project team is provided in the list below. The reasons why the points were considered important are underlined:

- Is there wet cleaning mid-shift? (i.e. is there splashing of *L. monocytogenes* from drains).
- Height of lowest section of the smoking racks (again drain or floor splash).
- Separation of raw and finished product (raw product is often contaminated with *L. monocytogenes*).
- Frequency of hand washing (Tomkin 2002; Klaeboe *et al*, 2010).
- Are fomites such as door handles cleaned and sanitised because handles have been shown to harbour *L. monocytogenes* in smoked fish plants.
- Are movements restricted from clean towards dirty ends of plants because employees and their equipment (aprons, knives) are also fomites.
- End of processing day cleaning frequency; how is it done, what chemicals, is there a routine change of active agent to prevent development of *L. monocytogenes* resistance. How is effectiveness monitored?
- Cleaning and sanitation of drains – how is it done (chemical and application) and how is effectiveness monitored?
- Cleaning and sanitation of brine injectors – how is it done, does it get inside the needles (for those plants that don't use just salt/ brine vats) how frequently is it undertaken? How is effectiveness monitored?
- Is brine recycled?
- How often is fresh brine made?
- Are waste dolavs sanitised before returned to plant?
- Are product crates reused? If so are they cleaned sanitised? Or are the crates lined? How is it done? How is effectiveness monitored?
- Separate crates for raw and finished product and never swapped from one end of the line to the other?
- Environmental swabs from hard to clean equipment prior to the commencement of days processing (i.e. not after cleaning) and the drains.
- Does the plant use high (not defined) numbers of casual workers – Cortesi *et al* 1997 report that this is a risk factor for *L. monocytogenes* contamination of smoked fish. Similarly, Rørvik *et al* 1997 report job rotation by full time staff is also a risk factor for *Listeria*.
- Copper drains release copper ions and significantly lower *L. monocytogenes* number in fish plants.

The CAC-derived points and literature review-identified issues were combined and a series of questions were phrased that aimed to determine how plants complied with the issues covered. The questionnaire is provided as Appendix C.

ENIVIRONMENTAL HEALTH OFFICERS

The opinions of lead food Environmental Health Officers (EHOs) who oversaw smoked fish plants as part of their professional duties were also sought. Since EHOs were given assurances of anonymity, the website did not save the identity of an EHO alongside any response made. Based on the number of unique logins to the site, which correlates exactly with the number of saved responses, EHOs from 18 local authorities in Scotland and 4 from England provided a response to the questionnaire. Including the face-to-face interviews, the total of responses obtained was 30.

The EHO opinions sought were focussed on *L. monocytogenes* contamination of smoked fish products along with more gen*eral microbiologi*cal hazards of fish curing and smoking. The opinions were solicited from two different viewpoints which were:

- How informed the EHOs thought the food business operators (FBOs) at the plants they visited were about a range of questions relating to *L. monocytogenes* and smoked fish
- How informed the EHOs felt they themselves, and their colleagues, were about the same topics

The EHO opinions were collected in one of two ways. EHOs were either interviewed face-to-face or their opinion were collected using a web-based online questionnaire with an email invitation to senior EHOs in Scotland for their opinions.

The questionnaire was constructed to capture the perceptions of EHOs regarding any areas of smoked fish inspection that they found difficult to inspect or enforce. In addition, the EHOs were asked to identify any specific areas which would benefit from more guidance. The questionnaire was designed to probe around some issues that had been raised with the project team in their other capacities when dealing with EHO enquiries in relation to food safety management in SMEs producing high-risk chilled foods.

The questionnaire was reviewed by all of the project industry collaborators and FSAS staff prior to being used. Both the face-to-face interviews and online questionnaires (see below for more detail) used the same basic set of questions ,which are provided as Appendix E.

An online version of the questionnaire was created using the C# programming language, the .Net framework v4 (Microsoft Corp., Redmond, Wash.), an FSA-sponsored server and a web address within the Agency's ukmeat.org website. The opinions questionnaire was password protected using standard Forms Authentication (Mitchell, 2008) to prevent unauthorised responses from being saved. A relational database (SQL Server 2008; Microsoft) was used to hold the entered EHO opinions. The majority of the source code used for the questionnaire form construction and the saving to the database of the entered responses was used as has been previously described by previously-funded Agency studies (FSA project MO1020; Hutchison *et al*, 2007). The online form validated the responses that were entered to ensure that appropriate entries were provided (e.g. no strange characters or script injections into the text boxes).

In order to make summarising of the provided responses possible, the majority of the questionnaire was designed to capture the EHO opinions as a series of numerical values. For the initial stages of the questionnaire, the EHOs were asked to score a value of 1 for a subject topic they considered to be low priority and 5 for something they considered to have a very high priority in the prevention of contamination of smoked seafoods by *L. monocytogenes*. For the later stages of the questionnaire, EHOs were asked to score a value of 1 for a topic that they considered to be poorly understood by EHOs or FBOs and 5 for a topic they considered that most would have good comprehension.

Two columns were provided for the EHO responses, one for their opinion in the context of FBOs and the other for their opinion in the context of EHOs. After the EHOs had finished the questionnaire, the EHOs clicked the button at the bottom of the screen to save their responses into the database. To encourage participation, EHOs were given assurances that their responses were completely confidential. Consequently, which EHOs had saved which responses was not recorded.

3.1.2 FARM AND PROCESSOR/SMOKEHOUSE VISITS

In total, three salt water fish farms were visited as part of this study. Two were salmon farms and one was a trout farm. Only the late growing stages in seawater were considered as part of the study and the questionnaire was developed with these stages in mind. No visit or discussions took place regarding any freshwater stages of the farming process.

For visits to food businesses premises, it was aimed to select businesses to visit that would provide a balanced picture of standard and non-standard processing practices relating to fish smoking. In total there were 23 visits to smoking plants (some of who undertook filleting and gutting on the same premises) and four to fish processors who exclusively undertook gutting and filleting. In addition, a single independent consultant was interviewed who was able to speak about multiple premises. Of all the businesses contacted, only two failed to respond to an initial round of 25 letters containing the invite to participate in the study. The breakdown of species, smoking type undertaken by the premises visited and business size is shown in Table 14. This classification was also carried out for the four processing companies that supplied some of the smokers (Table 15).

Two of the smoking plant visits were hosted by independent consultants who managed the microbiological aspects of smoking on behalf of the plant operators. Two of the businesses were in England. The visits were carried out between June and November 2011 by Norval Strachan, Ovidiu Rotariu (Mainland Scotland), Mike Hutchison (Southern Scotland and England) and Niall O'Rourke (Shetland Isles). Industry responses were collated and the results reported *en masse* to ensure confidentiality.

The purpose of the visits was to obtain information relating to:

- Practices in production and processing of smoked fish including cleaning and sanitation practices (CSP) and how industry undertakes monitoring of the effectiveness of CSP.
- Any specific issues felt by industry to be barriers for effective CSP with particular focus on the control of *Listeria* contamination.
- Any specific industry practices which promote the control or elimination of *Listeria*.

- Packing of smoked fish products and the use of modified atmospheres or vacuums and the reasons why different packs and atmospheres are used; again with a particular focus on the control of *Listeria* contamination
- How the shelf life of a product is determined and any assumptions made regarding chill chain performance, consumer handling and compliance with provided consumer handling instructions
- What industry perceives to be areas of weakness in relation to the effective control of *Listeria* during the processing of smoked fish
- Collation and analysis of industry data (where made available) to identify the stages in the production and processing chain where *Listeria* cross-contaminations are an issue.

The information was obtained both through observations made during touring the business and completion of the questionnaire during discussions with industry representatives.

Table 14 Visits and interviews with smoke houses (n=23) stratified by fish species and the number of plant employees (expressed in terms of full time equivalent posts [FTEP]).

	Number of plants producing each product			
Number of plant employees (FTE posts)	Cold Smoked Salmon	Hot Smoked Salmon	Smoked Mackerel /Herring	Smoked White Fish
Small (<5 people)	3	2	2	5
Medium (5-25 people)	6	4	2	4
Large (>25 people)	6	4	4	3

Table 15 Visits and interviews with fish processors (n=4) that supplied smokehouses.

	Number of plants processing each product		
Number of plant employees (FTE posts)	Salmon	Mackerel or herring	White fish
Small (<5 people)	0	0	0
Medium (5-25 people)	3	0	2
Large (>25 people)	4	1	2

3.2 OBSERVATIONS MADE DURING INDUSTRY VISITS

This section summarises the key observations made during the visits to the fish farms and also from the visits to processors and smokehouses. In the main, two types of smokehouses were visited, those that smoked farmed salmon or trout and those that smoked pelagic fish. Smaller processors tended to smoke the widest range of fish which could include small amounts of both fish types. The observations made from the two different types of businesses are presented separately for clarity. These observations relate only for the premises or farms visited and are not indicative of the entire sector.

3.2.1 FISH FARMS

ONGROWING

Farmed fish were exclusively found to be raised in cages. All of the farms visited used circular cages which were constructed using plastic tubes and copper oxide treated net. The bottom of the cages were either weighed down or anchored. As the fish grew and become larger, the size of the netting mesh was changed. Fish feeding was undertaken using automated feeding systems which conveyed the feed through pipes into the cages. Doppler effect-based systems were used to monitor the amounts of food that fell through the cage bottom in order to control feed supply rates and minimise wastage. The cage dimensions were similar in all of the farms visited at around 14 m depth with an 80-120 m circumference. The size of fish was monitored automatically (e.g. Vaki equipment for automated biomass measurement) or more simply by manually netting and weighing a sample of fish.

HARVESTING

As is common for most farmed animals, feed was withdrawn from fish prior to slaughter. For both salmon and trout, feed was removed around three days before harvest largely to empty the viscera and reduce the volumes of faecal material that could potentially be released during processing. Depending on the distance from the farm to the shore, the fish were either harvested by either pumping directly into a shore harvesting station or alternatively, fish could be pumped into well boats containing sea water and around 60 tonnes of fish per well. Alternatively, once the fish were transported close enough to the shore, they were subsequently pumped to the harvesting station. During harvest, the temperature of the fish was kept constant at approx. 6-7^{o}C. Also, the concentration of CO_2 was controlled (by pumping

air into the water containing the fish) to prevent the fish from becoming stressed. At this point, the fish were stunned either individually by a blow to the head or *en masse* by an electrical discharge into the water containing them. Stunned fish were bled using automated equipment or by manually cutting into one or both of the gill arches before there was recovery from being stunned. After harvest, the fish were manually sorted on the basis of size. Exsanguinated fish were stored on ice in bins, the largest of typically held around 800 kg of fish.

TRANSPORT TO PROCESSORS

The harvest bins were then typically loaded into trailers and transported usually by road, but sometimes by boat to a processing plant for further processing. The transport times experienced were variable with a typical range being between two and six hours.

3.2.2 SMOKED SALMON AND TROUT PROCESSORS/SMOKEHOUSES

The process for smoking farmed salmon and trout was generally as shown in Figure 1 in Section 8.3. For some of the food businesses there was an extra step not outlined previously which was a 'process and dispatch' step. This was where the farmed fish were delivered to a processor that carried out the filleting and gutting stage on behalf of the smokehouse, when it was not carried out in-house. Some observations made during the industry visits on the practices employed during the production stages for smoked fish manufacture from farmed salmon and trout are provided below.

PROCESSING AND DISPATCHING

The processing and dispatching stages at the premises visited involved several smaller steps which were:

- Tipping the fish and the ice from the transport bins into an alternative storage bin (using forklifts) which allowed the ice to drain away from the fish
- At this stage, optionally, the fish scales could be removed
- Gutting and removal of the viscera which was undertaken either using automated equipment or manually. Some automated equipment combined removal of the viscera and bones by removing the fish flesh as fillets from the rest of the carcass.

- Washing of the eviscerated carcass is the next stage. Washing is undertaken either automatically or by the use of a manual hose
- At a trout plant that was visited, pin bone removal (for non-filleted fish) was undertaken either automatically using a vacuum based deboning system or manually using boning pliers.
- The fish were then typically graded manually into superior, standard or outsize
- The fish were then boxed for export abroad before being weighed and labelled
- Ice was then applied to the top of the box. The mass used was typically 4kg of ice manually shovelled onto the top of each box
- The box lid was then manually placed onto the box and two plastic packing straps were automatically wrapped round the box
- The packed boxes were then manually stacked onto a pallet (e.g. at one plant, 21 boxes were manually lifted onto pallet as a 3x7 stack which was wrapped in cling film before a forklift was used to transported the pallet onto a lorry for further transport
- At all of the plants visited, further transport tended to be refrigerated. At one plant, each lorry was able to take up to 33 pallets. Once all the pallets were loaded, the lorry doors were closed and locked and the refrigeration system switched on. The fish at this stage went either to a smoking plant or to retail unit for the direct sale of fresh fish to the final consumer.

FILLETING

If the fish had not been filleted previously, the filleting stage involved manual head removal, automated washing of the carcass, automated splitting into fillets and a final manual trim.

SALTING AND RINSING

Although potentially, salting can be undertaken using either crystal salt or immersion of the fish flesh in vats of salted water/direct injection into the flesh, the reality in the plants visited was that for all of the large throughput processors, crystal salt was applied to fillets on racks. When asked about the apparent strong preference for crystal salt, it transpired that most of the processors knew about the ability of *L. monocytogenes* to contaminate brine injectors. The use of crystal salt in most plants was a considered choice that had been implemented (at least partly) to remove a potential source of *L. monocytogenes*.

Prior to the application of salt (or immersion in a brining vat with/without direct injection of brine), the fillets were washed using a hose fitted with a shower head-style arrangement. Fillets were allowed to drip dry for a few minutes before the application of salt (either automated or manual). The fillets were then manually stacked onto wire racks which allowed the free drainage of water drawn from the fish flesh by the salt. After salting, there was a further water wash to remove the excess salt. At this stage the fish were allowed to dry in a chiller for up to 24 hours

SMOKING

As was described previously (section 0), smoking in larger businesses typically uses hardwood chips (beech, oak, juniper, birch and old whisky barrels) which is lit under conditions which cause it to smoulder and the smoke is allowed to permeate into the kilns. The temperature for cold smoking can vary between 21 to 28^{o}C for a time period of 4 to 8 hours. Hot smoking is typically undertaken at 60-80^{o}C for up to 2 hours. It was apparent that some businesses smoke both hot and cold smoked under the same set of conditions (i.e. under cold smoke conditions) and then create the hot smoked product by heating the cold smoked one in a standard oven (without smoke) long enough to coagulate the fish protein (typically 1 hour at 80^{o}C).

SLICING AND CUTTING

Cold smoked fish was routinely sliced. However, none of the plants which were visited sliced their hot smoked products. It was considered by staff in a number of the plants visited that slicing of hot smoked product would happen only rarely, if at all. Slicing and cutting was preceded by blast chilling at <4^{o}C for 4 hours, manually deboning those fillets not previously deboned and automated skinning using rotating knifes.

Automated slicing was observed using a variety of equipment all of which had been designed to be easy to strip down and clean/sanitise. Some slicing equipment used lasers to detect the fish thickness and automatically adjust the knife height. Pinned conveyor belts to hold the fillets in place were observed.

PACKING

The sliced product was manually trimmed and weighed after slicing before being packed. It was common for the smoked fish products to be packed under vacuum. Almost every business visited had vacuum-packing facilities regardless of business size.

STORAGE

Smoked salmon was observed as being kept in a chiller at <4°C for a day or two before being transported to the customer. One business claimed to make 90% of its sales in the run up to the Christmas period and so froze almost all of its entire year's production for the period of their greatest sales.

TRANSPORT

Smoked salmon and trout in the UK appeared to be exclusively transported using refrigerated lorries. The majority of lorries inspected had automatic monitoring of the temperatures in the load area and were able to alert the driver if the temperature of the load became too high. Typical refrigeration temperatures for fish during transport were 0-4°C with an alarm being triggered if the transport chamber became warmer than 6-8°C.

POTENTIAL MARKETS

The businesses visited sold their products to consumers in a variety of ways. Products were frequently sold on site direct to consumers. Local restaurants and canteens were supplied by some of the smaller producers. The medium to large retailers supplied retailers and supermarkets or exported their products.

Some of the products were further processed before being sold (e.g. creation of cold smoked salmon parcels containing cream cheese and cold smoked salmon trim).

3.2.3 SMOKED PELAGIC FISH AND WHITEFISH

The various processing stages required to manufacture smoked pelagic or whitefish are similar to that shown in Figure 1 (Section 2.3.3), with some minor amendments. Pelagic and whitefish are landed at market instead of being grown on farms. In addition, these types of fish can either be transported to a food business where they are gutted (if evisceration has not already been carried out on the fishing vessel). Observations made during the industry visits on the practices employed during the production stages for smoked fish manufacture from wild pelagic and whitefish are provided below.

FISH LANDING AND TANSPORT

The fish are caught and are commonly degutted on the boat; although boat-based degutting is not universal. In either event, the fish are stored on ice in standard fish crates which can hold either 20kg or 50kg. Typically, boats hold their caught fish on ice for not more than 3 days. Once boats return to port, the fish were sent to an auction market or cold storage units in the ice-packed crates.

TRANSPORT

Refrigerated trailers were found to be used to transport fish which was packed in fresh ice to the processing factories in all cases.

EVISCERATING AND /OR FILLETING

As was mentioned previously, in a significant proportion of cases, the fish can be gutted and de-headed on the boat which caught them. For those boats that do not part-process their catch at sea, these stages would be undertaken at a processing plant. A number of fish processors in Scotland have automated systems from a variety of manufacturers which can de-head, eviscerate and remove the skeletal bones from fish. Although there are automatic machines available, filleting in smaller throughput businesses still tends to be undertaken manually. Some fish (e.g. kippers) are not filleted.

GRADING

Grading eviscerated fish or fillets on the basis of size or visible quality (e.g. a lack of visible bruising or blood spots) was done most of the time manually even in large throughput plants. Either immediately before or after grading, the fish were briefly washed as basic preparation for brining or salting.

BRINING/SALTING AND WASHING.

After an optional skinning (typically using a skinning machine) the fish were either treated by injecting liquid brine (2-3 minutes), soaking in brine vats (up to two hours) or using salt crystals (30 minute to one hour). At those few plants where liquid brining was undertaken, the salt solution was saturated (~13% w/v at 5^{o}C). Fish that were cured using salt crystals were normally rinsed by immersion into a volume of water. Fish cured in brine were generally not rinsed but hung to dry before smoking. In all cases, the fish are allowed to drip dry period of at least 30 minutes before the commencement of smoking.

SMOKING

Smoking of pelagic and whitefish was not observed to be significantly different to the processes already described for farmed fish (section 0). As before, smoking was using wood chips (beech, oak, juniper, birch and old whisky barrels) which is burned in kilns. The temperature for cold smoking varied between 21-28^{o}C for a time period of 4 to 8 hours and sometimes overnight for a stronger flavour. After smoking, cold smoked filets/fish were refrigerated. Hot smoking was done at approx. 70^{o}C for up to 2 hours. Before chilling the fish were cooled to ambient temperature for 30 minutes to 1 hour.

CUTTING

As before, there were no significant differences between the slicing of smoked fish as compared with the farmed fish. Fillet slicing of cold smoked products is commonly undertaken using automated knives (e.g. Marel, M Series 3000). Trimming of products was observed to be an exclusively manual task.

PACKING, STORAGE AND TRANSPORT

Vacuum packing was the most commonly used technique for packing smoked white fish (e.g. using “Multivac” vacuum packing machines). However, simply packing in foam or cardboard trays and freezing were also found to be common storage solutions. It was found that immediately after packing, it was common to trim (if that was not done at the cutting stage) and weigh the packed product. Smoked salmon was found to be kept in a chiller at <4^{o}C until it was transported to the customer. Refrigerated lorries were used exclusively for the transport of final smoked product.

SALE OF PRODUCT

The markets for Scottish smoked fish were reported to be local restaurants and canteens (practiced by small producers), major retailers and a healthy export market. A number of smokers had a small retail shop which sold direct to the public.

3.3 QUESTIONNAIRE RESULTS

3.3.1 FISH FARMS

There was a positive response to the project from fish growers and three visits to farms were conducted. Since the number of visits to growers were low, the questionnaire results are presented in descriptive form for each questionnaire section rather than tabulated as practices tended to be the same across the farms due to standard on-growing procedures employed by the industry.

FARM AND INFRASTRUCTURE

Two of the farms visited produced salmon, and one was a trout farm. All had cages located either in a sea loch or coastal bay. There was little direct evidence during the visits that the coast near the farms was used for cattle or sheep grazing. However it was considered likely at all three locations that there would be sheep grazed on the hillside slopes at some times during the year. Heavy rainfall did not influence when fish were harvested at the farms. One of the farms swabbed the surfaces of the live fish for absence/presence of *Listeria* directly before they were transported into a harvesting unit and these tests very occasionally gave positive detections.

Potential sources of *Listeria* at the farm were from bird faeces and also runoff from areas upstream of the farm site where there may well have been wild or farmed animals. The fish feed was not considered to be a source because it was heat treated and on one of the farms it was routinely tested for *Salmonella*.

HARVESTING

Both farms withdrew feed from the fish prior to harvest (e.g. 3-5 days). Fish were transferred from the cages by pumping them directly onto a wellboat before being transported to shore (or directly pumped into a shore-based harvest station). The wellboat journey could take from < 1 hour to several hours. After the fish were transferred from the well boat, the fish transport water was taken out to sea and discarded. After each trip the well boat was cleaned and disinfected (e.g. with a degreaser and then sanitised). Heavy antecedent rainfall did not influence when the fish were harvested.

Fish were harvested adjacent to the landing site and were slaughtered by automated mechanical stunners and bleeders (a priest and knives were also used as backup stunners). At one site the fish were allowed to bleed into tanks of iced water (potable) in the other they were allowed to bleed on the table before being washed and put into a transport bin lined with a polythene liner. The fish for all companies were transported whole from the harvesting site.

Cleaning of the harvesting stations involved washing down of gross debris by harvesting staff immediately after harvest. Staff (specialised hygiene for one of the farms) come in and rinsed the surfaces before spraying with degreaser (which was left for approx. 20 min) before manually scrubbing using brushes. The degreaser was then rinsed off and sanitizer applied. Equipment (manual stunners, knives etc) are cleaned and disinfected after the harvest.

All of the companies tested equipment for *Listeria monocytogenes* (pipes, bins, table surfaces, stunning equipment etc.). All of the testing was presence/absence and results were monitored by trending positive isolations over time. Any positive test result was taken as out of specification. After positive testing, there would be re-cleaning and disinfection of equipment followed by re-testing.

When asked if there were any problems that had arisen that the industry could learn from. One company said that they had not experienced any problem but that if there was a problem that the killing station should be the first place to be investigated. The second company said that there had been an issue previously with a harvesting/slaughtering site. Once improved cleaning regimes had been put into place the problem then resolved.

STAFF

The majority of staff at all three farms largely had defined roles which were rigidly adhered to. However a change of job duties could occur if there was a requirement (e.g. staff shortages). If colleagues were unable to work due to illness, then there were return to work policies. There was evidence that temporary staff received the same training as the rest of the staff. All the companies had sanitary staff toilets.

3.3.1.1 SUMMARY AND DISCUSSION OF FISH FARM QUESTIONNAIRE

Observations apparent to the members of the project team who undertook the visits was that there is a great deal of effort being made by industry to minimise the risk of *Listeria* contamination of farmed fish. There are well established microbiological testing programmes for the fish entering smoking plants and microbiological monitoring of cleaning and sanitation throughout the food chain so that if problems do arise then there can be a relatively rapid response.

It is generally considered that farmed salmon can acquire *Listeria* while in the cages and that an initial contamination could lead to an infection of the GI tract and/or the gills (Miettinen and Wirtanen (2005)). Waterborne *Listeria* were considered by industry to be the main source of *L. monocytogenes* contamination of the well boat and eventually the slaughter plant. The widely held opinion is strongly supported by the peer review literature (Gram, 2001). Once processing environments are contaminated it is well established that some *Listeria* species have the potential to survive and multiply as biofilms which contaminate large quantities of fish which are subsequently processed.

SUMMARY OF KEY FINDINGS

In summary the main findings identified during the visits and questionnaires for salmon growers were:

- The fish farmers consider that it is important to maintain strict cleaning regimes underpinned by microbiological (or other) monitoring to ensure cleaning effectiveness throughout the farming process.
- In Scotland, there is routine monitoring for *Listeria* on the fish and on the equipment used to handle the fish (e.g. fish pumps, well-boat, killing plant. The testing regimes were established by industry as their response to the potential issue without a need for regulation.
- If a problem did arise with *L. monocytogenes* contamination (e.g. associated with the killing station) then sensible and proportionate responses were undertaken by the farm staff. These included checking the cleaning and disinfection effectiveness and re-cleaning and re-testing if necessary.

- Testing generally is for the presence of *Listeria*. If there was a positive detection then the isolate would be routinely classified to the species level (e.g. *L. monocytogenes*).

3.3.2 SALMON AND TROUT PROCESSORS/SMOKEHOUSES

RAW FISH

A summary of the results gathered from the section relating to practices concerning the the raw ingredient ('Raw Fish' section) of the questionnaire is shown in Table 16. All of the companies except for one undertook some checking of the raw fish delivered to the plant as a way of ensuring that fish were transported in a manner that prevented their warming (Q1). The fish were transported on ice and frequently in polystyrene insulated containers.

A number of companies (particularly the large and medium enterprises) either measured the temperature of the incoming fish or since most fish were on ice, more simply checked a random selection of boxes or crates to ensure that there was still significant quantities of visible ice present. A number of the larger companies also did a more advanced visual inspection, checking for physical damage to the fish and the presence of parasites and physiological abnormalities. The large and medium sized companies also had programmes in place which monitored the numbers of microbiological indictors on the fish (some passed on by the grower and some done by themselves or frequently both). The indicators included numbers of total aerobic mesophiles and *E. coli* as well as detections of *Listeria* prevalence. The indicators were not used as the basis of rejecting a batch of fish. Indicators were monitored for process control purposes and used to indicate potential issues. Consequently, if an indicator count was significantly higher than expected, it would lead to a discussion with the grower or supplier to try and resolve the concern. Two companies (one small and one medium) did not have any microbiological criteria in place for accepting fish (Q.12). Most companies that purchased frozen fish thawed at ambient temperature (Q.9); but at least two processors thawed in chillers. Boxes that were used for fish were generally kept in designated storage areas inside the larger factories (Q.7). For smaller factories, boxes tended to be stored outdoors near the areas where fish were delivered.

Most of the companies that used polystyrene boxes disposed of them after a single use (frequently they were broken down for recycling). However, two companies did reuse some or

Table 16 Smoked fish processor assessment questionnaire: responses to the raw fish section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
1. Are fish transported in a manner which prevents their warming up?	6	0	0	5	1	0	4	0	0
2. Are transport crates/boxes/trays designed to permit easy and thorough cleaning and sanitation?	2	1	3	4	0	2	3	0	1
3. Are transport crates/boxes/trays cleaned and sanitised at the plant before being reused?	1	2	3	2	1	3	1	1	2
4. Are transport crates/boxes/trays cleaned and sanitised somewhere else before being reused?	3	0	3	4	0	2	3	0	1
5. Are they ever used for a purpose other than transporting fish?	0	3	3	2	4	0	1	1	2
6. Is any transport crates/boxes/tray cleaning and sanitation regime validated as effective in controlling *L. monocytogenes* (Plant may not know if it happens offsite)	0	0	6	0	1	5	0	2	2
8. Is there physical damage (e.g. scrapes and scores) in the surfaces of transport crates that could come into contact with raw fish?	3	0	3	2	2	2	3	0	1
9. If frozen fish is used, is it thawed at below 7^{o}C before being processed?	2	0	4	0	5	1	0	0	4

10. Are fish stored under refrigeration (i.e. in ice or in a chiller) prior to processing and smoking?	5	0	1	5	1	0	4	0	0
11. Is the incoming temp of the fish checked?	4	0	2	3	2	1	0	4	0
12. Does the FBO have any criteria for accepting fish? (e.g. does he check the listeria status of the fish coming in/ask the farm to supply data/ ask the farm what controls they take/ take account of environmental conditions when the fish were harvested)	6	0	0	5	1	0	3	1	0
13. Does waste removal (e.g. melted water, fish detritus) from the fish unloading and storage areas happen on a continual or near continual basis?	5	1	0	5	1	0	3	1	0

*NA is Not applicable; DK is Don't know.

all of their polystyrene boxes. One processor used the same boxes that a batch of fish arrived in to send the fish on after they had been eviscerated and filleted. The boxes were not cleaned by the processor, but they were lined with a food grade polythene liner before reuse. The second company was a smoking plant operator who cleaned the boxes that the raw fish arrived in before reuse for smoked vacuum packed RTE fish product. Where fish were transported in plastic boxes or crates, these often had scratches on them which made them more difficult to clean and disinfect effectively. Cleaning of re-useable crates was performed using appropriate cleaners and sanitisers (e.g. Chloromor) but none of the plants asked had performed any validation on whether their cleaning and sanitation regimes for crates were effective for removing contamination by *Listeria*. However, one factory representative was aware that the sanitiser being used for crates had been validated by the chemical manufacturer as effective for killing *Listeria* on food contact surfaces. As a general rule, all crate cleaning was done in a physically separate area that was distinct from the fish processing areas

PLANT INFRASTRUCTURE

Opinions gathered on plant infrastructure are presented in Table 17. The plant infrastructures of the factories visited were generally found to be in good repair (Q14, 15). However a minority had infrastructure issues which may lead to poor hygiene i.e. floor surfaces, wall and roof defects. All factories had removable grates (Q16) on their drains apart from one which had a fixed shower-type cover. A number of factories claimed to have slow-dissolving sanitiser blocks in their drains (Q17) but physical observations of such blocks was only observed on a few occasions. Those factories that did not use these sanitising blocks indicated that during cleaning of the factory the drains were dosed with cleaning chemicals. None of the factories currently used copper linings in their drains, although one plant claimed to have replaced their old copper lined drains with steel ones less than two years previously. A number of plants said that they believed that copper could react with the chemicals that were being used in the factory. Once oxidised however, copper oxide is fairly inert. A minority of medium and small factories had pipe work and ducting in their ceiling spaces which could accumulate dust (Q20). The project team noted that a number of factories had condensation problems – in particular in the chills. As is the case in a number of food processing plants, there were particular problems noted near the exhaust outputs from the ceiling-mounted refrigeration units. A common solution throughout the food industry is to fit a drip cover underneath the condenser coils of the units to stop any condensate dripping onto product and another did not store fish directly under the

Table 17 Smoked fish processor assessment questionnaire responses to the plant infrastructure section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
14. Is the plant infrastructure intact? i.e. no holes in the walls, broken windows damaged doors which allow insects and vermin into the plant	6	0	0	6	0	0	4	0	0
15. Are plant floors hard surface, non-absorbent and adequately drained (i.e. no standing water)?	5	1	0	6	0	0	4	0	0
16. Do the drains have removable grates?	6	0	0	6	0	0	3	1	0
17.Are the drains fitted with slow-dissolving blocks of sanitiser chemicals?	2	4	0	1	5	0	1	3	0
18. Do the drains have copper linings (or another source of copper ions such as a copper grating)?	0	6	0	0	6	0	0	4	0
19. Are the plant walls smooth, waterproof, light coloured (to easily see if they're dirty) and readily cleanable?	6	0	0	6	0	0	2	2	0
20. Are the ceilings constructed in a manner that prevents accumulation of dust, condensation and growth of microorganisms? (look out for pipes and electrical ducting)	4	2	0	3	3	0	1	3	0

21. Are the premises well ventilated (i.e. is there enough airflow to prevent formation of ceiling condensation)? Ask do you ever get condensation dripping off the ceiling?	5	1	0	6	0	0	1	3	0
22. Is there physical separation of raw product receiving area and product preparation, processing and packing areas?	6	0	0	3	3	0	2	2	0
23. Are refuse and processing by-products stored in a physically separate location to raw fish and final product?	4	1	1	5	1	0	2	1	1
24. Does the plant have plentiful supplies of hot and cold potable water? Ask: do you use mains water? How is it heated?	6	0	0	6	0	0	4	0	0
25. If borehole water is used, how is the water made potable? (e.g. chlorine dioxide addition)	0	0	6	1	0	5	0	0	4
26. Is the ice made by the plant made from potable water or sea water?	3	0	3	2	0	4	1	0	3
29. Does the plant have a non-potable water source (e.g. estuary or coastal water) and if so, are there protections to prevent non potable water from contaminating edible product?	0	6	0	1	5	0	0	4	0
30. Are the food contact surfaces in the plant hard, impermeable to water, free from cracks and pitting and not visibly corroded?	6	0	0	5	1	0	4	0	0
31. Are the food contact surfaces capable of withstanding repeated cleaning and sanitation?	6	0	0	6	0	0	4	0	0

32. Are the vats/other containers used for brining or salting vats free from corrosion, scoring and pitting and constructed in a manner that permits easy cleaning and complete drainage?	0	0	6	4	1	1	4	0	0
33. Are there knife sterilisers (e.g. >80oC water baths) at any filleting and evisceration stations?	0	5	1	0	6	0	0	4	0
34. Is a two-knife system in use? (one knife in steriliser, the other in use; knives periodically exchanged)	0	5	1	0	6	0	0	4	0
36. Are there low pressure (i.e. mains pressure) hoses in the processing area?	4	2	0	6	0	0	4	0	0
37. Are there high pressure (i.e. jet washers) hoses in the processing area?	1	5	0	1	5	0	1	3	0
38. Is the temperature monitored in processing/storage areas?	6	0	0	4	2	0	3	1	0

*NA is Not applicable; DK is Don’t know.

units. Staff in the plants with chiller problems, did not have an awareness of what had caused the issue or how best to fix it.

The large and medium sized factories tended to have ventilation systems that appeared to operate well whereas the smaller factories had no proper means of ventilation (Q.21). Separation of raw and processed product was readily achievable in the large factories. However, due to size restraints this was not possible for 50% of the small and medium sized factories (Q.22).

Most factories stored waste in a separate area from the raw fish and final product (Q.23). However, this was not universally the case. Although it is widely considered to be a poor practice to store waste with raw materials, and raw materials with finished product, those three businesses which found it unavoidable acknowledged that it was not a good practice. Each had tried to minimise the risk of cross-contamination from waste by bagging or otherwise sealing both the waste and the raw materials and finished product. All of the factories visited except two were on a mains water supply. The exceptions used hypochlorite to treat their water to render it potable before use (Q.25). The majority of the food contact surfaces were capable of withstanding repeated cleaning and sanitation and were in good condition apart from one company where the cutting boards were badly marked through intensive use (Q.30/31).

The vats used for brine were generally in good condition except for one factory which used plastic trays that were badly scuffed (Q.32). In the factories, no knife sterilisers (at 80^{o}C) were used and a two knife system was not seen to be in operation (Q.33/34). Knives were generally cleaned at end of shift (or more frequently if required). A number of factories had the individuals responsible for cleaning their own knives and effectiveness of cleaning was periodically checked.

A range of automated equipment was seen in the factories including machines that de-headed, split, filleted and skinned the fish (Q.35). There were also automated salting machines (a rotating plastic dispenser deposited a set amount of salt onto the surfaces of every fish that passed) and slicing machines. Detritus was generally removed as it accumulated using water hoses as was required. The water pressure in the hoses was usually low pressure but high pressure hoses were also observed. Foaming chemical cleaners appeared to be widely used followed by a sanitizer application which was processor-specific. At least one company sanitised

machines with a solution containing hypochlorite (bleach). Some of the smaller and medium sized companies had virtually no automated equipment. All of the processing operations were undertaken by hand.

For more thorough clean downs between processing shifts or at the end of processing days, most companies used low pressure hoses (Q.36/37). However, high pressure hoses were also used in some plants although management insisted that high pressure would be only used during the general cleaning of the factory once a day's processing was completed. A key observation made by the project teams was that high pressure hoses tended to be used in low risk areas with the low pressure hoses being more common in high risk areas, indicating an understanding of the hazards of high pressure water by plant technical staff. The temperature (Q.38) in the processing area was monitored in most of the factory processing areas, although two medium processors and one small did not monitor temperatures.

PROCESSING STAFF

Responses to queries on process staffing are shown in Table 18. Most factories had SOPs which detailed the minimum standards of cleanliness required for processing staff (Q.39). The exceptions dealt with unacceptable staff cleanliness by enforcing changes of clothing and hand washing prior to entering the processing area. A number of plants had included staff cleanliness into their HACCP manuals. In exception, in one company, a member of staff used the same clothes for all aspects of the work and had them laundered daily. Almost all of the medium and large factories had in place a return to work procedure for processing staff returning from illness (Q.40). Smaller organisations tended to not have a formal protocol but trusted their staff to come back to work when they were feeling better, or after soliciting a medical opinion.

All of the factories visited had sanitary toilets. They also all had facilities to wash hands in the factory (although one single smoker had no hand drying facility). It was noted that most of the larger factories had hand washing at the entrance of the processing area whereas for small factories this was where the sink happened to be (Q.41/42). Boot cleaning equipment and boot washes were also routinely encountered.

In most factories, gloves were changed at appropriate times (e.g. end of shift or when changing tasks Q.44). However in one incident it was noted that personnel wore the same gloves when carrying out several tasks which could have led to contamination of the final RTE product. In the

Table 18 Smoked fish processor assessment questionnaire: responses to the processing staff section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
39. Does the plant have an SOP which describes minimum standards of cleanliness for processing staff?	5	1	0	5	1	0	3	1	0
40. Does the plant have a return to work procedure for processing staff (known or suspected of) recovering from gastroenteritis?	5	1	0	4	2	0	2	2	0
41. Does the plant have sanitary toilets which allow employees to wash and dry their hands?	6	0	0	6	0	0	4	0	0
42. Are there facilities which allow employees to wash and dry their hands in the processing hall?	6	0	0	5	1	0	4	0	0
44. Are gloves changed at appropriate times (i.e. after touching something else etc..)	5	0	1	3	0	3	1	2	1
45. Are staff movements restricted in the direction of clean (i.e. the smoker) end of the process towards dirty (i.e. the raw fish receiving) end of the process?	5	1	0	3	3	0	2	2	0
46. Are different areas of the plant physically segregated?	6	0	0	5	1	0	4	0	0
47. Does the plant use casual labour?	3	3	0	2	4	0	0	4	0
48. Do plant employees change their jobs frequently (every few days or more frequently) to prevent boredom?	0	5	1	2	4	0	3	1	0

*NA is Not applicable; DK is Don't know.

majority of factories (especially the larger ones) staff movements were restricted so that people did not move from low risk to high risk areas (Q.45). In the larger factories, staff working in different areas wore different colours of clothing so that their presence in an inappropriate area was obvious. In the small and medium sized factories, it was quiet common for a change of clothing to be required between the different processing areas. The approach is widespread in the food processing sector.

PROCESSING PRACTICES

A summary of the responses to each of the questions in the processing practices section is provided as Table 19. Fish were generally washed on receipt (Q.49). The exceptions were when fish arrived in the form of fillets and some companies purchased whole fish that were washed after only gutting. A number of companies either did not or did not need to de-scale the salmon (Q.50). Those that did predominantly washed the fish afterwards. Those factories that gutted fish also washed the carcass cavity afterwards (Q.51). The factories exclusively used mains water to wash the fish (Q.52).

The processing in the majority of factories was carried out at ambient temperature (Q.53). However, particularly for the medium and large sized factories there was temperature control (varying by factory from 4-12^{o}C).

Salt was generally stored outside of the production environment in dry areas in sealed bags that were brought into the factory as required (Q.54). Salting for the vast majority of smoked salmon is using salt crystals and not brine. However, five companies that were visited did use liquid brine for smoked salmon (Q.60). These companies tended to be smaller or medium sized. Brine was usually made daily but one factory made it up once per week (Q.55) and at two of the small factories fatty scum and deposit sludges in the brine was observed (Q.56). Most of the brining occurred at temperatures <10^{o}C and the remainder at ambient (Q.57). Recycling of brine for different batches of fish occurred most frequently for smaller companies (Q.59).

When using salt crystals, the fillets were generally placed on wire grid racks on a trolley. Thus water drawn from the fish flesh was free to drain (Q.61) and would drip onto a fish lower down on the rack and eventually to the floor. One company salted fish on a polythene bag on top of a table that did not have drainage. However, it was assumed that excess salt would mop up any liquid drip. None of the companies restacked the fish during the salting treatment (Q.62).

There was no standing water (Q.64) and detritus was removed regularly or at end of shift (Q.65). Excess salt was washed off the fillets using a low power hose at the end of the maturation process.

A variety of kiln designs were used and most used traditional burning or smouldering hardwood (Q.66) as the smoke source. The kilns were operated using chipped wood (beech, oak, juniper and whisky barrels), sawdust, off-cuts from the local joiner etc. The majority of plants used AFOS kilns with an external smoke box. However, there were also brick built kilns which could be more than 50 years old observed in routine use in some plants. There was a tendency for the smaller plants to operate manual kilns which provided little or no control of the smoking process. The newer kilns (mostly in medium to large sized factories) had some level of automatic monitoring and control over smoke, control of the draft, oven temperature (Q.68). In the largest throughput plants the degree of automation included control over external smoke box airflows and reproducible cooling of the product after hot smoking. The frames/racks to support the fish were made of a material that could be readily cleaned apart from one company that used wooden sticks as part of a traditional method of smoking fish (Q.70). When trolleys were used to transport racks of fish into kilns, a small number of companies had splash guards fitted to protect low-lying fish but the majority did not (Q.71).

The final packing materials were stored under dry conditions in all companies except for one business which had a leaking roof (Q.75). All of the companies used vacuum packing and one used MAP packing for some ready to eat un-smoked salmon (Q.78). The final product was stored separately from the raw product for the large and medium sized companies but this was not the case for the smaller companies (Q.80). This was predominantly due to having only one main chill/refrigerator room. When companies were forced to use a single chiller for raw and final products, most attempted to segregate the chill space into specific sections for each commodity.

In most factories, safeguards were in place to ensure that boxes used to transport fish were not used for raw product (Q.81). Prevention of inappropriate reuse was achieved by a variety of means; for example, if the incoming boxes were polystyrene they were immediately disposed of. Alternative strategies such as colour coding of boxes or using cardboard boxes for final product only were also observed.

Table 19 Smoked fish processor questionnaire: responses to the processing practices section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
49. Are fish washed on receipt and what is the source (stored, mains etc) and type (SW, FW, potable, clean?) of water used?	2	4	0	5	1	0	2	2	0
50. Are fish washed after descaling?	0	0	6	3	1	2	0	1	3
51. Are fish washed after gutting? (if applicable)	1	0	5	5	0	1	0	0	4
54. Is salt stored under dry conditions which prevent its contamination?	5	1	0	5	0	1	3	1	0
55. If brine is used, is it made fresh every day (or more frequently)?	0	0	6	3	1	2	3	1	0
56. Does the brine ever accumulate fatty scum or deposit sludges of solid salt mixed with fish residue in the brining container?	0	0	6	0	4	2	2	2	0
57. Is brining undertaken at 3^{o}C or lower?	0	0	6	0	4	2	0	4	0
59. Is fresh brine used for each batch(YES) of fish or is it recycled for more than one batch(NO)?	0	0	6	3	1	2	1	3	0
61. If salt crystals are used, is the brine that forms free draining? (i.e. the fish don't sit in the created brine)	6	0	0	4	1	1	1	0	3
62. If salt crystals are used, are the fish restacked part way through the salt treatment?	0	6	0	0	5	1	0	1	3
64. Is the dripping/drying area well drained (i.e. no standing water on the floor)	6	0	0	5	0	1	4	0	0

65. Does waste removal (e.g. melted water, fish detritus) from the processing area happen on a continual or near continual basis?	6	0	0	5	1	0	4	0	0
66. Does the plant use automated smoke generation (YES) or traditional burning/smouldering hardwood(NO)	1	5	0	1	5	0	0	4	0
67. If hardwood is used for smoking, does it ever have traces of soil on the wood's surface?	0	4	2	0	3	3	0	2	2
68. Is the smoking process instrumented to monitor and control the smoking process?	6	0	0	6	0	0	2	2	0
70. Are the frames/racks/tenters used to support the fish during smoking constructed from corrosion-resistant material which is water impermeable and designed to be readily cleaned and sanitised?	6	0	0	5	0	1	3	1	0
71. Do the racks etc, if moveable have adequate splash protection to protect fish from spray from wet floors/environment?	3	3	0	0	3	3	1	2	1
75. Are final product packing materials stored under dry conditions which prevent contamination?	5	0	1	5	0	1	3	1	0
77. Are finished products stored in a manner which prevents their direct contact with melted ice?	0	0	6	3	0	3	3	0	1
78. Are final products vacuum packed?	6	0	0	6	0	0	4	0	0
79. Are final products modified atmosphere packed and if so what gas mix is used and what ratio of product volume to gas volume is used?	1	5	0	0	6	0	0	4	0
80. Are final products and raw fish held in physically separate locations?	6	0	0	6	0	0	1	3	0

81. Are there safeguards in place (e.g. different colours/types of container) to ensure crates/boxes/trays used for transport of raw fish are not used for finished product?	3	1	2	3	3	0	4	0	0
82. Do you test the final product for *L. monocytogenes*?	5	1	0	4	2	0	0	4	0
83. Is the testing presence/absence(NO) or numbers(YES)?	4	1	1	3	1	2	0	1	3
84. Do you ever get out of specification (OOS) results?	3	2	1	2	2	2	0	1	3
86. Is testing of the processing environment carried out (and as above – what methods are used) and where do they sample from?	6	0	0	5	1	0	0	4	0
87. Is any sampling data kept and used (e.g. to trend historical data and identify when conditions are moving out of spec)	6	0	0	3	2	1	0	0	4
88. Does the FBO determine product characteristics, particularly water activity and pH?	5	0	1	4	2	0	1	3	0
90. Would guidance on shelf life determination be useful?	2	1	3	6	0	0	2	2	0
91. Have you heard of the BRC/CFA/FSA 2010 Guidance ('Shelf Life of Ready to Eat Food in Relation to *L. monocytogenes* – Guidance for Food Business Operators'	4	1	1	2	4	0	0	4	0
93. Is there product traceability?	5	0	1	5	1	0	4	0	0
94. Are they aware of the microcriteria regulations?	5	0	1	0	4	2	0	3	1
95. Is there a HACCP plan?	6	0	0	5	1	0	3	0	1

*NA is Not applicable; DK is Don't know.

A number of companies obtained further information on their product that included pH, water activity and salt content (Q.88). Most companies thought that further guidance on shelf-life determination would be useful (Q.90). Some of the larger companies (e.g. those supplying supermarkets) did not require further advice as they already had this well in hand as part of their conditions of supply to various retailers. However, some of the smaller companies did not have the resource or expertise readily available to conduct shelf-life trials on their products. It was worth noting that shelf-life of a product could vary depending on the demands of the customer (e.g. export could require longer shelf lives).

Only a number of the larger and medium sized companies had working knowledge of BRC/CFA/ FSA 2010 guidance and only the larger companies were aware of the microcriteria regulations (Q.93/94). All of the companies except one had HACCP plans which ranged from basic off the shelf adaptions to extensive plants which were customised to individual processes. The sole exception was one small company that had only working SOPs in place.

Furthermore, nine of the companies specifically tested for the presence of *Listeria monocytogenes* (Q.82). Out of these nine, six additionally enumerated for *L. monocytogenes* (Q.83). The criteria used for an out of specification (OOS) result was variable between plants. Examples of the criteria used included a simple positive detection or, for quantitative testing, obtaining a count of >100 cfu/g on a final product. One company had obtained a *L. welshimera* strain, which it treated as OOS. Two companies reported that they obtained *L. monocytogenes*-positive test results rarely although one had found a count of >100 cfu/g on a finished product which was still in the factory. The prevalence of *Listeria* was found to vary considerably from company to company (Table 20).

Table 20 Prevalence of *Listeria* in raw and smoked products

Processor	Fish species/product	Prevalence of *Listeria* spp.
1	Salmon fillet (raw)	0% (out of 180 sampled)
2	Salmon trimmings	12%(only occasionally are isolates *L. monocytogenes*)
3	Salmon (raw)	0.5% on incoming fish; <2% on product; rarely >100 cfu/g
4	Smoked salmon	0.2%
5	Smoked salmon	11% (9% *L. monocytogenes*)
6	Smoked salmon	0.1 to 2%
7	Herring (raw)	5% (out of 20 sampled)
8	Smoked herring	0% (out of 300 sampled)

CLEANING AND SANITATION

A summary of the reponses to the cleaning and sanitation section of the questionnaire is provided in Table 21. Three of the companies (one small and two large) said that they wet cleaned mid-shift. The cleaning was undertaken using low power hoses (Q.96). One of the smaller companies regularly hosed down the processing area during the working day. For the larger companies the mid shift cleans were either for low care or specific areas in high care where there was a build-up of fish waste that needed to be cleaned away. It is worth noting that some companies brushed this waste away using squeegees in order to keep the high care part of the factory as dry as possible.

A minority of the small companies and one of the medium sized companies did not have arrangements in place for microbiological analysis (Q.98). The smaller companies tended to depend on the EHO's who took samples for analysis. The cleaning and sanitation were regularly checked by all of the large and most of the medium sized factories. These checks included using ATP tests or taking swabs followed by microbiological analysis or the use of protein detection sticks. Smaller companies reported that they would visually check for cleanliness before the commencement of processing. Only one of the larger companies changed their chemicals regularly to prevent the establishment of resistant bacterial populations. A further company

reported that they had been informed by the sales agent for the chemical company that there was no need for periodic chemical change.

Table 21 Smoked salmon fish processor questionnaire: responses to the cleaning and sanitation section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
96. Is there a wet cleaning mid- shift? (i.e. is there splashing of *L. monocytogenes* from drains)	4	1	1	6	0	0	2	2	0
98. Does the plant have equipment washing and cleaning SOPs that are undertaken outside of processing and which are validated as effective for the control of *L. monocytogenes*?	4	1	1	2	3	1	1	3	0
101. Does the plant have an arrangement in place which allows for microbiological testing?	6	0	0	5	1	0	2	2	0
102. Is the effectiveness of cleaning and sanitation periodically checked?	6	0	0	5	1	0	1	3	0
105. Are cleaning and sanitisers changed periodically to prevent the establishment of plant persistent bacterial populations which are resistant to long time use chemicals?	1	5	0	0	6	0	0	4	0

*NA is Not applicable; DK is Don't know.

3.3.2.1 SUMMARY AND DISCUSSION OF SALMON AND TROUT PROCESSOR/SMOKEHOUSE QUESTIONNAIRE

The chain for production of smoked fish can be complex and frequently involves a number of companies. For example, in the case of cold smoked salmon, one company may grow the fish, harvest and then pre-process (gut, wash and put on ice). The fish are then transported and another company may fillet the fish. The fillets are then further transported to another company which undertakes smoking, slicing and packing of the fish. The final product may then be sold direct to the public, to a retailer in the UK or be destined for export.

In several discussions with factory managers it was thought that the main route of *Listeria* into the factory was on the surfaces of incoming fish. Although there is some scientific evidence to broadly support such an opinion, the precise sources of plant persistent *Listeria* still require further study for absolute confirmation. A number of studies have shown that some *Listeria* which have colonised the plant environment are most likely to be isolated from the final product. For that reason, it is not always clear that the strains on incoming fish are the same as those on the final product (Dass *et al* 2010). In general, the biosecurity in the factories visited appeared to be of a high standard. However there is a need to monitor *Listeria* in other products used for preparation of smoked (and marinaded) fish products. For example one of the companies had isolated *Listeria* from a bag of herbs (dill) and another had been troubled with *Listeria*-contaminated cream cheese used as filling for smoked salmon parcels.

Washing of fish (e.g. gutted) was deemed to be an important processing stage for the removal of some (but not all *L. monocytogenes*). A number of plant operators thought that processing aids could play an important role in this stage of the process. Although chlorinated water washes can be used under specific circumstances (currently they are not allowed if the wash is to specifically decontaminate food), chlorine is allowed to be added to water to render it potable. The use of hypochlorite or chlorine dioxide added to borehole water or to mains water, which has been stored in tanks, *to render the water potable* for use as wash water (which happens to have a mild antimicrobial effect) is widespread in the fresh produce and meat sectors in the UK.

It was unclear whether manual or automated filleting of fish was likely to lead to a higher prevalence of *Listeria* contamination of fish flesh. In essence, it was felt most likely to depend on how easy and frequently the automated equipment was cleaned. Some automated equipment had continuous washing of blades and the rubber wheels used to move the fish through the equipment.

Most of the smoked salmon was salted by application of crystals to the surface of the fillet. A machine that was commonly used in factories had a set of plastic fingers set just above the conveyor. As the fillets went by the fish pressed on the fingers and salt was then released. If these fingers became contaminated from one fillet then there was the possibility of spreading the contamination to fillets further down the line. After maturation, excess salt was washed off the fillets using a low pressure hose. Depending on how these washes were undertaken, the

water poured from the upper fillets on the trolley onto the lower ones. The practice certainly has the potential to enable cross-contamination. Even if each tray of fillets was washed individually (away from the trolley) they would then be placed on the trolley and drip onto other fillets.

Where segregation was possible, the larger factories had designated "low care" and "high care" areas. High care started after the product was smoked and this area was generally kept as dry as possible. Usually different coloured or types of boxes for carrying fish and different colours of boots/overalls were used and staff could not move directly between the two areas (particularly for large and medium sized factories) without going through a hygiene barrier. There were definite advantages of having a kiln with an entrance on the low care side and an exit into the high care area because the arrangement ensured that there was separation between "raw" and "processed" product. If such an arrangement was not possible the events surrounding opening the kiln door were usually set as a CCP. This was to ensure it was cleaned after the fish had been put into the kiln. Incorporation of splash guards on the trolleys also helped to protect the product.

None of the factories that were asked had determined the effect of the smoking process on *Listeria*. The peer-reviewed literature suggests that cold smoking tends not to cause large reductions to the numbers of *Listeria* on the product, but a number of authors have reported apparently conflicting outcomes. In contrast, a number of authors have reported that for hot smoking (or baking after cold smoking), oven temperatures of >60^{o}C can cause significant reductions in numbers of *Listeria*. However more than 70^{o}C for two hours was required for effective control such that any contamination issues that arose could be only a consequence of cross contamination (Kolodziejska *et al*, 2002).

Cold smoked fillets were further processed at many of the plants including skinning, slicing and trimming. These processes involved the use of knives and, frequently, machinery. One company shared that they had isolated *Listeria* from the motor assembly of one of their machines. Another considered that the skinning machine was high-risk equipment because it operated on fish which may have been surface contaminated with *L. monocytogenes*. Another company considered that the Whizzards (a hand held device for trimming salmon fillets) may have been a risk for cross contamination between fillets and also required these to be cleaned thoroughly after use. Some technical managers shared that they had hygiene concerns with the

design of some the equipment they had inherited. One company had a slicing machine (D-slices) which had plastic pins on the feed conveyor from which fish flesh was difficult to remove during cleaning. The plant's experience with that equipment was that *Listeria* could survive, or they speculated; grow in a biofilm because the belt was hard to clean. There are reports in the literature that *L. monocytogenes* can re-appear sporadically from such niches to contaminate a factory (Porsby *et al*. 2008). Most technical managers recognised the importance of having machines that could be easily stripped down and properly cleaned, or that were designed as 'clean in place' (CIP).

Challenge tests as the basis of shelf life calculation were not deemed useful by industry – only two companies had done such tests and for both, *Listeria* grew in the product under conditions of adequate refrigeration. Both companies felt that the finding did not adequately mimic industrial processing conditions and natural contamination.

Condensation in chillers and near refrigeration devices in a number of factories that are areas for concern is acknowledged by industry, and should be addressed to help prevent the spread of *L. monocytogenes* to raw fish and the packaging of final product. The source of the condensate is the evaporation coils of the refrigeration units. A number of plants felt that advice on prevention of condensation would be helpful. Finally, a lack of slow dissolving sanitisers in drains should be considered as once drains are free from *L. monocytogenes*, block sanitiser can help prevent recolonisation. There is always a concern that splash back from drains during washing down of the factory can recontaminate food contact surfaces.

The European Salmon and Trout Smokers Association (ESTSA) has developed an IT system for monitoring the quality of raw fish in the EU. The ESTSA system is essentially a *Listeria* database which records the presence/absence of *Listeria* in raw fish prior to smoking. The ESTSA system combines test result data from samples taken at dispatch from the farm and also arrival at the smokers' factory. There is a modest cost for smokers to join the association and if members use the database, there is an obligation for companies to provide their *Listeria* results. The main advantage ESTSA system for the smokers is that they have an overview of the *Listeria* status of fish from different growers and hence can act accordingly. It appears that joining the scheme would be beneficial for smokers of salmon and trout. A potential drawback is the cost to join (a few hundred Euros) which may be prohibitive for the smallest companies.

SUMMARY OF KEY FINDINGS

In summary, the key findings from visits to processors and smokehouses and from the questionnaires were as follows:

- Problems exist with condensation, particularly in chillers
- There was an absence of drain sanitisers/disinfectants in factory drains which may increase the likelihood of these harbouring *L. monocytogenes*.
- A number of plant technical managers/ FBOs were strongly in favour of 'official' guidance describing how to react if a factory had a problem with *Listeria* (e.g. a number of *Listeria* positives in a product range). 'What to do' is a particular concern for the smaller companies who do not have ready access to microbiological expertise.
- A number of medium-sized fish smoking companies use external consultants to provide microbiological support. These could play an important role in communicating and applying any information or guidance.
- Challenge tests (where fish were inoculated with lab-cultured *L. monocytogenes*) were perceived to be expensive and were the results were not viewed as being typical of naturally contaminated product by industry.
- Accurate enumeration of *Listeria* was felt to be a problem by a number of the fish smokers.
- Minced smoked salmon was generally considered to have a higher contamination rate than sliced product.
- High pressure hoses continued to be used in a number of factories, despite their proven role in assisting the spread on microorganisms around food processing premises.
- A number of companies mentioned that they would at some point require larger premises and had questions regarding key elements for the design of new factories.
- Shelf-life varies greatly and is often apparently dependent on the customer/market
- Some of the larger companies exchange data on *Listeria* prevalences in raw fish and share experiences with (for example) shelf life testing through the European Salmon and Trout Smokers Association.
- Larger smokers were concerned that the biotypes of *Listeria* associated with fish may not actually be the same types which tended to cause disease in humans.

3.3.3 PELAGIC FISH PROCESSORS/SMOKEHOUSES

RAW FISH

A summary of the responses from the pelagic smoked fish processor questionnaire relating to questions on raw fish is shown in Table 22. Fish were transported to the processors either on ice or frozen (Q.1). The shipping containers used for the fish were either plastic or disposable (cardboard or polystyrene; Q.2). All non-disposable boxes were washed on site by automatic machinery or off-site by a commercial crate washer for the larger companies (Q.3/4). Smaller companies the tended to wash crates at sinks in yards with hoses. The plastic boxes were invariably scratched making them more problematic to clean effectively (Q.8).

Frozen fish were usually thawed at ambient temperature (Q.9). Fish were stored in the chill/refrigerator when they arrived, and five of the companies checked the temperature using an electronic probe at this stage. The remaining companies checked visually (presence of ice on fish or if there were signs of thawing for frozen product; Q.11). Waste material from fish processing was cleaned regularly or at least on a per batch basis (Q.13).

Table 22 Pelagic smoked fish processor questionnaire: responses from raw fish section

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
1. Are fish transported in a manner which prevents their warming up?	3	0	1	2	0	0	2	0	0
2. Are transport crates/boxes/trays designed to permit easy and thorough cleaning and sanitation?	2	0	2	2	0	0	1	0	1
3. Are transport crates/boxes/trays cleaned and sanitised at the plant before being reused?	1	0	3	0	0	2	1	0	2
4. Are transport crates/boxes/trays cleaned and sanitised somewhere else before being reused?	1	0	3	0	0	2	0	0	2
5. Are they ever used for a purpose other than transporting fish?	0	3	1	0	2	0	0	1	1
6. Is any transport crates/boxes/tray cleaning and sanitation regime validated as effective in controlling *L. monocytogenes* (Plant may not know if it happens offsite)	1	0	3	0	1	1	0	1	2
8. Is there physical damage (e.g. scrapes and scores) in the surfaces of transport crates that could come into contact with raw fish?	3	0	1	1	1	0	1	0	1
9. If frozen fish is used, is it thawed at below 7^{o}C before being processed?	1	2	1	0	2	0	0	0	2

10. Are fish stored under refrigeration (i.e. in ice or in a chiller) prior to processing and smoking?	4	0	0	2	0	0	2	0	0
11. Is the incoming temp of the fish checked?	3	1	0	1	0	1	1	1	0
12. Does the FBO have any criteria for accepting fish? (e.g. does he check the listeria status of the fish coming in/ask the farm to supply data/ ask the farm what controls they take/ take account of environmental conditions when the fish were harvested)	3	1	0	2	0	0	1	1	0
13. Does waste removal (e.g. melted water, fish detritus) from the fish unloading and storage areas happen on a continual or near continual basis?	3	0	1	2	0	0	2	0	0

*NA is Not applicable; DK is Don't know.

PLANT INFRASTRUCTURE

It is important that the infrastructure of the plant is intact enough to physically exclude vermin and insects, and also that it is maintained to prevent cracked and damaged areas becoming bacterial niches. The general fabric (Q.14) of the pelagic plants was generally observed to be in intact condition. Only one of the processors used slow dissolving blocks of sanitizer in the drains (Q.17) and none of the drains were copper lined (Q.18). As was observed for the farmed fish processors, in some of the pelagic factories there were issues with condensation particularly in chills. In extreme cases, walls were visibly wet with excessive drippage from ceilings. It was common for there to be pipes on walls and ceilings which could accumulate dust/dirt. It was noted in one plant there were problems keeping ceiling clean adjacent to kiln because of tar build up. A common fix for the condensation issue was to fix panels under the refrigeration units in the chill to direct drip away from product. Four of the companies appeared not to have adequate ventilation which was probably one of the main reasons for the condensation problems (Q.20/21; Table 23).

Most of the factories were able to keep separate the raw and processed products within the factory (Q.22). However, some of the kilns had only one entrance where raw fish came in and product came out. The organisational arrangement was not seen as a major problem as the products affected tended not to be RTE. In those plants what had single entrance access to kilns, the area outside the kiln was cleaned between batches. Only one of the companies had a non-potable supply which was treated and tested bi-monthly for *E. coli* (Q.29). None of the companies used knife sterilisers at the filleting/cutting stations (Q.33/34). Knives were washed at end of shift and before breaks (and also when deemed necessary by the user). Low pressure hoses were used in all of the factories. High pressure hoses were used only in the larger companies, presumably because of the larger areas that required cleaning and the extra costs of purchase and use of a power hose. One company used high pressure hose for cleaning the offal bins (which was done outside). Another company used them only at the end of the week as part of a “deep clean” and another to remove exceptionally stubborn detritus from machinery at the end of the working day.

Table 23 Pelagic smoked fish processor questionnaire: responses from plant infrastructure section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA Or DK	Yes	No	NA or DK
14. Is the plant infrastructure intact? i.e. no holes in the walls, broken windows damaged doors which allow insects and vermin into the plant	3	1	0	2	0	0	2	0	0
15. Are plant floors hard surface, non-absorbent and adequately drained (i.e. no standing water)?	4	0	0	2	0	0	2	0	0
16. Do the drains have removable grates?	4	0	0	2	0	0	2	0	0
17.Are the drains fitted with slow-dissolving blocks of sanitiser chemicals?	1	3	0	0	2	0	1	1	0
18. Do the drains have copper linings (or another source of copper ions such as a copper grating)?	0	4	0	0	2	0	0	2	0
19. Are the plant walls smooth, waterproof, light coloured (to easily see if they're dirty) and readily cleanable?	3	1	0	2	0	0	0	2	0
20. Are the ceilings constructed in a manner that prevents accumulation of dust, condensation and growth of microorganisms? (look out for pipes and electrical ducting)	3	1	0	1	1	0	0	2	0
21. Are the premises well ventilated (i.e. is there enough airflow to prevent formation of ceiling condensation)? Ask do you ever get condensation dripping off the ceiling?	2	2	0	2	0	0	0	2	0
22. Is there physical separation of raw product receiving area and product preparation, processing and packing areas?	2	2	0	2	0	0	2	0	0
23. Are refuse and processing by-products stored in a physically separate location to raw fish and final product?	4	0	0	1	1	0	1	0	1

24. Does the plant have plentiful supplies of hot and cold potable water? Ask: do you use mains water? How is it heated?	4	0	0	2	0	0	2	0	0
25. If borehole water is used, how is the water made potable? (e.g. chlorine dioxide addition)	0	0	4	1	0	1	0	0	2
26. Is the ice made by the plant made from potable water or sea water?	3	0	1	0	0	2	0	0	2
29. Does the plant have a non-potable water source (e.g. estuary or coastal water) and if so, are there protections to prevent non potable water from contaminating edible product?	0	4	0	1	1	0	0	2	0
30. Are the food contact surfaces in the plant hard, impermeable to water, free from cracks and pitting and not visibly corroded?	4	0	0	2	0	0	2	0	0
31. Are the food contact surfaces capable of withstanding repeated cleaning and sanitation?	4	0	0	2	0	0	2	0	0
32. Are the vats/other containers used for brining or salting vats free from corrosion, scoring and pitting and constructed in a manner that permits easy cleaning and complete drainage?	3	0	1	2	0	0	2	0	0
33. Are there knife sterilisers (e.g. >80^{o}C water baths) at any filleting and evisceration stations?	0	4	0	0	2	0	0	2	0
34. Is a two-knife system in use? (one knife in steriliser, the other in use; knives periodically exchanged)	0	4	0	0	2	0	0	2	0
36. Are there low pressure (i.e. mains pressure) hoses in the processing area?	4	0	0	2	0	0	2	0	0
37. Are there high pressure (i.e. jet washers) hoses in the processing area?	3	1	0	0	2	0	0	2	0
38. Is the temperature monitored in processing/storage areas?	4	0	0	2	0	0	1	1	0

*NA is Not applicable; DK is Don’t know.

PROCESSING STAFF

All of the companies had SOPs which described standards of cleanliness for processing staff except for one (Table 24; Q.39). All of the plants visited had sanitary toilets and washing facilities. Hand washing facilities were found in all of the processing halls (Q.41/42). Gloves, when used by personnel handling fish were changed every 3 hours or at breaks and also at the discretion of workers. Four of the companies did not restrict staff movement in the direction from raw to processed. In terms of staff logistics, one company had individual staff do several jobs throughout the process and hence it was not feasible to restrict staff movements. The larger companies where staff movement was not restricted occurred because of a combination of the layout of the factory and also the fact that they were not producing RTE products. These were also the reasons why some of the factories did not have segregation between areas.

Table 24 Pelagic smoked fish processor questionnaire: responses from processing staff section.

	Large			Medium			Small		
Question	Yes	No	NA or DN*	Yes	No	NA or DN	Yes	No	NA or DN
39. Does the plant have an SOP which describes minimum standards of cleanliness for processing staff?	3	1	0	2	0	0	2	0	0
40. Does the plant have a return to work procedure for processing staff (known or suspected of) recovering from gastroenteritis?	3	0	1	2	0	0	1	1	0
41. Does the plant have sanitary toilets which allow employees to wash and dry their hands?	4	0	0	2	0	0	2	0	0
42. Are there facilities which allow employees to wash and dry their hands in the processing hall?	4	0	0	2	0	0	2	0	0
44. Are gloves changed at appropriate times (i.e. after touching something else etc..)	2	1	1	2	0	0	0	1	1
45. Are staff movements restricted in the direction of clean (i.e. the smoker) end of the process towards dirty (i.e. the raw fish receiving) end of the process?	1	3	0	2	0	0	1	1	0
46. Are different areas of the plant physically segregated?	3	1	0	2	0	0	2	0	0
47. Does the plant use casual labour?	1	3	0	1	1	0	0	2	0
48. Do plant employees change their jobs frequently (every few days or more frequently) to prevent boredom?	1	3	0	1	1	0	2	0	0

*NA is Not applicable; DK is Don't know.

PROCESSING PRACTICES

A summary of the responses to each of the questions in the processing practices section is provided as Table 25. All the companies visited brined their fish by immersion rather than injection. Four of the companies visited (two very large and two very small) did not wash the fish before brining as the raw material arrived already gutted, filleted and washed (Q49-51). One of the smaller companies occasionally received fish pre-gutted but not filleted and would routinely wash such fish after filleting and before brining. All of the medium-sized companies washed fish prior to brining irrespective of its evisceration or filleted status.

As for the farmed fish, a mixture of kilns were used for the smoking process with a number of companies using automated AFOS-style kilns (of a variety of vintages) and some still using traditional brick built kilns (Q.66). There were no traces of soil on any of the wood or chips that was inspected before smoking (Q.67). All of the kilns could be controlled to varying extents except for one traditional kiln used by a small company that had to be monitored directly by staff (Q.68). In those smokers that used trolleys to transport fish to the kiln splash guards were universally absent (Q.71).

Final packing materials were all stored under dry conditions (Q.75). Most of the final products were vacuum packed (Q.78) and MAP packing was not used by any of the processors for pelagic or white fish (Q.79). In all factories visited except for one small one, it was possible to store raw and final products in different chillers (Q.80). In general different styles and colours of boxes were used for product and raw materials (Q.81).

Four of the companies tested the final product for *Listeria*. The ones that did not explained that this was because their final product was not RTE and one of the small companies said that the EHO had samples tested from time to time (Q.82). Only one of the companies enumerated for *Listeria* (Q.83). Two of the companies had obtained OOS results at some point in the past (Q.84) and this led to retraining of staff and a review of processes in one company whilst in the other no action was deemed necessary as this was in the incoming fish and not on the smoked product (Q.85). The large and medium sized companies periodically sampled the effectiveness of cleaning in their processing environments (e.g. swab based ATP test, swabbed surfaces for testing to determine numbers of total aerobic mesophiles and/or *Enterobacteriaceae*) but the small companies did not (Q.86). Data were kept for monitoring trends and the identification of problematic areas for cleaning (Q.87).

Table 25 Pelagic smoked fish processor assessment questionnaire: responses to the processing practices section.

	Large			Medium			Small		
Question	Yes	No	NA or DK*	Yes	No	NA or DK	Yes	No	NA or DK
49. Are fish washed on receipt and what is the source (stored, mains etc) and type (SW, FW, potable, clean?) of water used?	2	2	0	2	0	0	0	2	0
50. Are fish washed after descaling?	1	0	3	1	0	1	0	1	1
51. Are fish washed after gutting? (if applicable)	1	0	3	1	0	1	0	0	2
54. Is salt stored under dry conditions which prevent its contamination?	4	0	0	1	0	1	2	0	0
55. If brine is used, is it made fresh every day (or more frequently)?	3	0	1	1	1	0	2	0	0
56. Does the brine ever accumulate fatty scum or deposit sludges of solid salt mixed with fish residue in the brining container?	1	1	2	0	2	0	1	1	0
57. Is brining undertaken at 3^{o}C or lower?	1	2	1	0	2	0	0	2	0
59. Is fresh brine used for each batch of fish(YES) or is it recycled for more than one batch(NO)?	0	3	1	1	1	0	1	1	0
61. If salt crystals are used, is the brine that forms free draining? (i.e. the fish don't sit in the created brine)	1	0	3	1	0	1	0	0	2
62. If salt crystals are used, are the fish restacked part way through the salt treatment?	0	1	3	0	1	1	0	0	2
64. Is the dripping/drying area well drained (i.e. no standing water on the floor)	3	1	0	1	0	1	2	0	0

65. Does waste removal (e.g. melted water, fish detritus) from the processing area happen on a continual or near continual basis?	4	0	0	2	0	0	2	0	0
66. Does the plant use automated smoke generation(YES) or traditional burning/smouldering hardwood(NO)	1	3	0	1	1	0	0	2	0
67. If hardwood is used for smoking, does it ever have traces of soil on the wood's surface?	0	1	3	0	2	0	0	1	1
68. Is the smoking process instrumented to monitor and control the smoking process?	4	0	0	2	0	0	1	1	0
70. Are the frames/racks/tenters used to support the fish during smoking constructed from corrosion-resistant material which is water impermeable and designed to be readily cleaned and sanitised?	4	0	0	2	0	0	1	0	1
71. Do the racks etc, if moveable have adequate splash protection to protect fish from spray from wet floors/environment?	1	3	0	0	2	0	0	2	0
75. Are final product packing materials stored under dry conditions which prevent contamination?	2	0	2	1	0	1	2	0	0
77. Are finished products stored in a manner which prevents their direct contact with melted ice?	0	0	4	2	0	0	2	0	0
78. Are final products vacuum packed?	2	2	0	2	0	0	2	0	0
79. Are final products modified atmosphere packed and if so what gas mix is used and what ratio of product volume to gas volume is used?	0	4	0	0	2	0	0	2	0
80. Are final products and raw fish held in physically separate locations?	4	0	0	2	0	0	1	1	0

81. Are there safeguards in place (e.g. different colours/types of container) to ensure crates/boxes/trays used for transport of raw fish are not used for finished product?	1	0	3	1	1	0	2	0	0
82. Do you test the final product for *L. monocytogenes*?	2	2	0	1	1	0	1	1	0
83. Is the testing presence/absence(NO) or numbers(YES)?	0	2	2	1	0	1	0	1	1
84. Do you ever get out of specification (OOS) results?	1	1	2	1	0	1	0	1	1
86. Is testing of the processing environment carried out (and as above – what methods are used) and where do they sample from?	3	1	0	2	0	0	0	2	0
87. Is any sampling data kept and used (e.g. to trend historical data and identify when conditions are moving out of spec)	2	1	1	2	0	0	0	0	2
88. Does the FBO determine product characteristics, particularly water activity and pH?	1	2	1	1	1	0	0	2	0
90. Would guidance on shelf life determination be useful?	0	2	2	2	0	0	2	0	0
91. Have you heard of the BRC/CFA/FSA 2010 Guidance ('Shelf Life of Ready to Eat Food in Relation to *L. monocytogenes* – Guidance for Food Business Operators'	3	1	0	1	1	0	0	2	0
93. Is there product traceability?	3	0	1	2	0	0	2	0	0
94. Are they aware of the microcriteria regulations?	2	1	1	0	1	1	0	1	1
95. Is there a HACCP plan?	4	0	0	2	0	0	1	0	1

*NA is Not applicable; DK is Don't know.

All of the small and medium sized companies indicated that advice on shelf-life would be helpful (Q.90). Only the majority of the larger companies had heard of the BRC/CFA/FSA guidance on *Listeria* and the microcriteria regulations (Q.91/93); the document was not well known by the small and medium sized operations. All companies had procedures to ensure traceability and all except one had HACCP plans (Q.95).

CLEANING AND SANITATION

The majority of the factories cleaned mid-shift, usually with low power hoses; the remainder cleaned at either the end of the shift or the end of the day (Q.96). Only the largest factories validated their cleaning by taking swabs for *Listeria* (Q.98). However, the companies used cleaning reagents that were claimed by the manufacturer to be effective against *Listeria* and a number used ATP test to check the efficacy of the cleaning process. All of the large and medium sized factories had SOPs and cleaning schedules in place. All of the plants had an arrangement in place for microbiological testing (Q.102). None of the plants changed their sanitizers regularly (Table 26).

Table 26 Pelagic smoked fish processor assessment questionnaire: responses to cleaning and sanitation section.

	Large			Medium			Small		
Question	Yes	No	NA or DK	Yes	No	NA or DK	Yes	No	NA or DK
96. Is there a wet cleaning mid- shift? (i.e. is there splashing of *L. monocytogenes* from drains)	2	2	0	2	0	0	1	1	0
98. Does the plant have equipment washing and cleaning SOPs that are undertaken outside of processing and which are validated as effective for the control of *L. monocytogenes*?	2	2	0	1	1	0	1	1	0
101. Does the plant have an arrangement in place which allows for microbiological testing?	4	0	0	2	0	0	2	0	0
102. Is the effectiveness of cleaning and sanitation periodically checked?	3	1	0	2	0	0	1	1	0
105. Are cleaning and sanitisers changed periodically to prevent the establishment of plant persistent bacterial populations which are resistant to long time use chemicals?	0	4	0	0	2	0	0	2	0

*NA is Not applicable; DK is Don't know.

3.3.3.1 SUMMARY AND DISCUSSION OF PELAGIC FISH PROCESSORS/SMOKERS QUESTIONNAIRE

The majority of the factories visited produced products that were required to be cooked prior to consumption and as such there was less microbiological sampling particularly for *Listeria* compared with the cold smoked salmon plants justified on the basis of risk. There was a need, particularly for the small companies to be given guidance on what to do if they had an issue with a *Listeria* isolation. Based on the small smoker interviews, small companies were found to be heavily dependent on the advice given by the local EHOs.

It would also be useful to provide advice on how to check the efficiency of cleaning for factories and also what sort of microbiological samples should be taken (and how frequently). This would be most important for the small to medium sized factories that do not have such expertise at hand. The Agency has provided similar guidance previously to the meat sector which could be modified for fish smokers.

A number of the medium to larger factories had some degree of accreditation and were part of schemes such as SALSA (Safe and Local Supplier Approval) which involved being audited on an annual basis. The largest factories supplied supermarkets and were tied into their supply requirements/ assurance schemes for ensuring the safety and quality of products.

SUMMARY OF KEY FINDINGS

A summary of the key findings, discussed above, from the questionnaires and visits to processors nad smokers of pelagc fish are presented below:

- It not unusual for the fish coming into a plant to be contaminated or infected with *Listeria*.
- Despite a requirement for testing for *L. monocytogenes* for ready to eat foods under the auspices of 2073/2005 EC, there is little information available describing the prevalence of *L. monocytogenes* on final smoked products.
- Advice on shelf life determination using naturally contaminated product would benefit small to medium sized fish smokers
- Some of the companies were unclear as to what course of action they should take if there was an isolation of *L. monocytogenes* from a raw material batch, the plant environment and the final product.

- Much of the thawing of frozen fish was done at ambient temperature
- Companies generally did not use drain sanitisers/disinfectants
- Some factories had condensation problems particularly associated with their chills.
- Liquid brining was also frequently carried out at ambient temperature

3.3.4 ADDITIONAL INFORMATION IDENTIFIED DURING INDUSTRY VISITS AND QUESTIONNAIRE DISCUSSIONS – INFORMATION SOURCES

Although not part of the questionnaire, it was noted through converstations with the food businesses visited that the businesses obtained advice regarding *Listeria* and hygiene from a number of different sources, some of which were reliable. The sources included:

- Colleagues in the industry (even if those colleagues worked for competitor organisations)
- Local EHOs
- Staff at microbiological testing laboratories
- External consultants
- In-house expertise
- Customers
- Suppliers of cleaning chemicals
- Trade organisations and accreditation bodies – e.g. Campden BRI, SALSA

Where a company obtained its information tended to be dependent on an organisation's size. Some of the smaller companies had little expertise and were quite dependent on views from their local EHO. Medium-sized companies often relied primarily on consultants who worked as little as one day per month for the company. The larger companies frequently had knowledgeable in-house expertise. Larger smokers also had on-going discussions with their customers (which were primarily supermarkets). As a result of the variable quality of the advice provided from a diverse range of sources, the majority of plant staff asked felt it was important that any information and guidance generated by the FSA on *Listeria* needed not only to be targeted to the smokers but the list of bodies detailed above so that there was a standardisation of opinion. A concern was that retailers would continue to stipulate their own supply requirements which potentially could undermine or contradict any guidance.

3.3.5 ENVIRONMENTAL HEALTH OFFICERS (EHO)

Due the large amount of data generated during the questionnaires aimed at EHOs, to ease presentation and discussions, the responses to each question have been grouped in to related key food safety principle areas as shown in Table 27.

A summary of the numbers of actual responses recorded on a question-by-question basis, the average response score and a ranking of question importance is shown as Table 28 and Table 29. Table 28 shows the areas, ranked in order of importance, where the responses received by EHOs indicated that additional training or guidance could be of benefit to food business operators (FBOs). Table 29 shows, in ranked order, the key areas where the responses received from EHOs indicated that they themselves could benefit from further information. It is interesting to note that there are differences in what is it is perceived that the FBOs require compared to what the EHOs felt that they needed. There are differences in the numbers of responses made for each question.

Overall, the EHO feedback (Table 28) emphasised the need for robust FBO guidance describing effective cleaning and sanitation. Other areas that the EHOs felt could benefit from additional guidance were the control of key hazard organisms such as *L. monocytogenes* and how to effectively monitor salt application and brining of the fish (some EHOs are under the mistaken belief that high salt concentrations can be used as a critical or secondary control point for *L. monocytogenes*). Other highly ranked subject areas were how to appropriately handle the product post-process and how best to determine shelf life.

Table 27 Grouping of questionnaire questions in to related subject areas

Subject area	Corresponding questionnaire question
Key Food Safety Principles	
Control of key hazard organisms	2
Monitoring salt	3
Changing product formulation	4
Water brine quality	12
Plant clean and sanitise	13
Post process handling	15
HACCP-related	
HACCP principles	1
Plant physical separation	9
Plant high care	10
Plant high risk	11
Process monitoring	14
Personnel issues	
Food handler basic training	5
Return to work procedures after illness	6
Supply chain	
Raw materials sourcing	7
Raw materials acceptance	8
Shelf life determinations	16
Know shelf life testing consequence	18
Regulatory	
Understanding 2073/2005 EC	17
L. monocytogenes ISO test method (or validated equivalent)	19

Table 28 A summary of the EHO's perceived FBO requirements and areas of concerns.

Question name	Mean response score	Number of responses	Ranking
Plant clean and sanitise	4.58	**26**	1
Control of key hazard organisms	4.42	**26**	2
Monitoring salt etc	4.40	**25**	3
Post process handling	4.32	**25**	4
Shelf life determinations	4.32	**25**	4
Process monitoring	4.23	**26**	6
Plant high risk	4.16	**25**	7
Plant physical separation	4.08	**25**	8
Plant high care	4.04	**26**	9
Raw materials acceptance	3.88	**25**	10
HACCP principles	3.85	**26**	11
Return to work procedures after illness	3.81	**26**	12
Food handler basic training	3.50	**26**	13
Water brine quality	3.44	**23**	14
Raw materials sourcing	3.24	**25**	15
Changing product formulation	2.68	**25**	16
Understanding 2075 EC	2.15	13	17
Know shelf life testing consequence	1.69	13	18
L. monocytogenes ISO test method (or validated equivalent)	1.31	13	19

Mean question scores have been ranked by overall importance on a question-by-question basis.

Table 29 A summary of the EHO's perceptions on their own requirements and areas of concerns.

Question name	Mean response score	Number of responses	Ranking
Monitoring salt etc	4.61	18	1
Post process handling	4.40	15	2
Plant clean and sanitise	4.35	17	3
Shelf life determinations	4.27	15	4
Process monitoring	4.13	16	5
Control of key hazard organisms	4.11	18	6
Plant physical separation	4.06	16	7
Plant high risk	4.00	17	8
Plant high care	3.88	17	9
Water brine quality	3.88	16	10
Food handler basic training	3.82	17	11
HACCP principles	3.72	18	12
Raw materials acceptance	3.65	17	13
Raw materials sourcing	3.41	17	14
Understanding 2075 EC	3.29	17	15
Return to work procedures	3.28	18	16
Changing product formulation	3.11	18	17
Know shelf life testing consequence	3.06	17	18
L. monocytogenes ISO test method (or validated equivalent)	2.12	17	19

Mean question scores have been ranked by overall importance on a question-by-question basis.

At least 25 out of the 30 EHOs responded to those questions highlighted in bold in Table 28. In contrast only 13 responded to questions relating to understanding of the EU microbiological criteria for foodstuffs regulation 2073/2005, on shelf life testing and *L. monocytogenes* laboratory testing methodology. The observation provides some fairly robust evidence that the EHOs asked have a strong bias towards helping FBOs implement immediately-practical control measures rather than an insistence that FBOs are aware of and comply with the minutiae of regulations. Each of the subject areas (as outlined in Table 27) are considered in more detail in

the following sections. The responses to what EHOs perceived were the needs or concerns of FBOs and their own are considered separately.

3.3.5.1 EHO PERCEPTION OF FBO NEEDS OR CONCERNS

3.3.5.1.1 KEY FOOD SAFETY PRINCIPLES

Monitoring key hazard organisms

Compliance with a HACCP scheme within a food processing environment demands a clear understanding of all of the potential hazards associated with the food being manufactured, control of such hazards and critical tolerance limits determined by periodic monitoring. Since most FBOs in the UK operate to at least HACCP principles, a high rating (ranked second out of all issues) flagging a need for guidance regarding the control of key hazard microorganisms is disconcerting. Given the basic nature of the observation, the first potential explanation discussed by the project team was that there may have been some misunderstanding of what was being asked. However, a number of the comments made by the EHOs in response to the questions "Is there anything else you consider important for basic food safety?" broadly support that the EHOs understood what was being asked of them and that the observation is legitimate. A selection of the comments made which support a lack of knowledge of key hazard microorganisms were:

> *"In my experience, manufacturing SMEs have limited knowledge of food safety issues affecting their process which is compounded by a lack of HACCP knowledge."*
>
> *"FBOs need to ensure that there is someone within the business who has a good basic understanding of microbiology and food science principles as they relate to the products they are producing."*
>
> *"Some of the businesses I visit don't even know what a microorganism is."*

Based on the experiences of the EHOs, it seems that there is a lack of fundamental microbiological knowledge in some processing environments. It is an issue of some concern that some producers of high risk foods apparently have gaps in their basic knowledge. The project team consider the shortcoming serious and should be addressed satisfactorily to ensure

the manufacture of consistently safe food (which complies with basic food hygiene legislative requirements).

Monitoring salt

Guidance on the monitoring of salting effectiveness (as a rough measure of water activity (a_W)) ranked 3 overall. As was stated in the introduction to this report, *L. monocytogenes* is exceptionally halotolerant (Niedziela *et al*. 1998) and some strains can multiply at a relatively low a_W of 0.92, which equates to almost 13% w/v NaCl. At refrigeration temperatures, a 13% NaCl solution is saturated and requires near-constant agitation to keep the salt from precipitating out of solution. In those plants where liquid brine was observed to be used, the salt concentration of the brine was around 13% (i.e. a saturated solution). Niedziela *et al*. (1998) report that growth of some strains of *L. monocytogenes* is apparently unaffected by 13% NaCl. Therefore salt concentrations routinely found in brine are not reliable, effective controls for *L. monocytogenes.* Levels of salt that would effectively prevent *L. monytogenes* growth in ready to eat smoked fish is impractical for organoleptic reasons.

However, it is noted that salt at concentrations regulary found in brine/brining can be effective in preventing toxin production by non-proteolytic *Clostridium botulinum* (Lalitha and Gopakumar 2007), which is another food safety concern in vacuum-packed foods. Salt and a_W monitoring is therefore of general relevance from a broad microbiological viewpoint.

There would appear to be a misperception amongst a minority of EHOs that brine with a high salt concentration can be an effective control for *L. monocytogenes*: a selection of EHO comments relating to salting/brining made in response to the question "Is there anything else you consider important for basic food safety" were:

> *"A simple tool for determining a_W from the amount of sodium chloride, sugar and other potentially effective agents used in the production of cold smoked fish would be of value."*
> *"Think it would be beneficial if there was more research into the combination of factors that control all hazards inc. botulinum."*

There are widely available tables (e.g. http://www.bccdc.ca/NR/rdonlyres/E41F3065-A61C-41FF-B358-66255D2DDA4F/0/Water_Activity_of_Sucroseand_NaCl_Solutions.pdf) which

provide the a_W for a range of salt and sugar solutions. In addition, it is a relatively straightforward process to calculate a_W for any salt or sugar solution. Resnik and Chirife (1988) report a suitable, general formula. Whether such resources are accessible enough to be used by EHOs or FBOs is not clear.

If pH, a_W and storage temperatures are known, the free modelling software at www.combase.cc can be used to predict growth of time for a range of pathogens including *L. monocytogenes* and non-proteolytic *C. botulinum*. Raising EHO awareness of the availability of this tool may be of value following a qualification that training is required for effective usage.

Changing product formulations

Ranked as the 20th most important factor, the perceived lack of importance associated with making changes to product formulations may reflect the adherence of FBOs to relatively few 'tried and tested' product formulations and the lack of technical knowledge with regards to the control of *L. monocytogenes*. An EHO comment which summarises the situation in smaller plants was:

> *"Most small producers are not relying on technical support. They have a "recipe" approach. Little is spent on product testing."*

Water brine quality

The microbiological quality of the water used for making brine and the quality of the brine itself were not rated by EHOs as being of high importance (ranked 17 overall). Crucially, a consequence of the inability of high salt concentrations to prevent the growth of *L. monocytogenes* in brine means that brine can become a reservoir of contamination and a major source of cross-contamination, especially in those plants where brine is recycled and used for multiple batches of fish. An awareness of the documented role of brine, and associated brining equipment such as injectors, in the spread of *L. monocytogenes* in smoking plants should be highlighted to EHOs and FBOs as a matter of priority.

Plant clean and sanitise

Under the auspices of article 5 of the 2073/2005 EC regulation, there is a legal requirement for producers of ready-to-eat foods capable of supporting the growth of *L. monocytogenes* to verify

the effectiveness of their cleaning and disinfection procedures by testing food processing environments for *L. monocytogenes* (or *Listeria* spp.). Although the regulation stipulates that the testing is to be undertaken, no specific instructions relating to sample collection are provided and thus the procedure was the focus of a number of specific EHO comments. In general, EHOs believed that either the results of such testing were of variable quality, or that a proportion of smaller smoked fish processors were ignoring the requirement to test food contact surfaces. The area of concern related principally to smaller producers given there is a strong audited emphasis on microbiological monitoring and trending by those smokers who supply to major retailers.

Furthermore, Table 28 clearly shows that EHOs perceive FBOs key area responsibility and concern is effective plant cleaning and sanitation. EHOs expressed this is the area that FBOs need clear guidance on. The question responses were rated as either a four or a five by all 26 of the EHOs who answered the question. Clear guidance describing how to effectively clean and disinfect was the most highly-ranked perceived need. Although it is an important finding, it is not a surprising one. In other food sectors, issues surrounding effective cleaning and disinfection are also frequently flagged as important. For example, the public enquiry lead by Pennington in 1996 into the verocytotoxigenic *E. coli* outbreak centred on Wishaw made recommendations focussing on effective cleaning and disinfection. A similar recommendation was repeated in the Pennington-led public enquiry into the 2005 South Wales *E. coli* O157 outbreak.

Given the importance of effective cleaning and sanitation, the FSA in England in 2003 commissioned research into the feasibility of guidance for the selection and use of disinfectants in food processing and preparation areas (FSA project PAU166) and on the feasibility of producing a listing of disinfectant suppliers (FSA project PAU167). The outcome of both of these wide-scoping studies was that, although challenging, there were no insurmountable barriers to the production and publication of such guidance by the FSA. To date, the project team are not aware of any further progress towards the preparation of FSA guidance. However, the governments of a number of countries including the USA, Canada, Australia, New Zealand, Finland and Hong Kong have issued straightforward general guidance (i.e. guidance which is not specific for smoked fish plants) describing effective cleaning and disinfection in food processing and preparation premises. The Finnish guidance was issued in 2003 and an unofficial English translation is available (Goodburn, 2003). In combination, these broadly-equivalent documents

from overseas governments could be used rapidly and cost-effectively as the basis of good practice cleaning and sanitation information for FBOs and EHOs.

A selection of the comments made by EHOs relating to cleaning and disinfection in response to the question "Is there anything else you consider important for plant infrastructure and hygiene?" were:

> *"GMP and type of cleaning chemicals and methods."*
> *"Environmental swabbing is not practiced by most producers to verify effectiveness of cleaning routine."*
> *"Small fish smokers may not have the financial input nor space to provide completely separate areas within their establishment. In such cases, separate areas within the production room may be provided, with an area designated to raw product preparation and another to ready to eat product processing. In this case I believe that separate washing up sink provision should be made and separate wash hand basins for cleaning hands, with adequate separation of work utensils, equipment and surfaces, cleaning."*

Post process handling

Ranked joint fourth overall and recognising the need to prevent contamination post-process, a single EHOs commented that (s)he had concerns relating to EHO rather than FBO training. The comment was:

> *"A food hygiene training course specific to fish smokers would be most beneficial, also a HACCP training course specific to fish smokers. The current basic food hygiene qualification is not specific enough for fish smokers. As the fish smoking is a skilled process, most EH staff may not be aware of the actual process i.e. smoking times as this may be done on a visual perception of the product. A course available to EH staff on the actual process, along with specific FH/HACCP training would be beneficial."*

Shelf life determinations and consequences

Ranked joint fourth overall, the EHOs who were asked perceived that FBOs required some assistance when determining shelf life. The finding broadly agrees with project team discussions held with some of the smaller processors. It is apparent that many processors have never formally assessed their product's shelf lives. During project team plant visits, a number of FBOs stated that their products' shelf life was known to them historically as the length of time before the product went 'off'. Although shelf lives are required by 2073/2005 EC to have a sound scientific basis, many smaller operators lack the technical knowledge to determine shelf lives themselves and they lack the funds to pay a testing laboratory to undertake the work on their behalf. A common approach to circumvent a scientific determination of shelf life appears to be to sell product with a short shelf life.

The consequences of exceeding the 100 cfu *L. monocytogenes* /g limit at any point within the product shelf life was ranked 22nd overall. Consequently, the EHOs thought the issue would be of low priority to FBOs. We note that the question relating to the issue was only answered by 13 out of the 30 EHOs. This finding may indicate that microbiological testing data are not being gathered, reviewed or applied in contravention of 2073/2005 EC.

Although there is merit in reducing the length of time that *L. monocytogenes* has to multiply under refrigerated conditions, the high risk nature of these foods and their potential non-refrigerated distribution (e.g. through the post) means that the approach may not provide adequate consumer safeguards under imperfect refrigeration conditions.

The present situation regarding shelf life determination is neatly summarised by a single EHO comment which was:

> *"Most small producers content with relatively short shelf life and are unwilling to spend on product testing."*

A recent project report for FSA study B13006 (Peck *et al*., 2006) summarised the shelf lives of comparable foods including cold- and hot-smoked fish in the UK, the rest of the EU and internationally. The shelf lives of cold-smoked fish on the UK market were generally in the range 18-21 days. Although shorter than those documented overseas (where up to 120 days

were reported), the interval is substantially longer than for other chilled prepared foods (which are generally limited to shelf lives of 10 days in the UK). Smoked fish products therefore are not only high risk because they can support the growth of human pathogens, but their extended shelf lives provide significant opportunity for growth.

Although the root of the problem for small smokers is cost, the Agency has already provided resources in the form of free guidance on the assessment of shelf life in relation *to L. monocytogenes* , which has been available from FSA and other parties (CFA *et al*., 2010) since March 2010. Given a joint fourth overall ranking of the perceived need for guidance in this area, it would appear that the FSA guidance document needs to be promoted to both FBOs and EHOs.

The only other EHO comment of note in this region of the questionnaire was:

> *"Further research to see if the smoking process has an inhibitory effect on bacterial growth? SMEs in this area need to have a good underpinning knowledge of the microbiology and the food science involved, or access to those who can help them to gain this."*

There is a summary of the research to date on the microbiological consequences of smoking in the literature review section of this report. Arrangements are already in place to supply the participating EHOs with copies of this report which will help address the issue raised by the comment.

3.3.5.1.2 HACCP-RELATED ISSUES

Overall, the average mean score for issues relating to the physical segregation of various processing stages, the separate cold storage of raw and processed products and process monitoring were scored above 4.08 out of 5.0 (Table 28). The finding indicates that EHOs perceived HACCP refreshment of FBOs to be an area of high importance.

HACCP principles

The majority of EHO responses ranked the ability of some FBOs operate their businesses to HACCP principles as an area of moderate concern, the preparation of guidance in this area was

also given a medium level priority (11th, Table 28). There were specific comments however that indicated that at least some EHOs prioritised adherence to HACCP as a key area of concern. A selection of these comments were:

> *"Some SMEs find the concept of HACCP hard to understand and put into practice. Further training opportunity in this area would be welcomed."*
> *"[There is a] lack of [a] prescriptive requirement for HACCP training."*
> *"A template food safety system for FBOs and also for enforcement officers would be most beneficial. There are many small fish smokers who do not have the technical expertise nor the knowledge to develop their own system without great input from EH."*

Plant physical segregation, plant high care and plant high risk

Segregation of ready to eat (RTE) and non-RTE foods and equipment is widely recognised as important in reducing potential for microbiological cross contamination. The recognition of the importance of segregation has given rise to the concepts of high care areas (HCA) and high risk areas (HRA) in food processing premised. A HCA is defined as an area designed to a high standard of hygiene where practices relating to personnel, ingredients, equipment and environment are actively managed to minimise microbiological contamination of a ready-to-eat or ready-to-reheat product which contains uncooked ingredients. If best practices are being followed, **cold-smoked fish** should be produced in a HCA, separated from raw material intake and final packaged product. High Risk Areas (HRA) are defined as areas designed to high standards of hygiene where practices relating to personnel, ingredients, equipment and environment are managed to minimise microbiological contamination of a ready-to-eat or ready-to-reheat product comprising only cooked ingredients. Hot-smoked fish should therefore be manufactured in a HRA, again separated from raw material intake, final packaged product and any unpackaged raw/cold-smoked foods.

High scores of >4.0 were achieved for the EHOs perception of the importance of HRA and HCA and reflecting the need for guidance on achieving the appropriate degree of segregation. A number of EHO comments were made regarding the confined spaces in some plants which made segregation difficult.

Process monitoring

The need for FBO guidance relating to monitoring of the preparation and smoking processes was ranked as the seventh most important area of focus. A number of the EHOs who commented had particular concerns about small smokers which are concisely summarised by the comment:

> *"Traditional small smokers use a fire pit and have no control over temperature or smoke concentrations."*

The concerns of the EHOs are, to a large extent, mirrored by the project team who encountered a number of small smokers who undertake a 'HACCP test' for hot smoked products. The test typically involves looking through the edges of a batch of smoked fish for one which looks like it is the least well-cooked. Plant employees then taste the fish to ensure it has been cooked to their satisfaction. If the fish chosen is too raw, the entire batch is subjected to further cooking.

It may be relevant to note that no single step in the production of cold-smoked fish will result in a six log reduction of *L. monocytogenes* numbers, which emphasises the need for any secondary control measures to be put in place and their effectiveness monitored.

3.3.5.1.3 PERSONNEL ISSUES

The survey revealed EHOs perceived that FBOs held food handler training (score, 3.50; response, 26; rank 16) and return to work procedures (score, 3.80; response, 26; rank 15) as a matter of moderate to low importance. Although not perceived as key elements, we note that most EHOs thought that the FBOs would prioritise guidance describing how employees were returned to work after a period of infectious disease. Regarding the basic training of food handlers, one EHO felt that there was work to be done with FBOs in:

> *"Ensuring they are aware and understand the importance that they play in terms of producing safe food".*

3.3.5.1.4 SUPPLY CHAIN

The majority of EHOs asked did not believe that the source of the raw fish was a consideration that would be made by FBOs as part of their *L. monocytogenes* control strategy (Raw materials sourcing, score 3.24, rank 18; Raw materials acceptance score 3.88, rank 12). In addition, a number of EHO comments reflected the complexity of implementing sourcing controls where non-dedicated suppliers were used:

> *"The availability and cost of fish e.g. salmon varies throughout the year meaning that processors may source fish from various suppliers. Unless fish is sourced directly from fish farms, the processor will have little control over sourcing criteria i.e. controls in place on farms, environmental conditions etc. If fish is sourced from fish distributors/ processors then it is most likely that such fish will come from various sources. At the fish processing stage it is likely that batches will be mixed."*

Although there were only two questions relating to raw materials, the provision of common acceptance criteria scored and was ranked substantially higher than sourcing criteria indicating the possible opportunity for the creation of guidance criteria. Many of the points made apply to smaller smokers. It is apparent from the project team visits to larger throughput plants that the supply into those plants is tightly controlled and that some fish suppliers actively select source farms on the basis of recent rainfall and other criteria which may positively influence the *L. monocytogenes* infection or contamination status of the fish. Tight control of fish supplies is particularly noticeable for integrated operations (i.e. when the farms and processor are owned by the same company). One EHO felt that:

> *"Temperature control between harvest, slaughter and final processing is critical as is the time between these stages. They don't always take place in the same area and transportation becomes critical."*

3.3.5.1.5 REGULATORY

Legal compliance with shelf life and other requirements of 2073/2005 (e.g. the methodology to be used for *L. monocytogenes* testing in the laboratory) were rated by the EHOs as of low importance to the FBOs (Table 28; Understanding 2073/2005 EC; score, 2.15; rank, 21; *L. monocytogenes* ISO Test Method score, 1.31; rank 23). The finding is likely to be a consequence of the plant operators' priorities. Most plant operators will be familiar with some form of HACCP and its prerequisite programmes. Some EHOs were of the opinion that FBOs would focus their efforts on their HACCP schemes as a way of controlling microbiological hazards and achieving compliance with the *L. monocytogenes* criteria. Although as a general approach the strategy has merit; it is important that FBOs recognise the high risk nature of cold smoked fish and the lack of critical control points in the cold smoking process. When assessed with typical relatively long shelf lives and a potential for no or an imperfectly refrigerated distribution (e.g. through the post) it becomes apparent that there is a requirement for an application of all controls in combination with process monitoring from raw material sourcing throughout the entire process.

3.3.5.2 SUMMARY OF KEY FINDINGS FROM THE EHO QUESTIONNAIRES RELATING TO FBOS

The following is a summary of the main findings in terms of EHO perceived areas of importance and concerns for FBOs. In the main, the areas of concern apply mostly to small and medium sized fish smoking businesses. In the smaller businesses which were visited, both EHOs and the project team perceived that there were issues of insufficient money and a general lack of physical space which would prevent significant changes to operations of small smokers in the short term. The key findings were:

- There is a need for FBO guidance on effective cleaning and sanitation of food contact surfaces and the plant environment generally and the importance of verifying cleaning effectiveness.
- Guidance information relating to the role of salt in control of *L. monoytogenes* and effective salting and brining is needed.
- EHOs felt that FBOs would benefit from advice on best practices for preventing smoked fish from becoming contaminated after processing and during storage. Of particular concern was the role of condensation and drippage during refrigerated storage.

- Larger businesses have concerns about the methodologies used by some testing labs and question whether the behaviour of lab-cultured *L. monocytogenes* can be extrapolated as being the same behaviour of a natural *L. monocytogenes* fish contaminant.
- Information for FBOs describing basic shelf life determinations with a basic protocol for use by smaller processors and a best practice protocol which identified and used naturally contaminated fish were thought to be important by the EHOs.
- EHOs felt there would be significant benefit in providing basic microbiological guidance for small plant FBOs.
- Largely aimed at smaller processors, some refresher material for the operation of smoked fish businesses along HACCP principles was felt to be important.
- Again, largely aimed at smaller processors, information describing the operation of High Care Areas for cold smoked product, and High Risk Areas for hot smoked product was perceived to be required for the FBOs.

3.3.5.3 EHOS' PERCEPTIONS OF THEIR OWN GUIDANCE NEEDS

EHOs were separately canvassed to determine how EHOs perceived their own and their colleagues professional knowledge. EHOs were also asked if there were areas of where extra advice and guidance that would be of benefit to them. EHOs' provided less feedback on their own perceived guidance needs (Table 29) compared with that submitted for the FBOs. However, at least 15 out of the 30 EHOs answered some questions relating to EHOs. Critical inspection of the (anonymised) data saved showed that most EHOs had answered only selected specific EHO-related questions. It was not the case that 50% of the EHOs had declined to make any comment on their aspects of the questionnaire.

The maximum number of EHOs giving a score to any element was 18 out of 30 and the minimum number of respondents was 15. In summary, the EHOs declared a need for guidance on the monitoring of salt concentrations (with the caveat that some EHOs mistakenly believe high salt can reliably reduce numbers of *L. monocytogenes*), appropriate post-process handling, effective cleaning and sanitation, and shelf life determination were the top four rated elements.

As before, the elements were analysed after being grouped by topic according to the scheme outlined in Table 27.

3.3.5.3.1 KEY FOOD SAFETY PRINCIPLES

The food safety question grouping included five of the six highest-ranked responses. Food safety was the area where most EHOs thought guidance would be most beneficial for EHOs. The relative rankings for each of the questions are shown in Table 29.

The effective monitoring of salt was ranked the highest of all topics on which EHOs felt that guidance or additional information would be beneficial. Given that some EHOs believe salt to be a critical control point for *L. monocytogenes*, there must be a benefit for guidance which dispels that myth and perhaps highlights the importance of salt for the control of toxin production for non-proteolytic *C. botulinum*.

Maintaining the integrity of finished products is critically important as are the storage conditions. A number of Scottish fish smokers who were visited by the project team make more than 90% of their sales in the run up to the Christmas period. Their products are frozen for most of the year and there may be benefit in providing EHO guidance for minimum standards of the physical condition of some of the freezer units. For those EHOs who monitor smokers that chill rather than freeze, there may well be a benefit in providing guidance which explains how chillers work and the management of moisture inside chillers as a way of preventing condensation and drippage on chiller ceilings. Drippage issues were observed in a number of UK chillers for both small and large processors. Closely linked with post process handling, shelf life determinations were ranked fourth as a topic which EHOs felt their knowledge was weak and that they would benefit from some guidance.

In addition to the perceived need by EHOs for FBO plant cleaning and sanitation guidance, the EHOs also felt that they themselves would benefit from guidance in this area. Given that there is a perceived need by both EHOs and FBOs for the development of cleaning guidance, and that there is an obvious hole in the UK which has been addressed by other governments, this area should be given quite a high priority.

Provision of basic control information was ranked 2nd overall and with a score of 4.42. It is plausible that the perceived need is a consequence of the fact that best practice guidance for the control of *L. monocytogenes* does not tend to be freely available having been generated by larger businesses who would tend to protect the information as their intellectual property.

The microbiological consequence of changing product formulations was poorly ranked for importance by the 18 out of 30 EHOs. Based on some of the EHO comments made, it is considered likely that the poor rating may reflect the fact that many plant operators do not change their product formulations frequently. A number of EHOs commented that across the industry there was a "recipe-style approach" to fish smoking.

EHOs ranked the need for guidance on the consequences of exceeding the 100 cfu/g *L. monocytogenes* limit throughout shelf life 18th out of 19 topics. The finding mirrored the low ranking given to the perceived need for FBO guidance on this issue. However it is not clear from the comments made whether the EHOs consider they have an excellent understanding of the issue and thus do not require further guidance or if they simply don't consider it to be important.

3.3.5.3.2 HACCP RELATED ISSUES

After food safety, HACCP-related issues were ranked by the EHOs as the next most important area overall where guidance would be beneficial, for example processing monitoring (score, 4.13; rank, 5) plant high risk (score, 4.0; rank, 8), plant high care (score, 3.82; rank, 9), plant physical separation (score, 4.06; rank, 7) and HACCP principles (score, 3.72; rank, 12).The nature of the EHO concerns around HACCP are best summarised by the following two EHO comments:

> *"As the fish smoking is a skilled process, most EH staff may not be aware of the actual process i.e. smoking times as this may be done on a visual perception of the product. A course available to EH staff on the actual process, along with specific FH/HACCP training would be beneficial."*
> *"A template food safety system for FBOs and also for enforcement officers would be most beneficial."*

As might be expected, most of the EHOs who responded appeared to be relatively confident of their understanding of HACCP principles. This topic was rated 12 out of 19 indicating the fact that a small number of EHOs felt there would be a benefit for some reiteration.

The topics of physical separation of process stages inside the plant, high risk and high care were ranked 7th, 8th and 9th overall. These closely-related topics received a number of comments from EHOs which shed some light on the basis of their concerns. Two key comments were:

> *"Small fish smokers may not have the financial input nor space to provide completely separate areas within their establishment. In such cases, separate areas within the production room may be provided, with an area designated to raw product preparation and another to ready to eat product processing. In this case I believe that separate washing up sink provision should be made and separate wash hand basins for cleaning hands, with adequate separation of work utensils, equipment and surfaces, cleaning."*
>
> *"A lot of small processors don't have the space to physically separate process stages. They don't have the money to extend/create new buildings either. What's needed is clear advice on the best way to make use of existing spaces"*

Both of the above comments point towards the fact that EHOs acknowledge the importance of physical separations and the benefits of a high care/risk strategy; however from a practical viewpoint, the businesses they visit have space constraints and it appears that at least some EHOs are uncertain of the best ways to advise FBOs.

FSA guidance and supporting questions and answers for FBOs and EHOs (FSA, 2011) on the prevention of cross-contamination, although focusing on *E. coli* O157 and primarily targeting butchers selling RTE food, may provide useful information that could be used as the basis of advice to EHOs on managing separation in premises with space constraints.

3.3.5.3.3 PROCESS MONITORING

A number of EHOs felt that process monitoring was an area that would benefit from some additional guidance. Process monitoring was ranked 5th overall for EHO guidance provision.

A single EHO commented that *"Traditional small smokers use a fire pit and have no control over temperature or smoke concentrations."* which provides some clues as to the nature of the

concerns. Although some small smokers change the position of the fish midway through smoking, it is relatively straightforward and cost effective to implement checks using meat temperature probes to establish a basic temperature monitoring program.

As was discussed for the FBO section of the questionnaire, the manufacture of cold-smoked fish contains no process stages which can effectively control the *L. monocytogenes* hazard. Consequently, there seems little choice but to pursue a strategy of installing of multiple hurdles; each of which reduces, but does not reliably inhibit, *L. monocytogenes* growth on fish as a way of keeping final product contamination to an absolute minimum.

3.3.5.3.4 PERSONNEL ISSUES

On the matter of personnel issues, EHOs ranked the need for further guidance as moderate to low. For example food handler basic training had a mean response score of 3.83 (rank 11th). Return to work procedures after infectious illness , scored 3.75, was ranked 16th out of 19. Given that the comments above suggest that the poor rankings of personnel issues are not because EHOs feel well informed, the finding may indicate a need for greater consideration of this aspect of product safety by EHOs.

EHO comments regarding food handler training reflected some recognition of the importance of the subject area. Typical comments made were:

> *"Basic FH training should be specific to operations carried out."*
> *"High Risk food handlers should be trained above basic and specifically on L. monocytogenes."*
> *"Ensuring they are aware and understand the importance that they play in terms of producing safe food."*

3.3.5.3.5 SUPPLY CHAIN

The EHOs who responded to the questions relating to the supply chain did not perceive there to be any information gaps in their knowledge. Thus EHOs did not feel there would be much benefit of guidance in this area and most EHOs ranked supply chain issues as having low importance.

3.3.5.3.6 REGULATORY

Further guidance in regulatory issues in relation to the implementation of the EU Microbiological Criteria Regulation 2073/2005 were ranked as of low importance from the EHO viewpoint (rank, 15th; mean response score, 3.29). Similarly, more information on the ISO-based laboratory test method stipulated for use by Regulation 2073/2005 was poorly ranked for importance at position 23 with a response score of 1.31 (Table 28).

A single EHO comment was made regarding the 2073/2005 regulation which was:

> *"Ambiguity regarding frequency of end product testing required in terms of EC Reg 2073."*

The single issue is straightforwardly addressed by existing guidance on the implementation of 2073/2005 (including end product testing frequencies) which is freely available from a number of sources including the Chilled Food Association (CFA, 2005).

3.3.5.4 SUMMARY OF KEY FINDINGS FROM THE EHO QUESTIONNAIRES RELATING TO EHO NEEDS

As a professional body, EHOs perceived themselves to be better informed that most FBOs. However, there was a quite large overlap between the guidance that the EHOs felt would be important for them and their colleagues and what was perceived to be important for FBOs. The following lists the topics the EHOs prioritised as being least informed and thus with the most benefit were more guidance be made available to them. The key findings were:

- Information on effective salting and brining and the role of salt and the hurdle approach in controlling *L. monocytogenes* is required for EHOs.
- EHOs also felt it would be of benefit to be provided with guidance describing best practices for the post processing storage of smoked fish, including effective freezing and the operation of condensation-free chillers.
- Determination of shelf life as a guarantee that the *L. monocytogenes* criteria for smoked fish described by 2075/2005 were being met was also perceived to be important for EHOs.

- A best practice guidance document describing effective plant cleaning and disinfection would also be deemed to be of benefit by some EHOs.
- Although EHOs, generally believed they were knowledgeable about the operation of a food processing premises using HACCP principles, they felt that extra information on process monitoring specific to the ready-to-eat smoked fish sector would benefit them in their role as professional enforcers.

3.3.5.5 GUIDANCE FORMAT AND TYPE

At the end of the questionnaire, EHOs were asked ot identify what they felt was the preferred format for any advice or guidance that may be produced as a result of the findings of this report. All thirty EHOs expressed a view on their preferred format for guidance, reflecting the importance of getting the provision correct. In keeping with the findings of a number of Agency-funded studies (project codes MO1020, MO1017, B17007), free interactive web-based guidance was narrowly preferred over formal training courses or workshops (Table 30).

Table 30 EHO preferences for the format of any guidance

Guidance format	Mean score	Overall ranking
Web, interactive	4.230	1
Training course/workshop	4.000	2
Web, written	3.870	3
Booklet	2.870	4

Given resource pressures in both local authorities and FBOs, provision of easily-accessible information and guidance on the web, possibly akin to FSA's (EHO) training and knowledge assessment material on vacuum packing and MAP (www.food.gov.uk/vacuumpackingtraining) would be expected to be the lowest cost to utilise and deliver the highest impact. The other offered formats of static web pages or pamphlets/booklets were the least popular (Table 30). None of the EHOs suggested any alternative ways of delivering the guidance.

The EHO comments provided for guidance format showed consideration for both EHOs and rural FBOs. Examples of the points EHOs felt were important were summarised as:

"Some fish smokers may be rural and unable to travel to central points for training courses/workshops therefore I am of the opinion that they should be available over the internet or via conference calling facilities. The cost should also be taken into consideration as many smokers are small businesses who do not have the financial resources of larger businesses. Training provision should also be made for EH personnel for this quite unique process."

"If smoked fish are an emerging hazard for Listeriosis then special training for fish smokers is required."

"High Risk food handlers should be trained above basic and specifically on L. monocytogenes."

4 ANALYSES OF A GIFTED DATASET OF *LISTERIA* TEST RESULTS

Although not originally part of the original project scope, test results describing the qualitative prevalence of *Listeria* were obtained from a salmon grower/primary processor for the period February 2006 to August 2011. The information provides an opportunity to closely analyse data that would not normally have been made available to the FSA.

At this processors approximately 13,500 *Listeria* test samples were collected from key points along the processing chain over the 5.5 year period, including fish at receipt in the processing factory (Figure 10 a) and gutted fish at the exit of the processing factory (Figure 10 b). Fish were sampled both from their external and internal surface by swabbing. These datasets obtained contained additional supplementary information including the sampling date and the type of *Listeria* if a positive sample was detected.

A. B.

Figure 10 Sampling for *Listeria* at this processors was carried out a) at receipt of fish at processing factory and b) and at boxing of gutted fish

A total of 1052 (7.8%) isolates were obtained of which 217 (1.6%) were *Listeria monocytogenes*. Statistical analysis of the prevalence of *Listeria* spp. along the processing chain was performed using the data analyses pack plug in for Excel 2003 (Microsoft) and statistical significance (P-values) was determined using the Excel add in for Fisher's exact test (www.obertfamily.com/software/fisherexact.html). A number of questions were addressed and these and results are detailed below.

TEMPORAL VARIATION IN PREVALENCE

The prevalence of *Listeria* changed over time at both sampling points (receipt and boxing), being significantly higher (Odds ratio and Fisher's Exact Test; $P<0.001$) in 2007 and 2008 compared with the other years. Fish at receipt had the highest prevalence in 2008 (24.1%), compared with 3.1% in 2010. The average over the whole time-period was 11.2%. Fish at boxing sampled from their internal surfaces had the highest prevalence in 2008 (6.1%), compared with 1.6% in 2010. The average was 2.2% over the whole time period. Although the cause of the increase in 2007-2008 is unknown, we note that there was above average rainfall in 2007-2008 in many regions of the UK (shown in Figure 11). Given there are previously-discussed reports of rainfall causing increases in the numbers of *L. monocytogenes* present in surface waters (Miettinen and Wirtanen 2005), it does not seem unreasonable to speculate that extended periods of exceptionally wet weather may have been a contributory factor.

SEASONAL VARIATION IN PREVALENCE

The prevalence of *L. monocytogenes* was significantly higher (Odds ratio and Fisher's Exact Test $P<0.001$) in summer compared with the rest of the year for 2007 and 2008. For example, the prevalence in fish at receipt was 40.3% in summer and 21.9% in winter, with an average of 25% over these two years. However, the seasonality was not observed for those other years (2006 and 2009 to 2011) for which data was available. During 2006 and 2009 to 2011 then the summer prevalence in fish at receipt (3.7%) was broadly comparable with the prevalence in the winter period (2.4%). Similar trends were observed for fish at boxing sampled either internally or externally.

INTER-FARM VARIATION IN PREVALENCE

The prevalence of fish at receipt by farm varied between 2.5% to 66.7% for 2007 and 2008. The range of variation was less pronounced for the rest of the period (0 % to 8.7%). The higher prevalence rate in 2007/8 was seen across the majority of farms indicating that one particular farm was not the cause.

VARIANCE IN PREVALENCE ALONG THE PROCESSING CHAIN

Along the processing chain, the fish entering the factory (comprising whole fish at receipt) had a significantly elevated (Odds ratio and Fisher's Exact Test) prevalence of *Listeria* (11.2%) compared with the fish which exited (after washing and gutting) the factory (4.7% for fish

sampled external i.e. on the external skin) and 3.7% for the fish sampled internal i.e. from within the body cavity). The finding and its strong statistical significance suggests that one or more of the processing practices inside the factory has caused a marked reduction to the prevalence of *Listeria* on the fish. The prevalence found in the raw salmon in this plant was higher than has been previously reported (Table 20). However, it must be borne in mind that there will be other factors such as sampling and microbiological methods that may vary between plants. Ideally, it would be useful to follow the prevalence (and also the numbers) along the production chain. However, this is not all that easy to do because the types of samples that are taken vary along the chain (e.g. swabs or pieces of fish used for analysis).

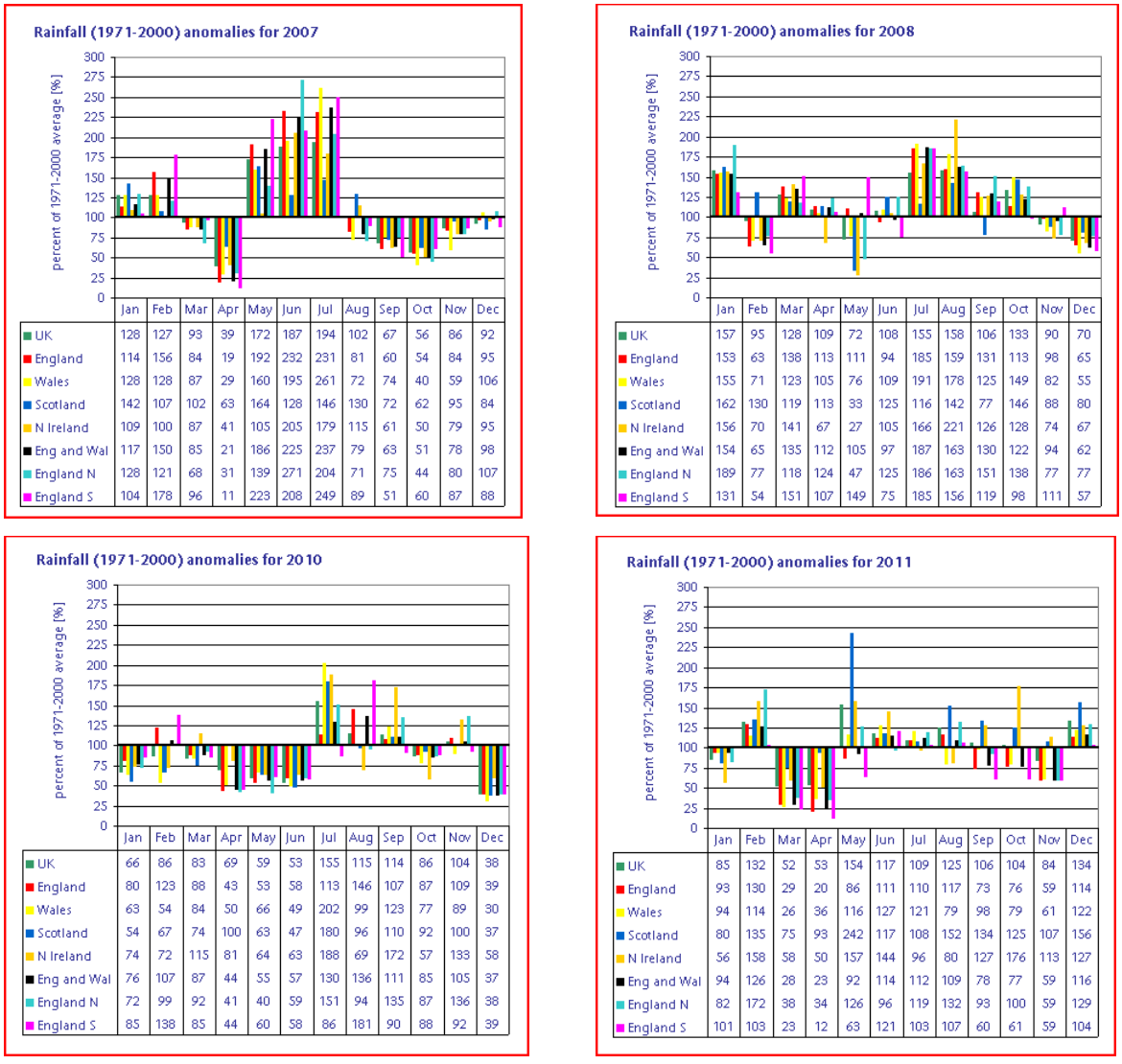

Figure 11 Rainfall across the UK in 2007, 2008, 2010 and 2011.

Rainfall graphs were obtained from the Met office website (http://www.metoffice.gov.uk/climate/uk/anomalygraphs/ Accessed 10/01/12).

5 STUDY RECOMMENDATIONS

Based on the review of the published, peer-reviewed scientific literature, the visits to commercial smoking premises and discussions with plant staff, the opinions of EHO enforcement staff and their perceptions of technical weakness both in the smoke plants and within the EHO community a number of recommendations have been presented below. The topics are arranged to account of the different needs of smaller and larger throughput plants, and with the issues of highest priority or greatest benefit in terms of consumer protection listed first. Larger throughput fish processors have expended considerable effort and finds in their attempts to control *L. monocytogenes* and have generally been very successful in their endeavours. Therefore the majority of the recommendations are aimed at smaller enterprises. Any references referred to as 'guidance' in the lists below should follow the preferences provided by the EHOs (section 3.3.5).

Recommendation	Target audience (in order of priority)
1A. Guidance on the importance and purpose of testing the processing environment, how sampling should be carried out, where sampling should best be directed and how frequent (Section 3.3.5.1.1)	SMEs EHOs
1B Information improving knowledge of how the principles of HACCP should be used to minimise food safety risks from *Listeria*, aimed at small plant FBOs. This could also include information covering general good microbiological practices or basic guidance on the fundamental principles of microbiology (Section 3.3.5.1.1).	SMEs
1C. Information on how sourcing of high quality raw ingredients, covering auditing suppliers as well as providing information on risk factors that may increase the chances of contamination on the raw fish and how to best to test raw fish for *Listeria*, aimed at small FBOs and EHOs (Section 2.3.2.2)	SMEs EHOs
1D. Guidance on cleaning and sanitation of food contact surfaces and the plant environment generally and further information describing the	SMEs

importance of verifying cleaning effectiveness (Section 3.3.5.3)	
2. A document which listed *L. monocytogenes* control best practices that would reduce the chances of final product contamination by introducing multiple hurdles throughout the entire process (Section 3.3.5.1.1)	SMEs EHOs Larger FBOs
3A. The preparation of guidance for on best practices for preventing post-process contamination of smoked fish (Section 3.3.5.1.1)	SMEs
3B. Guidance on how chillers work and how to manage moisture inside chillers as a way of preventing condensation and drippage (section 3.3.5.3).	SMEs EHOs Larger FBOs
4. Advice on shelf life determination using naturally contaminated product (Section 3.3.5.3).	SMEs
5. Information regarding the monitoring of various stages of the smoking process, particularly salting and brining, and the operation of these premises using HACCP principles (3.3.5.3.2, 3.3.5.1, and 3.3.5.4).	EHOs
6. Guidance on physical separation of process stages and the benefits of a high care/risk strategy when there are space constraints (Section 3.3.5.3.2)	SMEs EHOs

6 AREAS FOR POSSIBLE FUTURE RESEARCH

In addition to the key recommendations that were identified during the course of this review, several gaps in the knowledge base were identified and these have been collated and presented here as potential areas for further research.

At the primary production level (e.g farm level) the following areas could be investigated further:

- It is unclear exactly how fish farms become contaminated with *Listeria*. Although, it is widely accepted that the source is from runoff from adjacent land (section 11.6.4.4.2). Microbial source tracking (using whole genome information) could be applied to confirm a terrestrial source for *L. monocytogenes*. Quantitative testing would also allow the analysis of temporal data to determine whether the seasonality of *Listeria* positive samples is associated with air/water temperature and rainfall events and the comparison of different farm sites to determine if there were geographical factors that may influence the prevalence of *Listeria* on farmed fish. From this it may be possible to identify farm sites that could be at higher risk of *Listeria* contamination
- Currently all on-farm testing is for presence or absence of *L. monocytogenes*. Further work could be commenced to evaluate the feasibility of switching to quantitative testing. Numerical testing would allow the construction of a quantitative risk assessment that would allow the modelling of the numbers of *Listeria* through the processing chain.
- Typing of *L. monocytogenes* farm isolates and slaughter plant isolates and comparison with strains on the final product would clarify the role of fish-associated *L. monocytogenes* in the food chain.

Post-harvest and during processing/smoking, the following further work has been identified as necessary:

- Full genome sequencing of Isolates from smoked fish products (from both farmed and sea fish) and human clinical isolates with a view to determining possible source attribution. Approaches could be made to industry for access to stored isolates if possible. Source attribution techniques could be applied to such information in

combination with similar data from other food/animal vehicles/reservoirs as has already been done successfully for *Campylobacter*.

- Statistical analysis to determine whether there is a significant difference between the prevalence and/or numbers of *L. monocytogenes* inside the fish GI tract compared with the skin to indicate whether mitigation measures on the skin surface may be helpful in reducing levels. The comparison could be done on the samples at the end of the gutting process prior to boxing (or transfer to filleting line).
- It may be possible to generate "natural" growth challenge tests if companies were to share likely or known positive fish samples. If the first half of the sample was tested to be positive, then the second half could be stored and tested at end of shelf life.
- Work to determine the efficacy of initial washes, using either potable or water that has been rendered potable on incoming raw fish for reducing the load of *Listeria*.
- Fully quantitative risk assessment to identify the risks of cross contamination at each point in the processing line. Quantitative risk assessment has been used successfully in a number of areas (e.g. *Campylobacter* in poultry) to identify the importance of various stages of the production chain in controlling (or increasing) the risk of contamination as well as modelling the impact of interventions. A farm to fork risk assessment, populated and validated with microbial data would provide similar benefits for the fish smoking industry.

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8 APPENDIX A: SEARCH TERMS FOR LITERATURE REVIEW

Identification of relevant literature relating to *Listeria* in raw fish, fishing environments and fish farms

In order to determine:

"What is the contamination or infection prevalence (i.e. established infection/contamination) of wild or farmed fish by *L. monocytogenes*" and "What factors affect incidence (i.e. the rate of new infections)?", three electronic databases were searched from their inception dates until the end of March 2011. The databases included were: Thompson ISI Web of Science from 1899- March 2011; Thompson ISI MEDLINE from 1950- March 2011 and PubMed.Net from 1950– March 2011. The bibliographic databases used included food safety and processing, public health and agriculture or aquaculture subject areas.

The initial set of key words used was the same for all three databases with minor variations in syntax. For Thompson ISI Web of Science the search string was "*Listeria monocytogenes*" "incidence or prevalence" "fish OR trout OR salmon OR eel OR herring OR mackerel OR hake OR anchovy OR carp OR mussel OR shellfish OR whitefish OR sablefish OR swordfish OR dolphinfish OR dolphin fish"

For PubMed.Net the search was ("listeria *monocytogenes*"[MeSH Terms] OR ("listeria"[All Fields] AND "*monocytogenes*"[All Fields]) OR "listeria *monocytogenes*"[All Fields]) AND (("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "incidence"[All Fields] OR "incidence"[MeSH Terms]) OR ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms])) AND (contamination[All Fields] OR ("infection"[MeSH Terms] OR "infection"[All Fields] OR "communicable diseases"[MeSH Terms] OR ("communicable"[All Fields] AND "diseases"[All Fields]) OR "communicable diseases"[All Fields])) AND (("fishes"[MeSH Terms] OR "fishes"[All Fields] OR "fish"[All Fields]) OR ("trout"[MeSH Terms] OR "trout"[All Fields]) OR ("salmon"[MeSH Terms] OR "salmon"[All Fields]) OR eel[All Fields] OR herring[All Fields] OR ("perciformes"[MeSH Terms] OR "perciformes"[All Fields] OR "mackerel"[All Fields]) OR ("gadiformes"[MeSH Terms] OR "gadiformes"[All Fields] OR "hake"[All Fields]) OR anchovy[All Fields] OR ("carps"[MeSH Terms] OR "carps"[All Fields] OR "carp"[All Fields]) OR ("bivalvia"[MeSH Terms] OR "bivalvia"[All Fields] OR "mussel"[All Fields]) OR ("shellfish"[MeSH Terms] OR "shellfish"[All Fields]) OR ("salmonidae"[MeSH Terms] OR "salmonidae"[All Fields] OR "whitefish"[All Fields]) OR sablefish[All Fields] OR swordfish[All Fields] OR ("perciformes"[MeSH Terms] OR "perciformes"[All Fields] OR ("dolphin"[All Fields] AND "fish"[All Fields]) OR "dolphinfish" OR "dolphin fish"[All Fields]))

For MEDLINE the search was "*Listeria monocytogenes*", "incidence or prevalence", "fish OR trout OR salmon OR eel OR herring OR mackerel OR hake OR anchovy OR carp OR mussel OR shellfish OR whitefish OR sablefish OR swordfish OR dolphinfish OR dolphin fish"

Identification of relevant literature relating to *L. monocytogenes* and smoked fish

In order to answer the questions "What are the risks of human Listeriosis from smoked fish?" and "What are the processing risk factors and interventions for human Listeriosis from smoked fish?" the same three databases and date ranges were searched as described for *L. monocytogenes* contamination of raw fish.

As for the raw fish search, the initial set of key words used was the same for all three databases with minor variations in syntax. For Thompson ISI Web of Science the search string was "*Listeria* OR listeriosis", "fish OR trout OR salmon OR eel OR herring OR mackerel OR hake OR anchovy OR carp OR mussel OR shellfish OR whitefish OR sablefish OR swordfish OR dolphinfish"; "smok?". (the '?' character is a wild card and allows for multiple variations such smoke, smoking, smoked, smoker etc).

For PubMed.Net the search was ((Topic=(*Listeria*) OR MeSH Heading:exp=(*Listeria*)) OR(Topic=(listeriosis) OR MeSH Heading:exp=(*Listeria* Infections))) AND((((((((((((((Topic=(fish) OR MeSH Heading:exp=(Food)) OR(Topic=(trout) OR((MeSH Heading:exp=(Food)) AND(MeSH Heading:exp=(Seafood))))) OR(Topic=(salmon) OR((MeSH Heading:exp=(Food)) AND(MeSH Heading:exp=(Seafood))))) OR(Topic=(eel) OR MeSH Heading:exp=(Eels))) OR(Topic=(herring) OR((MeSH Heading:exp=(Food)) AND(MeSH Heading:exp=(Seafood))))) OR(Topic=(mackerel) OR((MeSH Heading:exp=(Food)) AND(MeSH Heading:exp=(Seafood))))) OR(Topic=(hake) OR MeSH Heading:exp=(Seafood))) OR(Topic=(anchovy) OR MeSH Heading:exp=(Fishes))) OR(Topic=(carp) OR MeSH Heading:exp=(Carps))) OR(Topic=(mussel) OR MeSH Heading:exp=(Shellfish))) OR(Topic=(shellfish) OR MeSH Heading:exp=(Shellfish))) OR(Topic=(whitefish) OR MeSH Heading:exp=(Salmonidae))) OR(Topic=(sablefish) OR MeSH Heading:exp=(Perciformes))) OR(Topic=(swordfish) OR MeSH Heading:exp=(Perciformes))) OR(Topic=(dolphinfish) OR MeSH Heading:exp=(Perciformes))) AND Topic=(smok*). (The asterisk is the wild card character for PubMed.Net)

For MEDLINE the search was (listeria OR listeriosis) (fish OR trout OR salmon OR eel OR herring OR mackerel OR hake OR anchovy OR carp OR mussel OR shellfish OR whitefish OR sablefish OR swordfish OR dolphinfish) smok?.

9 APPENDIX B: CAC GUIDANCE POINTS INCLUDED IN QUESTIONNAIRE USED TO GATHER OPINIONS FROM FISH SMOKERS

The key sets of relevant points with the reasons why points from the Codex Alimentarius Commission (CAC) were included in the development of the industry questionnaire, and the reasons they were included (underlined) are shown below:

- Floors should be hard-surfaced, non-absorbent and adequately drained.
- Drains should be of an adequate size, suitable type, equipped with traps and with removable gratings to permit cleaning.
- Internal walls should be smooth, waterproof, resistant to fracture, light coloured and readily cleanable.
- Ceilings should be so designed, constructed and finished as to prevent accumulation of dirt and minimize condensation, mould development and flaking, and should be easy to clean.
- Premises should be well ventilated to prevent excessive heat, condensation and contamination with obnoxious odours, dust, vapour or smoke
- Areas where raw materials are received, stored or handled should be separated from the areas in which product preparation, processing and packaging are conducted
- A separate refuse room or other equally adequate offal storage facilities should be provided on the premises
- An ample supply of cold and hot potable water and/or clean sea water under adequate pressure should be available at numerous points throughout the premises at all times during working hours. (for cleaning and rinsing purposes)
- When in-plant chlorination of water is used, the residual content of free chlorine should be maintained at no more than the minimum effective level for the use intended.
- Ice should be made from potable water or clean sea water and should be manufactured, handled and stored so as to protect it from contamination.
- Where a non-potable auxiliary water supply is used, it should be stored in separate tanks, carried in separate lines, identified by contrasting colours, labelled and have no cross-connections or back-siphonage with the lines carrying potable water or clean sea water.
- Proper facilities for washing and disinfection of equipment should be provided.
- Adequate and conveniently located toilet facilities should be provided facilities should be available in the processing areas for employees to wash and dry their hands and for disinfection of protective hand coverings.
- Salt and other ingredients used in curing of fish should be stored dry and in a manner to prevent their contamination.

- Storage facilities should be available for the proper dry storage of packaging materials.
- All working surfaces, equipment and utensils used in food handling areas and which may contact food should be made of material which does not transmit toxic substances, odours or tastes, is non-absorbent, is resistant to corrosion and is capable of withstanding repeated cleaning and disinfection. Surfaces should be smooth and free from pits and crevices. The use of wood and other materials which cannot be adequately cleaned and disinfected should be avoided except when their use would clearly not be a source of contamination.
- Boards and other surfaces on which fish are cut should be made of impervious materials which meet the physical requirements for cutting surfaces
- The use of properly designed machines for cutting, washing, splitting, skinning, staking, brining and tentering (hooking onto smoking frames) and other similar operations for fish is to be encouraged.
- Brining and salting vats should be made of suitable corrosion resistant material and should be so constructed as to permit easy cleaning and complete drainage.
- Fish transport vehicles should be designed to protect fish from warming up during transportation, and should be of such material and construction as to permit easy and thorough cleaning.
- The use of automated smoke generators is strongly recommended (because mechanical smokers give a more consistently reproducible smoking process).
- Instrumentation to monitor and control the smoking process should be applied wherever possible (for the same reason as above).
- Equipment used for hanging or laying out of fish during smoking should be constructed of suitable corrosion-resistant material and should be designed so as to be readily cleanable.
- Equipment used in "electrostatic" smoking should be managed by skilled operators and should be controlled by automatic regulators of temperature, humidity and smoke density.
- Fish intended for smoking should always be treated in a hygienic manner.
- The building, equipment, utensils and other physical facilities of the plant should be kept clean, in good repair and should be maintained in an orderly and hygienic condition.
- Splitting and cutting boards should be frequently and thoroughly scrubbed and treated with disinfectant. Wherever practicable, the boards should be continuously flushed with running potable water or clean sea water during use.
- All machines used for cutting, scaling, washing, filleting, splitting, tentering or other processing equipment used in similar operations, should be thoroughly cleaned, disinfected and rinsed during rest or meal breaks and before resumption of production following other work stoppages.
- Utensils and food-contact surfaces of equipment should be protected from contamination.
- Cleaning of smoking equipment should be made into a regular routine.

- Brine used in brining of fish should be changed as frequently as necessary to prevent accumulation of fatty scum and deposition of sludge of solid salt mixed with fish residue and other foreign matter.
- Only new and clean boxes, cartons and wrapping material should be used for the transport and distribution of smoked fish and similar products. Where returnable boxes are used, they should be corrosion-resistant material and should be thoroughly cleaned and disinfected after each use
- Water used for washing or conveying raw materials, including sea water for conveyance of fish and other marine products into the plant, should be from such a source, or suitably treated, as not to constitute a public health hazard.
- Removal of solid, semi-solid or liquid wastes from fish unloading, holding and processing areas should be on a continuous or near continuous basis using water and/or appropriate equipment so that these areas are kept clean and there is no danger of contaminating the product.
- All persons working in a smoked fish plant should maintain a high degree of personal cleanliness while on duty and should take all necessary precautions to prevent the contamination of the fish or fish products or ingredients with any foreign substance.
- No person who is known or suspected to be suffering from, or who is a carrier of a disease likely to be transmitted through food, or has an infected wound or open lesion, should be engaged in the preparation, handling or transporting of fish or fish products.
- Conveyances used for transporting fish should be cleaned and disinfected immediately after each use and should be so maintained as not to constitute a source of contamination for the product.
- All fish, fish products and ingredients used in fish-smoking establishments should be free from spoilage and contamination and should be safe for human consumption.
- Fresh fish which cannot be processed immediately on arrival should be chilled in clean containers and stored in specially designated areas within the plant where they will be protected from heat and weather conditions and will not become contaminated by dust, insects or vermin. Where possible, the iced fish should be stored in a chill room, the temperature of which is just above that of melting ice.
- All fish should be carefully inspected, sorted or culled before they are processed. Any damaged, contaminated or otherwise unacceptable fish should be discarded.
- All fish should be thoroughly washed before processing or immediately after operations like scaling or gutting.
- Where fish are being gutted, heated, skinned, boned or portioned, these operations should be carried out in a clean and hygienic manner.
- When frozen fish is used the temperature in any part of the thawed product should not rise above $7^{o}C$ before being processed. If processing cannot commence immediately the thawed material should be kept chilled.
- Brining should be carried out with the full understanding of the effect on the quality of the final product and should be done under strict hygienic conditions.

- Large fish should be split or cut or scarified before brining or dry-salting to allow for more effective and uniform salt penetration.
- Fresh brine should be prepared at least each day before the start of operations.
- During pickling (brining) fish should be kept at a temperature below 3^{o}C.
- The ratio of brine to fish should be at least 1:1 by weight when using a saturated brine.
- "Dripping" and drying of fish prior to smoking should be carried out under controlled conditions and in a hygienic manner.
- Fish for dry-salting should be properly arranged to ensure uniform conditions and proper drainage.
- Fish which is dry-salted in piles should be re-stacked periodically with the addition of fresh salt to ensure uniform curing conditions and pressing.
- For salting of small fatty fish, such as anchovy or small herring, dry-salting (known as Kench curing if the drawn liquid is allowed to freely drain) may be used; and for large fish, pickling (dry salted fish allowed to soak in the brine created by the salt) or brining should be used in preference.
- For smoke production, the wood, wood shavings or sawdust should be dry and free from soil dust and harmful substances such as wood preservatives and paint.
- In preparation for smoking, care should be taken to arrange the fish on tenters, hooks or on trays in such a manner as to provide for uniform smoke absorption, temperature exposure and dehydration.
- After completion of smoking and before packaging the warm products should be immediately cooled to the ambient temperature or lower. After packaging the product should be cooled further without delay.
- Vacuum or gas packed products should be stored at an appropriate safe temperature (3^{o}C)
- Smoked fish and similar products which are not frozen should be packed for transport so that there is no direct contact with ice or melt water.
- In addition to any control by the official agency having jurisdiction it is desirable that each smoked fish processing plant in its own interest should have access to laboratory control to establish hygiene and quality of the products processed and to monitor the hygiene of processing.

10 APPENDIX C: THE QUESTIONNAIRE USED TO GATHER OPINIONS FROM FISH SMOKERS

Colour codes: The Qs in blue don't need to be asked; and should be able to be assessed from just walking round the plant. The Qs in black should be asked to the plant staff.

Raw Fish
Are fish transported in a manner which prevents their warming up?
Are transport crates/boxes/trays designed to permit easy and thorough cleaning and sanitation?
Are transport crates/boxes/trays cleaned and sanitised at the plant before being reused?
Are transport crates/boxes/trays cleaned and sanitised somewhere else before being reused?
Are they ever used for a purpose other than transporting fish?
Is any transport crates/boxes/tray cleaning and sanitation regime validated as effective in controlling *L. monocytogenes* (Plant may not know if it happens offsite)
How are containers coming into contact with fish stored if kept on site?
Is there physical damage (e.g. scrapes and scores) in the surfaces of transport crates that could come into contact with raw fish?
If frozen fish is used, is it thawed at below 7°C before being processed?
Are fish stored under refrigeration (i.e. in ice or in a chiller) prior to processing and smoking?
Is the incoming temp of the fish checked?
Does the FBO have any criteria for accepting fish? (e.g. does he check the listeria status of the fish coming in/ask the farm to supply data/ ask the farm what controls they take/ take account of environmental conditions when the fish were harvested)
Does waste removal (e.g. melted water, fish detritus) from the fish unloading and storage areas happen on a continual or near continual basis?
Plant infrastructure:
Is the plant infrastructure intact? i.e. no holes in the walls, broken windows damaged doors which allow insects and vermin into the plant
Are plant floors hard surface, non-absorbent and adequately drained (i.e. no

standing water)?
Do the drains have removable grates?
Are the drains fitted with slow-dissolving blocks of sanitiser chemicals?
Do the drains have copper linings (or another source of copper ions such as a copper grating)?
Are the plant walls smooth, waterproof, light coloured (to easily see if they're dirty) and readily cleanable?
Are the ceilings constructed in a manner that prevents accumulation of dust, condensation and growth of microorganisms? (look out for pipes and electrical ducting)
Are the premises well ventilated (i.e. is there enough airflow to prevent formation of ceiling condensation)? Ask do you ever get condensation dripping off the ceiling?
Is there physical separation of raw product receiving area and product preparation, processing and packing areas?
Are refuse and processing by-products stored in a physically separate location to raw fish and final product?
Does the plant have plentiful supplies of hot and cold potable water? Ask: do you use mains water? How is it heated?
If borehole water is used, how is the water made potable? (e.g. chlorine dioxide addition)
Is the ice made by the plant made from potable water or sea water?
How is ice stored and used?
What happens to waste ice?
Does the plant have a non-potable water source (e.g. estuary or coastal water) and if so, are there protections to prevent non potable water from contaminating edible product?
Are the food contact surfaces in the plant hard, impermeable to water, free from cracks and pitting and not visibly corroded?
Are the food contact surfaces capable of withstanding repeated cleaning and sanitation?
Are the vats/other containers used for brining or salting vats free from corrosion, scoring and pitting and constructed in a manner that permits easy cleaning and complete drainage?
Are there knife sterilisers (e.g. >80^{o}C water baths) at any filleting and evisceration stations?
Is a two-knife system in use? (one knife in steriliser, the other in use; knives

periodically exchanged)
Are there machines for: **Cutting fish** **Washing fish** **Splitting fish** **Skinning fish** **Brining (injectors)** **Are these machines specifically designed to be easily cleaned and sanitised? Ask if there are bits of fish detritus trapped inside the machines at the end of a day's processing**
Are there low pressure (i.e. mains pressure) hoses in the processing area?
Are there high pressure (i.e. jet washers) hoses in the processing area?
Is the temperature monitored in processing/storage areas?
Processing staff
Does the plant have an SOP which describes minimum standards of cleanliness for processing staff?
Does the plant have a return to work procedure for processing staff (known or suspected of) recovering from gastroenteritis?
Does the plant have sanitary toilets which allow employees to wash and dry their hands?
Are there facilities which allow employees to wash and dry their hands in the processing hall?
How often do staff handling fish wash their hands/change their gloves?
Are gloves changed at appropriate times (i.e. after touching something else etc..)
Are staff movements restricted in the direction of clean (i.e. the smoker) end of the process towards dirty (i.e. the raw fish receiving) end of the process?
Are different areas of the plant physically segregated?
Does the plant use casual labour?
Do plant employees change their jobs frequently (every few days or more frequently) to prevent boredom?
Processing practices
Are fish washed on receipt and what is the source (stored, mains etc) and type (SW,

FW, potable, clean?) of water used?
Are fish washed after descaling?
Are fish washed after gutting? (if applicable)
What the source and type of water used after any of the above processes?
What is the overall processing environment temperature?
Is salt stored under dry conditions which prevent its contamination?
If brine is used, is it made fresh every day (or more frequently)?
Does the brine ever accumulate fatty scum or deposit sludges of solid salt mixed with fish residue in the brining container?
Is brining undertaken at 3°C or lower?
If liquid brine (i.e. not salt crystals) is used, what is the ratio of fish to brine?
Is fresh brine used for each batch of fish or is it recycled for more than one batch?
What fish species are processed in liquid brine?
If salt crystals are used, is the brine that forms free draining? (i.e. the fish don't sit in the created brine)
If salt crystals are used, are the fish restacked part way through the salt treatment?
What fish species are processed in salt crystals?
Is the dripping/drying area well drained (i.e. no standing water on the floor)
Does waste removal (e.g. melted water, fish detritus) from the processing area happen on a continual or near continual basis?
Does the plant use automated smoke generation or traditional burning/smouldering hardwood
If hardwood is used for smoking, does it ever have traces of soil on the wood's surface?
Is the smoking process instrumented to monitor and control the smoking process?
Need a Q here on electrostatic processing
Are the frames/racks/tenters used to support the fish during smoking constructed from corrosion-resistant material which is water impermeable and designed to be readily cleaned and sanitised?
Do the racks etc, if moveable have adequate splash protection to protect fish from spray from wet floors/environment?
What is the height of lowest section of the smoking rack that can hold fish? (in cm)
How does the plant ensure uniform smoke absorption, temperature exposure (if applicable) and dehydration of the fish during smoking?
After hot smoking (if applicable) how are the fish cooled to ambient temperature before chilling?

Are final product packing materials stored under dry conditions which prevent contamination?
What is the storage temperature of the finished product prior to shipping?
Are finished products stored in a manner which prevents their direct contact with melted ice?
Are final products vacuum packed?
Are final products modified atmosphere packed and if so what gas mix is used and what ratio of product volume to gas volume is used?
Are final products and raw fish held in physically separate locations?
Are there safeguards in place (e.g. different colours/types of container) to ensure crates/boxes/trays used for transport of raw fish are not used for finished product?
Do you test the final product for *L. monocytogenes*?
Is the testing presence/absence or numbers?
Do you ever get out of specification (OOS) results?
If applicable, what corrective actions do you take when you get an OOS result?
Is testing of the processing environment carried out (and as above – what methods are used) and where do they sample from?
Is any sampling data kept and used (e.g. to trend historical data and identify when conditions are moving out of spec)
Does the FBO determine product characteristics, particularly water activity and pH?
How is shelf life determined?
Would guidance on shelf life determination be useful?
Have you heard of the BRC/CFA/FSA 2010 Guidance ('Shelf Life of Ready to Eat Food in Relation to *L. monocytogenes* – Guidance for Food Business Operators'
What happens to fish waste? Is this collected sensibly and removed regularly with containers emptied and cleaned?
Is there product traceability?
Are they aware of the micro criteria regulations?
Is there a HACCP plan?
Cleaning and sanitation
Is there a wet cleaning mid- shift? (i.e. is there splashing of *L. monocytogenes* from drains)
What is the cleaning regime in place?
Does the plant have equipment washing and cleaning SOPs that are undertaken outside of processing and which are validated as effective for the control of *L.*

***monocytogenes*?**
If applicable, do these SOPs cover: **Cutting boards** **Knives** **Fish skinning machines** **Brine injectors (if applicable)** **Fish slicing machines** **Frames/racks/tenters used for smoking** **The inside of the kiln** **Chillers** **Drains** **Employee PPE (gloves and aprons)** **Door handles and equipment controls (e.g. electrical switches and kiln instrument controls)**
If applicable, what is the frequency of cleaning/sanitation for: **Cutting boards** **Knives** **Fish skinning machines** **Brine injectors (if applicable)** **Fish slicing machines** **Frames/racks/tenters used for smoking** **The inside of the kiln** **Chillers** **Drains** **Employee PPE (gloves and aprons)** **Door handles and equipment controls (e.g. electrical switches and kiln instrument controls)**
Does the plant have an arrangement in place which allows for microbiological testing?
Is the effectiveness of cleaning and sanitation periodically checked?
If applicable, how are these checks done? (swabbing and micro lab testing; what bacteria? ATP machine? Protein residue sticks?)
If applicable, when are the cleaning efficiency checks done? (e.g. after cleaning or before commencement of the day's production?)
Are cleaning and sanitisers changed periodically to prevent the establishment of plant persistent bacterial populations which are resistant to long time use

chemicals?
What water source is used for cleaning and how is the water delivered (e.g. low pressure hose)?

11 APPENDIX D: THE QUESTIONNAIRE USED TO GATHER OPINIONS FROM FISH GROWERS

Farm and Infrastructure	
1.	Is the farm in fresh, salt or estuary water?
2.	If fresh or estuary; does the supply water catchment pass through land used for growing crops, or farm animals or has poor water qualty? (may not know answer)
3.	If an estuary, do the tidal water movements cause water to wash against bare soil (e.g. river banks) in the vicinity of the farm?
4.	Does the farm have any earth-banked fish ponds?
5.	Does the farm harvest fish during/ immediately after periods of sustained rainfall?
6.	Does the farm test the supply water for *L. monocytogenes*? (if No go to Q7)
	If so, is the testing presence/absence or quantitative?
	If so, what criteria are used for in spec/out of spec
	If so, are out of specification (OOS) test results ever obtained?
	What corrective actions are undertaken for an OOS water result?
7.	Does the farm test the fish for *L. monocytogenes*? (If No go to Q8)
	If so, at what point in the process & where are the fish sampled? (skin swabs, gills etc)
	Is the testing presence/absence or quantitative?
	What criteria are used for in spec/out of spec
	Are OOS test results ever obtained?
	What corrective actions are undertaken for an OOS fish test result?
8.	Do you have any views on how listeria may get into the fish farm?
Harvesting	
1.	Are fish starved prior to slaughter? If so, for how long?
2.	How are the fish moved from the cages to shore?

3.	What equipment is used and has it been cleaned? If so, describe the cleaning process.
4.	Is any of the equipment tested for *L. monocytogenes*? (If No go to Q5)
	If so, is the testing presence/absence or quantitative?
	If so, what criteria are used for in spec/out of spec
	If so, are out of specification (OOS) test results ever obtained?
	What corrective actions are undertaken for an OOS result?
5.	How are the fish moved from boat to shore?
	What equipment is used and has it been cleaned? If so, what procedure is used for cleaning?
	Is any of the equipment tested for *L. monocytogenes*? (If No go to Q6)
	If so, is the testing presence/absence or quantitative?
	If so, what criteria are used for in spec/out of spec
	If so, are out of specification (OOS) test results ever obtained?
	What corrective actions are undertaken for an OOS result?
6.	Are fish slaughtered at landing site (go to 7) or transported elsewhere?
	If transported elsewhere, explain how fish are transported, details of equipment used and if it is cleaned.
	Describe the cleaning process.
	Is any of the equipment tested for *L. monocytogenes*? (If No go to Q7)
	If so, is the testing presence/absence or quantitative?
	If so, what criteria are used for in spec/out of spec
	If so, are out of specification (OOS) test results ever obtained?
	What corrective actions are undertaken for an OOS result?
7.	Describe how the fish are input into the slaughter line.
8.	Describe the stunning, bleeding and icing process.
9.	Describe the source of ice and water (potable – is it tested for *Listeria* or indicator organisms)?
	Describe the cleaning regime for the equipment.
	Describe the containers the fish are put into, whether they are cleaned.
	Is any of the equipment/surfaces tested for *L. monocytogenes*? (If No go to Q10)
	If so, is the testing presence/absence or quantitative?
	If so, what criteria are used for in spec/out of spec
	If so, are out of specification (OOS) test results ever obtained?

	What corrective actions are undertaken for an OOS result?
10.	Explain how the fish are transported to the factory/next stage of processing (duration and how chilled temperature is maintained).
11.	Do you have any particular concerns regards *Listeria* contamination of the harvesting process?
12	Are there any lessons you can tell where there has been a problem with listeria and how you managed to get round this.
Staff	
1.	Does the plant have an SOP which describes minimum standards of cleanliness for processing staff?
2.	Does the plant have a return to work procedure for processing staff (known or suspected of) recovering from gastroenteritis?
3.	Does the plant have sanitary toilets which allow employees to wash and dry their hands?
4.	How often do staff handling fish wash their hands/change their gloves?
5.	Does the plant use casual labour?
6.	Do plant employees change their jobs frequently (every few days or more frequently) to prevent boredom?

12 APPENDIX E: THE QUESTIONNAIRE USED TO GATHER OPINIONS FROM EHOS WHO VISIT FISH-SMOKING FBOS AS PART OF THEIR PROFESSIONAL DUTIES

Basic Food Safety Principles	Rating (1=low, 5=high)	
	Answer for FBOs	Answer for EHOs
1. HACCP principles		
2. Characteristics and control of key hazard organisms such as *Listeria monocytogenes*		
3. Understanding, controlling and monitoring salt, pH, temperature and the use of vacuum/modified atmospheres as multiple interventions that can be used in combination to help prevent the growth of *L. monocytogenes*		
4. Assessment of the impacts of changing product formulation using predictive modelling tools		
Anything else you consider important for basic food safety (500 character limit)		

Food handlers	Rating (1=low, 5=high)	
	Answer for FBOs	Answer for EHOs
5. Enhanced training specific to *L. monocytogenes* that should be completed by smoked food handlers		
6. Return to work procedures for food handlers known or suspected to be recovering from an infection by an agent capable of causing foodborne disease		
Anything else you consider important for food handlers (500 character limit)		

Raw materials	Rating (1=low, 5=high)	
	Answer for FBOs	Answer for EHOs
7. Raw materials sourcing criteria (e.g. controls in place on farms, environmental conditions during harvest)		
8. Common raw material acceptance criteria on delivery (e.g. *Listeria* status of the fish, physical condition, odour)		

Anything else you consider important for raw materials (500 character limit)	

Plant infrastructure and hygiene	Rating (1=low, 5=high)	
	Answer for FBOs	Answer for EHOs
9. Requirements for physical separation of raw product receiving area and product preparation, processing, final product storage and packing areas		
10. High care area requirements for cold-smoked fish		
11. High risk area requirements for hot-smoked fish		
12. Water quality and brine in contact with the product		
13. Management of cleaning and sanitation and the frequency and type of monitoring effectiveness		
Anything else you consider important for plant infrastructure and hygiene (500 character limit)		

Processing and shelf life	Rating (1=low, 5=high)	
	Answer for FBOs	Answer for EHOs
14. Effective monitoring and control of smoking processes		
15. Requirements for post process handling of products		
16. Determination of shelf life including frequency of day of production and end of life sampling for *L. monocytogenes*		
Anything else you consider important for processing and product shelf life (500 character limit)		

Statutory testing for *L. monocytogenes*	Rating (1=poor understanding, 5= excellent understanding)	
	Answer for FBOs	Answer for EHOs
17. Do you think that staff have a good working understanding of EC 2073/2005 and *L. monocytogenes* testing for smoked fish which is a ready-to-eat food?		
18. Do you think that staff in your region know that if they have not undertaken work to show that *L. monocytogenes* numbers stay below 100 cfu/g fish for the entire shelf life of		

the product, that the testing they should do is to confirm the absence of *L. monocytogenes* in 25g of fish at the end of manufacture?

19. Do you think that staff in your region know that the statutory test method for *L. monocytogenes* numbers is ISO 11290-2; and for presence/absence testing is ISO 11290-1 and if a lab uses a different test method, the method has to be validated against the reference test method?

Not answered

In what format should any guidance be provided?	Rating (1=low, 5=high)
Web-based written guidance	
Web-based interactive guidance (decision support tools, videos etc)	
Training courses and workshops	
Booklets or pamphlets	
Another format you think would be best (500 character limit)	

How many approved smoked fish plants do you estimate to be in your region?