Risk from Listeria monocytogenes in ready to eat smoked fish: Exposure assessment

Production processes and risk pathway

L. 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 (Thomas et al., 2012). Living fish encounter the pathogen in their natural environment (water, soil, decaying vegetation etc), however it has been reported in literature that *L. monocytogenes* primarily enters food products via cross-contamination in production plants (Jami et al., 2014). For the purposes of this risk assessment, the principal stages of the production of RTE smoked fish products were examined to understand the risk pathway for this organism in this commodity.

Principle stages of the production of RTE smoked fish products and associated risks for *Listeria monocytogenes*

Harvested fish are transported to primary processors where they are slaughtered and gutted. Slaughtering can occur once the fish have arrived to the primary processor (on shore) or slaughtering can occur in a killing vessel (off shore), where the fish are killed and bled. The culled fish are then transported to the processing premises, where they are gutted and packed (information obtained by FSS as part of investigations of an incident).

Upon reception at the primary processor, the fresh fish are washed to remove the mucus on the fish skin (this mucus can be a source of contamination for *L. monocytogenes)*. Then the fish are eviscerated. During evisceration, contact between flesh and the skin of other fish or waste (viscera, heads) is avoided as the skin, gills and intestines are recognised as the most contaminated parts of the fish. Fish are placed on a conveyor belt with skin against the belt at a speed preventing accumulation of fish to avoid any cross contamination. Correctly carried out early evisceration is crucial to prevent contamination of the flesh with parasites or any bacterial proliferation (incomplete evisceration can be a source of bacterial contamination). The eviscerated fish are then packed and transported (either fresh or frozen to a secondary processor). Note the heads of eviscerated fish are not removed at this point (head on gutted) (European Salmon Smokers Association, 2018).

Figure 1 is adapted from The European Salmon Smokers Association guide to good practice for manufacture of smoked fish products, and outlines the main stages in secondary production. It is noted that there are various both smoked and non-smoked RTE fish products which undergo the same general secondary processing steps, and that this adapted figure includes smoked and/or salted and/or marinated fish.

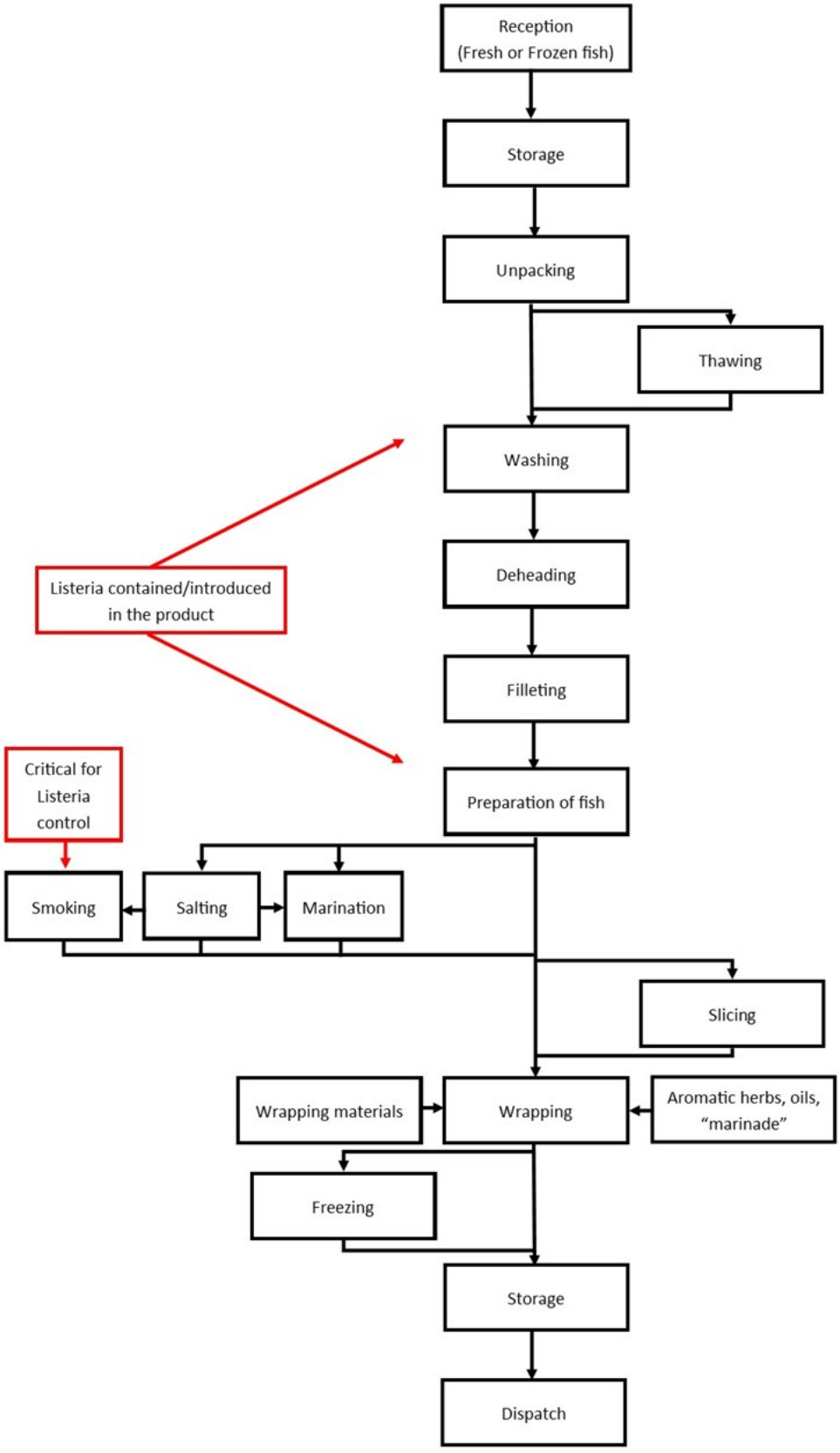
For the purposes of this risk assessment, the critical stages where *L. monocytogenes* can be introduced and/or controlled during secondary processing are indicated in red in Figure 1. This indicates that *L. monocytogenes* can be introduced through any stage of secondary processing

from “reception” of the gutted fish, “storage”, to “dispatch” to the consumer. *L. monocytogenes* can be contained within the raw product and proliferate throughout secondary processing. *L. monocytogenes* contaminated product can cross-contaminate other fish products or contaminate the processing premises. *L. monocytogenes* can live and persist within the secondary processing environment and contaminate each new batch of product. The stages of “deheading” and “filleting” are where *L. monocytogenes* on the fish carcass is most likely to cross-contaminate other products, if good manufacturing procedure is not followed correctly (Rotariu et al., 2014; Aalto-Araneda et al., 2019). There is one possible critical control point (CCP), which can result in batch fish being rendered free of *L. monocytogenes* and that is the heating step process of “hot smoking” (see section 4.1.1.1). Regarding the secondary processing of other RTE smoked fish products, there is not a single processing control step where *L. monocytogenes* can be completely eliminated.

Figure 1. Principal stages of production for smoked and/or marinated fish products (adapted from European Salmon Smokers Association, 2018) (accessible version)

1. Reception (fresh or frozen fish)
2. Storage
3. Unpacking
4. Thawing
5. Washing (Listeria contained/introduced in the product)
6. Deheading (Listeria contained/introduced in the product)
7. Filleting (Listeria contained/introduced in the product)
8. Preparation of fish (Listeria contained/introduced in the product)
9. Smoking, Salting, Marination (smking is critical for Listeria control)
10. Slicing
11. Wrapping materials, wrapping, aromic herbs, oils marinade’
12. Freezing
13. Storage
14. Dispatch

Figure 1. Principal stages of production for smoked and/or marinated fish products (adapted from European Salmon Smokers Association, 2018)



As mentioned above, smoked fish processing is split between primary and secondary processing where:

- **Primary processing** is slaughtering and gutting.
- **Secondary processing** is filleting, fillet trimming, portioning, producing different cuts such as cutlets, smoking, making ready meals or packing with modified atmosphere.

For the purposes of this risk assessment, the salmon primary and secondary production cycle were examined in further detail. This is due to: 1) information for salmon processing being the most abundantly available evidence and 2) that salmon is the most sold (both fresh and chilled) fish in the UK, which likely reflects consumer preference for this type of fish (Sandercock, 2019; White, 2019).

According to “Salmon Farming Industry Handbook 2019 by M?WI” the salmon farming production cycle is approximately 3 years. In the first production year, fish eggs are fertilised and fish are grown to 100-150 grams in a controlled freshwater environment. Then the fish are transported to seawater cages, where they continue to grow for 12-24 month until they reach 4-5 kg. Once grown to harvest size, the fish are transported to processing plants where they are slaughtered and gutted. Most salmon is sold to secondary processors gutted and transported on ice in a box (Mowi, 2021).

A basic overview flow chart from primary processing through secondary processing and manufacture of cold-smoked and hot-smoked salmon is shown on Figure 2 (Adapted from (FAO, 2006)). The critical stages where L. monocytogenes can be introduced and/or controlled during secondary processing are indicated in red in Figure 2.

It should be noted that there is variation between the specific operations of different FBOs. For example, the time it takes for the cutting, trimming, skinning, and slicing will differ from business to business. Additionally, the salting process varies significantly between different processors – all of the following can be used: dry salting, wet salting, brine injection or submersion in brine. The processes of smoking, for example hot smoking versus cold smoking are completely different (examined in further detail below – see 4.1.1.1 and 4.1.1.2). And each cold smoking and hot smoking process also varies from business to business. Furthermore, the characteristics of the final fish product can also vary significantly between hot smoked and cold smoked products. The hot-smoked product is typically sold and advertised as portions or “chunks” whereas the cold-smoked product is marketed sold as a sliced product or trimmings product. The cold smoked product can also be distributed as whole fillets, which undergo further slicing at the retail establishment. A portion of the hot- and cold-smoked salmon can be also further processed to produce “minces”, “spreads”, and “seafood salads” (FAO, 2006; FAO and WHO, 2006).

L. monocytogenes can be introduced at primary or secondary processing of salmon products. The stages of “butchering/cutting/splitting” are of particular risk for *L. monocytogenes* where contamination may spread from one contaminated fish to other fish or the processing equipment, if good manufacturing procedure is not followed correctly (Figure 2). Hot smoking offers a critical control step for the elimination of *L. monocytogenes* (Figure 2). The process of cold smoking could impact the growth and proliferation of *L. monocytogenes,* but it cannot remove it. Therefore, correctly controlling the cold smoking process and correct shelf-life determination are important for controlling Listeria growth.

Figure 2. An overview from primary processing through secondary processing and manufacture of cold-smoked and hot-smoked salmon (adapted from FAO, 2006) (accessible version)

1. Primary production (capture/harvest) (slaughter)

There are two separate processes:

1. Refrigerated transport.
2. Receiving fresh

3. Storage refrigerated.
4. Rinsing

1. Frozen transport
2. Receiving frozen
3. Storage frozen
4. Thawing

The process then joins up again with the following steps:

1. Butchering/Cutting/Splitting
2. Brining/dry salted
3. Rinsing/Draining
4. Racking/Hanging

Listeria potentially contained/introduced in the product during Brining, dry salted, rinsing and draining stages. The process then separates again for hot smoked and cold smoked fish:

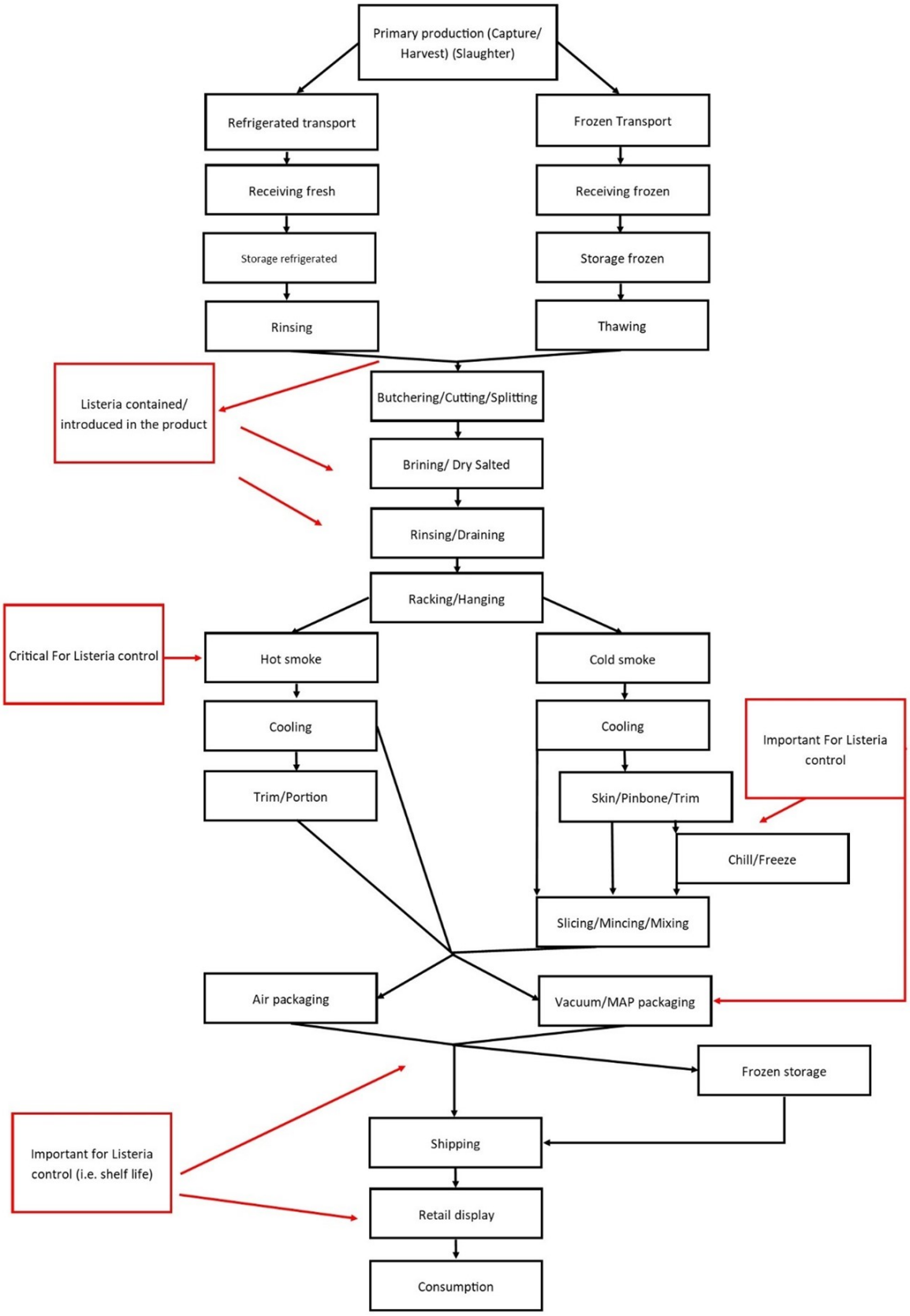
Hot smoke:

1. Hot smoke (critical for Listeria control)
2. Cooling
3. Trim/Portion
4. Air packaging or Vacuum/MAP packaging (important for Listeria control and shelf life)
5. Frozen storage
6. Shipping
7. Retail display
8. Consumption

Cold smoke:

1. Cold smoke
2. Cooling
3. Skin/Pinbone/Trim
4. Chill/Freeze (important for Listeria control)
5. Slicing/Mincing/Mixing
6. Air packaging or Vacuum/MAP packaging (important for Listeria control and shelf life)
7. Frozen storage
8. Shipping
9. Retail display
10. Consumption

Figure 2. An overview from primary processing through secondary processing and manufacture of cold-smoked and hot-smoked salmon (adapted from FAO, 2006)



Control points in the hot smoking process

A typical hot-smoking process uses temperatures of 30-40°C to dry the product, then a hot-smoking period of 2-3 h at 60-70°C, followed by a second drying period (FAO and WHO, 2006). The hot smoking process meets the criteria for a CCP according to the FAO guidelines (FAO,

1997).
If a critical temperature is achieved for a set time across all of the fillets during the hot smoking process, this can result in a batch of fish being rendered completely free of *L. monocytogenes* (Jemmi and Keusch, 1992; Branciari et al., 2016).

However, the critical temperature has not been definitively determined and there are a number of sources advising different targets. The UK Food Standards Agency, European Salmon Smoker's Association guidance, FAO guidance, Canadian government and the USDA/US-FDA do not specify a particular temperature that should be achieved for the control of *L. monocytogenes* in hot smoked fish (FSS, "Safe Smoked Fish Tool"). Food Standards Australia advises *L. monocytogenes* cannot survive >75°C, (with no specific duration stated) and industry associations such as a working group created by the US National Fisheries Institute and US National Food Processors Association advise that temperature of 145°F (62.8°C) for 30 minutes as sufficient as act as a control point. They do not specifically comment on any effect of smoke on the efficacy of this temperature as a control (National Fisheries Institute and National Food Processors Association, 2002; Food Standards Australia New Zealand, 2016).

The sources cited above agree that unless the level of *L. monocytogenes* on the fish prior to smoking is extraordinarily high, an adequate hot smoking process should eliminate the pathogen. Thus, any *L. monocytogenes* present in salmon product after the hot-smoking step is most likely due to recontamination of the product. The effect of this contamination in terms of the frequency of contamination or the levels of Listeria present, depends on the how the product is handled during final manufacturing, distribution, display on retail, and how it is handled by the consumer at home.

Control points in the cold smoking process

A typical cold smoking process involves adding salt and lactic acid to the salmon fillets via injection and/or dry salting. Then there is an equalisation process for 4 hours at maximum of 5°C. Following this, there is a drying period of approx. 5 hours and smoking period for approx. 3.5 hours (note these durations will vary between different businesses). Both drying and smoking are generally performed at temperatures of around 23°-28°C. The salmon is chilled after smoking to a core temperature of 0°C (FAO and WHO, 2006; Porsby et al., 2008; Rasmussen et al., 2017).

There is agreement between both the academic and industry guidance sources cited above that the temperatures used to cold-smoke salmon are insufficient to eliminate *L. monocytogenes* (FAO and WHO, 2006; Porsby et al., 2008; Rasmussen et al., 2017). Additionally, there are no steps in cold-smoked salmon production after the cold-smoking process that would eliminate *L. monocytogenes.*

However, the FAO advises that the steps of the cold-smoking process (salting, smoking, drying etc.) can reduce the levels of *L. monocytogenes* by 90 – 99%, if it was present on the raw salted fish (FAO and WHO, 2006). This is supported by academic studies where, if considered together, the processing steps involved in cold-smoking of salmon are able to reduce the levels of *L. monocytogenes* but do not eliminate it (Rørvik, 2000; Porsby et al., 2008).

Thus, if any *Listeria monocytogenes* is present within the product after the cold smoking process it is important that appropriate measures are in place (preventing cross-contamination, chilling, cold storage etc.), and that the shelf life is set correctly, to avoid growth of the microorganism. The frequency of contamination in final product, or the levels of *L. monocytogenes* present in contaminated product, depends on the food safety management system- how the product is handled during final manufacturing, distribution, display on retail, and how it is handled by the consumer at home.

Unfortunately, there is currently no market data available to indicate what proportion of smoked RTE fish products on retail in the UK are hot smoked vs cold smoked. The section above examined smoked RTE salmon products, as they have been reported to be most sold in the UK (Chilled Seafood in Multiple Retail, 2021). It is also unclear what proportion of other RTE fish are hot or cold smoked. However grey literature sources indicate that the majority of mackerel is hot smoked, whereas trout can be hot or cold smoked ('Hot smoked, cold smoked - what's the difference?', 2019; Hot smoked mackerel; Everything You Need to Know About … Smoked Mackerel).

Other control points

pH and salt content

Although limited, there is a small amount of data describing typical pH and water activity values for smoked seafood sold in the UK (Table 8), obtained during research done for the FSS smoked fish tool (Safe Smoked Fish Tool). In the UK, the salt contents of cold smoked salmon sampled at retail were 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. With the reported salt concentrations and available water (aw) typical of smoked fish in the UK, it is considered likely for *L. monocytogenes* to survive or even grow, as it would not be significantly impacted by these physicochemical properties (Table 8). Regulation 2073/2005 defines characteristics of RTE food which would not support the growth of *L. monocytogenes* as products with pH ? 4.4 or aw ? 0.92, products with pH ? 5.0 and aw ? 0.94, and products with a shelf-life of less than five days. Thus, the UK figures confirm that smoked fish products can support the growth of *L. monocytogenes* and are unlikely to fall under this definition.

Table 8. Typical properties of various cold smoked fish products sold in the UK

Product	VP/MAP	NaCl	Shelf life (chilled)	Process	Notes
Cold smoked salmon	VP VP or MAP	Aqueous >3.5% from top to bottom of salmon side Unknown 3%	16 days 1 to 6 weeks 10 days	22 to 30 degrees 12 to 24 hours	UK major multiple International (range)
Cold smoked salmon side	VP	2.2%	>14 days	22 to 30 degrees 12 to 24 hours	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 to 30 degrees 12 to 24 hours	UK Shelf life limited in practice by oragnoleptic quality

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

Modified atmosphere packaging (MAP) and vacuum packing (VP)

Literature review analysis conducted for the FSS smoked fish tool has demonstrated that naturally present *L. monocytogenes* can multiply under vacuum pack conditions in smoked fish. Although heavy salting in combination with some smoke residues has been reported to significantly delay growth and possibly even cause partial *L. monocytogenes* death, vacuum packing in itself is not an effective measure for controlling *L. monocytogenes* growth or eliminating it entirely during cold storage prior to consumption (Safe Smoked Fish Tool).

Production processes and risk pathway: conclusions

From primary to secondary processing of smoked RTE fish products, there is not a single CCP (apart from effective hot smoking), which fully eliminates *L. monocytogenes.* If FBOs are following the outlined guidelines by competent authorities and industry associations, the processing steps in the secondary processing of both hot- smoked and cold -smoked salmon should control the growth of *L. monocytogenes* and maintain it below the legal limit (100 CFU/g) or allow the FBO to demonstrate no detection in 25 grams at the end of secondary processing. This has been previously described as a “hurdle approach”, where multiple controlling factors are implemented to reduce the risk of pathogen growth (FAO, 2006; Tocmo et al., 2014; European Salmon Smokers Association, 2018). However, at any stage from “reception” of the raw fish to its “dispatch” to retail there are points, where the product can either be contaminated with *L. monocytogenes* or if it naturally contains it, there is possibility for the pathogen to grow during the production steps and during its display at retail.

Consumption data

Consumption data of smoked fish in the UK was determined from the National Diet and Nutrition Survey (NDNS), run jointly by UKHSA and the FSA, using data collected from 2008 to 2019 (National Diet and Nutrition Survey). The NDNS is a dietary survey covering a representative sample of around 1000 people per year in the UK. It is a snapshot over 4 days, so food that is regularly but infrequently consumed may not be reported. Table 9 presents results showing consumption of uncooked smoked fish, including smoked cod, smoked haddock, smoked mackerel, and smoked salmon. The NDNS does not have details on if participants fall within a vulnerable group other than age. Due to the way the data is collected, the adult population figures (table 9) include the overall adult population 16+ without an upper age bracket- for example the 65+ population is included in these figures. As the NDNS specifically excludes pregnant women in its data collection, the consumption habits of women aged 16 to 49 years old were used as a proxy for pregnant women.

Fewer women of child-bearing age report eating smoked fish compared to the general population and they also eat slightly less per day compared to the other groups. Conversely, more participants in the over 65 group report eating smoked fish compared to the general population and they also report eating slightly larger servings. However, the difference in consumption between each vulnerable group included in Table 9 and the general adult population is minimal.

Table 9. Number of people reporting consumption of uncooked smoked fish in the UK and the amount eaten.

Population Group	% reporting (n/total respondents)	Average g/person/day	Max g/person/day
16 to 49 (women only)	4.03% (103/2556)	73	300
65+	6.11% (94/1538)	82	300
Adult population (16+)	5.28% (404/7653)	77	300

Growth and survival of *L. monocytogenes*

Growth in naturally contaminated fish

Studies using both inoculated and naturally contaminated fish are available, with natural contamination giving a more realistic indication of the potential for survival and growth of *L. monocytogenes.*

Using a broad Pubmed search of "(*Listeria monocytogenes*) AND (fish) AND (growth)" just two studies were identified which used naturally contaminated fish to assess growth of *L. monocytogenes*. Lappi et al. (2004) analysed smoked salmon samples from two processing plants who reported detection of Listeria spp. in 12% and 14% of product respectively in the previous year, however, Listeria spp. was only detected in 5 of the 72 samples tested, and *L. monocytogenes* in 1 sample. The authors did not comment on this reduction in prevalence, other than that zero samples from plant 3 (where previously 14% of samples had tested positive for Listeria spp.) were positive in this study (Lappi et al., 2004). Each 500g sample was divided into 4 portions with one tested on day zero, and the others vacuum packed, stored at 4°C for 7, 14 or 28 days respectively (Lappi et al., 2004). The one sample for which enumeration was possible (>10 CFU/g) recorded 46 CFU/g at day 7 and 52 CFU/g at day 28, whereas days zero and 14 were recorded at <10 CFU/g, this isolate was identified as Listeria seeligeri. For the one sample where L. monocytogenes was detected, this was only at days 0 and 7 (<2 CFU/g) (Lappi et al., 2004).

Ultimately Lappi et al. (2004) does not provide data to support understanding of the growth of *L. monocytogenes* in naturally contaminated smoked salmon. Instead, the authors support the findings collated elsewhere in this assessment, that contamination is likely to be at low levels and heterogeneously distributed even within the same piece of fish. In a study of French cold-smoked salmon, Beaufort et al. (2007) sampled 384 vacuum packs within 7 days of processing, then re-vacuum packed the product and stored it at 4°C for 8-15 days, and 8°C for the final 7 days to simulate the likely temperature of a consumer's fridge. The initial contamination levels of *L. monocytogenes* were relatively low with the pathogen not detected in 54% of samples (LOD = 0.2 CFU/g), detected at <1 CFU/g in 34% of samples and >1 CFU/g in 12% of samples (Beaufort et al., 2007). The highest recorded level of contamination was 7 CFU/g (Beaufort et al., 2007). At the end of the study *L. monocytogenes* was not detected in 31% of samples, < 1 CFU/g in 18% of samples and >1 CFU/g in 51% of samples (Beaufort et al., 2007). Over half the samples where *L. monocytogenes* was detected at >1 CFU/g had levels greater than the legal limit for the end of shelf life (100 CFU/g), and the highest level enumerated was 2800 CFU/g (Beaufort et al., 2007). These results give an indication of the growth of natural contamination over a realistic shelf life and temperature regime. However, they cannot be considered to be truly representative given the wide variation in types of smoked salmon, their processing conditions and product formulation.

Artificial inoculation based growth studies

Scientific literature was examined for artificial inoculation of Listeria monocytogenes growth studies in terms of RTE fish processing conditions and their effect on Listeria growth. The PubMed database was searched using terms "(*Listeria monocytogenes*) AND (fish) AND (inoculated)". This produced a total 112 results, out of which 29 were deemed relevant based on information available in the abstract and taken into further consideration. Additional PubMed searches using terms "(*Listeria monocytogenes*) AND (salmon) AND (inoculated)", "(*Listeria monocytogenes*) AND (trout) AND (inoculated)", "(*Listeria monocytogenes*) AND (mackerel) AND (inoculated)" identified 5 papers not found in the first search, which were taken into consideration based on information available in the abstract. Overall, 34 papers were examined in detail and appropriate information about the effect of RTE fish processing on the growth of Listeria monocytogenes was extracted. Full text was accessed via the NHS Scotland OpenAthens service.

Although the inoculation studies were carried out in different ways and with different physicochemical parameters, some broad patterns can be pulled out from them. Academic studies using fish inoculated with *L. monocytogenes* supported the data presented in section 4.1.1.2 using industry data, that appropriate combinations of smoking and brining can reduce the level of contamination in these products. For example, in laboratory conditions it was reported that smoking (liquid smoke) or salting individually had little impact on the level of *L. monocytogenes*, but that their combined effect significantly reduced the *L. monocytogenes*

population by 1.6 log CFU/g (Neunlist et al., 2005). This was supported by a further study that found that the combination of brining, liquid smoke and drying resulted in a 1-2 log reduction in the level of *L. monocytogenes* compared to the initial inoculum level (Porsby et al., 2008). The combined importance of heat and smoke in reducing contamination levels was highlighted in a study that found a 10-25 fold reduction in *L. monocytogenes* from the initial inoculum level when cold smoking took place at 17.2-21.1°C, but that little change in inoculum level was seen when smoking took place at higher temperatures (22.2-30.6°C) (Eklund et al., 1995). The importance of the combination of temperature and smoke was also reported for hot smoked salmon where a minimum hot smoking temperature of 67.2°C was required to inactivate *L. monocytogenes* in inoculated salmon fillets, and that heating to 82.8°C was required to inactivate *L. monocytogenes* in the absence of smoke, demonstrating the role that both heat and smoke play in the elimination of *L. monocytogenes* in hot smoked salmon (Poysky et al., 1997). It is noted that the older studies referenced tend to use initial inoculum levels which are not necessarily representative of natural contamination or prevalence which might be expected in fish processing facilities (Eklund et al., 1995; Poysky et al., 1997). This being taken into consideration, the newer studies using lower levels of initial inoculum, show that the cold smoking conditions (in specific process combinations) can reduce the levels of *L. monocytogenes* (Neunlist et al., 2005; Porsby et al., 2008).

The impact that consumer behaviour may have on the growth of *L. monocytogenes* contamination was also illustrated by other studies using inoculated salmon. It was reported that *L. monocytogenes i*noculated into hot smoked salmon after the smoking process was detected at the same level after 20 days of incubation at 4°C, but where the incubation was at 8-10°C levels significantly increased from the initial inoculum level of 31 most probable number (MPN)/g up to 10,000,000 MPN/g (Jemmi and Keusch, 1992), demonstrating the importance of appropriate fridge temperature in controlling growth of *L. monocytogenes* in contaminated product. Additionally, it was shown that where smoked salmon samples were inoculated with low (6 CFU/g) or high (600 CFU/g) levels of inoculum, after 4 weeks incubation at 4°C that *L. monocytogenes* level in some low inoculum samples was equal to or greater than that in high inoculum samples, demonstrating that low levels can multiply substantially (Rørvik, Yndestad and Skjerve, 1991). It was also highlighted that even at high levels of contamination, the sensory quality of the salmon was not affected (Rørvik, Yndestad and Skjerve, 1991), suggesting that consumers are unlikely to be able to detect even very high levels of contamination by any change in the properties of the smoked fish.

Modelling of *L. monocytogenes* growth in smoked salmon

So far this section has covered the growth of natural contamination of RTE smoked fish products and how industry processing conditions may impact the growth of artificially inoculated *L. monocytogenes.* This next part will aim to model the growth of *L. monocytogenes* in cold smoked salmon, under conditions in which a consumer could reasonably be expected to keep it, to help understand the possible growth of contamination after the point of purchase.

To model growth, the online growth curve predictor ComBase was used (ComBase). As cold smoked salmon is typically a chilled product which prescribes storage in refrigerated conditions before consumption, the model assumed storage in a consumer fridge.

There were two independent variables used in the model (to make four models). The first, concentration values of 1 CFU/g (L1) and 10 CFU/g (L10) (Lindqvist & Westöö, 2000) of *L. monocytogenes* in the product immediately after manufacture were used. (Note: concentration of 1 CFU/g is the lowest possible concentration to use in ComBase). The second, two reasonable consumer behaviours were modelled: consumer 1 (C1) had a median performing fridge temperature of 8.1°C (Brennan et al., 2013) and consumed the product immediately after removing from refrigeration on the last day of shelf life. Consumer 2 (C2) had a fridge which performed in the upper 75% percentile of 8.7°C (Brennan et al., 2013), and consumed the product after allowing it to warm up to room temperature over 30 minutes before consumption, also on the

last day of shelf life. The independent variables used for each model are summarised in Table 10. The independent variables used are based on information from published academic studies and information from FSA “Kitchen life” study (Brennan et al., 2013).

Table 10. The independent variables used to create the four models of *L. monocytogenes* growth in cold smoked salmon

Variables	1 CFU/g (L1)	10 CFU/g (L10)
Consumer 1 (fridge 8.1 degrees, eats immediately) (C1)	L1/C1	L10/C1
Consumer 2 (fridge 8.6 degrees, eats after 30 minutes) (C2)	L1/C2	L10/C2

The dependent variables which were used across all four models were as follows. The physicochemical characteristics of the smoked salmon were taken from a median value from a range of published literature (shown in the Appendix). The available water (aw) was 0.955, the pH was 6.04. The median shelf life used was 16 days (see Appendix 2). To model the time-temperature combinations, the values shown in Table 11 were used (based on data from historical incidents and personal communications with Campden BRI).

Table 11. The time/temperature combinations used in the modelling, factoring in the shelf life of 16 days (384 hours), with differences due to the consumer fridge temperature and behaviours before consumption

Process	Time (hours)	Time (hours cumulative)	Temperature (degrees celsius)
End manufacture	0	0	22
Blast chilled	0.25	0.25	4
Transport from manufacturer to retailer display	48	48.25	4
On display in retailer	72	120.25	5
Consumer picking product, transport from retailer to consumer home	2	122.25	22
Storage in consumer fridge	263.75 (C1) or 263.25 (C2)	384 (C1) or 383.5 (C2)	8.1 (C1) or 8.6 (C2)
Removal of product to warm up (C2 only)	0 (C1) or 0.5 (C2)	384	22

Table 12. The final concentration of L. monocytogenes indicated by modelling for the consumer behaviour/pathogen concentration value combinations

-	1 CFU/g (L1)	10 CFU/g (L10)
Consumer 1 (fridge 8.1 degrees, eats immediately) (C1)	3.03 log CFU/g	4.02 log CFU/g
Consumer 1 (fridge 8.6 degrees, eats after 30 minutes) (C2)	3.47 log CFU/g	4.46 log CFU/g

The modelling (shown in Appendix 1) predicted that, in all four models, the product would have been non-compliant by the end of shelf life (being above 100 CFU/g, or 2 log CFU/g, Table 12). However, the ComBase model does not take into account the effect of smoke or other inhibitory substances, that the organism may be exposed to, thus is not a reliable indicator of levels likely to occur at the end of shelf life. An additional limitation of the model is that it does not take into account the effect of lactic acid bacteria growth and its possible competitive inhibition of *L. monocytogenes* - another reason why the model can overestimate growth.

Prevalence of *L. monocytogenes* in RTE Smoked fish

To assess typical prevalence of *L. monocytogenes* contamination in RTE smoked fish products, a dual approach was used to identify relevant studies. A literature search of Pubmed using the search terms Listeria AND smoked fish was used to identify surveys looking at prevalence of *L. monocytogenes* in smoked fish products, the date range was limited to 2000 to present. The search term was kept intentionally wide to ensure that any relevant studies could be identified. In total 201 studies were recovered by the search, and 23 of these were identified as potentially relevant. This data was considered alongside UK data identified with the help of FSA and FSS colleagues. Full text was accessed either via publicly available full-text, or via the NHS Scotland OpenAthens service.

Prevalence in cold smoked or hot smoked product

The last large UK wide survey of *L. monocytogenes* in smoked fish was carried out in 2006 with the results published in 2008 (FSA, 2008). Both cold and hot smoked fish were included in this survey, with *L. monocytogenes* detected in 17.6% of cold smoked fish samples (236/1344) and 3.4% of hot smoked fish samples (66/1878). The fish species represented in each category were not detailed (FSA, 2008).

Although there are a range of publications reporting prevalence of *L. monocytogenes* in smoked fish in retail samples from various European countries, only one peer-reviewed paper was identified that specified if the smoked fish product sampled was hot or cold smoked, and provided results for both (Lambertz et al., 2012) (discussed below). In this survey carried out in Sweden in 2010 *L. monocytogenes* was detected in 15.5% of cold smoked fish samples (n=206), and 1.8% of hot smoked fish samples (n=113) and these figures are broadly similar to those reported for the UK (FSA, 2008; Lambertz et al., 2012).

The data reported by Lambertz et al. (2012) was a combination of both a larger national survey and a smaller set of samples taken to contribute to the EFSA study ‘Analysis of the baseline survey on the prevalence of Listeria monocytogenes in certain ready-to-eat foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates’ which was published in 2013 (EFSA, 2013). Countries responding to this survey (26 EU Member States, which included the UK at that time, and 1 non-member) were asked to specify if prevalence data was from cold or hot smoked fish. The prevalence of *L. monocytogenes* in cold smoked fish was recorded as 17.4% (104/599) and 6.3% in hot smoked fish (33/525), although for an additional 1625 samples the type of smoking was unknown (EFSA, 2013). This data supports the conclusion of the information on production processes presented in section 4.1, that hot smoked fish is less likely to be contaminated with *L. monocytogenes*, but that it cannot be assumed to be absent, possibly due to contamination following the smoking process.

The Scottish Food Sampling Database (SFSD) collates results from Local Authority testing in Scotland. The results for *L. monocytogenes* testing in smoked fish (2014-2021) suggests a lower prevalence than the 2008 FSA survey, with 4.7% in cold smoked fish (6 /129 samples) compared to 0.7% for hot smoked fish (2/267 samples) and 2.5% (17/690) where the smoking method was not specified. SFSD can be considered a composite database comprising data from various different sampling programmes including FSS surveillance and Local Authority enforcement and surveillance sampling. The sampling effort is therefore variable from year to year and will also change in terms of how targeted the sample collection is, depending on the type of survey/sampling being conducted and for what reasons. For this reason these data cannot be seen as representative, and results broken down by year have not been presented as, due to the nature of the dataset, any annual trends in prevalence are unlikely to be meaningful.

Effect of fish species on prevalence

The FSA survey did not define which species of smoked fish were included in the UK-wide study (FSA, 2008), but information on species was provided in other publications identified as part of

the literature search. However, it is still difficult to summarise if there is any effect of species of fish on the prevalence of *L. monocytogenes* detected. For example, the prevalence of L. monocytogenes in cold smoked trout was reported to be both higher and lower than that in cold smoked salmon: 26.3% (10/38 samples) compared to 17.7% (16/90), and 0% (0/15 samples) compared to 19% (8/42) (Dominguez, Gomez and Zumalacarregui, 2001; Van Coillie et al., 2004) or *L. monocytogenes* was reported to be absent in both cold smoked trout and salmon (González-Rodríguez et al., 2002). One issue in identifying any effect of fish species on prevalence of *L. monocytogenes* is the very small sample numbers included in some studies, and the tendency for the vast majority of samples to be cold-smoked salmon (or salmon where hot or cold smoking is not defined). For example, in their EU-wide survey, EFSA reported that 71% of cold smoked samples were salmon, 9% mackerel, 4.4% herring and 15% other or mixed fish (EFSA, 2013). As illustrated for trout above, where authors indicate higher prevalence of *L. monocytogenes* in smoked fish products from some species over others, care must be taken to be aware of the sample numbers. For example, Van Coillie et al. (2004) reported that the prevalence of *L. monocytogenes* in smoked salmon was 19% (8/42 samples) which is broadly consistent with other studies identified, but that 33.3% (6/18 samples) of smoked halibut samples contained detectable levels of *L. monocytogenes*. However, in their conclusions Van Coillie et al. (2004) indicate that at least 4 of the 6 positive halibut samples come from the same producer over a 4 month period suggesting a persistent strain in one processing facility, as opposed to a particular risk associated with smoked halibut.

For products such as smoked eel where prevalence of 50% was reported (2/4 samples), or swordfish where prevalence of 25% was reported (1/4 samples) no meaningful conclusions can be drawn around the true levels of prevalence in these more unusual smoked fish products due to the low sample size (Uyttendaele et al., 2009; Acciari et al., 2017).

Effect of country or manufacturer on prevalence

As suggested by the study from Van Coillie et al. (2004) above, one supplier experiencing contamination problems can give an artificially high indication of prevalence or give a distorted picture of the real risk consumers are exposed to across smoked fish products from different manufacturers, and this was recognised by other authors. Acciari et al. (2017) report significant differences in prevalence of *L. monocytogenes* in smoked salmon between both different countries and different manufacturers, with frequency of detection from different manufacturers varying from 0 to 76.9%. Products from twelve different European countries were captured as part of their sampling, and they reported significant differences between prevalence of *L. monocytogenes f*rom different countries, although no details were given of countries with higher or lower prevalence so this cannot be accounted for in considering the risk to UK consumers (Acciari et al., 2017). Another study (Swedish) also reported significant variation between prevalence of product from different countries and manufacturers, and highlighted that prevalence was far higher in samples processed overseas (45%) as opposed to domestically (Sweden, 8%), but that these figures were skewed by roughly two-thirds of overseas samples coming from one manufacturer where 50% of samples tested positive (Lambertz et al., 2012). Although Lambertz et al. (2012) stated that this manufacturer was an approved establishment in an EU Member State, no further details are given to help identify the significance of this large manufacturer to the UK consumer. The findings of these studies are supported by the reported outbreaks in Section 3.3, which affected consumers in multiple EU countries, adding weight to the consideration that contamination at one large processor could skew prevalence data and affect a large number of UK consumers.

Additionally heterogeneous distribution of *L. monocytogenes* contamination within batches was highlighted by a number of authors who tested duplicate sealed packets at the start and end of shelf life, indicating that truly representative sampling of batches is likely to be difficult for businesses to achieve (Uyttendaele et al., 2009; Lambertz et al., 2012; Acciari et al., 2017).

Effect of packaging or further handling on prevalence

Some evidence was found on the effect of packaging or further handling on the prevalence of *L. monocytogenes i*n smoked fish products. Dominguez et al. (2001) reported a prevalence of *L. monocytogenes* of 17.7% (16/90 samples) in vacuum-packed smoked salmon, but that this rose to 28.5% (12/42 samples) where packaging had been opened prior to sale (for example for sale on a deli counter). Similarly, Uyttendaele et al. (2009) reported detection of *L. monocytogenes* in 27.8% (25/90) of packaged smoked fish samples, compared with 56.9% prevalence (33/58 samples) in smoked fish used as a raw material in deli-salad preparation, although the breakdown of smoked fish species included in these numbers were not specified. Although a small sample size, Van Coillie et al. (2004) reported that *L. monocytogenes* was detected in 3 of the 6 smoked salmon salads that they tested, in comparison to 19% (8/42) of packaged smoked salmon samples. A similar pattern to these European findings was recorded in Singapore, where *L. monocytogenes* was detected in 21.6% (37/171) packaged smoked salmon samples but 86.7% (13/15) of samples taken from unsealed product from salad bars (Chau et al., 2017). The smoked salmon sampled in Singapore originated from Australia, Chile, Denmark, Ireland, Korea, New Zealand, Norway and Scotland (Chau et al., 2017). Although limited in scope and scale, the evidence presented here suggests that the likelihood of detection of *L. monocytogenes* in smoked fish in deli settings for salad preparation is higher than for vacuum-packed smoked fish, and that this category may represent a further risk to vulnerable consumers. This higher prevalence may be linked to suboptimal storage of the product, additional handling leading to further scope for contamination or lower quality product being diverted into the deli sector, but it is suggested that the initial contamination is likely to originate from manufacture (Dominguez, Gomez and Zumalacarregui, 2001; Van Coillie et al., 2004; Uyttendaele et al., 2009; Chau et al., 2017).

Level of *L. monocytogenes* in products

Due to differing research questions, and differences in the way data is presented, it is not possible to provide a simple comparison of the frequencies of different levels of *L. monocytogenes* as presented by different authors. Recognising these difficulties, data has been complied in Table 13 below where authors have provided information on enumeration of samples.

Table 13. Prevalence of *L. monocytogenes* reported by authors and enumeration of levels >100 CFU/g

Reference	Country	Species	Detection	Enumberation >100 CFU/g
Uyttendaele et al., 2009	Belgium		27.8% (25/90)	4/25
Acciari et al., 2017	Italy		20.2% (157/778)	26/157
Dominguez et al., 2001	Spain		22.3% (38/170)	20/38
Lambertz et al., 2012	Sweden		Overall 12% (66/558), Cold smoked fish 15.5% (32/206), gravad fish 14.4% (28/194), hot smoked fish 2% (2/113). Plus 4 positive results where smoking process was unknown.	3/558
Van Coillie et al., 2004	Belgium`		23% (20/87)	4/20

The studies included in Table 13 distinguished where samples had a level > 100 CFU/g, linking this to the requirement specified in Regulation 2073/2005. However, the presentation of results beyond this threshold varied per author. Acciari et al. (2017) reported maximum levels of contamination of 1.3 x 10^6 at the end of shelf life, and 1.0 x 106 at the start of shelf life (it is unclear if these are for smoked salmon or smoked swordfish samples, although the study included 774 salmon and 4 swordfish samples). Dominguez et al. (2001) reported that of 20 positive samples that contained levels > 100 CFU/g, 18 were between 100 - 1000 CFU/g with the level in one cold smoked salmon sample recorded as 1,100 CFU/g, and one cold smoked trout as

1,700 CFU/g. Lambertz et al. (2012) recorded 3 samples with levels of *L. monocytogenes* > 100 CFU/g, one cold smoked salmon sample at 130 CFU/g, and 2 gravad salmon samples at 350 and 2500 CFU/g.