

Listeria monocytogenes in Aquatic Food Products—A Review

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Abstract: With the increased demand for lightly preserved and/or ready-to-eat (RTE) food products, the prevalence of the foodborne pathogen *Listeria monocytogenes* has increased, which is a public health concern. The goal for this review is to discuss the incidence, epidemiological importance, and contamination routes of *L. monocytogenes* in various aquatic ecosystems, seafood products, and processing environments and to summarize the data obtained since the 1990s. *L. monocytogenes* primarily enters the food-production chain by cross-contamination in production plants, making this pathogen a major threat to the seafood industry. This pathogen generally contaminates food products at low or moderate levels, but the levels involved in listeriosis outbreaks are significantly higher. The majority of isolates from aquatic products belong to serotype 1/2a, and outbreaks have been linked to highly similar or even indistinguishable strains. Several seafood-processing plants are colonized by specific “in-house” flora containing special DNA subtypes of *L. monocytogenes*. In such cases, *L. monocytogenes* populations can persist and/or multiply despite the inherent obstacles to their growth in food preservation and manufacturing operations. Therefore, food-processing facilities must be designed carefully with an emphasis on effective cleaning and disinfecting operations in the production line.

Keywords: listeriosis, *Listeria monocytogenes*, prevalence, RTE, seafood

Introduction

The consumption of seafood products has increased recently because of increased consumer awareness of nutrition and food quality. Nutritionists recommend seafood because of its high nutritional value. Seafood is an excellent source of high-quality protein and contains lipids with high levels of unsaturated fatty acids, which are claimed to reduce the risk of cardiovascular disease. In addition, seafood is tender, easily digested, and a good source of many important vitamins and minerals (Ghanbari and others 2013). In part because a large proportion of seafood products originate in developing countries, there are significant food safety concerns, which have led to the creation of official standards and enforcement of regulatory procedures for seafood production. The global proliferation of pathogens through seafood is a major hazard. To ensure good economic returns, improvement of seafood quality and safety is paramount for processing companies and exporting countries (Norhana and others 2010). Seafood frequently triggers regulatory alerts in importing countries. The Rapid Alert System for Food and Feed (RASFF) in the EU has indicated that compared with other food product categories, seafood is second only

to vegetables in the number of alerts activated between 2009 and 2012. Seafood import rejections by the RASFF comprised approximately 15% of the total product rejections in 2012 (Anonymous 2013). Based on the number of such cases in the United States, EU, and Japan, foodborne bacterial pathogens are the principle cause of product rejection and detention in the international seafood trade (Ababouch and others 2005). The bacterium *Listeria monocytogenes* has caused product detention in 4% of the cases recorded worldwide (Huss and others 2004). Furthermore, contamination with *L. monocytogenes* has also led to product recalls (Zhu and others 2005; Norhana and others 2010). Therefore, *L. monocytogenes* contamination has a significant impact on seafood trade; it causes direct and indirect financial losses because it necessitates sample reinspection as well as analysis and review of records, which can lead to product expiration and introduce costs associated with product recalls (Norhana and others 2010).

There is no consensus on the “acceptable levels” of *L. monocytogenes* in food (Table 1). As shown in Table 1, the criteria differ significantly among regions and food authorities. Although the seafood production chain faces several threats, only a few microbial species, including *Listeria* spp., *Salmonella* spp., and *Vibrio* spp., have been thoroughly studied with respect to their prevalence and impact on regulations and public health (Ghanbari and others 2013). This review discusses studies of *L. monocytogenes* contamination at different stages of seafood production based on the farm-to-fork principle, including data from experimental studies and surveys from multiple sources (<http://www.sciencedirect.com> and <http://www.pubmed.com>). Studies including reviews and other publications between 1990 and 2013 were critically evaluated. The

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Table 1—Overview on microbiological criteria and guidelines for *Listeria monocytogenes* in RTE food products including seafood.

Country	Food category	Microbiological limit	Reference
Australia and New Zealand	Food Group 1—RTE foods that will support the growth of <i>L. monocytogenes</i> and have been stored prepared for more than 1 d.	Absence in 25 g ($n = 5, c = 0$)	FSANZ (2001)
	Food Group 2—RTE food that will not support the growth of <i>L. monocytogenes</i> and has been stored prepared for more than 1 d.	Absence in 25 g if present < 100 CFU* ($n = 5, c = 0$)	
	Food Group 3—RTE food that will be consumed immediately and has not been stored prepared for more than 1 d.	Absence in 25 g if present < 100 CFU* ($n = 5, c = 0$)	
Canada	Category 1 (high priority for oversight)—RTE fish products (the growth of <i>L. monocytogenes</i> can occur and could exceed 100 CFU*/g before the end of the stated shelf life), for example, refrigerated seafood pâtés or mousses.	Absence in 25 g ($n = 5$)	Health Canada (2011b)
	Category 2A (medium to low priority for oversight)—RTE fish products (the growth of <i>L. monocytogenes</i> can occur but would not exceed levels greater than 100 CFU*/g before the end of the stated shelf life), for example, smoked salmon, sushi.	< 100 CFU*/g in 10 g ($n = 5$)	
	Category 2B (low priority for oversight)—RTE fish products (the growth of <i>L. monocytogenes</i> cannot occur throughout the shelf life), for example, frozen shrimp, pickled herring.	< 100 CFU*/g in 10 g ($n = 5$)	
China	For food under refrigeration (excluding frozen food) or food intended for infants.	Absence in 25 g	Anonymous (2007)
	For other RTE foods.	< 20 CFU*/g	
EU	RTE foods intended for infants and RTE foods for special medical purposes.	Absence in 25 g ($n = 10, c = 0$)	(EC 2005) amended by (EC) No. 1441/2007
	RTE foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes.	100 CFU*/g ($n = 5, c = 0$) Absence in 25 g ($n = 5, c = 0$)	
	RTE foods unable to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes.	100 CFU*/g ($n = 5, c = 0$)	
United States	RTE seafood products.	Absence in 25 g	FDA (2011)

m = Threshold below which all results are considered satisfactory.

n = Number of sample units drawn from a lot.

M = Limit above which sampling results are unacceptable.

c = Number of units in the sample giving values between *m* and *M*; the sample being considered acceptable if the values of the other sample units are = or < *m*.

RTE = Ready-to-eat * Colony-forming unit.

present work is the first review focused on listeriology in seafood and aims to describe the possible trends of occurrence and the nature of *L. monocytogenes* in aquatic food products in different environments as well as processing and storage conditions.

Characteristics and Epidemiological Importance of *L. monocytogenes*

Although the bacterial genus *Listeria* currently comprises 10 species, human cases of listeriosis are caused almost exclusively by the species *L. monocytogenes* (European Food Safety Authority 2013). *L. monocytogenes* is an ubiquitously growing Gram-positive bacterium, which occurs naturally in the terrestrial environment, fresh and salt water, livestock manure, decaying plant materials, and in many raw foods associated with these environments (Gram 2001; Zhu and others 2005). Typical physiological characteristics of *L. monocytogenes* include a high tolerance to moderate and high levels of NaCl (up to 28% w/v) for short periods and resistance to freezing temperatures. Therefore, this organism can grow and multiply during refrigeration, whereas other competing organisms cannot, and it is also able to survive at relatively low water activity (Zhu and others 2005; Beaufort and others 2007; Calo-Mata and others 2008; Ghanbari and others 2013).

In the early 1980s, food was recognized as a primary source of *L. monocytogenes* infection in humans (Farber and Peterkin 1991; Latorre and others 2007). Currently, *L. monocytogenes* infection is an important public health concern because of the high mortality rate (20% to 30%) in immunocompromised individuals and the severity of the disease, which is often associated with septicemia, meningitis, gastroenteritis, pneumonia, and spontaneous abortion (Vazquez-Boland and others 2001). In developing countries, this species is one of the most important causes of death from foodborne infections (Jemmi and Stephan 2006). Several foodborne listeriosis outbreaks (in U.S.A., Japan, New Zealand, Germany, England, France, and other countries) have occurred over the past 2 decades, and although *L. monocytogenes* has been observed in a large variety of foods, the greatest threat of this pathogen is associated with refrigerated products that have a long shelf life and products that are generally eaten with little or no prior heating (Bremer and others 2003). Seafood is the first among these high-risk, ready-to-eat (RTE) products (Rocourt and others 2003; Reij and Den Aantrekker 2004). Considering the significant public health implications of listeriosis and the importance of seafood products as a vehicle for *L. monocytogenes*, it is important to examine the incidence of this pathogen (Table 2).

Table 2—Survey of seafood products implicated in human listeriosis outbreaks.^a

Product	Country	Year	Number of cases	Serotype	Reference
Herring cutlet marinated in oil	Germany	2010	8 cases, 1 death ^b		Aichinger (2010)
Fish, vacuum-packed (suspected) ^c	Finland	1999 to 2000	10 cases, 4 deaths	1/2	Hatakka and others (2000)
Smoked rainbow trout	Finland	1999	5 cases	1/2a	Miettinen and others (1999)
Tuna-corn salad	Italy	1997	1566 ^d	4b	Aureli and others (2000)
Imitation crab meat ^e	Canada	1996	2 cases	1/2b	Farber and others (2000)
Cold-smoked rainbow trout (suspected)	Sweden	1994 to 1995	9 cases, 2 deaths	4b	Ericsson and others (1997)
Smoked mussels	New Zealand	1992	3 cases ^f , 1 death	1/2a	Brett and others (1998); McLauchlin and others (2004)
Smoked mussels	Australia (Tasmania)	1991	4 cases	1/2a	Misrachi and others (1991); Mitchel (1991)
Shrimp	United States	1989	2 cases	4b	Riedo and others (1994)
Smoked cod roe	Denmark	1989	1 case	4b	Rocourt (1991)
Fish ^c	Italy	1989	1 case	4	Facinelli and others (1989)
Fish or molluscan shellfish	New Zealand	1980	22 cases, 7 deaths ^g	1b-1/2a ^h	Lennon and others (1984); McLauchlin and others (2004)

^aThere was very limited epidemiological evidence available to confirm seafood as an etiological agent for these cases.

^bThree of eight patients do not remember about the consumption of this particular fish; 1 fatal outcome could be directly linked to fish consumption 3 d before death.

^cnot specified in detail.

^dNon-invasive listeriosis. Possible cross-contamination from other untreated foods.

^eArtificially flavored Alaska pollock.

^fDNA analysis using pulsed-field gel electrophoresis (PFGE) showed that the PFGE patterns of isolates from patients 1 and 2 were indistinguishable from the isolates from the mussels. Patient 3 had a history of consuming mussels and PFGE analysis of isolates of *L. monocytogenes* serogroup 1/2 revealed that the isolates were indistinguishable from the isolates of patients 1 and 2 (Brett and others 1998).

^gThe link was on the basis of recall of food consumption, not microbiological testing.

^hWhile in the first report, the serovar 1b indicated for isolated *L. monocytogenes*, McLauchlin and others (2004) mentioned the serovar 1/2a for the pathogen.

The epidemiological patterns of human listeriosis include a background level of sporadic cases with occasional outbreaks (Gillespie and others 2010; Thomas and others 2012). Notably, reports indicate that fish and shellfish products are frequently contaminated with *L. monocytogenes*. However, compared to listeriosis outbreaks associated with other foods, a low number of cases have been linked to seafood (European Food Safety Authority 2013), potentially due to the following factors: 1) the low numbers of *L. monocytogenes* generally present in seafood, 2) the relatively low volumes of products and the nature of the distribution chain, 3) increased consumer awareness that seafood must be chilled compared to other products, 4) reduced consumption of raw fish, 5) decreased epidemiological tracking efficiency due to long incubation periods after the ingestion of contaminated food, and 6) decreased consumption of high-risk products, such as RTE seafood, by immunocompromised individuals. Scientific analysis of the link between *L. monocytogenes* epidemiological patterns and seafood and seafood-processing environments is important for understanding and managing this microbial risk.

Phenotypic or genetic characterization through subtyping analysis enables identification of the source of infection (Wagner and Allerberger 2003). *L. monocytogenes* is grouped into 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) (FDA 2011). Serotype classification is useful for tracking of *L. monocytogenes* strains linked to disease outbreaks. However, this classification provides limited discrimination during epidemiological investigations because the majority of human listeriosis cases and outbreaks are predominantly associated with 3 serotypes (4b, 1/2b, and 1/2a) (Liu 2006). Larger outbreaks have been primarily associated with the 4b serotype, whereas the serotype 1/2a has been linked to sporadic cases (Lianou and Koutsoumanis 2013). Based on other phylogenetic and subtyping analyses, *L. monocytogenes* isolates are classified into 4 distinct lineages (lineage I, II, III, and IV) (Orsi and others 2011). However, lineage III and IV isolates (mainly serotypes 4a and 4c) are generally underrepresented, potentially due to their attenuated pathogenic potential and different phenotypic properties compared to lineage I and II isolates (Liu 2006; Orsi and others 2011). The majority of human infections are linked to lineage I serotype 4b and 1/2b isolates,

although lineage II serotype 1/2a isolates have caused some listeriosis outbreaks (Orsi and others 2011; Cruz and others 2014). Based on the geographical and temporal distribution of outbreaks, *L. monocytogenes* has been further classified into epidemic clones (ECs) I (lineage I), II (lineage II), III (lineage I), and IV (lineage I). This differentiation enables discrimination of isolates from different serotypes based on their ecological compartments (Orsi and others 2011). Three highly clonal lineage I serotype 4b strains have caused recurrent global outbreaks (ECs I, Ia, and II) (Cruz and others 2014).

Specific *L. monocytogenes* lineages have infectious potential including stress-resistance (for example, in food-associated environments). This finding has led to studies of the virulence characteristics of this pathogen in relation to different food types. Internalins are responsible for the internalization of *L. monocytogenes* into epithelial cells (*InlA*); mutations in such virulence genes, which are components of the well-documented virulence cycle, might attenuate the virulence potential (Orsi and others 2011; Kovacevic and others 2013). Cruz and others (2014) screened for the presence of *inlA* mutations (premature stop codons [PMSCs]) in *L. monocytogenes* isolated from New Zealand (NZ) mussel-processing environments to estimate the risk to human health. PMSCs might impact the environmental survival of *L. monocytogenes*. This analysis revealed that lineage II serotype 1/2a strains, typically isolated from food and the environment, are more adapted to food-chain-related conditions than 4b serotypes with rare PMSC mutations (Kovacevic and others 2013). Notably, isolates from NZ individuals and seafood-processing environments also harbored *inlA* PMSC mutations. Therefore, the environmental strains associated with seafood processing are closely related to human isolates, posing a potential health risk to humans (Cruz and others 2014).

The diversity of *L. monocytogenes* strains is often specific to the processing environment (FDA 2011; Tocmo and others 2014). Furthermore, there is a significant association between serotypes and listeriosis in patients (McLauchlin 1990). Studies of *L. monocytogenes* isolated from seafood outbreaks and seafood-processing environments have shown that >90% of human listeriosis cases are caused by serotypes 1/2a, 1/2b, 1/2c, and 4b (Table 1). A Swedish survey of RTE retail food showed that

L. monocytogenes pulsotypes (PFGE) from gravad and smoked fish were the most common among human pulsotypes. This observation is consistent with the increased incidence of listeriosis in Scandinavian countries compared with European countries because of higher RTE-fish consumption rates (Lambertz and others 2013). Furthermore, the majority of isolated *L. monocytogenes* strains belong to serotype 1/2a, which is common in food isolates (Orsi and others 2011). Johansson and others (1999) reported that 86% of the *L. monocytogenes* strains isolated from smoked fish in Finland belonged to serotype 1/2a, whereas 14% of the isolates belonged to serotype 4b. In a Swedish survey conducted by Gudmundsdottir and others (2005), the serotypes 1/2a, 1/2b, and 4b were isolated from different locations in a fish-processing plant. Handa and others (2005) observed that the majority (80%) of *L. monocytogenes* isolated from the roe of cod and salmon belonged to serotype 1/2a. Corcoran and others (2006) observed that the predominant serotype of *L. monocytogenes* detected in smoked salmon in Ireland belonged to 1/2a (>95%), and the other isolates were serotypes 4b and 1/2c. Several studies have observed the prevalence of specific serotype patterns in different seafood products. Miya and others (2010) observed that 21 of 39 *L. monocytogenes* strains isolated from minced tuna, salmon roe, and cod roe predominantly belonged to the serotype 1/2a, followed by the serotypes 3a, 1/2b, 3b, and 4b. *L. monocytogenes* strains isolated from RTE seafood in Italy are predominantly grouped into 4 serotypes: in 15 studied strains, the highest percentage belonged to serotype 1/2a (73.33%), followed by serotype 4b (13.33%), 1/2b (6.67%), and 4d (6.67%) (Gambarin and others 2012). Among 101 *L. monocytogenes* strains isolated from different mussel-processing environments in NZ, 96% were assigned to the serotypes 1/2a or 3a, and 4% were identified as 1/2b, 3b, or 7. Further subtyping by PFGE revealed plant-specific clusters, and 1 persistent pulsotype was associated with 2 nonperinatal cases of listeriosis (Cruz and Fletcher 2011).

Prevalence of *L. monocytogenes* in Water, Aquatic Food Products, and Processing Environments

Although *L. monocytogenes* is not considered a marine organism, it can be isolated from marine water (most likely due to runoff from the land), vegetation, sewage, animal feeds, farm environments, and food-processing locations (Ben Embarek 1994; Gram 2001). Therefore, it is very likely that fish, fish-processing environments, and final seafood products are contaminated (Gram 2001).

Water

Run-off water, particularly water that has passed through agricultural areas, is proposed to serve as the main route for the influx of *L. monocytogenes* into surface waters such as lakes, streams, and rivers (Gram 2001; Lyautey and others 2007b). Other sources of contamination, such as animal feces, may also increase the abundance of the pathogen in aquatic systems because animals such as ruminants shed *L. monocytogenes* in their feces (Hutchison and others 2004; Lyautey and others 2007a, 2007b). Lyautey and others (2007b) observed a significant direct link between the number of dairy farms in a catchment and increased *Listeria* prevalence in surface waters. Upstream proximity to a dairy plant was observed as another risk factor, and the majority of *L. monocytogenes*-positive water samples were located within a few kilometers downstream of a dairy plant. The researchers concluded that agricultural activities, such as the use of livestock manure followed by soil disruption, affect the rate of *Listeria* prevalence in surface waters (Lyautey and others 2007a, 2007b). However, these data were inconsistent with

studies by Hansen and others (2006) and Gonzalez and others (1999), who did not observe *Listeria* in freshwater streams feeding into several Danish fish farms. These observations were consistent with studies of water samples collected from 9 rivers in Spain. Nevertheless, these data suggest that agricultural activity is a risk factor for the contamination of surface waters with this pathogen, but the increased risk is not necessarily correlated with farming (Jemmi and Keusch 1994).

Climate conditions have a potential effect on the prevalence of *Listeria* spp., particularly *L. monocytogenes*. Among different climate conditions, rainfall is associated with the largest increase in bacterial contamination of surface waters (Mallin and others 2009; Reifel and others 2009; Sinclair and others 2009; Stumpf and others 2010). Therefore, seafood farms have potentially greater risk of *Listeria* contamination because brooks, rivers, and other surface waters enter such farms after heavy rainfall (Miettinen and Wirtanen 2006). The authors speculated that both soilborne *Listeria* and *L. monocytogenes* commonly present in livestock manure are simply washed into rivers and surface waters upstream of the fish farms, which is consistent with the link between surface-water contamination and farming activity. In their comprehensive study, 510 rainbow trout samples from fish farms in lakes and sea areas around Finland were screened for the presence of *L. monocytogenes*. The authors accurately highlighted the sporadic and rapid appearance and disappearance of *L. monocytogenes* in aqueous environments during different seasons (Miettinen and Wirtanen 2005).

Although most studies have observed *L. monocytogenes* in freshwater ecosystems, El-Shenawy and El-Shenawy (2006) examined *L. monocytogenes* contamination in fish farmed in freshwater and those with a partial or complete life cycle in seawater. The authors observed a correlation between the rates of contamination of *L. monocytogenes* in the Agba and Suez Gulf and in the Red Sea and a trend toward higher contamination levels in urban areas or areas with industrial/tourism activities. These observations suggested that the discharge of untreated sewage into seawater was involved in contamination of fish with *L. monocytogenes* (El-Shenawy and El-Shenawy 2006). Recently, Thomas and others (2012) reviewed studies of the presence of *L. monocytogenes* in water and concluded “*L. monocytogenes* can be isolated from fresh surface waters, including 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.”

Fish

Listeria spp. are components of the indigenous microflora in surface water and other water bodies connected to rivers. Therefore, these microorganisms are most likely present on the external surface of fish that swim in contaminated water. *L. monocytogenes* has been observed on the fish surface and in the stomach lining, gills, and intestines, but the flesh is usually free of the organism unless it has been contaminated from different sources. In general, there are 2 possible routes for contamination of fish with *Listeria*: (1) the spread of *Listeria* from the intestinal contents to other fish tissues (including muscles), especially if the period between death and viscera removal is greater than a few hours; (2) cross-contamination (due to manipulation of fish using contaminated equipment and to inappropriate transport) (Gudmundsdottir and others 2006; Souza and others 2008). Therefore, contaminated raw materials can also affect the final products, especially RTE-seafood products (Miettinen and Wirtanen 2005). *L. monocytogenes* is prevalent in raw fresh fish in several countries, but the level of contamination

Table 3–Reported prevalence data of *L. monocytogenes* in raw fish, raw shellfish, and bivalves.

Reference	Country	Product	N*	P**	[%]***
<i>Fresh fish</i>					
Chen and others (2010b)	USA	Catfish fillet	30	13	43.3
Chou and others (2006)	USA	Channel catfish fillet	240	90	37.5
Parihar and others (2008a)	India	Marine fish	43	6	14.0
Miya and others (2010)	Japan	Minced tuna	116	14	12.1
Mena and others (2004)	Portugal	Raw fish	25	3	12.0
Beleneva (2011)	Japan and south China sea	Marin fish	44	5	11.4
Mora and others (2006)	Spain	Marin fish (fresh steak and fillet)	50	5	10.0
Medrala and others (2003)	Poland	salmon and sea trout	72	6	8.3
Swetha and others (2012)	India	Fish	25	2	8.0
Yadollahi and others (2013)	Iran	Fish	220	17	7.7
El-Shenawy and El-Shenawy (2006)	Egypt	Fish	40	3	7.5
Busani and others (2005)	Italy	Fish and fish products	3160	204	6.5
Wang and others (2011)	USA	Tilapia (fresh and frozen)	70	3	4.3
Thimothe and others (2002)	USA	Whole craw fish	78	3	3.8
Miettinen and Wirtanen (2005)	Finland	Rainbow trout pooled sample	510	15	2.9
Gawade and others (2010)	India	Finfish	69	2	2.9
Handa and others (2005)	Japan	Marin fish	109	3	2.8
Davies and others (2001)	Europe	Marine and fresh water fish	76	2	2.6
Molla and others (2004)	Ethiopia	Fish	48	1	2.1
Siriken and others (2013)	Turkey	Anchovy	50	1	2.0
Kwiatek (2004)	Poland	Raw fish	633	8	1.3
Dhanashree and others (2003a)	India	Marin fish	86	1	1.2
Soultos and others (2007)	Greece	Marine fish	120	1	0.8
Wang and others (2012)	Taiwan	Fish	30	0	-
Kuzmanovic and others (2011)	Serbia	Fish (sea and fresh water)	43	0	-
Stonsaovapak and Boonyaratanakornkit (2010)	Thailand	Fish	20	0	-
Jalali and Abedi (2008)	Iran	Fresh fish	61	0	-
Van Coillie and others (2004)	Belgium	Herring fillets	5	0	-
Laciar and de Centorbi (2002)	Argentina	Mackerel	26	0	-
<i>Shellfish</i>					
Cordano and Rocourt (2001)	Chile	Fresh shrimp	59	17	28.8
Gudmundsdottir and others (2006)	Iceland	Shrimp	43	9	20.9
El-Shenawy and El-Shenawy (2006)	Egypt	Shellfish	15	3	20.0
Beleneva (2011)	Russia	Shrimp	21	3	14.3
Parihar and others (2008a)	India	Crab and shrimp	11	1	9.1
Moharem and others (2007)	India	Fresh shrimp (<i>Paenus monodon</i>)	30	2	6.7
Pagadala and others (2012)	Australia	Raw carb	488	22	4.5
Gawade, Barbuddhe and Bhosle (2010)	India	Prawns	26	1	3.8
Thimothe and others (2004)	USA	Salmon	234	9	3.8
Inoue and others (2000)	Japan	Shellfish	253	7	2.8
Wang and others (2011)	USA	Shrimp	38	1	2.6
Yadollahi and others (2013)	Iran	Shrimp	40	1	2.5
Siriken and others (2013)	Turkey	Mussel	50	1	2.0
Wang and others (2012)	Taiwan	Octopus and shrimp	60	0	-
Stonsaovapak and Boonyaratanakornkit ((2010)	Thailand	Shrimp	20	0	-
Modaresi and others (2011)	Iran	Crustacean (<i>Astacus leptodactylus</i>)	12	0	-
Minami and others (2010)	Thailand	Shrimp	43	0	-
Mena and others (2004)	Portugal	Shellfish	8	0	-
Jalali and Abedi (2008)	Iran	Shrimp (fresh and frozen)	12	0	-
Handa and others (2005)	Japan	Shrimp	8	0	-
Dhanashree and others (2003a)	India	Crab	25	0	-
Busani and other (2005)	Italy	Crustaceans	496	0	-
<i>Bivalves</i>					
Di Ciccio and others (2012)	Italy	Salmon	21	5	23.8
Gawade and others ((2010)	India	Bivalves	16	2	12.5
Dhanashree and others (2003a)	India	Clams	24	1	4.2
Beleneva (2011)	Russia	Bivalves	74	3	4.1
Pinto and others (2006)	Portugal	Bivalves	75	3	4.0
Minami and others ((2010)	Thailand	Oyster	48	0	-
Handa and others (2005)	Japan	Bivalve and mollusks	37	0	-
Stonsaovapak and Boonyaratanakornkit ((2010)	Thailand	Squid	20	0	-
Laciar and de Centorbi (2002)	Argentina	Mussel	15	0	-

*Number of samples tested, **Number of positive samples, ***Percent positive samples.

tends to be low and varies between 0% and approximately 30% of the products (Miettinen and Wirtanen 2005; Thomas and others 2012; Table 3). Gram (2001) reviewed studies that observed large variations; 1% to 34% of the raw fish samples entering fish-processing plants were positive for *L. monocytogenes*. Davies and others (2001) observed that *L. monocytogenes* was present in 3% of European fish. The prevalence of *Listeria* spp. was 30% in freshwater fish samples and 10.4% in marine fish samples in Turkey

(Yucel and Balci 2010). *L. monocytogenes* (44.5%) and *L. murrayi* (83.5%) were the most commonly isolated species from freshwater and marine fish samples, respectively. Wang and others (2011) observed that the *L. monocytogenes* contamination rate in fresh fish (including salmon and tilapia) was 4.1% (4.8% in salmon and 4.3% in tilapia).

Notably, the rate of contamination of raw fish might vary among different geographical areas and processing plants. Furthermore,

unstandardized sampling procedures and different sampling techniques are among the most frequent factors affecting the reported prevalence of contamination. Jemmi and Keusch (1994) studied the water resource and management practices of 2 rainbow trout farms; in their study, *L. monocytogenes* was not observed in 30 fish skin samples or 30 fecal content samples from the farm using concrete-walled ponds and hygienic management practices. In contrast, in the farm that used river water and naturally (earth) walled ponds, 33.33% and 40% of skin and fecal samples, respectively, contained *L. monocytogenes* (Jemmi and Keusch 1994). However, no *L. monocytogenes* isolates were observed in samples obtained from salmon in a Norwegian coastal fish farm (Ben Embarek 1994). Mędrala and others (2003) observed that among 72 samples of Norwegian coastal cage-bred salmon and sea trout entering a Polish processing plant, only 6 samples contained *L. monocytogenes*.

The location of *Listeria* in fish carcasses and the place of sampling are considered to affect *Listeria* screening results. Several studies have used samples from the fish surface to generate prevalence data; however, this approach may not always yield accurate results. There is evidence that gill-filtered water containing small quantities of *L. monocytogenes* may concentrate the cells on the gas-exchange surfaces of the gill. When fish are contaminated with low numbers of *L. monocytogenes*, or when contamination is sporadic, selective sampling of the gills rather than the skin yields more accurate results (Miettinen and Wirtanen 2005; Thomas and others 2012). Miettinen and Wirtanen (2005) observed that among 510 fish, 43 gills tested positive for *L. monocytogenes*, whereas only 1 skin sample and 1 visceral sample tested positive for *L. monocytogenes*. Conversely, in Turkey, *L. monocytogenes* has been frequently isolated from both gill (25%) and skin (52%) samples of raw freshwater and marine fish ($n = 30$) (Yücel and Balci 2010), which included brown trout and horse mackerel.

At retailers, wholesalers, and importers, fresh and repackaged fish products may be susceptible to frequent *L. monocytogenes* contamination (Table 3). The pathogen may be acquired from food-contact surfaces and/or by secondary contamination from site equipment; the prevalence of this type of contamination varies from very low levels to 14% (Mena and others 2004; Handa and others 2005; Parihar and others 2008b). This difference is most likely caused by differences in sampling procedures and analytical methods. In a survey of minced tuna collected from retail stores in Japan between 2002 and 2003, *L. monocytogenes* was present in 14.3% of the raw material (Handa and others 2005). In several U.S. market surveys, the prevalence of *L. monocytogenes* ranged from 0% to 12.5% (Abeyta 1983; Pao and others 2008).

Shellfish

Shellfish are another important potential source of foodborne illness. Because of the manner in which they feed, shellfish can accumulate bacteria from polluted aquatic environments. Therefore, pathogens such as *Listeria* may be frequently present in shellfish (Berry and others 1994; Ghanbari and others 2013). The transmission of *L. monocytogenes* via shellfish, either as a carrier or a direct source, is a component of a proposed cycle for infection of humans with *L. monocytogenes* (Norhana and others 2010). *L. monocytogenes* is also associated with shrimp and shrimp products, and the contamination ranges from low levels to 50% (Table 3). Cordano and Rocourt (2001) detected *L. monocytogenes* in 28% of the fresh shrimp in Chile. In Iceland, *Listeria* was observed in 20.9% of the fresh shrimp studied (Gudmundsdottir and others 2006). However, several researchers (Dhanashree

and others 2003b; Moharem and others 2007; Jalali and Abedi 2008) did not observe *L. monocytogenes* in fresh shrimp samples. Although these products might contain *L. monocytogenes*, they do not pose a risk to the majority of consumers because they are generally cooked before consumption, similar to other aquatic food products.

Lobsters, crabs, and crawfish are sources of *L. monocytogenes* (Table 3). Thimothe and others (2002) monitored the presence of *L. monocytogenes* in raw and whole crawfish and in cooked crawfish meat in 2 processing plants. These authors observed that only 3 of the 78 raw material samples (3.8%) were positive for *L. monocytogenes*, and all the processed products sampled were negative for *L. monocytogenes*. The authors concluded that heat treatment of the raw material during processing and practices to avoid postprocessing recontamination could significantly decrease *Listeria* spp. contamination in the final product. Recently, another study observed that 4.5% of the raw crabs sampled in the U.S.A. contained *L. monocytogenes* (Pagadala and others 2012).

Among shellfish, mussels are the dominant source of *L. monocytogenes* (Brett and others 1998; Laciari and de Centorbi 2002; Pinto and others 2006). Pinto and others (2006) isolated *L. monocytogenes* from 4% of the bivalve mollusk samples marketed in Portugal. However, in a study conducted in Japan, most of the live mussels examined were *Listeria*-negative (Table 3) (Handa and others 2005).

Lightly preserved seafood

Lightly preserved seafood products (LPSPs) constitute a broad group of chilled, stored RTE foods with a pH > 5.0 and <6% NaCl in the water phase of the product (Lyhs and others 2002). Over the last decade, *L. monocytogenes* has been frequently isolated from RTE and LPSPs, including cold- and hot-smoked salmon, gravad salmon, fermented fish, and fish salads (Table 4 and 5). This observation highlights the high risk of transmission of *Listeria* spp.

Smoked seafood products are among the RTE foods identified as potential carriers of *L. monocytogenes* (Basti and others 2006) (see also Table 4). Several factors result in contamination of smoked seafood products: (a) the relatively high prevalence of *Listeria* spp. immediately after final packaging; (b) the ability of the pathogen to grow on smoked fish; (c) a production process providing multiple opportunities for contamination or recontamination; and (d) the tolerance of *Listeria* to refrigerated temperatures for long periods.

Because *L. monocytogenes* is a significant food safety concern, the prevalence of *L. monocytogenes* in smoked fish products in Europe has been extensively studied. The presence of *L. monocytogenes* in cold-smoked salmon has been examined by legislators, researchers, and food-control agencies. The overall reported prevalence rate for *L. monocytogenes* in cold-smoked salmon is approximately 10% (Rorvik and Yndestad 1991; Vogel and others 2001; Gram 2001; González-Rodríguez and others 2002; Azevedo and others 2005; Gudmundsdottir and others 2005; Miettinen and Wirtanen 2006); however, other researchers, including Di Pinto and others (2010) and Vitas and others (2004), observed a much higher prevalence (34.1% and 28%, respectively). The overall rate is consistent with studies (with reported incidence levels between 5% and 35%) in specific EU member states (European Food Safety Authority 2013). Different fish species and seafood products have different intrinsic characteristics (pH, water activity, salt concentration, and so on) that affect the presence and resistance of *L. monocytogenes* (Uyttendaele and others 2009); these

Table 4—Reported prevalence of *L. monocytogenes* in smoked seafood products.

Reference	Country	Product type	N*	P**	[%]***
<i>Smoked seafood</i>					
Eklund and others (1995)	USA	Cold-smoked salmon	61	49	80.3
Fletcher and others (1994)	New Zealand	Packed Cold-smoked salmon	13	10	76.9
Hudson and others (1992)	New Zealand	Smoked salmon	12	9	75.0
Medrala and others (2003)	Poland	Sliced VP cold-smoked salmon	44	27	61.4
(Fletcher and others (1994)	New Zealand	Hot-smoked fish	13	5	38.5
Jorgensen and Huss (1998)	Denmark	Cold-smoked fish	210	74	35.2
Di Pinto and others (2010)	Italy	Smoked salmon	132	45	34.1
Johansson and others (1999)	Finland	Cold-smoked and salted trout	62	21	33.9
Farber (1991)	Canada	Cold-smoked salmon	32	10	31.3
Amirkhanlou and others (2011)	Iran	Smoked fish silver carp ^a gutted	30	9	30.0
Peiris and others (2009)	Sweden	Cold-smoked salmon VP and MAP	25	7	28.0
Vitas and Garcia-Jalon (2004)	Spain	Sliced smoked fish salmon	100	28	28.0
Basti and others (2006)	Iran	Smoked fish	87	22	25.3
Salihu and others (2008)	Nigeria	Smoked fish	115	29	25.2
EFSA (2014)	Hungary	Smoked fish	173	39	22.5
EFSA (2014)	Germany	Cold-smoked fish	777	174	22.4
Dass and others (2011)	Republic of Ireland	VP-sliced cold-smoked salmon	120	26	21.7
Van Coillie and others (2004)	Belgium	Smoked fish salmon	81	17	21.0
Wagner and others (2007)	Austria	Cold-smoked fish	88	18	20.5
Cortesi and others (1997)	Italy	Cold-smoked salmon	165	31	18.8
Yamazaki and others (2000)	Japan	Smoked salmon	12	2	16.7
Heinitz and Johnson (1998)	USA	Cold and hot-smoked fish	525	71	13.5
Jemmi and others (2002)	Switzerland	Cold and hot-smoked fish	1285	171	13.3
EFSA (2014)	Poland	Smoked fish	9159	1208	13.2
EFSA (2014)	Lithuania	Smoked fish	56	7	12.5
Meloni and others (2009)	Italy	Smoked fish	50	6	12.0
EFSA (2014)	Spain	Smoked fish	166	19	11.4
Rorvik and others (1995)	Norway	Cold-smoked salmon	65	7	10.8
Latorre and others (2007)	Italy	Smoked salmon	104	11	10.6
Beaufort and others (2007b)	France	Cold-smoked salmon	1010	105	10.4
Lambertz and others (2012)	Sweden	Smoked fish Cold-smoked	340	32	9.4
FSA (2008)	UK	Cold and hot smoked salmon	3222	302	9.4
Rorvik and Yndestad (1991)	Norway	Smoked salmon	33	3	9.1
EFSA (2014)	France	Smoked fish	386	34	8.8
Loncarevic and others (1996)	Sweden	Cold-smoked salmon and trout	23	2	8.7
EFSA (2014)	Austria	Smoked fish	108	9	8.3
Cabedo and others (2008)	Spain	Smoked salmon	89	7	7.9
Couvert and others (2010)	France	VP cold-smoked salmon	551	42	7.6
Kramarenko and others (2013)	Estonia	Smoked fish 2008-2010	563	41	7.3
Garrido and others (2009)	Spain	Cold-smoked trout and salmon	142	10	7.0
Hartemink and Georgsson (1991)	Iceland	Cold-smoked salmon and trout	16	1	6.3
Inoue and others (2000)	Japan	Smoked salmon	92	5	5.4
EFSA (2014)	Germany	Hot-smoked fish	929	47	5.1
Gombas and others (2003)	USA	Smoked seafood	2644	114	4.3
Gudmundsdottir and others (2005)	Iceland	Cold-smoked salmon	125	5	4.0
Miya and others (2010)	Japan	Smoked salmon	33	1	3.0
Kuzmanovic and others (2011)	Serbia	Fillet smoked salmon. herring. trout	72	2	2.8
EFSA (2014)	Bulgaria	Smoked fish	50	1	2.0
Domenech and others (2012)	Spain	Smoked salmon and cod (industry and retail samples)	509	7	1.4
Lappi and others (2004a)	USA	Cold and hot-smoked salmon	72	1	1.4
Thimothe and others (2004)	USA	Smoked salmon	233	3	1.3
Kwiatek (2004)	Poland	Smoked fish	451	4	0.9
EFSA (2014)	Czech Republic	Smoked fish	60	0	-
EFSA (2014)	Cyprus	Smoked fish	45	0	-
EFSA (2014)	Belgium	Smoked fish	200	0	-
EFSA (2014)	Slovenia	Smoked fish	50	0	-
EFSA (2014)	Romania	Smoked fish	36	0	-
Pesavento and others (2010)	Italy	Smoked salmon	19	0	-
Dhanashree and others (2003a)	India	Smoked tuna	22	0	-

*Number of samples tested, **Number of positive samples, ***Percent positive samples, VP= Vacuum-packed, MAP= Modified atmosphere packaging.

^aHypophthalmichthys molitrix.

characteristics might underlie the differences in prevalence. The levels of contamination in smoked fish are low (<10 CFU/g), but retail samples might occasionally contain viable bacterial counts ranging from 10⁴ to 10⁶ CFU/g (Gombas and others 2003).

Another high-risk RTE food that can support the growth of *L. monocytogenes* is “gravad” fish (Tham and others 2000). Gravad (marinated) fish is cured in salt and sugar without any thermal treatment. The Swedish term “gravad” is derived from the artisanal method in which fish are wrapped in leaves and buried in sand or

peat. The fish are then filleted and salted, and spices are added (Lyhs and others 2002). In modern preparations, the fish are filleted, and salt, sugar, dill, and sometimes pepper and fennel are added. The fillets are then matured for 1 to 3 d. The finished product is sold sliced or as a fillet, packed in a cling film or under vacuum, and stored at chilled temperatures. Salmon (*Salmo salar*), herring (*Clupea harengus*), rainbow trout (*Oncorhynchus mykiss*), mackerel (*Scomber scombrus*), and Greenland halibut (*Reinhardtius hippoglossoides*) are most frequently used as raw materials. Fish products of

Table 5–Reported prevalence of *L. monocytogenes* in slightly preserved seafood products including Gravad products, seafood salad, and fish roe.

Reference	Country	Product type	N*	P**	[%]***
<i>Gravad</i>					
Jemmi and others (2002)	Switzerland	Marinated fish	125	48	38.4
Jorgensen and Huss (1998)	Denmark	Gravad fish	176	51	29.0
Loncarevic and others (1996)	Sweden	Gravad salmon and trout	58	12	20.7
Lambertz and others (2012)	Sweden	Gravad fish	200	28	14.0
Peiris and others (2009)	Sweden	Gravad salmon	31	4	12.9
Hartemink and Georgsson (1991)	Iceland	Gravad salmon	12	1	8.3
Kwiatek (2004)	Poland	Marinated fish	34	0	-
<i>Seafood salad</i>					
Van Coillie and others (2004)	Belgium	Seafood salad	45	16	35.6
Uyttendaele and others (1999)	Belgium	Fish and shrimp salad	362	98	27.1
Hartemink and Georgsson (1991)	Iceland	Seafood salad	29	7	24.1
Gombas and others (2003)	USA	Seafood salad	2446	115	4.7
Little and others (2007)	UK	Seafood salad	1418	54	3.8
<i>Fish roe</i>					
Handa and others (2005)	Japan	Fish roe	67	9	13.4
Miya and others (2010)	Japan	Salmon and cod roe	287	22	7.7
Miettinen and others (2003)	Finland	Fish roe	147	7	4.8
Kramarenko and others (2013)	Estonia	Caviar 2008-2010	44	0	-
<i>Salted</i>					
Basti and others (2006)	Iran	Salted mullet (<i>Liza aurata</i>)	40	24	60.0
Siriken and others (2013)	Turkey	Salted Anchovy	50	6	12.0
Kramarenko and others (2013)	Estonia	Salted fish 2008-2010	391	38	9.7
Kuzmanovic and others (2011)	Serbia	Salted fish	15	0	-
Cabedo and others (2008)	Spain	Salted herring and anchovies	27	0	-
<i>Dried</i>					
Miya and others (2010)	Estonia	Dried fish 2008-2010	89	0	-
Miya and others (2010)	Japan	Dried seafood	16	0	-
Dhanashree and others (2003a)	India	Dried fish and prawn	42	0	-
<i>Miscellaneous</i>					
El-Shenawy and others (2011)	Egypt	Cooked and fried seafood (sandwich)	71	10	14.1
Jamali and others (2013)	Malaysia	Fried and barbecue seafood	25	2	8.0
Kovacevic and others (2012)	Canada	RTE fish	40	2	5.0
Meloni and others (2009)	Italy	Cooked marinated products	42	2	4.8
Jorgensen and Huss (1998)	Denmark	Cured seafood ^a	191	8	4.2
Hosein and others (2008)	Trinidad and Tobago	RTE seafood	70	2	2.9
Kramarenko and others (2013)	Estonia	Heat-treated and non-heat treated fish products 2008-2010	596	17	2.9
Miya and others (2010)	Japan	Tuna block and sushi	74	1	1.4
Pagadala and others (2012)	Australia	Cooked crab meat	624	1	0.2
Fletcher and others (1994)	New Zealand	Marinated mussle	11	0	-
Kuzmanovic and others (2011)	Serbia	Heat-treated products and RTE fish	20	0	-
Thimothe and others (2002)	USA	Raw fish meat	78	0	-

*Number of samples tested, **Number of positive samples, ***Percent positive samples, RTE = Ready-to-eat; VP = Vacuum-packed; MAP = Modified atmosphere packaging.
^aCured seafood includes lightly preserved products such as brined shrimps and surimi, oil marinated shrimps, caviar and marinated herring, the latter being a semipreserved product.

this type are lightly preserved with an NaCl content of 3% to 6% (w/w) and a pH greater than 5, and the products are typically consumed without heat treatment (Lyhs and others 2002). Several studies have examined the prevalence of the pathogen in this subgroup of LPSPs (Table 5). In a Swedish study, Loncarevic and others (1996) isolated *L. monocytogenes* from 21% (12/58) of gravad fish samples. Mejlholm (2007) observed that 31.6% of gravad fish products ($n = 403$) contained *L. monocytogenes*. Among the contaminated samples, gravad trout had the highest contamination rate (36.6%), followed by gravad salmon (14%). Recently, Lambertz and others (2012) observed a prevalence of 14% in gravad fish samples in Sweden, which is similar to the prevalence in cold-smoked fish (14%). Several studies have observed large differences in the prevalence of *L. monocytogenes* in gravad and smoked product samples (Hartemink and Georgsson 1991, Loncarevic and others 1996, Jorgensen and Huss 1998). This difference might be caused by the lack of potential listericidal steps (such as smoking) during gravad production. Noriega Orozco (2000) proposed that the combination of smoking and inhibitory effects of lactic acid bacteria reduce the number of *L. monocytogenes* in cold-smoked salmon, but gravad production does not involve a smoking procedure. Consistent with this hypothesis, a high prevalence of *L. monocytogenes* was observed in gravad (28%; $n = 64$) and cold-smoked fish (18%; $n = 49$) samples in Finland (European Food Safety Author-

ity 2011). The high prevalence of *L. monocytogenes* in cold-smoked and gravad fish highlights the requirement for improved in-house control and surveillance systems to eliminate this bacterial hazard and to reduce the risk of human listeriosis.

RTE

RTE refrigerated food products are ready for consumption without additional treatment or cooking. Examples of RTE refrigerated foods include seafood salads, deli salads, soft cheeses, and prepacked fresh vegetables and fruits. The lack of a heating step before consumption necessitates hygienic preparation and appropriate storage conditions to ensure the safety of these foods throughout their shelf life. *L. monocytogenes* contamination is a major concern in refrigerated seafood salads (Table 5). The presence of *L. monocytogenes* in these products may result from contaminated raw materials or from cross-contamination during processing, packaging, or retail presentation (Little and others 2007). The safety of seafood salads can be ensured by a combination of refrigerated storage, preservative use, and addition of organic acids to decrease the pH of the final product. However, when the contamination involves a resistant/adapted pathogenic strain, this food-preservation strategy might be ineffective and thus compromise food safety (Foley and others 2005). Several Class I recalls by the U.S. Food and Drug Administration have involved

mayonnaise-based salads (including vegetable, meat, seafood, and pasta salads) contaminated with *L. monocytogenes* (Isonhood and others 2006).

Examination of 31000 RTE products for *L. monocytogenes* contamination between January 2000 and November 2001 in the United States revealed that the prevalence of the pathogen was highest in seafood salad samples. Among 2446 seafood salad samples, 4.7% tested positive for *L. monocytogenes*, with levels ranging from 0.04 to 10^3 CFU/g (Gombas and others 2003). Furthermore, a study performed on a smaller number of samples in Iceland indicated that *L. monocytogenes* was present in 16% of seafood salad samples (Hartemink and Georgsson 1991). Both salmon and shrimp salads were positive for *L. monocytogenes*, with a high incidence of *Listeria* spp. in the gravad-salmon salads (80%; half of the samples contained *L. monocytogenes*) (Hartemink and Georgsson 1991). *L. monocytogenes* was observed in 27% and 3.8% of seafood salad samples in Belgium (Uyttendaele and others 1999) and the United Kingdom (Little and others 2007), respectively. The prevalence of *L. monocytogenes* in seafood samples in the United States in 2000 to 2001 (4.7% seafood salads; Gombas and others 2003) was similar to the prevalence in the United Kingdom. A review by Mejlholm (2007) indicated a prevalence of *L. monocytogenes* of 6.6% ($n = 5294$; range: 0% to 50%) in mayonnaise-based products and smoked salmon and shrimp.

Fish eggs are initially sterile; however, after harvesting, roe is inevitably contaminated (Himelbloom and Crapo 1998; Table 5). Fish roe products, such as salted fish roe, are RTE food products, which are generally not cooked further before consumption. The only decontamination step in the preparation of these products is curing with salt, and these products do not undergo any heat treatment unless they are prepared in a shelf-stable form or included in a cooked entree. Pathogens, including *L. monocytogenes*, can contaminate roe during production because these microorganisms occur naturally in the environment, fish, and fish-processing factories (Miettinen and others 2003; Shin and Rasco 2007). The salt content of fish-roe products ranges from 2.5% to 25.5%, and the water activity and pH range from 0.96 to 0.98 and 5.5 to 6.3, respectively (Shin and Rasco 2007).

Because roe is normally eaten as a raw delicacy, and fresh or frozen-thawed roe are stored for different times before consumption, there is a risk of *L. monocytogenes* contamination and propagation (Miettinen and others 2003). Miettinen and others (2003) observed that *L. monocytogenes* was present in 2% to 8% of fish-roe samples. Among 147 samples from Finnish retail markets, *L. monocytogenes* was present in 4.7% of rainbow trout (*O. mykiss*), white fish (*Coregonus lavaretus*), vendace (*Coregonus albula*), and burbot (*Lota lota*) roe samples. Notably, the prevalence of *L. monocytogenes* in fresh roe was 20 times higher than the level in frozen and frozen-thawed roe products combined. Similar results were obtained in a Japanese study in which 4.8% (10 of 208) of frozen roe products from retail markets tested positive for *L. monocytogenes* (Handa and others 2005).

L. monocytogenes grows better on crabmeat compared to other seafood (Pagadala and others 2012). Few studies have examined the health concerns posed by *L. monocytogenes* in fresh crabs. Rawles and others (1995) observed *L. monocytogenes* in 7.9% of cooked and pickled blue crab meat samples collected from different processing facilities in the U.S.A. This observation indicated that the pathogen was able to grow in inoculated pasteurized crabmeat, even at refrigerated temperatures. In 2000, imitation crabmeat was identified as a source of a small listeriosis outbreak in which *L. monocytogenes* serotype 1/2b strains were isolated from healthy

individuals; these cases were traced to consumption of imitation crabmeat, which contained 2.1×10^9 CFU/g in a household refrigerator (Farber and others 2000). Pagadala and others (2011) observed *L. monocytogenes* in 6.1% of RTE crabmeat samples collected monthly from 7 processing plants during the plant operating season in the U.S.A. The same group performed a more comprehensive study using 1248 samples (environmental and product) and observed *L. monocytogenes* in 0.2% of cooked crabmeat samples and 2.1% of processing environment samples. In addition, the antimicrobial resistance profiles of these isolates revealed that more than 50% of the *L. monocytogenes* strains were resistant to at least 3 antibiotics (Pagadala and others 2012).

Processing environments

Although raw materials are the primary source of potential *L. monocytogenes* contamination in seafood products (Eklund and others 1995), the processing plant environment might also play an important role (Rorvik and others 1995, 2000; Reij and Aantrekker 2004). Most seafood products undergo heat treatment, which can eliminate *Listeria* before consumption; therefore, if recommended hygiene regimes and good hygiene practices are implemented, cross-contamination can be avoided, and contaminated raw materials would not contaminate the finished products. Different areas within processing plants may have significantly different levels of *L. monocytogenes* contamination (Table 6).

There is a link between contamination and cross-contamination of seafood, especially RTE seafood products, during processing (Farber and Peterkin 1991; Dorsa and others 1993; Tompkin and others 1999; Norton and others 2001; Thimothe and others 2002; Gudbjornsdottir and others 2004; Thimothe and others 2004; Lappi and others 2004b; Gudmundsdottir and others 2005; Soutos and others 2007).

It is extremely difficult to determine the specific source of environmental contamination in processing plants. Based on data from different types of RTE food-processing plants, knives, conveyor belts, and mainly drains and floors contained the highest levels of *Listeria* spp. and *L. monocytogenes* (Table 6). Floors and drains are particularly difficult to clean and maintain *Listeria*-free. Rorvik and others (1997) observed that 33% of salmon smokehouse plants ($n = 40$) were contaminated with *L. monocytogenes*, mainly because drains represent a niche of contamination. However, the authors did not observe a correlation between the contamination of the processing plants and the occurrence of *Listeria* spp. and *L. monocytogenes* in the final product. *Listeria* spp. were present in 15.4% of the drains ($n = 6/39$) and in 5.1% of the employee contact surfaces (gloves and aprons) (2/39) but in none of the samples from food contact surfaces. Gudbjornsdottir and others (2004) also observed that raw material and specific locations in a cold-smoked salmon-processing plant, specifically the floors and drains, were potential sources of *L. monocytogenes* in final products. In the article "Control of *L. monocytogenes* in the food-processing environment," Tompkin (2002) proposed that floor drains might contaminate equipment through aerosols, which are generated during sanitation or air currents. However, other studies did not observe *L. monocytogenes* in the drains of processing plants. Table 6 contains information regarding *L. monocytogenes* contamination via the environment and raw materials as well as during processing and in final products in different seafood-processing plants.

Molecular subtyping methods such as pulsed-field gel electrophoresis (PFGE) or PCR-based fingerprinting (for example, Rep-PCR, RAPD, and MLST) provide in-depth information on the routes of contamination of *L. monocytogenes* (Gasnov and

Table 6–Sources of contamination in the fish processing environment.

Source of contamination	Direct/indirect food contact surface	Processed product	Country	N*	P [%]**	Reference	
Conveyors	Direct	Smoked fish	Japan	7	5 (71.4)	Nakamura and others (2006)	
	"	Catfish fillet	USA	36	6 (16.6)	Chen and others (2010b)	
Spiral/Blast freezers	Direct	Blue crab	Australia	78	5 (6.4)	Pagadala and others (2012)	
Personnel and their protection clothing e.g. white coats	Direct	Smoked rainbow trout	Denmark	2	2 (100)	Hansen and others (2006)	
	"	Cold-smoked rainbow trout	Finland	19	6 (31.6)	Autio and others (1999)	
	"	Smoked fish	USA	135	14 (10.4)	Thimothe and others (2004)	
	"	Cooked shrimp. raw salmon or raw cod	Nordic countries	48	3 (6.3)	Gudbjornsdottir and others (2004)	
	"	Blue crab	Australia	78	1 (1.3)	Pagadala and others (2012)	
	Packaging equipment	Direct	Cold smoked salmon Plant 1 .1998-1999	Denmark	472	131 (27.8)	Fonnesbech Vogel and others (2001)
	"	Cold-smoked rainbow trout	Finland	84	20 (23.8)	Autio and others (1999)	
	"	Cold-smoked salmon	Norway	155	23 (14.8)	Klaeboe and others (2005)	
	"	Smoked fish	Japan	101	9 (8.9)	Nakamura and others (2006)	
	"	Cold-smoked salmon	USA	57	3 (5.3)	Hu and others (2006)	
"	Smoked fish	USA	125	6 (4.8)	Thimothe and others (2004)		
"	Cold smoked salmon Plant 2. 1998-1999	Denmark	346	9 (2.6)	Fonnesbech Vogel and others (2001)		
"	Cold-smoked salmon	USA	174	1 (0.6)	Hu and others (2006)		
"	Cold-smoked salmon	USA	113	1 (0.9)	Hu and others (2006)		
Skinning, slicing, blending equipment and smoking area	Direct	Catfish fillet	USA	45	7 (15.6)	Chen and others (2010b)	
Injection brines and other solutions	Direct	Cold-smoked rainbow trout	Finland	6	4 (66.7)	Autio and others (1999)	
	"	Cold smoked salmon	Iceland	14	3 (21.4)	Gudbjornsdottir and others (2005)	
	"	Cooked shrimp. raw salmon or raw cod	Nordic countries	23	2 (8.7)	Gudbjornsdottir and others (2004)	
Drains	Indirect	Cold-smoked fish	USA	128	80 (62.5)	Hoffman and others (2003)	
	"	Smoked fish	USA	131	31 (23.7)	Thimothe and others (2004)	
	"	Cooked shrimp. raw salmon or raw cod	Nordic countries	166	37(22.3)	Gudbjornsdottir and others (2004)	
	"	Catfish fillet	USA	9	2 (22.2)	Chen and others (2010b)	
	"	Cold smoked salmon	Iceland	137	29 (21.2)	Gudbjornsdottir and others (2005)	
Floors/gangways	Indirect	Cold-smoked rainbow trout	Finland	67	53 (79.1)	Autio and others(1999)	
	"	Smoked fish	Japan	48	26 (54.2)	Nakamura and others (2006)	
	"	Catfish fillet	USA	9	4 (44.4)	Chen and others (2010b)	
	"	Cold-smoked fish	USA	96	31 (32.3)	Hoffman and others (2003)	
	"	Smoked Rainbow trout	Denmark	29	7 (24.1)	Hansen and others (2006)	
	"	Smoked fish	USA	162	20 (12.3)	Thimothe and others (2004)	
	"	Blue crab	AUS	78	1 (1.3)	Pagadala and others (2012)	
Cleaning equipment	Indirect	Cold-smoked rainbow trout	Finland	12	2 (16.7)	Autio and others(1999)	

*Number of samples tested, **Number of positive samples (% positive).

others 2005; Miettinen and Wirtanen 2006; Chen and others 2010a; Jadhav and others 2012; Mędrala and others 2003; Zunabovic and others 2012; Schoder and others 2014). However, the transmission of the bacterium through the primary production areas is not well understood; this pathway is important to identify the etiology of contamination and outbreaks (Miettinen and Wirtanen 2006). It is unclear whether the same subtypes are present in the raw material, indoor environment during processing, and the final product. Few studies have observed the same subtype in the raw material and final product, indicating that raw material contamination is sometimes transmitted to the final product (Norton and others 2001; Vogel and others 2001; Nakamura and others 2006; Wulff and others 2006). By examining the steps between primary production and processing to the final product, Miettinen and Wirtanen (2006) detected 30 *L. monocytogenes* PFGE-pulsotypes in 15 Finnish rainbow trout farms and fish-processing plants; most of the isolated strains were the same pulsotypes as those from the processing area and the raw fish. Notably, only very few of the subtypes isolated in the indoor processing environment were de-

tected in the final product, but a persisting subtype was observed in the final product (Norton and others 2001; Vogel and others 2001; Nakamura and others 2006; Wulff and others 2006). Almost every plant has an “in-house” subtype of *L. monocytogenes* (Rørvik and others 1995; Miettinen and others 1999; Norton and others 2001; Thimothe and others 2004; Wulff and others 2006), which may persist inside the facility for prolonged periods. The reason for persistence of those *L. monocytogenes* subtypes is unclear. The ability to form biofilms has been proposed as a crucial factor affecting the establishment of *L. monocytogenes* in seafood-processing environments; furthermore, biofilm formation poses a challenge for bacterial elimination (Gandhi and Chikindas 2007; Srey and others 2013). For example, *L. monocytogenes* can produce strong biofilms on plastic surfaces (for example, the conveyor belts used in fish factories), which is also observed with weak-biofilm-producing strains (Srey and others 2013). The large amount of water used during processing (for example, in hatcheries) is a crucial factor contributing to biofilm formation because the water quality is impaired by the detachment of biofilm-forming

bacteria, irrespective of the type of water treatment (Seyr and others 2013). Hoffman and others (2003) examined contamination by *L. monocytogenes* in a smoked fish-processing environment. Ribotyping analysis was used to distinguish between raw material- and environment-associated contamination, and the authors concluded that environmental contamination independently introduced *L. monocytogenes* into seafood products. Furthermore, the automated ribotyping analysis revealed that raw fish contained a greater diversity of strains (16 of 46 isolates) compared to the processing environment (11 of 115 isolates), indicating separate bacterial populations (Hoffman and others 2003). These results were consistent with other studies, indicating that the processing environment possessed specific subtypes of *L. monocytogenes* (Rorvik and others 2000; Vogel and others 2001; Mędrala and others 2003; Orsi and others 2011).

In principle, cleaning and sanitizing procedures can eliminate the pathogen from the processing line and equipment, but recontamination may occur after processing (Eklund and others 1995). The sporadically occurring subtypes are often sensitive to cleaning and disinfection procedures, but the strains belonging to persistent subtypes are not eliminated by such procedures (Vogel and others 2001; Wulff and others 2006). Eklund and others (1995) observed that the external surfaces of fresh and frozen fish might act as inoculating agents that introduce *L. monocytogenes* into processing plants; this contamination is extended by further operations such as filleting, rinsing, and brining. Therefore, equipment, personnel, and surfaces might serve as secondary contamination sources of *L. monocytogenes*. A survey of smoked salmon preparation practices during 16 site visits observed that only the largest companies (44%) evaluated their washing and cleaning SOPs to control *L. monocytogenes* contamination, and only 1 company periodically changed the sanitizers. Furthermore, smaller companies even used household dishwashing detergents (Rotariu and others 2014).

The review by Carpentier and Cerf (2011) contained important conclusions regarding the control of *Listeria* spp. in manufacturing environments. An important rule is to avoid water in sites of product exposure; this restriction is not easy during the processing of raw materials, but it may be possible for RTE products after processing. Another important rule is to clean the floors surrounding the equipment to avoid contamination of equipment by bacteria dislodged from the floors. Furthermore, bacterial growth must be inhibited by temperature reduction, limitation of soiling, and drying.

Secondary contamination during preparation in a processing line or at a retail store plays an important role in the contamination and/or recontamination of products, based on much of the literature. Furthermore, moist areas must be monitored carefully during processing because these areas frequently harbor *L. monocytogenes*, which may adapt and spread through the processing units. It is important to consider factors such as appropriate design of food-processing equipment, detailed working instructions for employees, reasonable planned job rotations, and monitoring the effectiveness of cleaning and disinfecting procedures in the production facilities.

Considerations for Future Research

Food safety issues are of critical concern to society, governments, and industry. However, each of these stakeholders has different roles in preventing the risk to public health. Recent efforts by industrial and regulatory bodies have led to a substantial reduction in the incidence of listeriosis, but this disease remains a major

health issue, especially considering RTE food products. Although several studies have traced the route of *Listeria* contamination in seafood products, greater attention must be paid to understanding the mechanisms and intrinsic and extrinsic factors affecting and/or promoting the growth and/or the survival of *Listeria* and how this pathogen becomes associated with seafood. Improving the discriminatory capacity of current subtyping methodologies will enable tracking and elimination of contamination sources in the food industry. Several studies have observed significant strain/serotype heterogeneity in the virulence and pathogenicity of *L. monocytogenes* strains. Therefore, the virulence potential of a representative set of *L. monocytogenes* strains should be analyzed. Although significant effort is devoted to eliminating *L. monocytogenes*, some strains (for example, those isolated from food-processing plants) are less virulent than clinical isolates. Furthermore, the presence or even overrepresentation of *inlA* PMSC mutations does not necessarily indicate decreased *L. monocytogenes* virulence. Therefore, other pathogenicity markers must be characterized. Finally, new intervention strategies must be developed to control this microbe in seafood-processing environments.

Summary and Concluding Remarks

A large number of studies have examined the prevalence of *L. monocytogenes* in seafood products and related environments in several countries. RTE seafood has extensively been examined in several international studies, leading to a definitive identification of this food as a carrier of *L. monocytogenes*. However, the existing data are not fully consistent and not always comparable because some of the studies mentioned above and listed in Table 3, 4, 5, and 6 do not specify the fish species examined, whereas other studies did not specify the treatment procedure used, such as cold- or hot-smoking. Often, surveys conducted over the same periods in geographically proximal regions did not observe consistent prevalence rates. In several surveys performed repeatedly within the same region, there was no attempt to explain the marked differences. Although *L. monocytogenes* is highly prevalent in RTE seafood products, the total counts of *L. monocytogenes* are usually very low (<10 CFU/g). The diverse *L. monocytogenes* prevalence rates in RTE seafood samples most likely reflect geographical differences and differences in regulatory policies and control measures in different countries.

Whereas raw seafood entering processing facilities can be contaminated with *L. monocytogenes* to varying degrees, the subsequent processing environment is the major source of contamination. Studies of different food-processing facilities worldwide over the past 10 y have indicated that the food-processing environment is the principal source of *L. monocytogenes* contamination in foods prior to consumer purchase, preparation, and consumption. Therefore, *L. monocytogenes* biotypes isolated from the final product may be indistinguishable from the raw product strains, although this similarity occurs infrequently. In some cases, these biotypes persistently colonize the processing plant environment for several years, increasing the risk of (re)contamination of the final products.

To reduce and prevent contamination in the processing environment and products, it is important to detect the main sources of contamination and to understand the mechanisms underlying the persistence of different *L. monocytogenes* strains in the environment. The key areas where *Listeria* spp. have been detected or where greatest *Listeria* contamination has been observed in the processing environment can be identified. In particular, drains and difficult-to-clean skinning areas as well as brine injection and

slicing equipment are frequent reservoirs of persistent *L. monocytogenes* colonization in fish-processing plants; however, further research is required to confirm the original source of contamination. Cleaning and disinfection of the production plant should lower the prevalence of *L. monocytogenes* although the results of some studies are inconsistent with this hypothesis. Therefore, there is an urgent need to design sanitation strategies that precisely target persistent strains, for example, using sanitizers with active ingredients more suitable for biofilms. The processing companies must carefully consider factory design, good raw materials, staff training, good manufacturing and hygiene practices, and effective cleaning and sanitation to prevent contamination of the product. Inappropriate handling of food products by consumers can also play a major role in increasing the prevalence of *Listeria*, resulting in noncompliance with the Food Safety Objective in the final RTE seafood products. Therefore, it is important to improve consumer education regarding food safety practices during the purchase, transport, storage, and handling of food.

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