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Predicting growth rates and growth boundary of *Listeria monocytogenes* — An international validation study with focus on processed and ready-to-eat meat and seafood

Ole Mejlholm[a], Annemarie Gunvig[b], Claus Borggaard[b], Jesper Blom-Hanssen[b], Lyndal Mellefont[c], Tom Ross[c], Francoise Leroi[d], Tony Else[e], Diana Visser[e] and Paw Dalgaard[a, *]

[a] Seafood and Predictive Microbiology, Division of Seafood Research, National Food Institute (DTU Food), Technical University of Denmark, Søltofts Plads, Building 221, DK-2800, Kgs. Lyngby, Denmark
[b] Danish Meat Research Institute (DMRI), Roskilde, Denmark
[c] Tasmanian Institute of Agricultural Research (TIAR), University of Tasmania, Hobart, Tasmania, Australia
[d] Départment des Sciences et Techniques Alimentaires Marines, Ifremer, Nantes, France
[e] PURAC biochem b.v., Gorinchem, The Netherlands

[*] Corresponding author : Paw Dalgaard, Tel.: + 45 45252566; fax: + 45 45884774, email address : pad@aqua.dtu.dk

Abstract:

The performance of six predictive models for *Listeria monocytogenes* was evaluated using 1014 growth responses of the pathogen in meat, seafood, poultry and dairy products. The performance of the growth models was closely related to their complexity i.e. the number of environmental parameters they take into account. The most complex model included the effect of nine environmental parameters and it performed better than the other less complex models both for prediction of maximum specific growth rates (μ_{max} values) and for the growth boundary of *L. monocytogenes*. For this model bias and accuracy factors for growth rate predictions were 1.0 and 1.5, respectively, and 89% of the growth/no-growth responses were correctly predicted. The performance of three other models, including the effect of five to seven environmental parameters, was considered acceptable with bias factors of 1.2 to 1.3. These models all included the effect of acetic acid/diacetate and lactic acid, one of the models also included the effect of CO_2 and nitrite but none of these models included the effect of smoke components. Less complex models that did not include the effect of acetic acid/diacetate and lactic acid were unable to accurately predict growth responses of *L. monocytogenes* in the wide range of food evaluated in the present study. When complexity of *L. monocytogenes* growth models matches the complexity of foods of interest, i.e. the number of hurdles to microbial growth, then predicted growth responses of the pathogen can be accurate. The successfully validated models are useful for assessment and management of *L. monocytogenes* in processed and ready-to-eat (RTE) foods.

Keywords: Predictive models; Bias and accuracy factors; Correct prediction percentage; Growth/no-growth predictions; Psi (ψ) value

1. Introduction

Evaluation of predictive microbiology growth models includes a comparison of predicted growth responses with those observed in food. Typically, food data are obtained from challenge tests with inoculated products but data from naturally contaminated food are important and should be used when they can be obtained. These evaluations and the resulting indices of performance (e.g. bias and accuracy factors) are important to determine if a predictive model can be used with confidence. In addition, model evaluations help identify the range of products, product characteristics and storage conditions where predictions are sufficiently accurate to be useful (Augustin et al., 2005; Dalgaard and Jørgensen, 1998; Ross, 1996; te Giffel and Zwietering, 1999). For *Listeria monocytogenes*, the EU regulation EC 2073/2005 specifically indicates that predictive mathematical modelling can be used to document control of growth in ready-to-eat food (EC, 2005). The critical concentration is 100 CFU/g and food business operators must document the growth control of *L. monocytogenes* depending on measured product characteristics, different reasonably foreseeable storage conditions and within the shelf-life of products (EC, 2005, Annex II). Similar criteria were recently adopted by the Codex Alimentarius Commission (FAO/WHO, 2009). Clearly, models to accurately predict the combined effect of product characteristics (including antilisterial food additives) and storage conditions on growth and the growth boundary of *L. monocytogenes* hold great practical interest for the industry and for food inspection authorities.

Numerous models to predict the growth rate and the growth boundaries of *L. monocytogenes* in response to environmental conditions relevant to food are available in the scientific literature and several models have been included in user-friendly application software (McMeekin et al., 2006; Ross and Dalgaard, 2004; Tamplin 2009). The complexity of these models differs markedly. Some models include the effect of the storage temperature only (e.g. Delignette-Muller et al., 2006) whereas others take into account the effect of as many as 15 different environmental parameters

(Augustin and Carlier, 2000). *L. monocytogenes* growth models including the effect of temperature, pH and NaCl/a_w have been evaluated and some were successfully validated for cheese, meat and seafood products. However, the performance of models with a similar degree of complexity differed markedly. Those evaluation studies also suggested that more complex models were needed to accurately predict growth in cured, smoked and modified atmosphere packed food as well as to predict the growth boundary of *L. monocytogenes* (Augustin et al., 2005; Dalgaard and Jørgensen, 1998; Mejlholm and Dalgaard, 2007a; te Giffel and Zwietering, 1999). The degree of model complexity required to obtain accurate predictions remains a topic of controversy and discussion.

To reduce or prevent the growth of *L. monocytogenes* in food, lactic acid (naturally occurring or added as acid or as lactate) in combination with added acetic acid or diacetate has been successful for various meat and seafood products. When new hurdle like organic acids are added to a food product it becomes more complex, however, the inhibiting effect of these organic acids depends on other product characteristics and storage conditions. Importantly, several mathematical models have been developed to predict the combined effect of acetic acid/diacetate and lactic acid/lactate on growth and on the growth boundary of *L. monocytogenes* (Augustin and Carlier, 2000; Gunvig et al., 2007; Hwang and Tamplin, 2007; Legan et al., 2004; Le Marc et al., 2002; Mejlholm and Dalgaard, 2007a; Mejlholm and Dalgaard, 2009; Nerbrink et al., 1999; Pradhan et al. 2009; PURAC, 2007; Seman et al. 2002; Skandamis et al., 2007; Zuliani et al., 2007). These models differ in their structure (e.g. artificial neural network or cardinal parameter type models), complexity (the number of environmental parameters that they take into account when growth rates and/or growth boundaries are predicted) and in the ways they have been developed with data from liquid laboratory media or food. Some models have been evaluated and successfully validated for specific types of food and these models may contribute to management of the risk of *L. monocytogenes* in these foods as indicated in the EU regulation (EC, 2005) and by the USDA FSIS compliance

97 guidance documents (FSIS, 2008). Nevertheless, the performance of these complex models is likely
98 to differ in the same way as observed for simpler models including the effect of temperature, pH
99 and NaCl/$a_{w.}$ Therefore, a comparison of the performance of complex models including the
100 combined effect of acetic acid/diacetate and lactic acid for growth rates and the growth boundary of
101 *L. monocytogenes* in different foods is relevant and timely.
102 The objective of the present study was to evaluate the performance of existing *L.*
103 *monocytogenes* growth rate and growth boundary models with different degrees of complexity and
104 for a wide range of foods. Six models were evaluated that include the effect of between one and
105 nine environmental parameters. Growth responses and environmental parameters were collected for
106 1014 well characterized meat, seafood, poultry and non-fermented dairy products. These growth
107 responses were determined in many different laboratories and include 114 different isolates of *L.*
108 *monocytogenes* and more than 20 different types of food products.
109
110 **2. Materials and methods**
111 *2.1 Predictive models*
112 The performance of six predictive models, including five cardinal parameter or square-root
113 type models and one artificial neural network model, for growth of *L. monocytogenes* was evaluated
114 in the present study (Table 1). Models were evaluated by comparison of observed and predicted
115 maximum specific growth rates (μ_{max}-values) and growth/no-growth responses. In accordance with
116 FAO/WHO (2009) an increase in *L. monocytogenes* concentrations of ≥ 0.5 log CFU/g within the
117 experimental time was defined as “growth”. For all the evaluated models growth was predicted
118 without inclusion of a lag phase although several of the models also have the ability to predict
119 growth when a lag phase is taken into account.

120 The number of environmental parameters included in the models evaluated varied between
121 one and nine. The least complex models did not take into account the effect of acetic acid/diacetate
122 and lactic acid, although these are important preservatives added to some of the products considered
123 in this evaluation of models. The simple models were studied to determine the difference in model
124 performance when compared to the more complex models including the combined effect of these
125 organic acids and other product characteristics (Tables 2 and 3).
126 The square-root model of Delignette-Muller et al. (2006) included the effect of temperature as
127 the only environmental parameter (Eqn. 1). This model was developed as part of a quantitative risk
128 assessment of *L. monocytogenes* in French cold-smoked salmon (Pouillot et al. 2007). The effect of
129 environmental parameters other than temperature (e.g. pH, naturally occurring lactic acid, pH,
130 smoke components and water activity) was modelled as variability and taken into account by using
131 a Bayesian approach.

132
$$\mu_{\max} = \begin{cases} 0, & T \le T_{\min} \\ \mu_{ref} \cdot \dfrac{(T - T_{\min})^2}{(T_{ref} - T_{\min})^2}, & T > T_{\min} \end{cases} \quad (1)$$

133 where μ_{ref} is equal to the maximum specific growth rate (μ_{max}) at the reference temperature (T_{ref}) of
134 25 °C; *T* is the temperature (°C); *Tmin* is the theoretical minimum temperature (°C) preventing
135 growth of *L. monocytogenes*. Delignette-Muller et al. (2006) estimated μ_{ref} and T_{min} values of 6.24
136 1/d (0.26 1/h) and -2.86 °C, respectively, from growth of *L. monocytogenes* in cold-smoked salmon.
137 The cardinal parameter model of Augustin et al. (2005) included the effect of temperature,
138 a_w, pH, smoke components (measured as the concentration of phenol), nitrite, CO_2 and interactions
139 between these environmental parameters (Eqn. 2). This model was developed to predict growth and
140 the growth boundary of *L. monocytogenes* in dairy, meat and seafood products. Augustin et al.
141 (2005) estimated optimal specific growth rates (μ_{opt} values) for each of the product categories by
142 fitting their model to growth data obtained from dairy (n = 340), meat (n = 324) and seafood (n =

80) products. In the present study we used their μ_{opt} values of 1.168, 0.565, 1.168 and 0.742/h for meat, seafood, poultry and dairy products, respectively (Augustin et al. 2005).

$$\mu_{max} = \mu_{opt} \cdot CM_2(T) \cdot CM_1(pH) \cdot SR(a_w) \cdot SR(nit) \cdot SR(phe) \cdot SR(CO_2) \cdot \xi \qquad (2)$$

where a_w is the water activity; *nit* is the concentration (mM) of undissociated sodium nitrite; *phe* is the concentration (ppm) of smoke components (phenol); CO_2 is the CO_2 proportion; and ξ is the effect of interactions between the environmental parameters. $CM_n(X)$ is defined by Eqn. 3; $SR(a_w)$ by Eqn. 4; and SR(*nit*), SR(*phe*) and SR(CO_2) by Eqn. 5. Abbreviations CM_n and SR were included as used by Augustin et al. (2005).

$$CM_n(X) = \begin{cases} 0 & , \quad X \le X_{\min} \\ \dfrac{(X - X_{\max}) \cdot (X - X_{\min})^n}{(X_{opt} - X_{\min})^{n-1} \cdot [(X_{opt} - X_{\min}) \cdot (X - X_{opt}) - (X_{opt} - X_{\max}) \cdot ((n-1) \cdot X_{opt} + X_{\min} - nX)]} & , \quad X_{\min} < X < X_{\max} \\ 0 & , \quad X \ge X_{\max} \end{cases} \qquad (3)$$

where X is temperature or pH; X_{min}, X_{opt} and X_{max} are the theoretical minimal, optimal and maximal values of X for growth of *L. monocytogenes*.

$$SR(a_w) = \begin{cases} 0 & , \quad a_w \le a_{w\,min} \\ \left(\dfrac{a_w - a_{w\,min}}{a_{w\,opt} - a_{w\,min}} \right) & , \quad a_{w\,min} < a_w < a_{w\,opt} \\ 0 & , \quad a_{w\,opt} \le a_w \le a_{w\,max} \end{cases} \qquad (4)$$

where $a_{w\,min}$, $a_{w\,opt}$ and $a_{w\,max}$ are the theoretical minimal, optimal and maximal a_w values for growth of *L. monocytogenes*.

$$SR(c) = \begin{cases} 1 - \dfrac{c}{MIC} & , \quad c < MIC \\ 0 & , \quad c \ge MIC \end{cases} \qquad (5)$$

where MIC is the minimal inhibitory concentration of undissociated sodium nitrite (mM), phenol (ppm) or CO_2 (proportion) against *L. monocytogenes*; and *c* is the concentration of undissociated sodium nitrite (mM), the concentration of phenol (ppm) or the proportion of CO_2. The effect of interactions between the environmental parameters (ξ) in Eqn. 2 was modelled using the approach

of Le Marc et al. (2002). The value of ξ was calculated according to Eqn. 6, with contributions from the different environmental parameters as shown in Eqn. 7 and Eqn. 8. In Eqn. 8, the ψ-value is determined from sets of environmental parameters (e_i), and it describes how far specific combinations of product characteristics and storage conditions are from the predicted growth boundary (ψ = 1.0) (Le Marc et al., 2002).

$$\xi = \begin{cases} 1 & , \quad \psi \le 0.5 \\ 2(1-\psi), & 0.5 < \psi < 1 \\ 0 & , \quad \psi \ge 1 \end{cases} \tag{6}$$

where ξ is the term modelling the effect of interactions between the environmental parameters on μ_{max}.

$$\begin{aligned} \varphi(T) &= \left(\frac{T_{opt} - T}{T_{opt} - T_{\min}} \right)^3 \\ \varphi(pH) &= \left(\frac{pH_{opt} - pH}{pH_{opt} - pH_{\min}} \right)^3 \\ \varphi(a_w) &= \left(\frac{a_{w\,opt} - a_w}{a_{w\,opt} - a_{w\,\min}} \right)^3 \\ \varphi(nit, phe, CO_2) &= 1 - SR(nit) \cdot SR(phe) \cdot SR(CO_2) \end{aligned} \tag{7}$$

$$\psi = \sum_i \frac{\varphi_{e_i}}{2\prod_{j \neq i}(1-\varphi_{e_j})} \tag{8}$$

Cardinal parameter values for temperature, a_w and pH as well as minimal inhibitory concentrations (MICs) for phenol, nitrite and CO_2 were determined from growth data obtained in liquid laboratory media (Augustin and Carlier, 2000; Augustin et al., 2005). In the present study, the following of their cardinal parameter values and MICs were used for the model of Augustin et al. (2005): T_{min} = -1.72 °C, T_{max} = 45.5 °C, T_{opt} = 37.0 °C, $a_{w\,min}$ = 0.913, $a_{w\,max}$ = 1.000, $a_{w\,opt}$ = 0.997, pH_{min} *lactic*

178 *acid* = 4.71, pH_{max} = 9.61, pH_{opt} = 7.10, MIC (phenol) = 31.9 ppm, MIC (nitrite) = 25 mM
179 undissociated nitrite and MIC (CO_2) = 3.04 (corresponding to a partial pressure of CO_2 above
180 atmospheric pressure).

181 The cardinal parameter model of Zuliani et al. (2007) included the effect of temperature, a_w,
182 pH, acetic acid, lactic acid and interactions between these environmental parameters (Eqn. 9). This
183 model was developed to predict growth and the growth boundary of *L. monocytogenes* in pork
184 products, and its structure was derived from the model of Augustin et al. (2005). An optimal
185 specific growth rate (μ_{opt} value) of 0.85/h was determined from growth of *L. monocytogenes* in
186 ground pork (Zuliani et al. 2007).

187 $$\mu_{max} = \mu_{opt} \cdot CM_2(T) \cdot CM_1(pH) \cdot SR_1(a_w) \cdot SR(OA) \cdot \xi \quad (9)$$

188 where OA represents the concentration (mM) of undissociated acetic or lactic acid. Zuliani et al.
189 (2007) suggested that the antimicrobial effect of acetic and lactic acid should be modelled as the
190 effect of the dominating undissociated acid alone (Eqn. 10 or 11).

191 $$SR(AAC_U) = \begin{cases} 1 - \dfrac{[AAC_U]}{[MIC_{U\ aceticacid}]}, & AAC_U < MIC_{U\ acetic\ acid} \\ 0, & AAC_U > MIC_{U\ acetic\ acid} \end{cases} \quad (10)$$

192 $$SR(LAC_U) = \begin{cases} 1 - \dfrac{[LAC_U]}{[MIC_{U\ lacticacid}]}, & LAC_U < MIC_{U\ lactic\ acid} \\ 0, & LAC_U > MIC_{U\ lactic\ acid} \end{cases} \quad (11)$$

193 where $[AAC_U]$ and $[LAC_U]$ are the concentrations (mM) of undissociated acetic acid and lactic
194 acid, respectively; and $[MIC_{U\ acetic\ acid}]$ and $[MIC_{U\ lactic\ acid}]$ are the MICs (mM) of acetic acid and
195 lactic acid, respectively, that prevent growth of *L. monocytogenes*. The approach suggested by Le
196 Marc et al. (2002) was used to model the effect of interactions between the environmental
197 parameters (ξ) in Eqn. 9. The value of ξ was calculated according to Eqn. 6, with contributions
198 from the different environmental parameters as defined in Eqn. 7, 8 and 12.

199 $$\varphi(OA) = (1 - SR(AAC_U))^2 \ or \ (1 - SR(LAC_U))^2 \quad (12)$$

200 Cardinal parameter values for temperature, a_w and pH were identical to the ones used for the model
201 of Augustin et al. (2005) with the exception of pH_{min} for which pH_{min} *HCl* with a value of 4.26 was
202 used. Their MICs of 5.83 and 1.76 mM undissociated acetic acid and lactic acid, respectively, were
203 used to predict growth of *L. monocytogenes* by the model of Zuliani et al. (2007). These MIC values
204 were determined from growth of the pathogen in ground pork with different concentrations of the
205 two organic acids added.

206 The cardinal parameter model of PURAC has been developed to predict growth of *L.*
207 *monocytogenes* in cured and uncured cooked meat and poultry products and includes the effects of
208 temperature, NaCl/a_w, pH, acetic acid/diacetate and lactic acid (added as acid or as lactate) but not
209 the effect of interaction between these parameters. In addition, the effect of a fixed concentration of
210 nitrite can be included or excluded (Table 1; Legan et al. 2004; PURAC, 2007; Seman et al. 2002).
211 In the present study we used the model including the effect of nitrite as it resulted in the best model
212 performance for all types of products. The model is available free of charge from the PURAC
213 website as the Opti.Form Listeria control model 2007 and has been used as a flexible and
214 conservative tool to determine concentrations of acetic acid/diacetate and lactic acid required to
215 control growth of *L. monocytogenes* in meat and poultry products (PURAC, 2007). Equations and
216 parameter values underlying the Opti.Form Listeria control model 2007 have not been published
217 which makes an evaluation and comparison of this and other models interesting. Predicted doubling
218 time (h) was converted to maximum specific growth rate (μ_{max}, 1/h) for calculation of bias and
219 accuracy factors (See section 2.3).

220 The artificial neural network (ANN) model from the Danish Meat Research Institute (DMRI)
221 included the effect of temperature, pH, sodium chloride in the water phase, acetic acid/diacetate and
222 lactic acid in the water phase, sodium nitrite (ppm) added to the product and CO_2 in the packaging

atmosphere (%) and interactions between all these environmental parameters. The model was developed using 446 growth rates for *L. monocytogenes* in different meat products (Gunvig et al. 2007). The ANN contained an input neuron for each of the seven environmental parameters included in the model, three hidden neurons and a single output neuron for μ_{max} values. Training was performed using scaled input data and a back propagation of error algorithm. The ANN model (Eqn. 13-15) includes weights (w_{ij}) for each input neuron (x_i) and bias for the three hidden neurons (b_i) (Eqn. 14). The hidden neurons contained a sigmoid response function for the combined effect of inputs and biases (Eqn. 15). The trained ANN model includes 28 parameter values for the 7 x 3 weights (w), three weights (u) and four biases (b). Parameter values have not been published.

$$\mu_{max} = b_2 + \sum_{j=1}^{3} u_i \cdot \sigma(\omega_i) \quad (13)$$

where b_2 and u_i are bias and weights from the hidden layer to the output neuron.

$$\omega_i = \sum_{i=1}^{7} w_{ij} \cdot x_i + b_i \quad (14)$$

$$\sigma = 1/(1+e^{-\omega}) + 0.5 \quad (15)$$

The DMRI model is available on-line (See Table 1) and growth curves (log CFU/g) are simulated by using the Log-transformed three-parameter logistic model without lag-time and including a constant maximum population density of $10^{8.7}$ CFU/g.

The cardinal parameter model of Mejlholm and Dalgaard (2009) includes the effect of temperature, NaCl/a_w, pH, phenol, nitrite, CO_2, acetic acid, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid as well as interactive effects between all these parameters. However, in the present study, products with added benzoic, citric and/or sorbic acids were not considered and, thus, the performance of this model with nine rather than 12 environmental parameters was evaluated (Eqn. 16). The model of Mejlholm and Dalgaard (2009) was developed by adding terms for the

effect of acetic, benzoic, citric and sorbic acid, as determined in liquid laboratory substrates, to an existing growth and growth boundary model for *L. monocytogenes* (Mejlholm and Dalgaard 2007a, b). Importantly, the parameter values μ_{ref}, T_{min} and P_{max} in that model was determined by fitting growth rates (μ_{max} values) obtained for *L. monocytogenes* in lightly preserved seafood (n = 41) with well-characterised product characteristics and storage conditions (Mejlholm and Dalgaard, 2007a, b).

$$\mu_{\max} = \mu_{ref} \cdot \left[\frac{(T - T_{\min})}{T_{ref} - T_{\min})}\right]^2 \cdot \frac{(a_w - a_{w\min})}{(a_{w\,opt} - a_{w\min})} \cdot \left[1 - 10^{(pH_{\min} - pH)}\right] \cdot \left(1 - \frac{[LAC_U]}{[MIC_{U\ lactic\ acid}]}\right) \cdot \frac{(P_{\max} - P)}{P_{\max}} \cdot \left[\frac{(NIT_{\max} - NIT)}{NIT_{\max}}\right]^2 \cdot \frac{(CO_{2\max} - CO_{2\ equilibrium})}{CO_{2\max}} \cdot \left(1 - \sqrt{\frac{[DAC_U]}{[MIC_{U\ diacetate}]}}\right) \cdot \left(1 - \sqrt{\frac{[AAC_U]}{[MIC_{U\ acetic\ acid}]}}\right) \cdot \xi \quad (16)$$

where μ_{ref} is the reference specific growth rate with a value of 0.419/h for μ_{max} at the reference temperature (T_{ref}) of 25 °C; a_w is the water activity calculated from the concentration of NaCl in the water phase of the product; P_{max}, NIT_{max} and $CO_{2\ max}$ are the theoretical maximal concentrations (ppm) of smoke components (phenol), nitrite and CO_2, respectively, that allow growth of *L. monocytogenes*; P and NIT are the concentrations (ppm) of phenol and nitrite, respectively; $CO_{2\ \text{equilibrium}}$ is the concentration (ppm) of dissolved CO_2 at equilibrium; $[LAC_U]$, $[DAC_U]$ and $[AAC_U]$ are the concentrations (mM) of undissociated lactic acid, diacetate and acetic acid, respectively; and $[MIC_{U\ \text{lactic acid}}]$, $[MIC_{U\ \text{diacetate}}]$ and $[MIC_{U\ \text{acetic acid}}]$ are the MICs of undissociated lactic acid, diacetate and acetic acid, respectively, that prevent growth of *L. monocytogenes*. The effect of interactions between the environmental parameters (ξ) in Eqn. 16 was modelled using the approach of Le Marc et al. (2002). The value of ξ was calculated according to Eqn. 6, with contributions of the different environmental parameters as shown in Eqn. 8 and 17.

$$\varphi(T)=\left[1-\frac{(T-T_{\min})}{(T_{ref}-T_{\min})}\right]^2;\varphi(a_w)=\left[1-\sqrt{\frac{(a_w-a_{w\min})}{(a_{wopt}-a_{w\min})}}\right]^2;\varphi(pH)=\left[1-\sqrt{1-10^{(pH_{\min}-pH)}}\right]^2;$$

$$\varphi(P)=\left[1-\sqrt{\frac{(P_{\max}-P)}{P_{\max}}}\right]^2;\varphi(NIT)=\left[1-\frac{(NIT_{\max}-NIT)}{NIT_{\max}}\right]^2;\varphi(CO_2)=\left[1-\sqrt{\frac{(CO_{2\max}-CO_{2equilibrium})}{CO_{2\max}}}\right]^2; \quad (17)$$

$$\varphi([LAC],[DAC],[AAC])=\left\{1-\left[\left(1-\sqrt{\frac{[LAC_U]}{[MIC_{U\ lactic\ acid}]}}\right)\cdot\left(1-\sqrt{\frac{[DAC_U]}{[MIC_{U\ diacetate}]}}\right)\cdot\left(1-\sqrt{\frac{[AAC_U]}{[MIC_{U\ acetic\ acid}]}}\right)\right]\right\}^2$$

The model of Mejlholm and Dalgaard (2009) includes the following cardinal parameter values and MICs: T_{min} = -2.83 °C, $a_{w\ min}$ = 0.923, $a_{w\ opt}$ = 1.000, pH_{min} = 4.97, P_{max} = 32.0 ppm phenol, NIT_{max} = 350 ppm nitrite, $CO_{2\ max}$ = 3140 ppm CO_2, $MIC_{U\ lactic\ acid}$ = 3.79 mM undissociated lactic acid, $MIC_{U\ diacetate}$ = 4.8 mM undissociated diacetate and $MIC_{U\ acetic\ acid}$ = 10.3 mM undissociated acetic acid.

2.2 Data for evaluation of predictive models

1014 sets of environmental conditions and corresponding growth responses of *L. monocytogenes* in processed and RTE foods were collected to evaluate the performance of the six predictive models for growth of this pathogen (Table 1). Data for model evaluation were collected from 37 independent sources (publications, research institutes and companies) and represented more than 20 different types of meat, seafood, poultry and non-fermented dairy products (Table 2 and 3). A total of 737 data sets were supplied by the participants of the present study including both previously published and unpublished data (Table 2). In addition, data from 277 experiments were obtained from the literature of other workers (Table 3). The number of experiments involving products that were naturally contaminated with *L. monocytogenes* was 13, whereas for the remaining 1001 experiments the products were inoculated with the pathogen. For each of the 1014 experiments, information on growth of *L. monocytogenes* was obtained together with product characteristics and storage conditions of the specific product. Fifty percent of the products included added acetic acid/diacetate and/or lactic acid. Growth of *L. monocytogenes* was described by the

maximum specific growth rate (μ_{max}, 1/h) and by growth and no-growth responses. Growth rates (μ_{max}, 1/h) were obtained (i) directly from the reported data; (ii) by fitting growth curves of *L. monocytogenes* with the integrated and log-transformed form of the four-parameter Logistic model (Dalgaard, 1995) or (iii) by linear-regression using data from the exponential part of published graphs. To differentiate between growth and no-growth, the latter was defined as an increase in *L. monocytogenes* concentrations of less than 0.5 log cfu/g within the experimental time (FAO/WHO, 2009).

The collected data were divided into groups consisting of meat (n = 702), seafood (n = 193), poultry (n = 64) and non-fermented dairy (n = 55) products. If reported, measured water activity (a_w) values were used to predict growth of *L. monocytogenes*. Otherwise, a_w values were calculated from the concentrations of NaCl, acetate/diacetate and lactate using the PURAC calculator (PURAC, 2007). For the DMRI model concentrations of WPS in the products was used directly to predict growth of *L. monocytogenes* as specified in the software version of the model. For the model of Mejlholm and Dalgaard (2009), a_w was always calculated from the percentage of water phase salt (WPS) only, using the relationship $a_w = 1 - 0.0052471*WPS - 0.00012206*WPS^2$ (Chirife and Resnik, 1984). The model of Mejlholm and Dalgaard (2009) was developed using measured concentrations of WPS in products and therefore a_w calculated from WPS was also used when predictions were obtained. In the same way the Seafood Spoilage and Safety Predictor (SSSP) software, that includes this model, uses WPS rather than a_w to predict growth responses of *L. monocytogenes* (Table 1).

For 474 of the 1014 experiments one or more of the relevant environmental parameters were not reported (See Table 2 and 3). In those cases it was assumed that the products contained 3.5 % water phase salt (WPS) (n = 112), 0.70 % water phase lactic acid equivalent to 78 mM (n = 172) and had a pH value of 6.2 (n = 92). These values, however, were not used for the liquid non-

fermented dairy products where 0.9% NaCl and pH 6.7 were assumed (Table 3). For 160 experiments a concentration of 6.0 ppm phenol was assumed for products that typically are smoked (i.e. ham, sausages and smoked salmon) if no information on the content of smoke components was given. To establish this value for smoke components data from previous studies were considered and in addition various ready-to-eat meat products (i.e. ham and sausages) were analysed using a spectrophotometric method (Cardinal et al., 2004; Mejlholm and Dalgaard, 2007b). From a total of 12 samples, the average content of smoke components was determined as 5.8 ± 2.1 ppm phenol. For 90 experiments the content of nitrite was assumed to be 50 ppm if this preservative was described to be part of the product formulation, but no concentration was reported. If not measured, the equilibrium concentration of CO_2 in the headspace of modified atmosphere packed (MAP) products was calculated using the initial gas to product ratio and Henry's constant at the appropriate storage temperature (Ross and Dalgaard, 2004). Concentrations of diacetate were converted to equivalent concentrations of acetic acid to be used with predictive models including the effect of acetic acid but not diacetate (Table 1).

2.3 Indices of model performance

Predicted and observed growth rates (μ_{max}, 1/h) of *L. monocytogenes* were compared by calculation of bias and accuracy factors (Ross, 1996). The bias factor indicates a systematic over- or underestimation of growth rates and the accuracy factor is a measure of the average difference between observed and predicted μ_{max} values (Eqn. 18 and Eqn. 19). The bias factor values were calculated so that numbers higher than 1 always indicated that predicted growth was faster than observed growth (Eqn. 18). As an example, a bias factor of 1.25 indicates that predicted growth rates on average is 25% faster than observed growth rates.

$$Bias\ factor\ (\mu_{\max}) = 10^{(\sum \log(\mu_{\max}\ predicted\ /\ \mu_{\max}\ observed)\ /\ n)} \quad (18)$$

$$Accuracy\ factor\ (\mu_{\max}) = 10^{(\sum |\log(\mu_{\max}\ predicted\ /\ \mu_{\max}\ observed)|\ /\ n)} \quad (19)$$

To graduate the performance of the predictive growth rate models for *L. monocytogenes*, the following interpretation of the bias factor values were used (Ross, 1999): (i) 0.95-1.11 good; (ii) 0.87-0.95 or 1.11-1.43 acceptable and (iii) < 0.87 or > 1.43 unacceptable.

Graphic comparison of predicted and observed μ_{max} values were used to illustrate distribution of data and to evaluate model performance for different sub-sets of the dataset e.g. products with or without added acetic acid/diacetate and lactic acid. Square-root transformed μ_{max} values were plotted to avoid graphs with a large number of values that were too small to be evaluated visually.

Predicted and observed growth and no-growth responses were compared by calculating the percentage of all samples that were correctly predicted. Incorrect predictions were categorised as fail-dangerous (i.e. no-growth predicted when growth was actually observed) or fail-safe (i.e. growth predicted when no-growth was actually observed). For cardinal parameter type models including the effect of interactions between the environmental parameters (Augustin et al., 2005; Mejlholm and Dalgaard, 2009; Zuliani et al., 2007), the psi(ψ)-value was used to quantify the incorrectness of fail-dangerous and fail-safe predictions (Le Marc et al., 2002; Mejlholm and Dalgaard, 2009). The ψ-value is determined from sets of environmental parameters (Eqn. 8) and describes how far specific product characteristics and storage conditions are from the predicted growth boundary (ψ = 1.0). Thus, the closer products with fail-dangerous (ψ > 1.0) and fail-safe (ψ < 1.0) predictions are to the growth boundary, the better the performance of the model.

3. Results and discussion

3.1 Evaluation of predictive models

In the present study, six predictive models for *L. monocytogenes* were evaluated using bias and accuracy factors as performance indices for growth rates (μ_{max} values), and the percentage of correct, fail-dangerous and fail-safe predictions as performance indices for growth/no-growth responses. Models that predict growth to be too fast (bias factor > 1.0) should be used with caution e.g. for product development or risk assessment, as this might result in an excessive use of preservatives or an overestimation of the risk associated with *L. monocytogenes*. On the other hand, prediction of too slow growth (bias factor < 1.0) might result in foods that allow growth of *L. monocytogenes* or an underestimation of the risk. To be considered good or acceptable, growth rates should not be over- or under-predicted by more than 43% and 13%, respectively, corresponding to a bias factor of between 0.87 and 1.43 (Ross, 1999).

The model of Delignette-Muller et al. (2006) significantly overestimated growth rates of *L. monocytogenes* as shown by average bias and accuracy factors of 2.0 and 2.2 for all data (Table 4). The model predicted growth of *L. monocytogenes* for all experiments resulting in a high percentage of fail-safe predictions (Table 5). This model was developed to predict growth of *L. monocytogenes* in cold-smoked salmon. When evaluated for seafood in the present study (with 86% of the samples being cold-smoked salmon) predicted growth rates were 70% faster than the observed ones (Table 4). Delignette-Muller et al. (2006) developed this growth model for cold-smoked salmon without added acetic and lactic acid. An improved bias factor was therefore expected when the model was used exclusively for seafood without addition of these organic acids. However, for seafood without added acetic acid/diacetate and lactic acid (n = 121), bias and accuracy factors were 1.5 and 1.6, respectively, and the performance of this model was considered unacceptable. The model of Delignette-Muller et al. (2006) was developed using 96 growth curves from challenge tests with cold-smoked salmon. Differences between the product characteristics in those studies and of the products evaluated here (Table 2 and Table 3) may explain the average overestimation of growth

382 rates by this model. This is likely as the model exclusively takes into account the effect of storage
383 temperature on growth rates.
384 Average bias and accuracy factors of 1.8 and 2.3 were obtained for all data when the model of
385 Augustin et al. (2005) was used (Table 4). Seventy-six percent of the growth/no-growth responses
386 were correctly predicted with the incorrect ones being distributed as nine and 15% fail-dangerous
387 and fail-safe predictions, respectively. The conservative performance of this model was mainly
388 explained by the fact that it did not include the effect of acetic and lactic acid (Table 1). Dividing
389 the data set, average bias and accuracy factors of 1.2 and 1.9 were found for products without
390 addition of acetic acid/diacetate and lactic acid (n = 392) whereas corresponding values of 3.1 and
391 3.3 were determined for products added these two organic acids (n = 211) (Fig. 1). The model of
392 Augustin et al. (2005) was developed for growth of *L. monocytogenes* in dairy (n = 340), meat (n =
393 324) and seafood (n = 80) products without added acetic acid/diacetat and lactic acid. The present
394 study showed that the average performance of the model was acceptable for products without acetic
395 acid/diacetate and lactic acid whereas it should not be used to quantitatively evaluate growth of *L.*
396 *monocytogenes* in food in which these organic acids have been added (Fig. 1).
397 For the model of Zuliani et al. (2007), average bias and accuracy factors of 1.3 and 1.9 were
398 obtained for all data, and 85% of the growth/no-growth responses were correctly predicted (Tables
399 4 and 5). The better performance of this model as compared to the closely related one of Augustin
400 et al. (2005) was explained by the inclusion of acetic and lactic acid as environmental parameters
401 (Table 1). The model of Zuliani et al. (2007) was developed for ground pork meat. Importantly, its
402 performance was good or acceptable for the other product categories evaluated, with the exception
403 of (i) pork loin where predicted growth was a little too slow and (ii) ham/cold-cuts where predicted
404 growth was too fast (Table 4). The model does not take into account the effect of smoke
405 components, nitrite and CO_2. For products including these environmental parameters (n = 447)

406 growth rates of *L. monocytogenes* were overestimated by 40% compared to 30% for the entire data
407 set (Table 4). Thus, expanding the model of Zuliani et al. (2007) with one or more of these
408 environmental parameters will most likely improve its growth rate predictions. However, such an
409 expansion is also likely to increase the already high percentage of fail-dangerous predictions for
410 growth/no-growth responses (Table 5).

411 Average bias and accuracy factors of 1.3 and 1.7 were found for the PURAC model when all
412 data were evaluated. The PURAC model was originally developed for RTE cured and uncured meat
413 and poultry products. It also performed well for seafood and non-fermented dairy products but
414 predicted μ_{max} values were higher than observed for ham/cold-cuts (Table 4). This model did not
415 include the effect of smoke components and CO_2 (Table 1). Growth rates of *L. monocytogenes* were
416 overestimated by 51% for products including phenol and CO_2 in the packaging atmosphere (n =
417 449) whereas for all products μ_{max} values were only over-predicted by 29%. Consequently, an
418 expansion of the PURAC model with the effect of phenol and CO_2 would most likely improve its
419 performance for growth rate prediction in food products where these factors are relevant. Seventy-
420 one percent of the growth/no-growth responses were correctly predicted with zero and 29 percent of
421 the predictions being fail-dangerous and fail-safe, respectively (Table 5). The PURAC software
422 'Opti.Form Listeria control model 2007' allows growth to be predicted without lag time or with
423 90% and 95% lag time confidence intervals. Using the 95% lag time confidence interval seventy-
424 nine percent of the growth/no-growth responses were correctly predicted with 15 and six percent of
425 the predictions being fail-dangerous and fail-safe, respectively (Results not shown). Thus, by using
426 the 95% confidence interval for lag time the percentage of correct growth/no-growth response
427 increased but at the same time a higher percentage of fail dangerous predictions were obtained. The
428 decision to include or exclude the lag time should therefore be made cautiously. For three of the
429 other models evaluated the percent of correctly predicted growth/no-growth responses increased

when interaction between environmental parameters was taken into account (Table 6). In the same way, the PURAC model may be improved by modelling the effect of interaction between environmental parameters.

The DMRI model was developed for pasteurized RTE meat products but with average bias and accuracy factors of 1.2 and 1.6 its performance was considered good or acceptable for all product categories (Table 4). Eighty-three percent of the growth/no-growth responses were correctly predicted, whereas four and 13% of the predictions were fail-dangerous and fail-safe, respectively (Table 5). It should be noted that 418 growth responses in meat of the total of 1014 growth responses evaluated was used to develop the DMRI model. This may influence the evaluation of this model. However, when evaluated on the remaining part of the data (n = 596), the obtained performance indices for growth rates and growth/no-growth responses were almost identical to the ones for all data. The DMRI model did not include the effect of smoke components (Table 1). When products including phenol (n = 142) were evaluated alone, average bias and accuracy factors of 1.4 and 1.6 were obtained. This indicates that the already good performance of this model could be improved by including the effect of phenol and this may reduce the percentage of fail-safe predictions and increase the percentage of correct predictions. With average bias and accuracy factors of 1.3 and 1.6 the DMRI model predicted growth rates to be slightly higher than observed for liquid non-fermented dairy products whereas this was not the case for the five cardinal parameter models (Table 4). Growth of bacteria in liquid compared to solidified media has been observed to result in higher growth rates and less restricted growth limits (Koutsoumanis et al. 2004; Theys et al. 2008). Therefore, the DMRI model, developed using data for growth of *L. monocytogenes* in pateurized meat products (solid), would be expected to underestimate growth rates in liquid non-fermented dairy products. The opposite was observed and this may be due to the fact that the liquid dairy products, with high pH and no added salt, did not include the product

454 characteristics used to train the ANN model. Nevertheless, the performance of this model was
455 acceptable for liquid non-fermented dairy products (Table 4).
456 The model of Mejlholm and Dalgaard (2009) included the effect of more environmental
457 parameters than the other models in the present study (Table 1). On average it performed better than
458 the less complex models both with respect to prediction of growth rates (μ_{max} values) and
459 growth/no-growth responses of *L. monocytogenes* (Tables 4 and 5). Average bias and accuracy
460 factors of 1.0 and 1.5 were obtained for all data, and 89% of the growth/no-growth responses were
461 correctly predicted (Fig. 2, Tables 4 and 5). Five percent of the predictions were fail-dangerous and
462 six percent were fail-safe. This model was originally developed for processed and RTE seafood, but
463 importantly, it also performed well for meat, poultry and non-fermented dairy products (Table 4).
464 However, for pork loin where growth was predicted (n = 30), bias and accuracy factors of 0.8 and
465 1.5 indicated that predicted μ_{max} values were slightly lower than those observed (Table 4, Table 5).
466 The good overall performance of this model (Fig. 2) suggests that it contained the effect of relevant
467 environmental parameters and that its range of applicability includes temperature (2-25 °C); pH (5.4
468 to 7.7), nitrite (0 to 150 ppm), water phase lactic acid (< 6.1 % equivalent to < 677 mM) and water
469 phase diacetate (< 0.38% equivalent to < 32 mM), with the range of the other environmental
470 parameters being as reported previously (Mejlholm and Dalgaard, 2007a; Mejlholm and Dalgaard,
471 2009).
472
473 *3.2 Importance of smoke components for predicting growth*
474 When the effect of smoke components was ignored for the model of Mejlholm and Dalgaard
475 (2009), bias and accuracy factors increased to 1.4 and 1.7 for seafood. Interestingly, these values are
476 almost identical to the bias and accuracy factor obtained for seafood by the DMRI model, not
477 including the effect of smoke components (Table 4). This supports that smoke components had an

478 important and predictable inhibitory effect on growth of *L. monocytogenes* in the evaluated smoked
479 seafood (n = 148). For meat products, concentrations of smoke components have not typically been
480 reported in studies concerning growth of *L. monocytogenes*. However, smoked meat products can
481 contain from 2.6 to 37 ppm of phenol (Lustre and Issenberg, 1970) and smoke components
482 originating from wood smoke and corresponding to above ca. 10 ppm of phenol have an important
483 inhibitory effect on growth of *L. monocytogenes* (Mejlholm and Dalgaard, 2007a). Thus, one
484 explanation of why predicted growth rates in ham and cold-cuts were higher than observed (Table
485 4) could be that a significant number of these products actually contained wood smoke components
486 corresponding to more than 10 ppm of phenol. This suggests it is relevant, in future studies, to
487 further quantify, predict and evaluate the antilisterial effect of smoke components in meat products.
488 Recently, Hwang (2009a) suggested a polynomial model including the effect of smoke components
489 (phenol), temperature and NaCl on growth of *L. monocytogenes*. We evaluated this model but found
490 its performance inferior to the model of Mejlholm and Dalgaard (2009) (Results not shown).
491
492 *3.3 Interactions between the environmental parameters*
493 For three of the models evaluated in the present study, interactions between environmental
494 parameters were modelled using the approach of Le Marc et al. (2002) (Table 1). The importance of
495 including interactions between environmental parameters in order to predict the growth boundary of
496 e.g. *L. monocytogenes* has previously been documented (Augustin et al., 2005; Le Marc et al. 2002;
497 Mejlholm and Dalgaard, 2007a; Mejlholm and Dalgaard, 2009; Tienungoon et al., 2000) although
498 some controversy remains (Bidlas and Lambert, 2008). For the models of Augustin et al. (2005),
499 Zuliani et al. (2007) and Mejlholm and Dalgaard (2009) the percentage of correctly predicted
500 growth/no-growth responses increased when the effect of interactions between environmental
501 parameters was taken into account (Table 6). This was most pronounced for the model of Mejlholm

and Dalgaard (2009) where the percentage of correct predictions increased from 69 to 89. For all three models, the percentage of fail-safe predictions decreased and the percentage of fail-dangerous predictions increased but not proportionally, when interactions were taken into accounts. The ratio between the decrease in numbers of fail-safe predictions and the increase in numbers of fail-dangerous predictions was 5.6 for the model of Mejlholm and Dalgaard (2009). Corresponding ratios of 1.8 and 1.6 were determined for the models of Augustin et al. (2005) and Zuliani et al. (2007). The pronounced difference between the model of Mejlholm and Dalgaard (2009) and the two other models with respect to the impact of interactions was most likely caused by (i) the different numbers of environmental parameters included in the models and (ii) the use of different terms to model the contribution of the individual environmental parameters on the interactive effect (Eqn. 7, 12 and 17). However, further studies are required both on the mathematical terms needed to optimally model the quantitative effect of interactions between environmental parameters on growth responses and on the underlying genetic and physiological responses of *L. monocytogenes.* In this respect, the available models that allow quantification of the 'distance' to the growth boundary can be useful in design of experiments to improve understanding of the stresses and molecular aspects of *L. monocytogenes* close to the growth boundary and under no-growth conditions.

3.4 Distance to the growth boundary (psi-value)

The incorrectness of fail-dangerous and fail-safe predictions can be described quantitatively by the psi (ψ)-value. This value can express the 'distance' between combinations of environmental parameters and the growth boundary ($\psi = 1.0$). The closer the ψ-value of incorrect predictions lie to 1.0 the better the performance of the model (Mejlholm and Dalgaard, 2009). ψ-values are obtained directly from the model structure suggested by Le Marc et al. (2002) to take into account the inhibiting effect of interaction between environmental parameters. On the no-growth side of the

growth boundary, ψ-values are higher than 1.0 and on the growth side they are lower than 1.0. For meat sausages (5 °C, pH 6.5, 3.8 % water phase salt, 60 ppm nitrite and 9.4% CO_2 at equilibrium), the growth boundary of *L. monocytogenes* was calculated as a function of water phase concentrations of acetic and lactic acid and compared with 13 measured growth and no-growth responses of the pathogen in this product (Fig. 3). Nine of the growth/no-growth responses (69 %) were correctly predicted whereas four were fail-dangerous (i.e. no-growth predicted when growth was actually observed) with $\psi > 1.0$ (Fig. 3). However, the four fail-dangerous predictions were placed close to the growth boundary with an average ψ-value of 1.07 ± 0.05 (mean ± SD). These incorrect predictions can be due to limitations of the model and/or variability in product characteristics and storage conditions resulting in differences between the conditions used to obtain the predictions and conditions to which the growing cells of *L. monocytogenes* were actually exposed. For all data in the present study, the model of Mejlholm and Dalgaard (2009) gave 47 fail-dangerous predictions (5%) with an average ψ-value of 1.22 ± 0.31 (mean ± SD) whereas the 59 fail-safe predictions had an average ψ-value of 0.67 ± 0.18 (Table 6). Interestingly, these ψ-values are close to those previously obtained for much fewer seafood data by Mejlholm and Dalgaard (2009). The highest ψ-value for the 47 fail-dangerous predictions was 1.95 observed for an experiment with pork cold-cuts. This result suggests that combinations of product characteristics and storage conditions with ψ-values above 1.95 can be used to prevent growth of *L. monocytogenes*. It is note worthy that this value is within the 99% confidence interval for ψ-values of fail-dangerous predictions (1.22 + 2.6 x 0.31 = 2.03). Importantly, by using the ψ-value the effect of different environmental parameters on a products 'distance' to the growth boundary can easily be evaluated. To facilitate the practical use of this concept the Seafood Spoilage and Safety Predictor (SSSP) software v. 3.1 has been designed so that ψ-values are reported together with growth rates and lag times of *L. monocytogenes*. In addition, growth interfaces corresponding to specific ψ-

550 values, in the range 0.5-2.5, can be predicted. Thus, with a selected ψ-value of 2.0 the software
551 predicts combination of product characteristics and storage conditions that will prevent growth of *L.*
552 *monocytogenes* even when typical variation in the environmental parameters is taken into account
553 (SSSP, 2009). Such predictions seem important as both the EU regulations (EC, 2005) and the
554 criteria adopted by the Codex Alimentarius Commission (FAO/WHO, 2009) distinguish between
555 products in which *L. monocytogenes* is, or is not, able to grow. Alternatively, the growth boundary
556 of *L. monocytogenes* can be predicted by probability models typically relying on logistic regression.
557 Many probability models are available for the growth boundary of *L. monocytogenes* but several
558 include the effect of just a few of the environmental parameters known to influence growth of the
559 pathogen in food (Ross and Dalgaard, 2004). However, more complex models including the effect
560 of temperature, NaCl/a_w, acetic acid/diacetate, lactic acid, pH or liquid smoke have been developed
561 (Hwang, 2009b; Skandamis et al. 2007; Vermeulen et al., 2007). Probability of growth predicted by
562 these models did not closely correspond to the growth boundaries predicted be the model of
563 Mejlholm and Dalgaard (2009) (Fig. 4). The model of Hwang (2009b) predicted high
564 concentrations of phenol (smoke components) to limit growth *L. monocytogenes* much less than
565 predicted by the model of Mejlholm and Dalgaard (2009) (Fig. 4a). One reason for this difference is
566 most likely that the model of Hwang (2009b) was developed using data for cooked salmon with
567 added liquid smoke whereas the model of Mejlholm and Dalgaard (2009) was based on growth of
568 *L. monocytogenes* in seafood processed with wood smoke. The effect of a_w and acetic acid on the
569 growth boundaries predicted by the models of Vermeulen et al. (2007) and Mejlholm and Dalgaard
570 (2009) were more alike The main difference being that the model of Vermeulen et al. (2007)
571 predicted a stronger effect of a_w on growth/no-growth responses of *L. monocytogenes* (Fig. 4b). At
572 their present state of development, probability models for the growth boundary of *L. monocytogenes*
573 includes the effect of fewer environmental parameters and therefore seems less performant than the

available cardinal parameter models relying on the Le Marc approach to take into account the effect of interactions between environmental parameters on the growth boundary of the pathogen.

Comparison of environmental conditions to reduce and prevent growth of *L. monocytogenes*, determined from ψ-values as described above, with the conditions predicted by dedicated stochastic models would be of interest. Stochastic models with the ability to describe variability (natural heterogeneity) and uncertainty (lack of perfect knowledge e.g. due to measurement errors) are desirable for exposure and risk assessment studies (CAC, 1999). The present study suggests that rather complex models are needed to reduce bias when growth of *L. monocytogenes* in RTE foods is predicted. It remains a challenge to develop stochastic models with a comparable degree of complexity. Finally, when models for growth of *L. monocytogenes* are evaluated it must be kept in mind that growth to high concentrations can be influenced by the inhibiting effect of the dominating microflora in some foods (the Jameson effect). Both, deterministic and stochastic models are available that predict the antilisterial effect of other microorganisms in high concentrations (Delignette-Muller et al., 2006; Giménez and Dalgaard, 2004; Giuffrida et al., 2009; Hwang and Sheen, 2009; Mejlholm and Dalgaard, 2007b; Powell et al., 2006). Such models may be needed to predict growth responses of the pathogen for both fermented food and several lightly preserved products when naturally contaminated with *L. monocytogenes.* The limited amount of data for naturally occurring *L. monocytogenes*, in the present study, was due to difficulties in finding storage trials where both growth of the pathogen and the relevant product characteristics were quantified (See Code S1 in Table 2 and Table 4). The thirteen growth/no-growth responses studied for naturally contaminated cold-smoked salmon resulted in 62% correct and 38% fail-safe predictions for the model of Mejlholm and Dalgaard (2009). These fail-safe predictions support the conclusion, from previous more detailed studies of naturally contaminated products, that both the Jameson

effect and the lag phase of *L. monocytogenes* are important to accurately predict its growth in naturally contaminated cold-smoked salmon (Mejlholm and Dalgaard, 2007b).

4. Conclusion

The present study showed that growth rate and conditions that in combination prevent growth of *L. monocytogenes* in RTE foods can be predicted and that predictive models with a relevant degree of complexity can successfully predict growth responses of *L. monocytogenes* in fresh and processed RTE foods. In fact, predictions with good precision can be obtained when the complexity of the applied mathematical models match the complexity of the foods of interest with respect to the number of environmental parameters influencing growth of the pathogen. The model evaluations undertaken showed sufficiently complex models can predict growth responses accurately for fresh products without added antimicrobials as well as for products with salt, nitrite, organic acids and smoke components. However, simple models including the effect of a few environmental parameters were unable to accurately predict growth responses in complex foods. Importantly, models that were previously developed and successfully validated for a specific type of foods (e.g. meat or seafood) also performed well for other categories of products. This indicates that predictive models can be generally applicable when all relevant environmental parameters are taken into account. The successfully validated predictive models will be valuable for future assessment and management of *L. monocytogenes* in food.

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621

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Fig. 1. Comparison of observed and predicted maximum specific growth rates (μ_{max}, 1/h) of *L. monocytogenes* in meat, seafood, poultry and dairy products. μ_{max} values were predicted by the model of Augustin et al. (2005) for products with (closed symbols, n = 392) and without (open symbols, n = 211) addition of acetic acid/diacetate and lactic acid.

Fig. 2. Comparison of observed and predicted growth rates (μ_{max}, 1/h) of *L. monocytogenes* in meat, seafood, poultry and dairy products (n = 640) as predicted by the model of Mejlholm and Dalgaard (2009).

Fig. 3. Predicted growth boundary (solid line) and interfaces (dotted lines) for *Listeria monocytogenes* in meat sausages (5 °C, pH 6.5, 3.8 % water phase salt, 60 ppm nitrite and 9.4% CO_2 in the atmosphere at equilibrium) depending on water phase concentrations of acetic and lactic acids. The model of Mejlholm and Dalgaard (2009) was used to predict the growth boundary (ψ = 1.0) and interfaces with ψ values of 0.75 and 1.25. Observed growth (■ □) and no-growth responses (●) of *L. monocytogenes* in meat sausages (n = 13) were determined by DMRI (Table 4). Closed and open symbols represent correctly and incorrectly predicted growth and no-growth responses, respectively.

Fig. 4. Predicted growth boundaries for *Listeria monocytogenes*. Comparison of the models of Hwang (2009b) and Mejlholm and Dalgaard (2009) (a) and of the models of Vermeulen et al. (2007) and Mejlholm and Dalgaard (2009) (b).The models of Hwang (2009b) and Vermeulen et al. (2007) was used to predict growth boundaries as 0.5 (bold solid lines) and 0.01 (fine solid lines) probability of growth. Growth boundaries with ψ-values of 1.0 (bold dashed lines) and 2.0 (fine dashed lines) was predicted by the model of Mejlholm and Dalgaard (2009).

Figure 1 – Mejlholm et al.

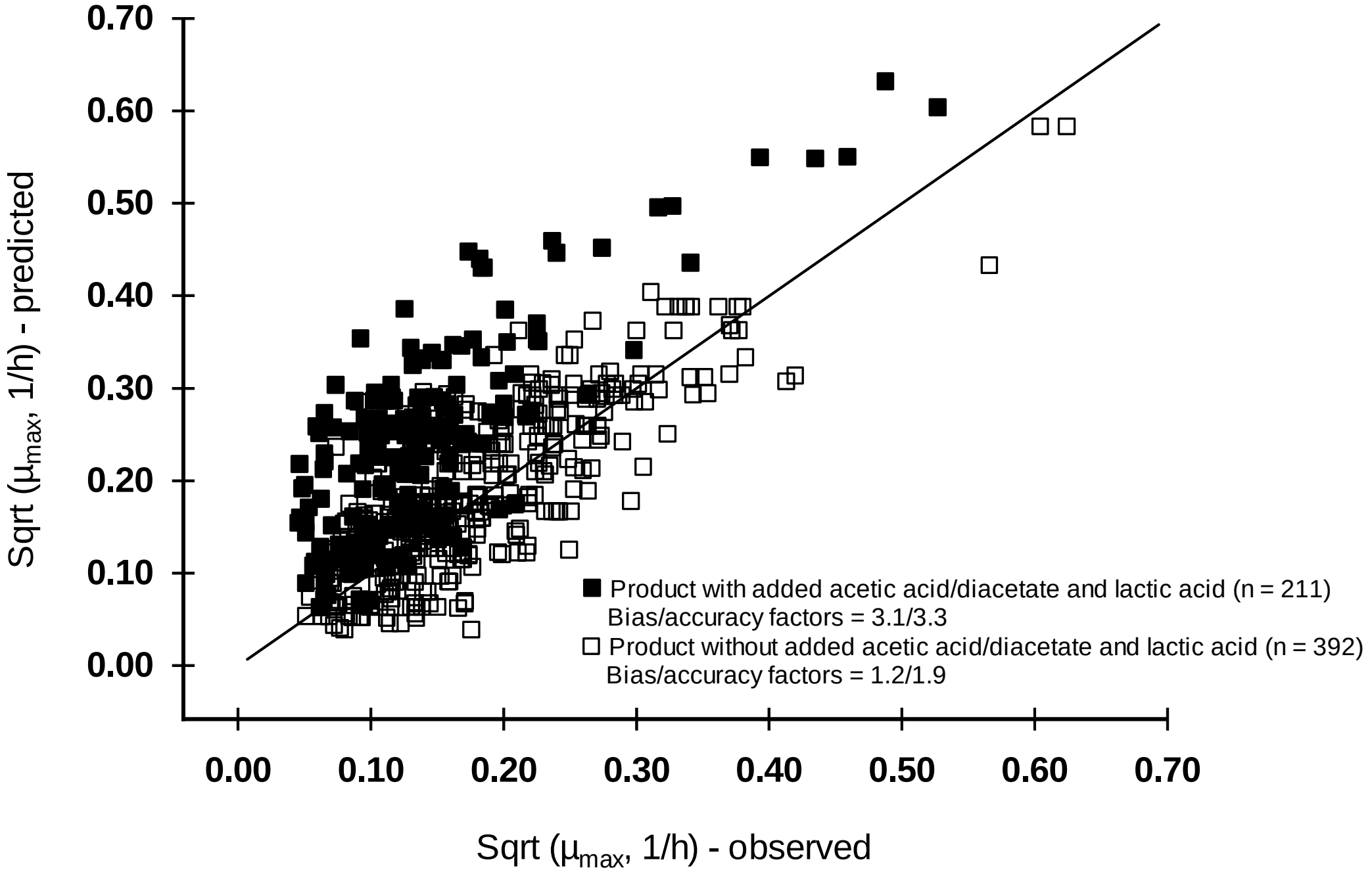

Figure 2 - Mejlholm et al.

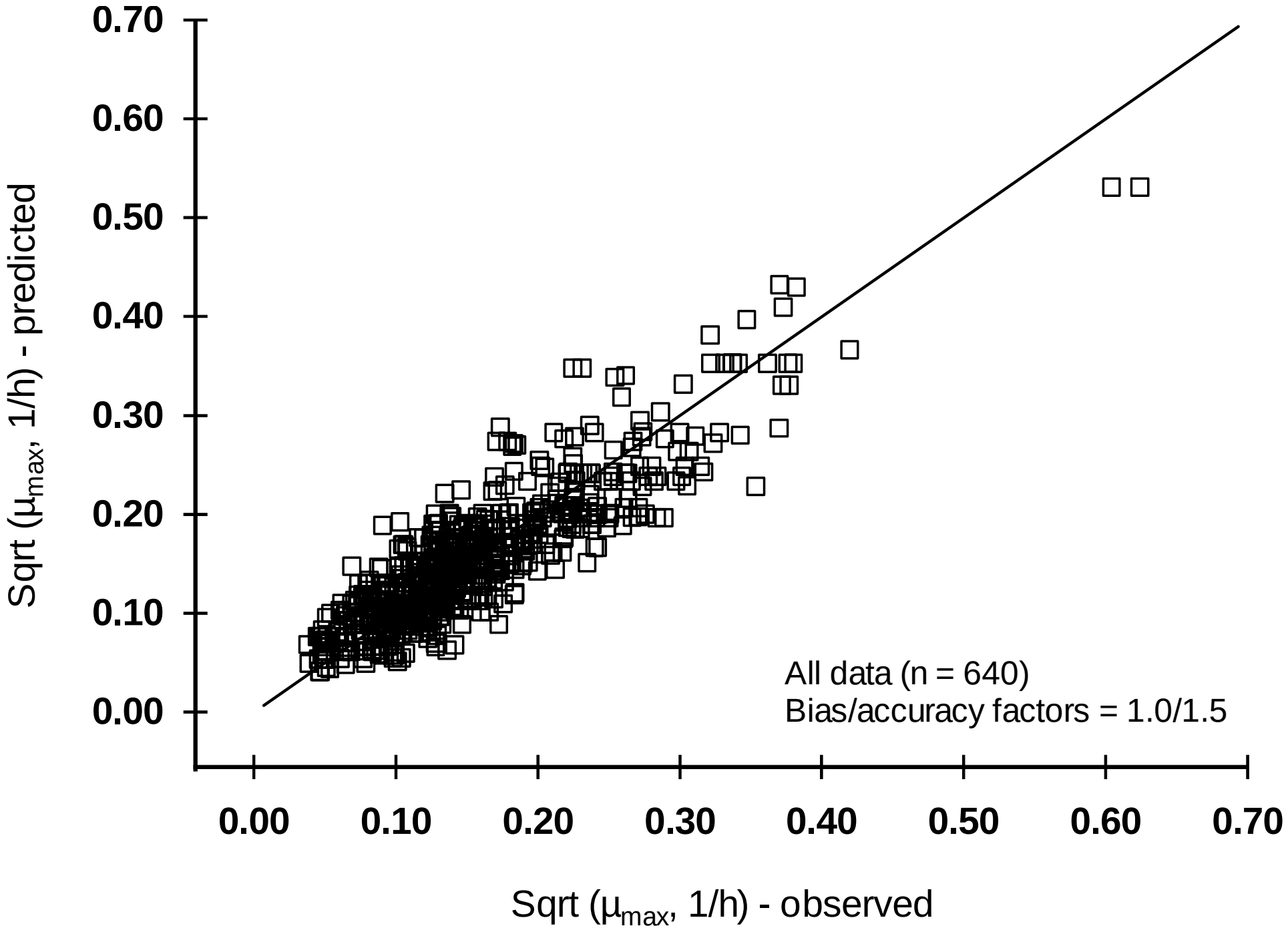

Figure 3 – Mejlholm et al.

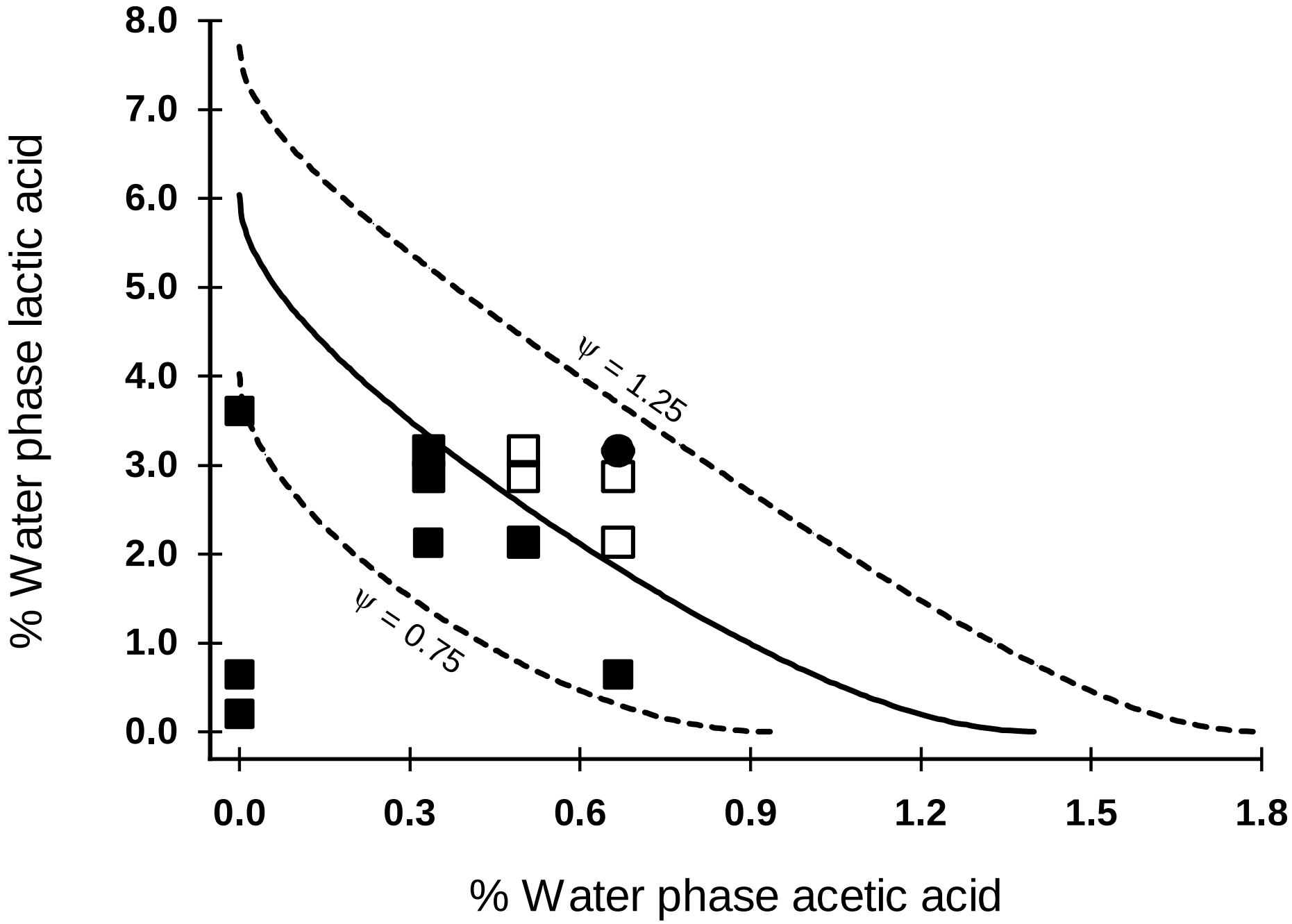

Figure 4 – Mejlholm et al.

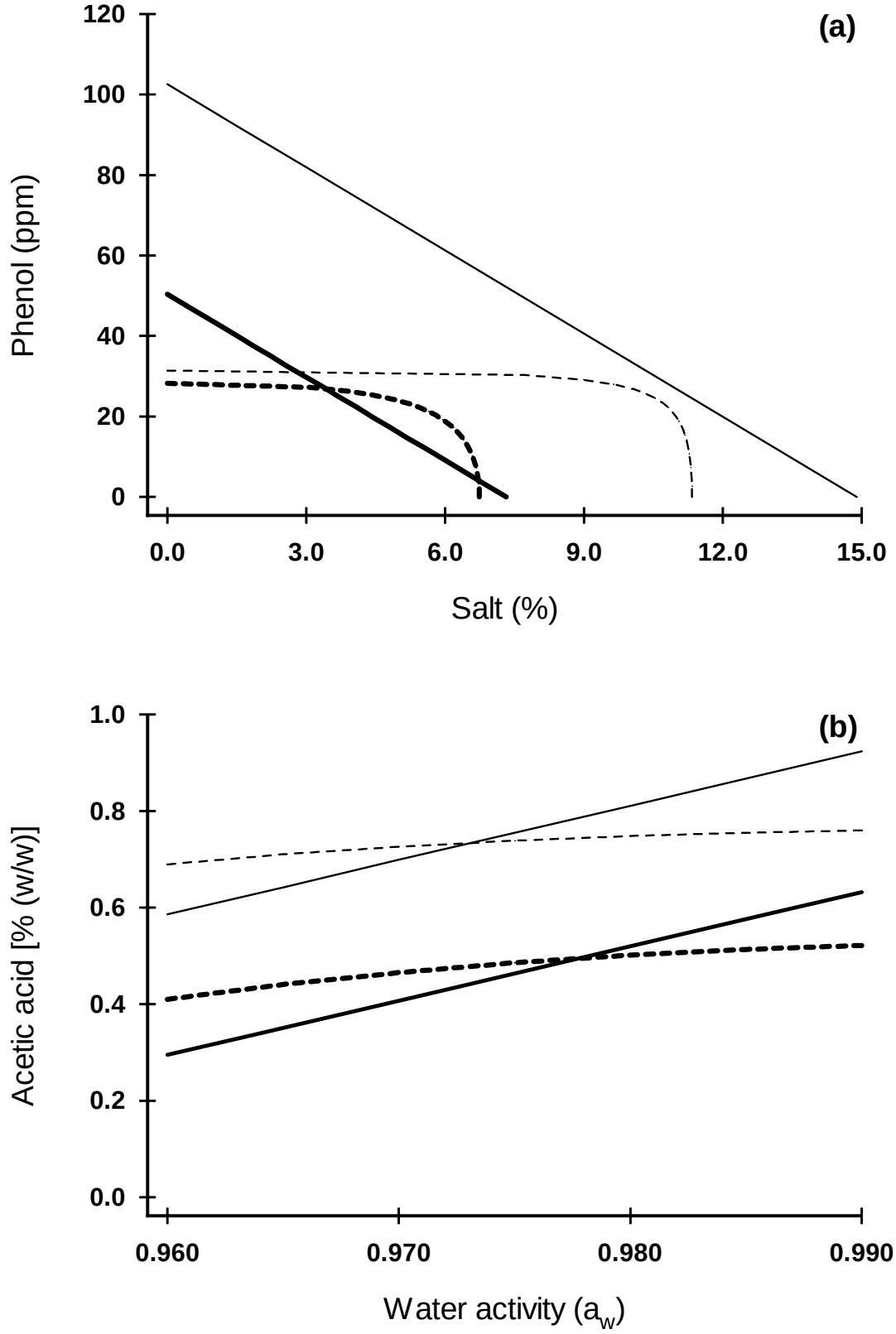

Table 1. Overview of the *Listeria monocytogenes* growth models evaluated in the present study

Models	Developed for	Type of model	Number of environmental parameters in the models	Specific environmental parameters in the models								
				Temp.	NaCl/a_w	pH	Phenol	Nitrite	CO_2	Acetic acid and/or diacetate	Lactic acid	Interactions between environmental parameters
Delignette-Muller et al. (2006)	Cold-smoked salmon	Square-root model	1	+	-	-	-	-	-	-	-	-
Augustin et al. (2005)	Dairy, meat and seafood	CPM[a]	6	+	+	+	+	+	+	-	-	+[b]
Zuliani et al. (2007)	Ground pork	CPM[a]	5	+	+	+	-	-	-	+	+	+[b]
PURAC (2007)[c]	Meat	CPM[a]	6	+	+	+	-	(+)	-	+	+	-
DMRI (2007)[d]	Meat	ANN model[e]	7	+	+	+	-	+	+	+	+	+
Mejlholm and Dalgaard (2009)[f]	Seafood	CPM[a]	9[g]	+	+	+	+	+	+	+	+	+[b]

[a] Cardinal parameter model.

[b] Interactions between environmental parameters was modelled by using the Le Marc approach (Le Marc et al. 2002).

[c] The Opti.Form Listeria control model 2007. Available from http://www.purac.com/EN/Food/Contact.aspx.

[d] The DMRI Listeria model. Available from http://1.test.dezone.dk/ (username: matmodel and password: listeria).

[e] Artificial neural network.

[f] Available as part of the Seafood Spoilage and Safety Predictor (SSSP) freeware (http://sssp.dtuaqua.dk/).

[g] The model includes the effect of 12 environmental parameters but only nine were used in the present study where products did not include benzoic, citric and sorbic acids.

Table 2. Product characteristics and storage conditions in experiments (n = 737) used for evaluation of *Listeria monocytogenes* growth models

Products and codes	Products	Country	Data source[a]	Reference	*n*[b]	No. of strains inoculated	Temp (°C)	Water phase salt (%)	a_w[c]	pH	Phenol (ppm)	Nitrite (ppm)	% CO_2	Acetic acid	Diacetate	Lactic acid
Meat products																
M1	Pork loin	Denmark	DMRI	-[d]	100	1 or 5	5.0-10.0	1.60-4.76	0.951-0.981	5.4-6.2	0-**6.0**[e]	0-150	0-48.4[f]	0-0.50	-	0.49-3.19
M2	Ham	Denmark	DMRI	-[d]	15	5	5.0-10.0	2.99-4.50	0.953-0.970	6.1-6.4	-	60	7.2-48.4[f]	0-0.50	-	0.97-3.19
M3	Ham	Australia	UTAS	Mellefont and Ross (2007)	36	5	4.0-80	**3.5**	0.953-0.975	6.17-6.30	**6.0**	50	12.3-15.3[f]	-	0-0.15	1.00-2.86
M4	Ham	Australia	UTAS	-[d]	23	2	4.0-8.0	**3.5**	0.963-0.972	6.14-6.48	**6.0**	50	12.3-24.7[f]	-	0-0.15	1.00-2.86
M5	Cold-cut, beef or pork	Denmark	DMRI	-[d]	27	5	5.0-10.0	3.5-4.5	0.949-0.968	5.8-6.4	0-**6.0**	0-60	0-9.4[f]	-	-	0.47-3.63
M6	Sausages	Denmark	DMRI	-[d]	338	5	2.0-12.0	1.5-5.8	0.938-0.982	5.7-6.6	0-**6.0**	0-150	0-76.0[f]	0-0.67	-	0.20-4.35
M7	Sausages	Netherlands	PURAC	-[d]	40	3	4.0-120	**3.5**-5.7	0.952-0.985	5.67-6.95	-	0-25	-	0-0.17	0-0.05	0.85-4.28
Seafood products																
S1	Cold-smoked salmon	Denmark	DTU Food	Jørgensen and Huss (1998)	13	NC[h]	5.0	3.5-8.9	0.932-0.966	6.1-6.3	**6.0**	-	-	-	-	0.67-1.11
S2	Cold-smoked salmon	Denmark	DTU Food	Gimenez and Dalgaard (2004)	5	4	2.0-25.0	4.9	0.960	6.03	12.6	-	-	-	-	0.37
S3	Cold-smoked salmon	Denmark	DTU Food	Lakshmanan and Dalgaard 2004	2	4	5.5-9.9	4.9	0.959	6.12	14.6	-	-	-	-	0.59
S4	Cold-smoked salmon	Denmark	DTU Food	Mejlholm and Dalgaard (2007a,b)	17	4	7.8-14.3	3.17-5.26	0.939-0.968	5.80-6.24	1,0-14,2	-	0-61[g]	-	0-0.30	0.50-4.35
S5	Cold-smoked salmon	France	Ifremer	-[d]	45	1 or 4-5	4.0-8.0	3.4-5.5	0.955-0.969	**6.2**	2,5-20,6	-	-	-	-	**0.70**
S6	Cold-smoked salmon	Australia	UTAS	-[d]	19	1 or 2	4.0-15.0	**3.5**	0.953-0.964	6.05	**6.0**	-	-	-	0-0.19	**0.70**-3.16
S7	Gravad salmon	Denmark	DTU Food	Mejlholm and Dalgaard (2007a)	2	4	14.3	2.95-3.12	0.968-0.970	6.11-6.26	4,8-5,0	-	25.0[g]	-	0-0.17	0.93-0.98
S8	Cold-smoked Greenland halibut	Denmark	DTU Food	Mejlholm and Dalgaard (2007a)	8	4	7.6-7.7	3.74-4.64	0.954-0.968	6.17-6.50	16.2-20.1	-	0-26.0[g]	-	0.20-0.23	0.14-3.16
S9	Marinated Greenland halibut	Denmark	DTU Food	Mejlholm and Dalgaard (2007a)	2	4	7.8	3.40	0.967-0.970	6.68-6.84	-	-	16.2[g]	-	0.05-0.15	0.25-0.89
S10	Brined shrimp	Denmark	DTU Food	Mejlholm and Dalgaard (2009)	6	4	5.0-7.0	1.46-2.85	0.973-0.980	6.20-6.35	-	-	0-31.0[g]	0.06-1.07	-	0.07-0.84
S11	Cooked and peeled shrimp	Denmark	DTU Food	Mejlholm et al. (2005)	6	4	2.0-8.0	1.90	0.981	7.7	-	-	25.0[g]	-	-	0.08
Poultry products																
P1	Chicken	Denmark	DMRI	-[d]	4	5	5.0-10.0	3.39-3.48	0.957-0.966	6.50	-	60	7.2-9.4[f]	0-0.49	-	0.96-2.74
P2	Chicken	Australia	UTAS	-[d]	8	5	4.0-8.0	**3.5**	0.966-0.972	6.18-6.27	-	-	22.0-27.4[f]	-	0-0.14	**0.70**-2.38
P3	Chicken sausage	Netherlands	PURAC	-[d]	3	1	4.0-12.0	2.5-3.8	0.956-0.973	5.9-6.3	-	-	-	0-0.12	-	1.70-2.86
P4	Cold-cut, chicken or turkey	Denmark	DMRI	-[d]	11	5	5.0-10.0	2.7-4.1	0.950-0.967	6.0-6.4	-	0-60	7.2-9.4[f]	0-0.50	-	0.48-3.69
P5	Turkey ham	Netherlands	PURAC	-[d]	6	3	4.0	2.5	0.962-0.977	6.1-6.3	-	-	-	0-0.21	-	**0.70**-2.43
P6	Turkey sausage	Denmark	DMRI	-[d]	1	5	10.0	3.7	0.965	6.1	-	-	9.4[f]	-	-	0.96

[a] Danish Meat Research Institute (DMRI), University of Tasmania (UTAS), PURAC biochem b.v. (PURAC), Technical University of Denmark (DTU Food), Département des Sciences et Techniques Alimentaires Marines (Ifremer).

[b] *n*, number of experiments.

[c] Measured or calculated from the concentrations of water phase salt, water phase sodium-lactate and water phase sodium-acetate using the PURAC calculator (http://www.purac.com/purac_com/a5348511153c582f5bd69fd6bd64bb49.php).

[d] Not previously reported in the literature.

[e] Bold type: Assumed values. See explanation in Section 2.2.

[f] Calculated equilibrium concentrations in head space gas using Henry's constant at the appropriate storage temperature. See section 2.2.

[g] Measured equilibrium concentrations in head space gas.

[h] NC, naturally contaminated.

Table 3. Product characteristics and storage conditions in experiments (n = 277) used for evaluation of the predictive *Listeria monocytogenes* models

Products and codes	Products	Country	Reference	n [a]	No. of strains inoculated	Temp (°C)	Water phase salt (%)	a_w [b]	pH	Phenol (ppm)	Nitrite (ppm)	% CO_2	Acetic acid	Diacetate	Lactic acid
Meat products															
M8	Ham	Norway	Blom et al. (1997)	4	3	4.9	2.5-3.0	0.960-0.971	**6.2**[c]	**6.0**	-	-	0-0.26	-	**0.70**-3.57
M9	Ham	US	Glass and Doyle (1989)	4	5	4.4	3.2-4.1	0.964-0.969	6.29-6.52	-	18.7-28.0	-	-	-	**0.7**
M10	Ham	Netherlands	Stekelenburg and Kant-Muermans (2001)	5	1	4.0	2.6-3.1	0.961-0.977	5.9-6.2	**6.0**	11	-	0.24-0.25	-	0.83-3.08
M11	Ham	US	Burnett et al. (2005)	3	3	5.0-10.0	**3.5**	0.964	6.2	-	**50**	-	-	-	**0.7**
M12	Ham	US	Glass et al. (2007a,b)	2	5	4.0	3.52	0.967	6.39	**6.0**	34.6	-	-	0-0.14	**0.70**-2.87
M13	Ham	US	Hwang and Tamplin (2007)	17	6	4.0-25.0	2.6	0.951-0.969	6.2	-	-	-	-	0.06-0.20	1.50-5.45
M14	Ham	US	Pal et al. (2008b)	18	3	4.0-12.0	3.6	0.957-0.967	**6.2**	-	**50**	-	-	0-0.24	**0.70**-2.69
	Sausages														
M15	Bologna	US	Glass and Doyle (1989)	4	5	4.4	4.1-4.9	0.959-0.964	6.09-6.45	-	16.7-38.0	-	-	-	**0.70**
M16	Pork bologna	US	Barmpalia et al. (2005)	10	10	4.0-10.0	3.6	0.954-0.967	6.34-6.60	-	**50**	-	-	0-0.38	**0.70**-3.33
M17	Bratwurts	US	Glass and Doyle (1989)	2	5	4.4	4.4	0.962	6.45-6.48	-	-	-	-	-	**0.70**
M18	Bratwurts	US	Glass et al. (2002)	8	5	3.0-7.0	2.9-3.5	0.944-0.967	6.0-6.1	0-**6,0**	-	-	-	0-0.17	**0.70**-6.07
M19	Frankfurters	US	Porto et al. (2002)	5	5	4.0-10.0	2.5	0.960-0.974	5.78-6.11	**6.0**	4.5	-	-	-	**0.70**-3.69
M20	Frankfurters	Netherlands	Stekelenburg (2003)	6	1	4.0	3.1-3.2	0.964-0.974	6.1-6.3	-	7.3-12.7	-	0.14-0.19	0.06-0.10	0.72-2.50
M21	Frankfurters	US	Pal et al. (2008a)	18	3	4.0-12.0	3.9	0.952-0.965	6.17	**6.0**	**50**	-	-	0-0.31	**0.70**-3.24
M22	Servelat	Norway	Blom et al. (1997)	4	3	4.0-9.0	2.5-3.0	0.960-0.971	**6.2**	-	-	-	0-0.26	-	**0.70**-3.57
M23	Wieners	US	Glass and Doyle (1989)	4	5	4.4	4.4-5.1	0.957-0.962	5.89-6.18	**6.0**	14.7-20.7	-	-	-	**0.70**
M24	Wieners	US	Glass et al. (2002)	9	5	4.5	2.8-4.2	0.944-0.963	5.9-6.4	**6.0**	2.0-31.0	-	-	0-0.38	**0.70**-5.88
Seafood products															
S12	Cold-smoked salmon	US	Yoon et al. (2004)	8	1	4.0-10.0	**3.5**	0.959-0.967	6.1	**6.0**	-	-	-	0-0.17	**0.70**-3.62
S13	Cold-smoked salmon	US	Burnett et al. (2005)	3	3	5.0-10.0	**3.5**	0.981	6.4	**6.0**	**50**	-	-	-	**0.70**
S14	Cold-smoked salmon	US	Hwang and Sheen (2009)	8	6	4.0-16.0	2.97	0.971	6.5	4.0	-	-	-	-	**0.70**
S15	Smoked salmon	France	Thurette (1995)	15	3	4.0-12.0	3.4-5.6	0.954-0.968	6.2	8.8-11.2	-	-	-	-	**0.70**
S16	Smoked salmon	Australia	Szabo and Cahill (1999)	3	7	4.0-10.0	**3.5**	0.967	6.3	**6.0**	-	0-81.4[d]	-	-	**0.70**
S17	Cold-process salmon	US	Peterson et al. (1993)	6	3	5.0-10.0	3.0-6.0	0.951-0.971	6.2	-	-	-	-	-	**0.70**
S18	Cold-process salmon	US	Pelroy et al. (1994a,b)	22	3	5.0-10.0	2.0-3.0	0.954-0.971	6.1	-	0-96.7	-	-	-	0.70-4.41
S19	Cooked and peeled shrimp	US	Paranjpye et al. (2008)	3	1	5.0-10.0	1.9	0.981	7.7	-	-	-	-	-	0.08
Poultry products															
P7	Chicken	US	Glass and Doyle (1989)	2	5	4.4	1.8-2.4	0.975-0.978	6.35-6.39	-	-	-	-	-	**0.70**
P8	Turkey breast	US	Glass and Doyle (1989)	4	5	4.4	1.8-3.6	0.967-0.978	6.26-6.52	-	-	-	-	-	**0.70**
P9	Turkey breast	US	Burnett et al. (2005)	3	3	5.0-10.0	**3.5**	0.971	**6.2**	-	-	-	-	-	**0.70**
P10	Turkey breast	US	Glass et al. (2007a,b)	2	5	4.0	2.3	0.972	6.42	-	-	-	-	0-0.27	**0.70**-4.96
P11	Turkey breast	US	Pal et al. (2008b)	17	3	4.0-12.0	2.85	0.962-0.972	**6.2**	-	-	-	-	0-0.24	**0.70**-2.69
P12	Turkey breast	US	Peterson et al. (2008)	1	1	10.0	**3.5**	0.971	**6.2**	-	**50**	-	-	-	**0.70**
P13	Turkey bologna	US	Wederquist et al. (1994)	2	7	4.0	3.1	0.946-0.963	6.58-6.63	-	-	-	0-0.57	-	**0.70**
Non-fermented dairy products															
D1	Ice cream	Greece	Gougouli et al. (2008)	8	1	4.0-16.0	**0.9**	0.957-0.965	6.50-6.67	-	-	-	-	-	-
D2	Vanilla cream	Greece	Panagou and Nychas (2008)	4	4	3.0-15.0	**0.9**	0.987	6.7	-	-	-	-	-	-
D3	Milk	UK	Combase database[e]	9	3	5.0-10.0	**0.9**	0.987	5.4-7.0	-	-	-	-	-	-
D4	Skim milk	US	Rosenow and Marth (1987)	13	4	4.0-21.0	**0.9**	0.987	**6.7**	-	-	-	-	-	-
D5	UHT cream	Poland	Combase database[f]	8	1	3.0-12.0	**0.9**	0.987	6.7	-	-	-	-	-	-
D6	Whipping cream	US	Rosenow and Marth (1987)	13	4	4.0-21.0	**0.9**	0.987	**6.7**	-	-	-	-	-	-

[a] n, number of experiments.

[b] Measured or calculated from the concentrations of water phase salt, water phase sodium-lactate and water phase sodium-acetate using the PURAC calculator (http://www.purac.com/purac_com/a5348511153c582f5bd69fd6bd64bb49.php).

[c] Bold type: Assumed values. See explanation in Section 2.2.

[d] Calculated equilibrium concentrations in head space gas using Henry's constant at the appropriate storage temperature. See section 2.2.

[e] Food Standards Agency funded data generated at Champden and Chorleywood Food Research Association, UK (Combase id: L168_1 - L168_9)(Combase, 2009).

[f] Data from Dairy and Quality Management, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland (Combase id: ALO_01 - ALO_08) (Combase, 2009).

Table 4. Comparison of observed and predicted maximum specific growth rates (μ_{max} values) of *Listeria monocytogenes* in processed and ready-to-eat foods (n = 1014)[a]

Code	Products	*n* [b]	Delignette-Muller et al. (2006)	Augustin et al. (2005)	Zuliani et al. (2007)	PURAC (2007)	DMRI (2007)	Mejlholm and Dalgaard (2009)
Meat products								
M1	Pork loin	100[c]	1.8/2.3	1.5/1.9	0.8/1.8	1.2/1.9	0.9/1.5	0.8/1.5
M2-M5, M8-M14	Ham/cold-cuts	154[d]	2.6/2.7	2.2/2.4	1.8/2.1	1.7/2.2	1.2/1.5	1.2/1.7
M6-M7, M15-M24	Sausages	448[e]	2.2/2.4	2.2/2.6	1.3/2.1	1.3/1.6	1.1/1.6	1.0/1.5
Average bias and accuracy factors (meat)			**2.3/2.4**	**2.1/2.5**	**1.3/2.1**	**1.4/1.8**	**1.1/1.5**	**1.0/1.5**
Seafood products								
S1-S19		193[f]	1.7/1.8	0.7/1.9	1.2/1.6	1.3/1.5	1.4/1.6	1.0/1.4
Poultry products								
P1-P13		64	1.5/1.9	2.0/2.1	1.0/1.5	1.0/1.5	1.2/1.5	0.9/1.5
Non-fermented dairy products								
D1-D6		55	0.7/1.6	0.9/1.3	1.0/1.3	0.9/1.3	1.3/1.6	0.9/1.3
Average bias and accuracy factors (all data)			**2.0/2.2**	**1.8/2.3**	**1.3/1.9**	**1.3/1.7**	**1.2/1.6**	**1.0/1.5**

[a] See Tables 2 and 3 for information on product characteristics and storage conditions of the experiments.

[b] *n*, number of experiments.

[c] All these data were used for development of the DMRI model.

[d] 42 of these 154 experiments were used to develop the DMRI model.

[e] 276 of these 448 experiments were used to develop the DMRI model.

[f] 41 of these 193 experiments were used to develop the model of Mejlholm and Dalgaard (2009).

Table 5. Comparison of observed and predicted growth/no-growth responses of *Listeria monocytogenes* in processed and ready-to-eat foods (n = 1014)[a]

			Observed		Number of fail-dangerous/fail-safe predictions					
Code	Products	*n* [b]	Growth	No-growth	Delignette-Muller et al. (2006)	Augustin et al. (2005)	Zuliani et al. (2007)	PURAC (2007)	DMRI (2007)	Mejlholm and Dalgaard (2009)
Meat products										
M1	Pork loin	100[c]	36	64	0/64	6/29	7/1	0/64	4/14	6/1
M2-M5, M8-M14	Ham/cold-cuts	154[d]	102	52	0/52	17/26	26/13	0/52	6/29	8/22
M6-M7, M15-M24	Sausages	448[e]	304	144	0/144	28/65	49/27	1/133	25/57	31/29
Seafood products										
S1-S19		193[f]	160	33	0/33	39/18	12/9	0/33	0/27	1/6
Poultry products										
P1-P13		64	50	14	0/14	1/13	10/0	0/14	1/2	1/1
Non-fermented dairy products										
D1-D6		55	55	0	0/0	0/0	0/0	0/0	0/0	0/0
All data		**1014**	**707**	**307**	**0/307**	**91/151**	**104/50**	**1/296**	**36/129**	**47/59**
		Correct predictions (%)			**70**	**76**	**85**	**71**	**83**	**89**
		Fail-dangerous predictions (%)			**0**	**9**	**10**	**0**	**4**	**5**
		Fail-safe predictions (%)			**30**	**15**	**5**	**29**	**13**	**6**

[a] See Tables 2 and 3 for information on product characteristics and storage conditions of the experiments

[b] *n* , number of experiments

[c] All these data were used for development of the DMRI model.

[d] 42 of these 154 experiments were used to develop the DMRI model.

[e] 276 of these 448 experiments were used to develop the DMRI model.

[f] 41 of these 193 experiments were used to develop the model of Mejlholm and Dalgaard (2009).

Table 6. Effect of interactions beween environmental parameters on performance indices

Predictive models	Correct	Fail-dangerous	Fail-safe
Augustin et al. (2005)			
Without interaction	68	0	32
With interaction	76	9	15
Psi (ψ)-value (mean $\pm$ SD)		1.28 $\pm$ 0.39	0.69 $\pm$ 0.16
Zuliani et al. (2007)			
Without interaction	80	5	15
With interaction	85	10	5
Psi (ψ)-value (mean $\pm$ SD)		1.35 $\pm$ 0.60	0.64 $\pm$ 0.20
Mejlholm and Dalgaard (2009)			
Without interaction	69	1	30
With interaction	89	5	6
Psi (ψ)-value (mean $\pm$ SD)		1.22 $\pm$ 0.31	0.67 $\pm$ 0.18