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Predictive modelling of the growth and survival of *Listeria* in fishery products

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Abstract

Predictive microbiology provides a powerful tool to aid the exposure assessment phase of 'quantitative microbial risk assessment'. Using predictive models changes in microbial populations on foods between the point of production/harvest and the point of eating can be estimated from changes in product parameters (temperature, storage atmosphere, pH, salt/water activity, etc.). Thus, it is possible to infer exposure to *Listeria monocytogenes* at the time of consumption from the initial microbiological condition of the food and its history from production to consumption. Predictive microbiology models have immediate practical application to improve microbial food safety and quality, and are leading to development of a quantitative understanding of the microbial ecology of foods.

While models are very useful decision-support tools it must be remembered that models are, at best, only a simplified representation of reality. As such, application of model predictions should be tempered by previous experience, and used with cognisance of other microbial ecology principles that may not be included in the model. Nonetheless, it is concluded that predictive models, successfully validated in agreement with defined performance criteria, will be an essential element of exposure assessment within formal quantitative risk assessment.

Sources of data and models relevant to assessment of the human health risk of *L. monocytogenes* in seafoods are identified. Limitations of the current generation of predictive microbiology models are also discussed. These limitations, and their consequences, must be recognised and overtly considered so that the risk assessment process remains transparent. Furthermore, there is a need to characterise and incorporate into models the extent of variability in microbial responses. The integration of models for microbial growth, growth limits or inactivation into models that can predict both increases and decreases in microbial populations over time will also improve the utility of predictive models for exposure assessment. All of these issues are the subject of ongoing research.

Keywords: Predictive modelling; *Listeria*; Fishery products; Growth; Survival

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1. Introduction

1.1. Microbial risk assessment and predictive microbiology

Under the SPS Agreement, to harmonise international trade in foods, formal risk assessment was suggested to assess the level of human health risk posed by those foods. To assess that risk, one must know how much food is ingested, how often that food contains harmful agents, and how heavily contaminated the food is at the time of eating. Within the risk assessment process, this task is called 'exposure assessment' (ICMSF, 1998; CAC, 1999).

Predictive microbiology provides a powerful tool to aid assessment of human exposure to microbial pathogens in foods. Models that have been successfully validated enable changes in microbial populations on foods, between the point of production/harvest and the point of eating, to be estimated from product, storage and processing parameters, e.g. storage or cooking temperatures, storage atmosphere, pH, salt/water activity, preservatives, smoking, etc. Predictive models can also be used to assess the microbiological consequences of fluctuating conditions. Thus it is possible to infer exposure to pathogens from the initial microbiological condition of the product and the history of the product between production and consumption.

Predictive microbiology involves the development of mathematical models to describe the effect of the most important environmental factors controlling the responses of microorganisms in foods. Predictive microbiology models have immediate practical application to improve microbial food safety and quality, and also can provide quantitative understanding of the microbial ecology of foods. Thus, predictive microbiology models can be used pragmatically, simply to describe and summarise microbial responses in foods. If properly formulated, however, they can also model the physical, chemical and biological processes that give rise to those responses.

1.2. Assessing health risks due to Listeria monocytogenes in fish products

The perceived risk of *Listeria monocytogenes* in foods, including fish products, has received much attention. Foods which do not undergo a listericidal treatment before eating, e.g. seafoods eaten raw, lightly preserved seafoods like cold-smoked salmon, or seafoods which are subject to a disinfection step but may subsequently become recontaminated (e.g. cooked shrimp, crabmeat) have received particular attention. The NACMSF (NACMSF, 1991) reviewed epidemiological and animal model data on the dose of *L. monocytogenes* required to cause infection. It was noted that very high numbers of *L. monocytogenes* are required to infect normal mice and monkeys, while sometimes very low numbers (10's or 100's) will infect immunocompromised animals. It was concluded that the infectious dose of *L. monocytogenes* for humans cannot be stated with precision, but it is high for most healthy people and human listeriosis does not usually occur in the absence of a predisposing risk factor. Subsequent studies (Hitchins, 1996; Buchanan et al., 1997a; Notermans et al., 1998) have supported this view. Consequently, quantitative information about the survival and growth potential of *L. monocytogenes* initially present in fish products, and during subsequent handling and processing, will be critical to assessing the risk to health from these products.

This paper discusses the microbial ecology of *L. monocytogenes* in foods, and its description by predictive models. Together with data for the prevalence and level of contamination of *L. monocytogenes* on fisheries products, these models can be used to assess exposure from fish products. The paper will identify data and models describing the rate of growth, limits to growth, and rates of death of *L. monocytogenes* in seafoods, with particular emphasis on the behaviour of *L. monocytogenes* in cold-smoked salmon products.

2. Microbial ecology of fish products

The potential responses of populations of microorganisms in any stable environment include growth, lag before growth, survival or death. In fluctuating environments these responses may occur sequentially. To predict the fate of an organism in food it is necessary to know the ranges of environmental factors over which the organism can grow. The region that encompasses all combinations of all environmental factors that permit growth has been termed the 'Hyperspace Cloud' (Boddy and Wim-

penny, 1992). Beyond the Hyperspace Cloud the organism may survive or, more probably, die. To determine the microbial safety of a food, it is also necessary to know the *rates* of growth and death, lag phase resolution, etc. for the microorganisms of concern. All of these properties are dependent on the environment. Thus, equally, it is necessary to characterise the environment that the food presents to the organism.

2.1. Physico-chemical properties of fish and fish products

The major factors controlling the fate of microbial populations in seafoods are temperature, pH and organic acid levels, water activity, and preservatives. Furthermore, many seafoods have a high redox potential (+ 100 to > + 300 mv) and high pH resulting in a short shelf life and storage at low temperatures is required. Clearly, it is important to understand the behaviour of *L. monocytogenes* under these conditions.

The natural pH of fish and aquatic invertebrates varies as shown in Table 1. Post-mortem, the pH of the fish muscle drops to ~6.0–6.4 due to the catabolism of muscle glycogen resulting in lactic acid production. At that pH, the muscle contains from 60 to 120 μg lactate/g fish, i.e. 65–130 mM (Sikorski et al., 1990). Lower pH is correlated to higher lactate concentration, and the lactate present will enhance the inhibitory affect of the reduced pH. However, it is noteworthy that most seafoods have substantially higher pH and contain less lactate than meat products.

Table 1
The pH of the edible portions of some fish and shellfish[a]

Product	pH
Fish muscle (most species)	6.6–6.8
Clams	6.5
Crabs	7.0
Oysters	4.8–6.3
Tuna	5.2–6.1
Shrimp/prawns	6.8
Salmon	6.1–6.3

[a] After Jay (1996).

The water activity (a_w) of fresh fish is in the range 0.98–0.999. Lightly salted fish products (e.g. smoked salmon) are in the a_w range 0.93–0.98 while low salt products (3–2% NaCl in the aqueous phase) might be expected to have water activity in the range 0.97–0.98. Heavily salted fish products, e.g. anchovies, have a_w values in the range 0.6–0.85 (Christian et al., 1980). Cold-smoked salmon typically contains 3–6% water phase salt (a_w ~0.983–0.964), has a pH of ~6, and is frequently stored and distributed in vacuum packs at 5°C (Dalgaard, 1997).

3. Growth limits for *Listeria monocytogenes*

The growth limits of *L. monocytogenes* are discussed in detail in a number of reviews (e.g. Ryser and Marth, 1991; ICMSF, 1996) as summarised in Table 2. These limits are not absolute, however, as is discussed below.

3.1. Temperature limits

L. monocytogenes was reported to grow at temperatures <0°C in laboratory media broth (Walker et al., 1990; Bajard et al., 1996), and in vacuum-packed foods including seafood (Hudson et al., 1994; Bell et al., 1995). However, other reports of the

Table 2
Growth limits for *L. monocytogenes* (summarised from Ryser and Marth (1991) and ICMSF (1996))

Environmental factor	Lower limit	Upper limit
Temperature (°C)	−2 to +4	~45
Salt (% water phase NaCl)	13–16	<0.5
(& corresponding a_w)	(0.91–0.93)	(>0.997)
pH (HCl as acidulant)	4.2–4.3	9.4–9.5
Lactic acid (water phase)	0	3.8–4.6 mM, MIC of undissociated acid[a] (800–1000 mM, MIC of sodium lactate[b])

[a] From Tienungoon (1998).
[b] From Houtsma et al. (1993).

minimum growth temperature for *L. monocytogenes* range from 0.5°C to 5.0°C in various broth media (Wilkins et al., 1972; Junttila et al., 1988; Duh and Schaffner, 1993; Gill et al., 1997) and from 3°C to >5°C in foods (Ross, 1993; Membré et al., 1997; Tienungoon, 1998; Dalgaard and Jørgensen, 2000). These inconsistent reports may suggest that there are important strain differences, that *L. monocytogenes* has specific nutritional requirements for growth at lower temperatures, or that the physiological state of the inoculum used in challenge studies influence the minimum growth temperature.

3.2. Water activity/aqueous phase salt limits

Several researchers (Tapia de Daza et al., 1991; Farber et al., 1992; Nolan et al., 1992) reported the minimum a_w (NaCl as the humectant) for growth of *L. monocytogenes* to be 0.91–0.93. Estimates of the *theoretical* minimum water activity (a_{wmin}, discussed further below) include 0.912–0.916 (Wijtzes et al., 1993), 0.92–0.93 (Ross, 1993), and 0.923–0.927 (Tienungoon, 1998).

3.3. pH limits

The minimum pH for the growth of *L. monocytogenes* was reported to be 4.3 using HCl as the acidulant (Farber et al., 1989; George et al., 1996). Tienungoon (1998) found *L. monocytogenes* Scott A and L5 were able to grow at levels as low as pH 4.23 and 4.25 respectively in HCl-acidified media. Organic acids accentuate the pH inhibition of growth rates and limits, the magnitude of that inhibition being most dependent on the concentration of the undissociated acid (Sorrells et al., 1989; Young and Foegeding, 1993; Presser et al., 1997). Consequently, it is important to consider the concentration of undissociated organic acid in predictions of the microbial ecology of foods.

3.4. Atmosphere

In general, the growth of *L. monocytogenes* is reported to be little affected by anaerobic, or oxygen reduced, atmospheres (Buchanan and Phillips, 1990; Pelroy et al., 1994; Buchanan and Golden, 1995; ICMSF, 1996), however, growth is reduced by CO_2 as used in modified atmosphere packaging (MAP). In fresh trout, MAP with 60% CO_2/40% N_2 or 40% CO_2/30% N_2/30% O_2 prevented growth at 5°C over 2 weeks of storage although under aerobic storage growth was detected after 4 days (Davies, 1997). In smoked blue cod growth, observed at −1.5°C under vacuum packaging, was prevented during 160 days by MAP with 100% CO_2. At 3°C, this MAP condition reduced both the growth rate and yield substantially (Bell et al., 1995). Similarly, Ingham et al. (1990) reported inhibition of growth by high levels of CO_2, but only at low temperatures. Szabo and Cahill (1998) tested the combined effect of storage atmosphere, temperature and nisin on *L. monocytogenes* growth. At 4°C and with 100% CO_2, growth rate in buffered tryptone soya broth was reduced, and lag times increased, but the addition of the bacteriocin ALTA 2341 was required to prevent growth for at least 21 days. Nilsson et al. (1997) had earlier reported similar effects of CO_2, nisin and temperature when the growth substrate was cold-smoked salmon.

3.5. Smoke components

Wood and liquid smoke influences growth rate, growth limits, and rates of death/inactivation of *L. monocytogenes*. Smoke components prevented growth of *L monocytogenes* inoculated on the surface of cold-smoked, whole salmon fillets stored in vacuum packs at 4°C for 28 days (Niedziela et al., 1998). Nevertheless, substantial growth occurred when inoculated on the sliced product stored at similar conditions (Rørvik et al., 1991; Hudson and Mott, 1993; Macrae et al., 1990). Smoke components are primarily deposited in the outer 0.5 cm of fish muscle during smoking (Chan and Toledo, 1975). This may explain the different growth of *L. monocytogenes* on whole and sliced smoked fish but further studies are required to identify inhibitory components in different types of smoke. Phenols in concentrations of 5–20 ppm reduced growth of *L. monocytogenes* markedly in liquid broth as well as in cold-smoked salmon (Membré et al., 1997; Thurette et al., 1998). However, other studies found only a weak antilisterial effect of phenols and even for the more effective compounds, including isoeugenol, tertiary butylhydroquinone and propylparaben, high levels (64–512 ppm) were required to inhibit growth of *L. monocytogenes* (Payne et al., 1989a; Payne et

al., 1989b; Faith et al., 1992; Niedziela et al., 1998). Recently, formaldehyde (50 ppm) was found to delay growth of *L. monocytogenes* by 2 weeks at 8°C and it may be an important inhibitory component of smoke (Niedziela et al., 1998). Eklund et al., (1995) found a 10- to 25-fold reduction of *L. monocytogenes* during cold-smoking of salmon at 17.2–21.1°C but no effect was observed at 22.2–30.6°C. Eklund et al. (1995), however, noted also that *L. monocytogenes* injected into the interior of the same portions increased from 2- to 6-fold at 17.2–21.1°C but 100-fold at 22.2–30.6°C, regardless of the presence of smoke. During hot-smoking of salmon the temperature required for inactivation of *L. monocytogenes* without smoke (82.8°C) was reduced to 67.2°C by smoke generated from sawdust and to 58.9°C with a high level of a commercial liquid smoke (Poysky et al., 1997).

3.6. Interaction of abiotic factors

There is an important distinction between conditions optimal for growth *rate* and conditions optimal for growth *yield*. That difference was discussed, and an explanation proposed, by Ross (1999a). For *L. monocytogenes* yield is maximal when the temperature is in the range 20–25°C, while the growth rate is fastest at ~37°C. For several food-borne pathogens, greatest tolerance to one sub-optimal condition is exhibited at conditions optimal for growth yield (George et al., 1996; Presser et al., 1998; Salter, 1998; Tienungoon, 1998). A corollary is that when several factors are sub-optimal for growth, the ranges of each tend to be reduced. There are exceptions to this behaviour. While slightly elevated salt concentration may inhibit growth rate, it has also been reported to increase the high temperature tolerance of many bacterial species, though the effect is not universal. In consequence, when describing limits to growth of an organism, it is necessary to describe all other conditions under which those limits were determined. Unfortunately, this is not always done.

Only a few models (Ratkowsky and Ross, 1995; Presser et al., 1998; Razavilar and Genigeorgis, 1998; Tienungoon, 1998; Bolton and Frank, 1999) are available to describe the growth limits of specific microorganisms when several factors interact to prevent growth. Such models quantify the Hurdle Concept (Leistner, 1985, 1994). Tienungoon (1998) studied the combined effects of temperature, pH, lactic acid and water activity (NaCl) on the potential for growth within 90 days of two strains of *L. monocytogenes*. Figs. 1 and 2 show the predicted boundary between conditions that *do* permit growth and those that *do not,* based on her studies, and compared to the observations of independent workers using different strains. Those figures also illustrate the interactions between factors.

4. Predictive microbiology

4.1. Growth rate models

The combination of factors required to prevent growth of *L. monocytogenes* suggest that growth will be possible in many fish products under normal storage conditions, other than frozen storage.

4.2. Sources of growth rate models and data

Much data (ICMSF, 1996) and many models for prediction of the growth rate of *L. monocytogenes* are available (Table 3). In general, *L. monocytogenes* responds to environmental factors with the same patterns of response as other vegetative microorganisms and can be described by the same forms of model that describe growth rate responses of other organisms (Ross, 1993; Wijtzes et al., 1993, Tienungoon, 1998). However, it is noted that the temperature–growth rate relationship of *L. monocytogenes* is not as well described by existing models as are other organisms, particularly at low temperatures that cause slow growth rates (Bajard et al., 1996; Ross, 1999b).

Currently, no single model includes the effect of all variables which may be of interest in all foods. Thus, users of models must be aware of the predictive limits of models, both in terms of the range of conditions that a model's interpolation region encompasses (Baranyi et al., 1996) and the variables that the model considers. 'Completeness error' arises in model predictions when the model does not explicitly consider the effect of factors in a food that will affect the growth response of the microorganism modelled. The models referred to in Table 3 were developed to test different modelling strategies or, in

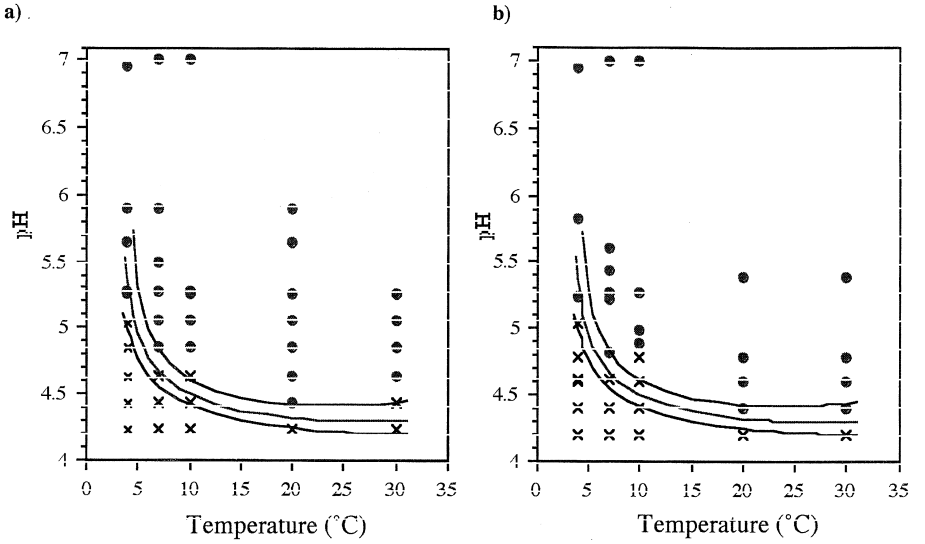

Fig. 1. Data of George et al. (1988) showing growth (●) or non-growth (×) of: (a) *L. monocytogenes* NCTC 10357; and (b) *L. monocytogenes* Scott A in TSB+1% glucose+0.3% yeast extract (a_w~0.995) compared to the growth limits (at three levels of confidence) predicted by the model of Tienungoon (1998). Lower line, 90% confidence that growth will never occur under those conditions; central line, 50% confidence; upper line, 10% confidence.

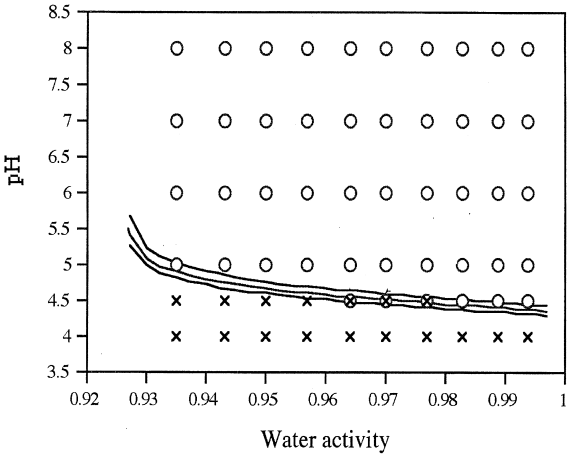

Fig. 2. Data of McClure et al. (1989) showing the effect of water activity and pH on the growth potential of *L. monocytogenes* NCTC 9863 and compared to the growth/no growth interface predicted by the model of Tienungoon (1998). Growth observed: (○); growth not observed (×). Lower line, model prediction at 90% confidence that growth will not ever occur under those conditions; central line, model prediction at 50% confidence; upper line, model prediction at 10% confidence.

the later published models, to include the effect of specific variables not included in earlier models. In theory, a single model could encompass all the variables of relevance in all foods. This is the ultimate aim of the scientific approach to predictive microbiology as the basis of a quantitative understanding of the microbial ecology of foods. However, creating such a model and scientific framework is time consuming. Alternatively, an iterative approach for the development of product oriented models, i.e. based on observations in a system closely related to the food of interest may satisfy the current technological needs of the food industry (Dalgaard, 1997; Dalgaard et al., 1997). When conducted rigorously, the different approaches all contribute to the longer term objectives of predictive microbiology. Under optimal conditions *L. monocytogenes* has a generation time in the range of 35–40 min (Ross, 1993; ICMSF, 1996; Tienungoon, 1998). Generation times of *L. monocytogenes* under a range of conditions can be estimated easily using models such as most of those listed in Table 3. One can easily incorporate a published model into spreadsheet software. As an example, indicative generation times of *L. monocytogenes* under conditions relevant to some seafood products and predicted by a model of the type presented as Eq. (1) (below) are shown in Table 4. That model can be considered as a series of terms, one for each environmental factor modelled. The

terms are multiplied together to give the overall prediction.

$$\begin{aligned}\text{Growth rate} = \text{constant}\\ &\times((\text{temperature} - T_{\min})\\ &\times(1 - \exp(\text{constant} \times (\text{temperature} - T_{\max})))^2\\ &\times((\text{water activity} - a_{w\min})\\ &\times(1 - \exp(\text{constant}\\ &\times(\text{water activity} - a_{w\max}))))\\ &\times(1 - 10^{pH_{\min} - pH})\\ &\times(1 - 10^{pH - pH_{\max}})\\ &\times\left(1 - \frac{\text{organic acid concentration}}{U_{\min} \times (1 + 10^{pH - pK_a})}\right) \qquad (1)\end{aligned}$$

where: temperature, pH and water activity have their usual meaning. $T_{\min}$ is a theoretical minimum temperature for growth of the modelled organism, determined by extrapolation of the modelled growth rate response to the sub-optimal temperature at which growth rate is predicted to be zero; $T_{\max}$ is a theoretical maximum temperature for growth of the modelled organism, determined by extrapolation of the modelled growth rate response to the super-optimal temperature at which growth rate is predicted to be zero; $a_{w\min}$, $a_{w\max}$, $pH_{\min}$ and $pH_{\max}$, and $U_{\min}$ are analogous terms describing the theoretical pH and water activity limits and theoretical minimum inhibitory concentration of a specific undissociated organic acid respectively; pK_a is the pH at which half of the specific organic acid is in the dissociated form.

In Eq. (1) the first line models the effect of

Table 3
Some predictive models for the growth rate of *L. monocytogenes*

Authors	Variables and ranges
Buchanan and Phillips, 1990	T (5–37°C); pH (4.5–7.5); NaCl (0.5–4.5%w/v); $NaNO_2$ (0–200 μg/ml).
Cole et al., 1990	T (5–30°C); pH (4.4–7.0); NaCl (0–18% w/v).
McClure et al., 1991	T (1.0–35°C); pH (4.4–7.4); NaCl (0.1–11.5% w/v).
Grau and Vanderlinde, 1993	Lean beef: T (0–43°C); pH (5.6–6.7). Fat beef: T (0–31°C); pH (5.6–5.7).
Ross, 1993	T (3–37°C); pH (5.6–7.0); NaCl (0.5–13% w/v); lactic acid (0 or 200 mM).
Duh and Schaffner, 1993	T (3–46°C).
Wijtzes et al., 1993	T (5–35°C); pH (4.6–7.4); NaCl (0.5–6%w/v).
Murphy et al., 1996	T (3–35°C); pH (4.5–7.5); NaCl (0–8%w/v),
George et al., 1996	T (1–20°C); pH (4.5–7.2); lactic acid (0–222 mM); acetic acid (0–10000 mg/l)
Farber et al., 1996	T (4–10°C); pH (5.5–6.5); CO_2 (10–90%).
Fernandez et al., 1997	T (4–20°C); pH (4.5–7.0); NaCl (0.5–8.0%); CO_2 (0–100%, balance N_2).
McClure et al., 1997	T (1–35°C); pH (4.0–7.2); NaCl (0.5–11.5%); $NaNO_2$ (0–200 mg/l)
Membre et al., 1997	T (4–12°C); NaCl (2–4% w/v); phenol (5–20 ppm)
Lebert et al., 1998	T (4–30°C); a_w (0.96–0.992); pH (5.4–7.0)
Tienungoon, 1998	T (3–37°C); pH (4.2–7.3); NaCl (0.5–13% w/v); lactic acid (0–450 mM)
Nerbrink et al., 1999	T (9°C); NaCl (1.0–4.0%), pH (5.5–6.5); sodium lactate (0.0–0.5%); sodium acetate (0.0–0.6%); sodium nitrate (70 ppm)

Table 4
Predictions of growth rate of *Listeria monocytogenes*: model of Tienungoon (1998)

Growth rate ([l/generations time (h)])			
Temperature	pH 7.0, a_w: 0.990	90 mM total lactate, pH 6.2, a_w: 0.990	90 mM total lactate, pH 6.2, a_w: 0.965
25	1.22	1.03	0.646
10	0.174	0.147	0.092
7	0.078	0.066	0.042
5	0.035	0.030	0.019
0	0.002	0.001	0.001

temperature on growth rate, the second line models the effect of water activity, the third line models the effect of pH and the fourth line models the effect of undissociated lactic acid. If one factor is held constant the corresponding term can be evaluated and replaced by a constant. Thus, the model is readily simplified and, once the parameters values are fitted, requires no special mathematical skills to use. That type of multi-factor model was first presented by McMeekin et al. (1987) and the approach has since been incorporated into the cardinal parameter family of models (Rosso et al., 1995) and the gamma model concept (Zwietering et al., 1996). An advantage of this type of approach is that it enables the relative effect of a change in the level of one variable to be readily calculated, without the need to re-evaluate the entire equation. In the context of risk assessment, the ability to calculate the relative effects of various changes may be useful for performing sensitivity analyses, for example, to evaluate potential risk mitigation strategies.

4.3. Evaluation of growth rate model performance

A number of authors have evaluated the reliability of predictive microbiology models for *L monocytogenes* growth rate and many have concluded that the models perform satisfactorily (Buchanan and Phillips, 1990; Wijtzes et al., 1993; George et al., 1996; Fernandez et al., 1997; McClure et al., 1997; te Giffel and Zwietering, 1999). The definition of 'satisfactory' performance was, in some cases, ill-defined. Ross (1996) introduced measures of model performance called the Bias factor (B_f) and Accuracy factor (A_f). Modifications to the factors were proposed by Baranyi et al. (1999). Ideally, predictive models would have $A_f = B_f = 1$ but, typically, the accuracy factor will increase by 0.10–0.15 for every variable in the model. Thus, an acceptable model that predicts the effect of temperature, pH and water activity on *Listeria* growth rate could be expected to have $A_f = 1.3–1.5$. Satisfactory B_f limits are more difficult to define because limits of acceptability are related to the specific application of the model. B_f is a measure of the extent of under- or over-prediction by the model of the growth rates observed. Thus, a bias factor of 1.1 indicates not only that the model is 'fail-dangerous' because it predicts longer generation times than are observed, but also that the observations exceed the predictions, on average, by 10% in terms of log CFU. Conversely, $B_f < 1$ indicates that a model is, in general, 'fail-safe'. Note, however, that when applied to *rate*-based data, $B_f > 1$ indicates the model *under*-predicts the observed rate, potentially leading to 'fail-dangerous' predictions.

Ross (1999b) recommended that for models describing *pathogen growth rate*, B_f in the range 0.9–1.05 could be considered good, in the range 0.7–0.9 or 1.06–1.15 considered acceptable, and less than ~0.7 or >1.15 considered unacceptable. Ross (1999b) considered that for pathogens, less tolerance should be allowed for $B_f > 1$ because B_f in excess of one corresponds to under-predictions of the extent of growth and could lead to 'fail-dangerous' predictions. Dalgaard (2000) suggested that B_f values for successful validations of seafood *spoilage* models should be in the range 0.8–1.3. Conversely, Armas et al. (1996) considered that B_f values in the range 0.6–3.99 were acceptable for the growth rates of pathogens and spoilage organisms when compared to independently published data.

Thurette et al. (1998) compared the predictions of the model of Membré et al. (1997) to the observed growth of *L. monocytogenes* in smoked fish products held at either 4, 8 or 12°C for 7, 14, 21 or 35 days. Predictions were considered satisfactory if the observed and predicted growth differed by <0.5 log CFU/g. The Membré et al. (1997) model was satisfactory in ~60% of cases, while predictions from the Food MicroModel software were satisfactory in ~22% of cases. It was notable that the Food MicroModel predictions became much less reliable as storage time increased. Further, Food Micromodel predictions overestimated the lag time duration, but underestimated the generation time. Under some conditions, these individual prediction errors counteracted one another to give a more satisfactory prediction of the 'time required for a 1000-fold increase' in the numbers of *Listeria* initially present, than either parameter estimated separately.

Dalgaard and Jørgensen (1998) compared the growth of *L. monocytogenes* predicted by four models with that observed in cured and non-cured seafoods inoculated with *L. monocytogenes* and with growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon. All models tested *over*-predicted the observed rate of growth, some with B_f in the range 3–4, indicating over-prediction of the

observed rate of growth by factors of 300 to 400%. The over-prediction was greater for naturally contaminated cold-smoked salmon. Jørgensen and Huss (1998), Thurette et al. (1998) and te Giffel and Zwietering (1999) also found that available models over-predicted the growth of *L. monocytogenes* in seafoods. However, growth responses predicted by some models are close to values obtained in challenge tests.

If terms for the effects of, e.g. lactate, smoke components and nitrate were included in those models, they likely could provide growth rate estimates that agree with those observed in challenge studies with both cured and non-cured seafood (Dalgaard and Jørgensen, 1998). Those authors suggested two reasons for the failure of the models tested to predict *L. monocytogenes* growth accurately in naturally contaminated product. The first was the possibility of microbial injury leading to very long lag times which were not accounted for by the models. The second is a consequence of the growth of other organisms on the product, a phenomenon termed the 'Jameson effect' by Stephens et al. (1997). Jameson (1962), in studies concerning the growth of *Salmonella*, reported the suppression of growth of all microorganisms on the food when the total microbial population achieved the maximum population density (MPD) characteristic of the food. The same effect has been reported for *S. aureus* in seafoods (Ross and McMeekin, 1991) and *L. monocytogenes* in meat products (Grau and Vanderlinde, 1992) as well as for co-cultures of *L. monocytogenes* and *Carnobacterium* spp. in laboratory broth, fish juice and seafood (Buchanan and Bagi, 1997; Duffes et al., 1999; Nilsson et al., 1999). *C. piscicola* make up a substantial part of the natural spoilage microflora in cold-smoked salmon (Paludan-Müller et al., 1998; Leroi et al., 1998) and this organism most likely contributes to the low MPD of *L. monocytogenes* observed in naturally contaminated cold-smoked salmon. *Carnobacterium* spp. produce bacteriocins, but this may not be responsible for the antilisterial effect observed in cold-smoked salmon (Nilsson et al., 1999). Detailed mathematical models can be developed for indirect interaction between microorganisms as caused by changes in the environment, e.g. pH, redox potential, substrates or specific metabolites. However, any model for growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon needs to explain both: (i) an initial phase of limited growth; and (ii) growth under conditions where *C. piscicola,* or other components of the natural microflora, are present in high levels and change micro-environments in the product. Conversely, the reduced growth of *L. monocytogenes* in cold-smoked salmon suggests that removal of contaminating organisms, e.g. by hot smoking, cooking or pasteurising, may render foods *more* susceptible to the consequences of contamination with *L. monocytogenes.* Observations that model predictions agree better with *Listeria* growth on heat-treated products, on which the contaminating microbiota would be expected to be greatly reduced (Dalgaard and Jørgensen, 1998; Jørgensen and Huss, 1998), and that model predictions were better for conditions at which *Listeria* grow more quickly relative to the indigenous microbiota (Thurette et al., 1998), are consistent with the Jameson effect.

A fundamental principle of formal food safety risk assessment under the Codex Alimentarius Commssion Framework (CAC, 1999; FAO, 1995) is that the assessment be science-based and defensible. From the foregoing discussion, it is apparent that before a predictive microbiology model can be used in risk assessment the validity of its predictions must be demonstrated in the product of concern under realistic conditions of contamination and handling. If it cannot, or has not been demonstrated, this applicability of the model should be identified explicitly as an assumption.

4.4. Lag time response

In effect, physiological lag times before exponential growth commences reduce the potential growth of an organism during a given period of time. Lag time duration has often been considered erratic, and evaluations of predictive models has shown that lag times are less reliably predicted than generation times. This has usually been attributed to the effect of the prior history of cells on the duration of the lag time. Robinson et al. (1998) formalised a concept of the lag time as being dictated by two elements: (i) the amount of work required of the cell to adjust to a new environment and/or repair injury due to the shift to the new environment; and (ii) the rate at which those repairs and adjustments can be made. The latter is presumed to respond to the environment

in the same way, relatively, that generation time does (i.e. if the environment causes the generation time to double the lag time will also double, etc.). A number of studies have now been published which systematically consider the effect of the prior history of the cell, including prior temperature and osmotic stresses on the duration of lag time when the cells are subsequently grown (Robinson et al., 1998; Bréand et al., 1997; Bréand et al., 1999). Other studies (Ross, 1999b) have considered the effect of stationary phase stresses and osmotic stresses on lag times. That information was combined with published observations to determine the distribution of lag times that are observed. When the lag time is expressed as an equivalent number of generation times of the organism in the same environment (i.e. lag time divided by generation time), the distribution of lag times observed has a sharp peak in the range 3–6. This suggests that in many situations there is a practical upper limit to the lag time duration (Ross, 1999b). This information can be exploited for 'exposure assessment' by enabling the effect of fluctuating environmental conditions on lag time duration to be estimated, as well as placing limits on the risk-mitigating effects of lag times.

4.5. Death rates

Beyond the biokinetic range, microorganisms will either survive or be inactivated. Inactivation usually follows log-linear kinetics, characterised by *D* and *z*-values, although the actual kinetics may be complex and involve several distinct phases, each with its own log-linear rate. Until recently, *D* and *z*-values were the primary methods of modelling thermal inactivation of microorganisms.

Several recent reports indicate that log-linear models, i.e. based on *D*-values, are inadequate to describe the death kinetics of *L. monocytogenes*, and that more complex (e.g. sigmoidal) functions are needed. Augustin et al. (1998) used the concept of heat resistance *distributions* to develop models. The issue of variability in responses between strains, or due to uncontrolled variables, is currently a major theme in 'predictive microbiology'. It is expected that predictive microbiology models will, increasingly, also incorporate such information and provide predictions not only of the most likely response, but also of the range of responses that may be expected.

The use of temperatures above the biokinetic range to inactivate microorganisms may be termed 'thermal' processes, while the use of other growth preventing conditions, e.g. high salt or low pH, which result in inactivation have been called 'non-thermal inactivation'.

4.5.1. Thermal inactivation

Ryser and Marth (1991) reviewed in detail the large literature on the thermal inactivation of *L. monocytogenes*. More recently, ICMSF (1996) provided extensive lists of thermal inactivation times under different conditions and food types. Those data do not support the often cited view that *L. monocytogenes* has unusually high thermal tolerance. Rather, *D*-values for *L. monocytogenes* are similar to those for *Salmonella* or *S. aureus.*

As with other organisms, heat tolerance of *L. monocytogenes* can be maximised by prior sub-lethal shocks, stress or having reached stationary phase. These effects, and effects on subsequent lag and growth, have been studied and modelled (Stephens et al., 1994; Bréand et al., 1997; Augustin et al., 1998; Bréand et al., 1999). Ben Embarek and Huss (1993) reported the heat resistance of *L. monocytogenes* was higher (D_{60}-values 4.23–4.48 min, $z=6.4$) in vacuum-packed sous-vide cooked (58–80°C) fillets of salmon than in cod fillets (D_{60}-values 1.95–1.98 min, $z=5.7$). Increased thermal resistance in the presence of fat has been widely reported for other foodborne pathogenic bacteria. *D*-values in seafood were consistent with those reported for other food products (e.g. Farber et al., 1989; Mackey and Bratchell, 1989; ICMSF, 1996).

4.5.2. Non-thermal inactivation

In some products, e.g. heavily salted, marinated, etc., the combination of environmental conditions may be beyond the Hyperspace Cloud for the growth of *L. monocytogenes*. Conditions which prevent growth of microorganisms ultimately lead to their inactivation. Low temperature seems to be an exception to the general rule that more extreme conditions accelerate rates of microbial inactivation. Lower temperatures reduce the rate of death when other factors prohibit growth; very low temperature is routinely used as a method of culture preservation. Non-thermal inactivation may be very slow. Seeliger (1961) reported that *L. monocytogenes* can survive

for up to 1 year in 16% NaCl (a_w: 0.883). The mechanisms of non-thermal inactivation are currently poorly understood but are being studied by several groups around the world, but frequently using pathogenic *Escherichia coli* as the test organism.

Buchanan and his colleagues have provided much non-thermal inactivation data for *L. monocytogenes* (e.g. Buchanan and Golden, 1994; Buchanan and Golden, 1995; Golden et al., 1995; Buchanan et al., 1997b). In most of those studies, organic acid was considered the main factor causing inactivation. A single predictive model encompassing much of the USDA data was presented in Buchanan et al. (1997b). The inactivation kinetics were not log-linear, but the model predicts the time required to reduce the original population by 99.99%, a time termed t_{4D}. The model includes the effects of temperature (4–42°C), pH (3–7), lactic acid (0–2%), NaCl (0.5–19%) and sodium nitrate (0–200 μg/ml). Buchanan et al. (1997b) compared the predictions of that model to published values for the inactivation of *L. monocytogenes* under analogous condition in foods. Model predictions typically *over*-estimated the observed t_{4D} by factors of 2 to 3, i.e. the predictions were too conservative, and it is probable that factors other than those included in the model are important in determining the rate of non-thermal inactivation. Performance criteria and limits for successful validation of inactivation models remain to be established. Nonetheless, the model and data provide useful information on the qualitative features of the responses to non-thermal lethal conditions in foods.

Data are also available for rates of radiation inactivation and are summarised in ICMSF (1996).

4.6. Predictive microbiology software resources

Some of the models referred to above are available in software packages. The Pathogen Modeling Program, available free from the Internet, includes the growth and non-thermal inactivation models developed by Buchanan and his colleagues. Food MicroModel, which may be leased on an annual basis, also includes models for the growth of *L. monocytogenes*. Neither software considers the effect of factors which may contribute to the Jameson effect. Nor can either make predictions for the effects of conditions which fluctuate over time, a technique generally described as time–environmental function integration. Temperature is the factor most likely to change over the storage life of the product. There are a number of 'time-temperature function integration' systems available or proposed (see McMeekin et al., 1993; Chapter 7), and other factors discussed above can also be incorporated into software, but none are yet publicly available.

5. Conclusions

5.1. Predictive microbiology: needs for assessment of the health risk of L. monocytogenes in fish products

Currently, there exists a wealth of data and models that can be used to predict the changes in the numbers of *L. monocytogenes* in fish products for human consumption. There is, as yet, no model that includes all the variables that may affect the growth, survival and/or death of *L. monocytogenes* in fish products such as temperature, pH, organic acid concentration, water activity, gaseous atmosphere and smoke components. It would be beneficial to draw together all the available data to generate models that encompass all factors that may affect the fate of *L. monocytogenes* in seafoods. Importantly, there has been very little work done to include in predictive models factors that contribute to the 'Jameson effect', i.e. the suppression of growth of all microorganisms on the food by high *total* microbial loads. In some products, this effect may greatly reduce the health risk from *L. monocytogenes* predicted on the basis of models currently available. For example, high levels of *L. monocytogenes* ($>10^5$ cfu/g) have not been observed in studies with naturally contaminated cold-smoked salmon. In fact, levels most often remain at 10^3–10^4 cfu/g or lower even after 1–2 months of storage (Teufel and Bendzulla, 1993; Cortesi et al., 1997; Jørgensen and Huss, 1998). This suggests that cold-smoked salmon is a practical example of the 'Jameson effect' and that while the presence of *L. monocytogenes* on this product is not uncommon, high levels of *L. monocytogenes* on this product may not often occur. Other factors that may have a large affect on predicted outcomes are the duration of the lag phase of *L. monocytogenes* in seafoods, and whether additional

lags may be induced by changes in the product during normal processing and handling. Furthermore, there is a need to characterise and incorporate into models the extent of variability in microbial responses. The integration of models for microbial growth, growth limits or inactivation into unified models that can predict both increases and decreases in microbial populations over time, will also improve the utility of predictive models for exposure assessment. All of these issues are the subject of ongoing research.

Growth, survival and death models for *L. monocytogenes* are now available, including models that accurately predict growth in challenge tests. Quantitative or semi-quantitative assessment of that risk may now be possible by using models that have been successfully validated ($0.7 < B_f < 1.15$) in products of similar microbial ecology to the product of interest. However, some models are too 'fail safe' to be useful. Clearly, the generally applicable and highly accurate predictive microbial models required for quantitative microbial risk assessment in seafood are not yet available. However, other types of information required for hazard identification, exposure assessment and hazard characterization are also incomplete, and this does not prevent application of risk assessment. Given that decisions related to food safety currently rely on data from challenge tests, those decisions may equally well rely on data from predictive models. Any such assessment, however, must explicitly recognise the limitations of the current generation of predictive microbiology models so that the risk assessment process remains transparent. One means to achieve this is to use the bias and accuracy factors as simple indices of performance for the models to be used in risk assessment. Indices of performance and specified critical limits for predictive microbial models and for other steps in risk assessment, e.g. dose-response modelling, will contribute to a transparent system where the weakest points can be identified and improved when required.

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