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# BAYESIAN MODELING OF BACTERIAL GROWTH FOR MULTIPLE POPULATIONS[1]

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Bacterial growth models are commonly used for the prediction of microbial safety and the shelf life of perishable foods. Growth is affected by several environmental factors such as temperature, acidity level and salt concentration. In this study, we develop two models to describe bacterial growth for multiple populations under both equal and different environmental conditions. First, a semi-parametric model based on the Gompertz equation is proposed. Assuming that the parameters of the Gompertz equation may vary in relation to the running conditions under which the experiment is performed, we use feedforward neural networks to model the influence of these environmental factors on the growth parameters. Second, we propose a more general model which does not assume any underlying parametric form for the growth function. Thus, we consider a neural network as a primary growth model which includes the influencing environmental factors as inputs to the network. One of the main disadvantages of neural networks models is that they are often very difficult to tune, which complicates fitting procedures. Here, we show that a simple Bayesian approach to fitting these models can be implemented via the software package `WinBugs`. Our approach is illustrated using real experimental *Listeria monocytogenes* growth data.

**1. Introduction.** The predictability of bacterial growth is of major interest due to the influence of bacteria on food safety and health. The evolution of microorganisms in food products can spoil the products or even cause pathogenic effects. Foods are ecosystems composed of the environment and the organisms that live in it. The food environment is composed of intrinsic factors inherent to the food (pH, water activity, nutrients) and extrinsic factors external to it (temperature, gaseous environment, bacteria). The interactions between the chemical, physical and structural aspects of a niche and the composition of its specific microbial population emphasize the dynamic complexity of food ecosystems [ICMSF (1980)]. Food may contain multiple microenvironments and can be heterogeneous on a micrometer scale [Montville and Matthews (2005)]. Products in the modern

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food supply are often preserved by multiple hurdles that control microbial growth, increase food safety and extend product shelf life [Leistner (2000)]. Salt, high- or low-temperature processing and storage, pH, redox potential and other additives are examples of hurdles that can be used for preservation [IOM, Institute of Medicine of National Academies (2010)]. The influence of pH on bacterial gene expression is a relatively new area [Montville and Matthews (2005)]. The expression of genes governing proton transport, amino acid degradation, adaptation to acidic or basic conditions, and even virulence can be regulated by the external pH. The influence of temperature on microbial growth is very important, both in growth rate and in gene expression. Cells grown at different temperatures express different genes (governing from motility to virulence) and are physiologically different [Montville and Matthews (2001)]. Salt is effective as a preservative because it reduces the water activity of foods (i.e., the amount of unbound water available for microbial growth and chemical reactions) by the ability of sodium and chloride ions to associate with water molecules [Fennema and Tannenbaum (1996); Potter and Hotchkiss (1998); IOM, Institute of Medicine of National Academies (2010)]. Adding salt to foods can also cause osmotic shock in bacteria cells, limit the oxygen solubility, interfere with cellular enzymes, or force cells to expend energy to exclude sodium ions from the cell [Davidson (2001); Shelef and Seiter (2005); IOM, Institute of Medicine of National Academies (2010)]. *L. monocytogenes* is able to grow over a wide range of temperatures ($-0.4$ to $45^\circ$C), pH values (4.39 to 9.4) and osmotic pressures (NaCl concentrations up to 10%). It is also facultatively anaerobic [Montville and Matthews (2005)]. Summarizing, all these factors can be manipulated to preserve food due to their influence on the microbial growth. However, even when it is well known that these factors affect bacterial growth, the kind of effects and the interactions of the factors are still unclear and need more research. Accurate models which describe the bacterial growth and the effect of environmental factors are very important to prevent diseases by determining the shelf life of perishable foods or by predicting the behavior of foodborne pathogens.

Starting from Gompertz (1825), various parametric growth models which describe the evolution of the population size directly as a function of time—called primary models—have been developed; see, for example, McKellar and Lu (2004) for a good comparison. These models perform well in describing the evolution of bacterial density under fixed experimental conditions. Nevertheless, as described before, bacterial growth is strongly affected by environmental conditions such as temperature, acidity or salinity of the environment and, therefore, when multiple bacterial populations are analyzed, it is important to account for these effects in growth curve modeling.

In predictive microbiology, models that describe the effect of environmental conditions on the growth parameters are called secondary models; see, for example, Ross and Dalgaard (2004). For example, the square-root model of Ratkowsky et al. (1982) was developed to describe the effect of suboptimal temperature on

growth rates of microorganisms. This initial approach was later extended to include other factors such as level of acidity, water activity and salt concentration in additive or multiplicative models; see, for example, McMeekin et al. (1987), Miles et al. (1997), Wijtzes et al. (1995, 2001). The most common secondary models are polynomial models [see, e.g., McClure et al. (1993)], which allow any of the environmental factors and their interactions to be taken into account but include many parameters without biological interpretation. Another important model class is the cardinal parameter models [see Rosso et al. (1995), Augustin and Carlier (2000) and Pouillot et al. (2003)], which assume that the effect of environmental factors is multiplicative.

A disadvantage of these models is that they assume simple parametric forms for the effects of the different environmental factors. Therefore, more recently, there has been interest in modeling bacteria growth curves using nonparametric approaches such as artificial neural networks; see, for example, Hajmeer, Basheer and Najjar (1997), Geeraerd et al. (1998) and García-Gimeno et al. (2002). One advantage of neural networks is their capability to describe very complex nonlinear relationships without imposing any structure on the relationship between the interacting effects. Furthermore, using a suitable (logistic) basis function which is of a similar shape to typical bacterial growth curves, neural networks can capture these curves without the necessity of using large numbers of nodes.

To achieve the general objective of a high level of protection of human health, food law shall be based on risk analysis [FAO/WHO (1995); NACMCF (1997); CEC (2002)]. Quantitative microbial risk assessment (QMRA) is the scientific evaluation of the known or potential adverse health effects resulting from human exposure to foodborne microbiological hazards. The objective of a QMRA is to derive a mathematical statement, based on the probability of certain events, of the chance of adverse health consequences resulting from exposure to a microbiological agent capable of causing harm [FAO/WHO (1995); CAC (1996); NACMCF (1997)].

In this paper, we shall develop two approaches which are applicable to growth curve estimation for bacterial populations under different environmental conditions. The first model is based on the Gompertz function where the dependence of the growth parameters on the environmental factors is modeled by a neural network. Second, we shall consider a direct nonparametric approach based on the use of neural networks as a primary growth model. An important feature of our approaches is that in cases where we observe bacterial growth in various colonies under possible different environmental conditions, we use hierarchical modeling to improve estimation of any single growth curve by incorporating information from the various different bacterial populations. Although hierarchical analysis of parametric bacteria growth models has been undertaken [see, e.g., Pouillot et al. (2003)], to the best of our knowledge, hierarchical analysis has not been combined with nonparametric approaches previously in this context.

In most empirical work the fitting of any secondary models is carried out in two steps. First, a primary growth model is fitted to estimate the growth parameters

and, second, a secondary model is fitted conditional on the estimated parameters to estimate the controlling factors. One problem with this strategy is that the estimated uncertainty of the first stage is not taken into account in the second stage and, therefore, a poor fit at the first stage could produce inaccurate estimations at the second stage. Second, most work in fitting such models has used classical statistical techniques such as least squares, which, as noted in Pouillot et al. (2003), may also underestimate uncertainty.

To overcome these problems, inference for our models is undertaken throughout using a Bayesian approach. In the case of the parametric primary model and neural network secondary model, the use of this approach avoids the problems inherent in the two-stage inference outlined previously. Furthermore, our Bayesian approach permits the prediction of unobserved growth curves and of growth curve values at future time periods. To our knowledge, neural networks techniques have not been used either in food risk analysis or with the objective of a QMRA procedure in mind. We have built a neural network risk model with direct application in food industry and using very well-known noncommercial software in the context of Bayesian analysis, because, although previously the implementation of Bayesian inference for neural networks models has required the use of complicated sampling algorithms [see, e.g., Lee (2004)], here, we show that inference can be carried out via the use of the well-known `WinBugs` software through the `R2WinBugs` interface.

The present work covers different issues related to bacterial dynamics: (i) the use of the hurdle technology with different combinations of temperatures, pH values and percentage of NaCl with great importance in ready-to-eat foods safety conditions and in food handling as part of the foodservice industry; (ii) the use of NN to model the selected combinations of hurdles because of its absence of imposed restrictions (i.e., a new approach to the variability of the bacterial behavior under different environmental conditions and its application to QMRA); (iii) predictions of new data (interpolate) from the experimental growth curves obtained in the laboratory (i.e., to obtain proper new data avoiding the time-consuming and expensive assays carried out in the laboratory); and (iv) the study of the behavior of *Listeria* for its application to ready-to-eat foods under the legal requirement of 100 CFU (colony-forming units)/g or ml established by the EU Regulation 2073/2005 [CEC (2005)] and the QMRA procedures widely applied in food industry.

We begin in Section 2 with a brief introduction to neural networks. In Section 3 we propose two alternative models for bacterial growth curves that include environmental conditions as influencing factors modeled by neural networks. In Section 4 we show how to undertake Bayesian inference for these models and then, in Section 5, we illustrate the models with an application to a database of *Listeria monocytogenes* growth curves generated under various experimental conditions. Finally, in Section 6, we present our conclusions and some possible extensions of our approach.

**2. Feedforward neural networks.** In many situations it is assumed that there are $q$ dependent variables, $(Y_1, \ldots, Y_q) = \mathbf{Y}$, and they can be modeled as an approximate linear or polynomial function of a set of explanatory variables, $(x_1, \ldots, x_p) = \mathbf{x}$, via, for example, multivariate regression. However, such a relationship may not always be appropriate and a more general functional relation between the dependent and independent variables must be assumed, say,

$$E[\mathbf{Y}|\mathbf{x}] = \mathbf{g}(\mathbf{x}),$$

where the functional form, $(g_1, \ldots, g_q) = \mathbf{g}: \mathbb{R}^p \to \mathbb{R}^q$, is unknown. One of the most popular methods of modeling the function $g$ is via neural networks; see, for example, Stern (1996). In particular, a feedforward neural network takes a set of inputs $\mathbf{x}$ and from them computes the vector of output values as follows:

$$\mathbf{g}(\mathbf{x}) = B \cdot \mathbf{\Psi}^T\big(\mathbf{x}^T \Gamma\big), \tag{1}$$

where $B$ is a $q \times M$ matrix with $q \in \mathbb{N}$ the number of output variables and $M \in \mathbb{N}$ the number of nodes and $\Gamma$ is a $p \times M$ matrix with $p \in \mathbb{N}$ being the number of explicative variables. The element $\gamma_{rk} \in \mathbb{R}$ is the weight of the connection from input $r$ to hidden unit $k$ and the element $\beta_{sk} \in \mathbb{R}$ is the weight connection from hidden unit $k$ to output unit $s$. Finally, $\mathbf{\Psi}(a_1, \ldots, a_M) = (\Psi(a_1), \ldots, \Psi(a_M))$, where $\Psi$ is a sigmoidal function such as the logistic function

$$\Psi(x) = \frac{\exp(x)}{1 + \exp(x)}, \tag{2}$$

which we will use here, as typically bacterial growth curves have an approximately sigmoidal form. Equations (1) and (2) define a feedforward neural network with logistic activation function, $p$ explanatory variables (inputs), one hidden layer with $M$ nodes and $q$ dependent variables (outputs) that can be illustrated as in Figure 1.

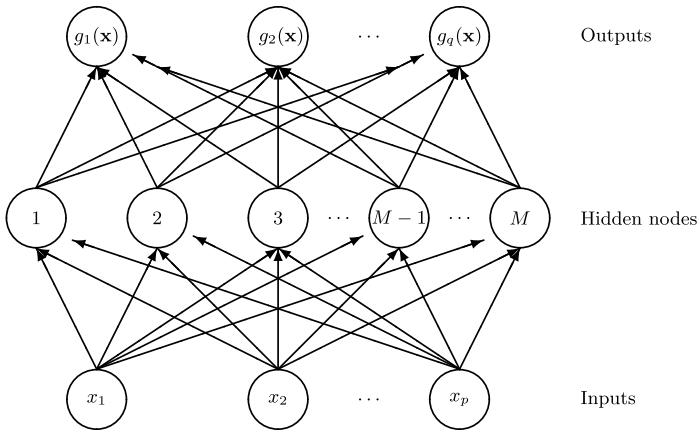


FIG. 1. *Neural network representation.*

Note that each output combines the node values in a different way. For practical fitting of neural networks models, it is typically assumed that the input variables are all defined to have a similar finite range, for example, [0, 1]. From now on, we shall assume this throughout.

**3. Neural network-based growth curve models.** Bacterial growth is very influenced by environmental factors. For example, bacteria grow in a wide range of temperatures, but in higher temperatures bacterial growth increases and in lower temperatures it decreases. In a similar way, changes on the level of acidity or salinity affect the growth of bacteria. The grade and the direction of the effect depend on the strain of bacteria and also on the level of the other factors. Figure 2 shows the different behaviors of *Listeria* growth under different environmental conditions.

To account for these effects, we develop growth curve models based on the use of neural networks.

3.1. *A neural network-based Gompertz model.* The bacterial growth process is typically characterized by three distinct phases, that is, the lag stage that reflects the adaptation of cells inoculated in a new medium; the exponential stage that represents the bacterial growth by binary fission; and, finally, the stationary stage which describes the decay of the growth rate as a consequence of nutrient depletion and accumulation of waste which is followed by death or decline of the population. Sigmoidal functions which account for these three phases have been typically used to model microbial growth; see, for example, Skinner, Larkin and Rhodehamel

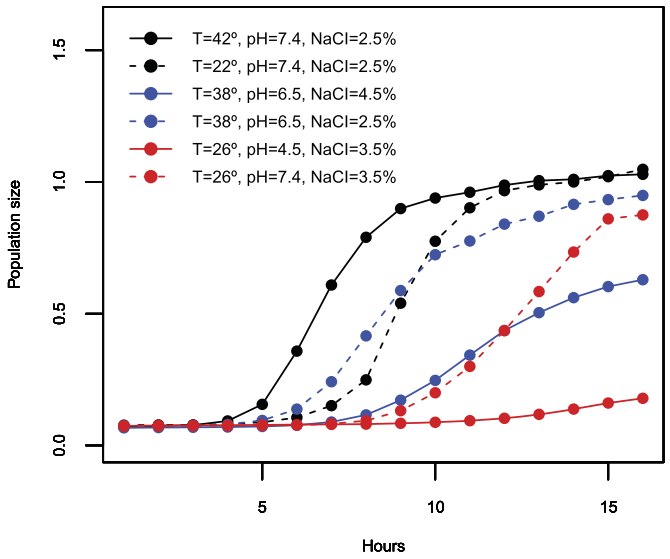


FIG. 2. *Bacterial growth under different environmental conditions.*

(1994). In particular, the Gompertz equation is a well-known model for bacterial growth over time and it has been used extensively by researchers to fit a wide variety of growth curves from different microorganisms; see, for example, Ross and McMeekin (1994) and McKellar and Lu (2004).

Here we consider a reparameterized Gompertz equation proposed by Zwietering et al. (1990). Let $N_t$ represent the population concentration of bacteria cultivated in a Petri dish experiment at time $t \geq 0$. Then the Gompertz equation is

$$E[N_t|N_0, D, \mu, \lambda] = g(t, N_0, D, \mu, \lambda)$$
$$\text{where } g(t, N_0, D, \mu, \lambda) = N_0 + D\exp\left(-\exp\left(1 + \frac{\mu e(\lambda - t)}{D}\right)\right), \tag{3}$$

where $e$ is Euler's number, $N_0$ is the initial bacterial density, $D$ is the difference between the maximum bacterial density, $\mu$ is the maximum growth rate and $\lambda$ is the time lag.

The primary growth model described in (3) does not allow for the case where we wish to study bacterial populations under a variety of controlled environmental conditions. Thus, suppose that we observe the growth of $I$ bacterial populations under similar initial conditions and that we have $J$ different environments determined by temperature, level of acidity (pH) and salt concentration (NaCl). Under fixed environmental conditions, it may be reasonable to assume that all replications have the same growth curve parameters. However, growth rates will vary under different conditions and, therefore, assuming a Gompertz model, we propose the use of neural networks to reflect the parameter dependence on the environmental factors. If $N_{tij}$ is the concentration in population $i$ under environmental conditions $j$ at time $t$, the Gompertz function is

$$E[N_{tij}|N_{0j}, D_j, \mu_j, \lambda_j] = g(t_{ij}, N_{0j}, D_j, \mu_j, \lambda_j), \tag{4}$$

where $g(\cdot)$ is as in (3), for $i = 1, \ldots, I$ and $j = 1, \ldots, J$. Now, we model the growth parameters $\mu$, $\lambda$ and $D$ as a function of the temperature, the level of acidity and the salt concentration by a feedforward neural network, that is,

$$\boldsymbol{\theta}_s = \sum_{k=1}^{M} \beta_{sk} \cdot \Psi(\mathbf{x}'\boldsymbol{\gamma}_k) \qquad \text{for } s = 1, 2, 3, \tag{5}$$

where $\boldsymbol{\theta}_s$ stands for the parameters $D$, $\mu$, $\lambda$ and $\mathbf{x} = (T, \text{pH}, \text{NaCl})$ is the vector of explanatory variables and $\Psi$ is the logistic function. Note that this network does not include an intercept term. In our practical experiments we have found that the addition of an intercept produces no significant differences to typical curve fits. The model defined in this section by expressions (4) and (5) will be referred to as the GNN model.

3.2. *A hierarchical neural network model.* Here, we generalize the previous model to a new one which does not assume any underlying parametric growth function. Instead, we propose a neural network as a primary model. The output of the network is the instantaneous reproduction rate per member of the population and the inputs are the current population size and the experimental conditions. Formally, we can write the model as

$$
\begin{aligned}
&E[N_{tij}|N_{(t-1)ij}, f_j, T_j, \mathrm{pH}_j, \mathrm{NaCl}_j] \\
&\qquad = N_{(t-1)ij} + N_{(t-1)ij} f_j(N_{(t-1)ij}, T_j, \mathrm{pH}_j, \mathrm{NaCl}_j),
\end{aligned} \tag{6}
$$

$$
\begin{aligned}
&f_j(N_{(t-1)ij}, T_j, \mathrm{pH}_j, \mathrm{NaCl}_j) \\
&\qquad = \sum_{k=1}^{M} \beta_{jk}\big(\Psi(\gamma_{1k} N_{(t-1)ij} + \gamma_{2k} T_j + \gamma_{3k}\mathrm{pH}_j + \gamma_{4k}\mathrm{NaCl}_j) \\
&\qquad\qquad - \Psi(\gamma_{2k} T_j + \gamma_{3k}\mathrm{pH}_j + \gamma_{4k}\mathrm{NaCl}_j)\big),
\end{aligned} \tag{7}
$$

for $i = 1, \ldots, I$ and $j = 1, \ldots, J$, and $f_j(\cdot)$ is the growth rate for populations with environmental condition $j$. As previously, we could consider adding an intercept term to the network. However, for the given model, given the addition of an error term as defined in the following subsection, when $N_{(t-1)ij} = 0$, then $N_{tij} = 0$, so that once the population has died out, then it remains extinct. Including an intercept would mean that this desirable property is lost. The model defined in this section by (6) will be referred to as the NN model.

3.3. *Error modeling.* In the previous subsections two approaches to modeling the expected population density have been provided. These models are completed by including an error term. Thus, in the case of the full neural network model, we assume that

$$
N_{tij} = N_{(t-1)ij} + N_{(t-1)ij} f_j(N_{(t-1)ij}, T_j, \mathrm{pH}_j, \mathrm{NaCl}_j) + \varepsilon_{tij}, \tag{8}
$$

where we assume that the error term is

$$
\varepsilon_{tij}|N_{(t-1)ij}, \sigma, v \sim \mathcal{N}\big(0, \sigma^2 N_{t-1}^{v}\big), \tag{9}
$$

where $\sigma^2 \geq 0$ and $v = 0.5$ so that the possibility that the error variance increases with population density is allowed for. Figure 3 illustrates different bacterial growth curves from Petri dish experiments under the same conditions. It can be seen that the curves are closer together initially when the population density is lower and diverge over time as the population density grows, which suggests that a model of this type is reasonable. Our empirical experiments suggest that the value of $v = 0.5$ is appropriate here, although, clearly, a prior distribution for $v$ could be considered. Following the same idea of increasing error variance, we assume for the GNN model that the error term is

$$
\varepsilon_{tij}|gt_{ij}, \sigma, v \sim \mathcal{N}\big(0, \sigma^2 g(t_{ij})^{v}\big), \tag{10}
$$

where $g(\cdot)$ is the Gompertz function evaluated at the current time point.

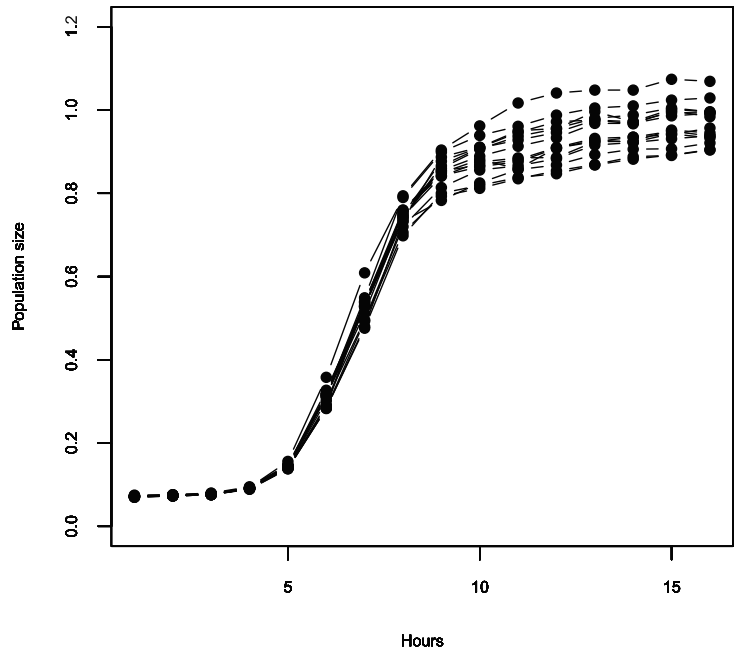


FIG. 3. 15 *replications of bacterial growth under* $T = 42°C$, $pH = 7.4$ *and* $NaCl = 2.5\%$.

**4. Bayesian inference for the neural network models.** Given a set of observed inputs and outputs from a neural network, say, $D = (x_1, y_1), \ldots, (x_N, y_N)$, inference can be carried out using a variety of approaches; see, for example, Neal (1996) and Fine (1999) for reviews. Here, we shall consider a Bayesian approach. To implement such an approach, we must first define suitable prior distributions for the neural network parameters $\beta$ and $\gamma$ and for the uncertainty. First, we suppose little prior knowledge concerning the variance and, hence, we propose a vague inverse-gamma prior distribution for it, $\sigma^{-2} \sim G(a/2, b/2)$. In neural network models, it is common to use relative uninformative prior distributions due to the scarcity of prior information about the parameters. For simplicity, we choose normal and gamma distributions with a hierarchical structure, that is,

$$\beta_{ik}|m_{i\beta}, \sigma_\beta^2 \sim \mathcal{N}(m_{i\beta}, \sigma_\beta^2),$$

$$\boldsymbol{\gamma}_k|m_\gamma, \sigma_\gamma^2 \sim \mathcal{N}(\mathbf{m}_\gamma, \sigma_\gamma^2 I),$$

where the subscript $i$ in the GNN model accounts for the growth parameters and in the NN model for the groups defined by the environmental conditions. The Bayesian approach is completed by vague, but proper prior distributions for the remaining hyperparameters as follows:

$$m_{i\beta}|\sigma_\beta^2 \sim \mathcal{N}\left(m_{0\beta}, \frac{\sigma_\beta^2}{c_\beta}\right),$$

$$m_{0\beta}|\sigma_\beta^2 \sim \mathcal{N}\left(0, \frac{\sigma_\beta^2}{e_\beta}\right),$$
$$\frac{1}{\sigma_\beta^2} \sim \mathcal{G}\left(\frac{d_{\beta 1}}{2}, \frac{d_{\beta 2}}{2}\right),$$
$$\mathbf{m}_\gamma|\sigma_\gamma^2 \sim \mathcal{N}\left(\mathbf{0}, \frac{\sigma_\gamma^2}{c_\gamma} I\right),$$
$$\frac{1}{\sigma_\gamma^2} \sim \mathcal{G}\left(\frac{d_{\gamma 1}}{2}, \frac{d_{\gamma 2}}{2}\right),$$

where $c_\beta, e_\beta, d_{\beta 1}, d_{\beta 2}, c_\gamma, d_{\gamma 1}$ and $d_{\gamma 2}$ are assumed known and fixed. Similar hierarchical prior distributions are typically used in Bayesian inference for neural network models; see, for example, Lavine and West (1992), Müller and Insua (1998) and Andrieu, de Freitas and Doucet (2001). For alternatives, see, for example, Lee (2004), Robert and Mengersen (1999) and Roeder and Wasserman (1997).

Usually, we will have good prior knowledge about the average initial population density, $m_0 = E[N0_i|m_0, s_0]$, and the variance, $s_0$, as typically Petri dishes are seeded with very similar quantities of bacteria close to a known theoretical level, so we shall typically assume that these are known. Otherwise, a simple noninformative prior distribution $f(m_0, t_0) \propto 1/t_0$, where $t_0 = 1/s_0^2$ can be used when, immediately, we have that given the observed set of initial densities, $\mathbf{N0} = (N0_1, \ldots, N0_I)$,

$$m_0|\mathbf{N0}, s_0 \sim \mathcal{N}\left(\overline{N0}, \frac{s_0^2}{I}\right),$$
$$s_0^2|\mathbf{N0} \sim \mathcal{I}G\left(I - 1, \sum_{i=1}^{I}(N0_i - \overline{N0})^2\right),$$

where $\overline{N0} = \frac{1}{I}\sum_{i=1}^{I} N0_i$ is the average initial density and $\mathcal{I}G$ means inverse gamma.

Given the above prior structure, a closed form for the posterior parameter distributions is not available. However, Markov Chain Monte Carlo (MCMC) techniques can be employed to allow us to generate an approximate Monte Carlo sample from the posterior parameter distributions; see, for example, Gilks, Richardson and Spiegelhalter (1996) for a full review. Various different MCMC algorithms have been proposed in the neural networks literature, but in general the efficiency of such samplers depends on the model; see, for example, Lee (2004).

As an alternative, here, we propose using the generic MCMC sampler, `WinBugs`, as developed by Spiegelhalter, Thomas and Best (1999), which is appropriate for hierarchical modeling situations, programmed in combination with `R`, via `R2WinBugs`.

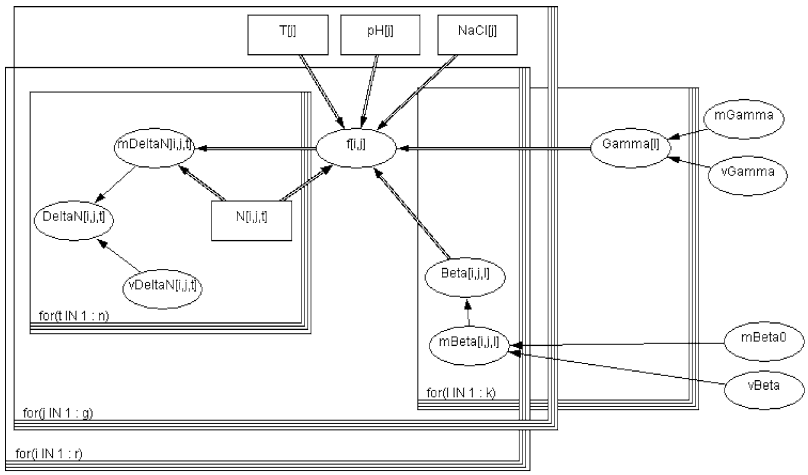


FIG. 4. *Dependence structure of the NN model.*

Figure 4 illustrates the dependence structure of the NN model in `WinBugs` style (although code cannot be constructed directly from this diagram). In the figure, random and logical nodes are represented by ellipses and fixed nodes (independent variables) are represented by rectangles. The arrows represent dependence relationships, with the single arrows showing stochastic dependence and the double arrows representing logical dependence. For more details see http://www2.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml.

As `WinBugs` is a generic approach to MCMC sampling, it is important to check on the convergence of the sampler. Various tools can be used to check the convergence. In particular, as well as standard graphical techniques such as looking at the trace, the evolution of the mean and the autocorrelations of the sampled output, we also use formal diagnostic techniques such as the modified Gelman–Rubin statistic, as in Brooks and Gelman (1998).

Note finally that the codes for running both models are available in the supplemental materials [Palacios et al. (2014a, 2014b)].

4.1. *Model selection.* Thus far, inference is conditional on the number of hidden nodes, $M$, being known. Various approaches to estimating $M$ may be considered. One possibility is to treat $M$ as a variable and, given a prior distribution for $M$, use variable-dimensional MCMC approaches to carry out inference; see, for example, Müller and Insua (1998) or Neal (1996). Another approach which we shall use in this article is to use an appropriate model selection technique to choose the value of $M$.

A number of criteria have been proposed for model selection in Bayesian inference. A standard Bayesian selection criterion which is particularly appropriate when inference is carried out using MCMC methods is the deviance information

criterion (DIC), as proposed in Spiegelhalter et al. (2002). However, in the context of neural networks, the possible lack of identifiability of the model or multimodality of the posterior densities make this criterium unstable. Many variants of the DIC have also been considered and, here, we prefer to apply the $\mathrm{DIC}_3$ criterion of Celeux et al. (2006). For a model $\mathcal{M}$ with parameters $\boldsymbol{\theta}$ and observed data $\mathbf{y}$, the $\mathrm{DIC}_3$ is defined as follows:

$$\mathrm{DIC}_3 = -4E_{\boldsymbol{\theta}}\big[\log f(\mathbf{y}|\boldsymbol{\theta})|\mathbf{y}\big] + 2\log \prod_{i=1}^{n} E_{\boldsymbol{\theta}}\big[f(y_i|\boldsymbol{\theta},\mathbf{y})\big].$$

In Celeux et al. (2006) this criterion is recommended in the context of latent variable models. Furthermore, Watanabe (2010) recommends the use of this criterion in the case of singular models such as neural networks.

An alternative approach which we also consider when comparing different models is the posterior predictive loss performance (PPLP) proposed by Gelfand and Ghosh (1998). Based on the posterior predictive distribution, this criterion consists in defining a weight loss function which penalizes actions for departure from the corresponding observed value as well as for departure from what we expect the replication to be. In this way, the approach is a compromise between the two types of departures: fit and smoothness. For squared error loss, the criterion becomes

$$\mathrm{PPLP} = \frac{k}{k+1}\sum_{i=1}^{n}(m_i - y_i)^2 + \sum_{i=1}^{n} s_i^2,$$

where $m_i = E[y_i^{\mathrm{rep}}|\mathbf{y}]$ and $s_i^2 = \mathrm{Var}[y_i^{\mathrm{rep}}|\mathbf{y}]$ are, respectively, the mean and the variance of the predictive distribution of $y_i^{\mathrm{rep}}$ given the observed data $\mathbf{y}$ and $k$ is the weight we assign to departures from the observed data. The first term of the PPLP is a plain goodness-of-fit term and the second term penalizes complexity and rewards parsimony.

**5. Application: *Listeria monocytogenes*.** In this section we analyze a data set taken from Petri dish experiments of one of the authors (EQ) and consisting of measures of the concentrations of *Listeria monocytogenes* bacteria in a Petri dish under several experimental conditions. A strain of *Listeria monocytogenes* previously isolated from poultry meat was provided by the Department of Animal and Food Sciences, School of Veterinary Medicine, Autonomous University of Barcelona, Spain, and used in the present study. *L. monocytogenes* growth data was obtained as reported by Eduardo et al. (2011). Briefly, an automated method (SLT 340 ATTC microplate reader, SLT Labinstruments, Austria) for the measurements of the optical density of a *L. monocytogenes* culture was used. Aliquots of the microorganism, previously cultured in nutrient broth at 31°C overnight and serially diluted, were inoculated into the microplate wells and read at a wavelength of 595 nm every 15 min. Optical density curves of bacterial growth were obtained. At the same time, aliquots were also spread onto Petri plates with nutrient agar and

cultured at 31°C overnight. The environmental factors taken into account are temperature, level of acidity and salinity. Temperatures range between 22°C and 42°C, pH between 4.5 and 7.4 and NaCl between 2.5% and 5.5%. There are 96 different combinations of environmental factors (we call groups) and for each group there are several replications. The number of observations per curve varies between 16 and 24, depending on the curve. We kept for the analysis 74 groups (excluding the cases with extreme values of factors which inhibit growth) and chose randomly 10 replications for each one so that the remaining curves could be used for cross-validation and prediction purposes and to reduce computational time. The temperatures selected cover the following situations in food handling: room temperature in northern countries (22 and 26°C); room temperature in warm countries (30 and 34°C); and inadequate reheating treatments of ready-to-eat foods previous to consumption (38 and 42°C). The selected pH values cover most of the range of the pH values tolerated by *Listeria*. The percentages of NaCl selected are well under the limits tolerated by *Listeria*, but it is very important to know their possible effects under a hurdle technology point of view combined with temperature and pH values. A reduced version of this data set including six groups under the same environmental conditions as the data illustrated in Figure 2 and each with ten replications is contained in the supplemental materials [Palacios et al. (2014a)].

Using the $\mathrm{DIC}_3$ criterion as outlined earlier, the optimum number of nodes for both models is 2. Temperature, pH and NaCl as inputs of the neural networks were previously scaled onto [0.1, 0.9] as recommended in Valero et al. (2007). In the implementation of the GNN model we keep the hyperparameters $m_{i\beta}$, $\sigma_\beta$, $m_\gamma$ and $\sigma_\gamma$ fixed at $m_{i\beta} = 0$, $\sigma_\beta = 10$, $\mathbf{m}_\gamma = (0, \ldots, 0)'$ and $\sigma_\gamma = 10$. Regarding the error variance, we choose $a = 0.2$ and $b = 0.2$. In the NN model the highest level of hyperparameters were set to $c_\beta = 10$, $e_\beta = 10$, $d_{\beta 1} = 0.1$, $d_{\beta 2} = 0.01$, $c_\gamma = 10$, $d_{\gamma 1}$ and $d_{\gamma 2} = 0.01$.

For both models, we generated chains with random initial values and 200,000 iterations each, including 100,000 iterations of burn-in. To diminish autocorrelation between the generated values, we also used a thinning rate of 1000. Trace plots and autocorrelation functions were used to check convergence in the predictions and in all cases it was found that the burn-in period of 100,000 iterations was reasonable. Furthermore, the Gelman–Rubin statistic was equal or very close to 1 for predictions, being a good indicator of convergence.

In order to have a benchmark for the comparison of models, we also fit two different simple models, the independent Gompertz model and the pooled Gompertz model. The first one implies that each observed curve, including the replications, is independent and therefore has its own Gompertz growth parameters. Independent normal prior distributions with mean zero and variance 100 are assumed for these parameters. In contrast, the pooled model assumes that the replications under a fixed set of environmental conditions are samples from a unique underlying growth curve for that set of conditions. Normal priors are then placed on the parameters of this growth curve as for the independent model. For both benchmark

TABLE 1
*Model comparison*

| Model | $DIC_3$ | PPLP |
|---|---|---|
| Independet Gompertz | −19,136 | 781 |
| Pooled Gompertz | −39,420 | 211 |
| Gompertz & NN | −40,099 | 41 |
| Neural Networks | −58,492 | 28 |

models the errors are the same as in the GNN case with a $\mathcal{G}(0.1, 0.1)$ prior distribution for the error variance.

The $DIC_3$ and the PPLP criteria were computed to compare the different models under consideration and Table 1 shows the estimated values for all of these models. As is expected, the pooled model performs better than the independent one since the assumption of independence for all the curves is somewhat extreme. Therefore, it seems reasonable to assume different curves under different environmental conditions, but under equal conditions we assume a common curve and this is the approach we choose for the proposed models. But the problem with this model is that it does not explain the effect of the environmental factors and it is needed to estimate one model for each group of conditions. Then, regarding our proposed models which incorporate the environmental factors as explanatory variables, the results show that the hierarchical neural network model outperforms the Gompertz model with neural networks for the parameters. The $DIC_3$ and the PPLP values are lowest for the former model.

Figure 5 shows for a particular curve ($T = 34°C$, pH = 6.5 and NaCl = 5.5%) the fitting of both models. On the left, the Gompertz model with neural networks explains the dependence of the growth parameter on the environmental factor and on the right the fitting of the hierarchical neural network model. The observed values are represented by points, the estimated growth curves are represented by the solid line, and the dashed lines represents the 95% credible interval computed from the posterior distributions. It can be observed that the fit is good in both cases and the credible intervals included all the true observations. Nevertheless, note that the NN model overestimates the lag period. In the remaining curves (replications and different group conditions), we also found good fits for both models. Similar results are observed in the fitted plots for all the groups.

Additionally, with the GNN model we can make predictions of the growth parameter values for a certain level of environmental factors. Based on previous works, it should be expected that an increase of temperatures and a decrease in pH values kills a foodborne pathogen. However, predictions from our model show an interesting behavior of *Listeria* under several environmental conditions. The impact of temperature on growth is not the same when considering different pH values, changing even the direction of the effect. On the other hand, the effects

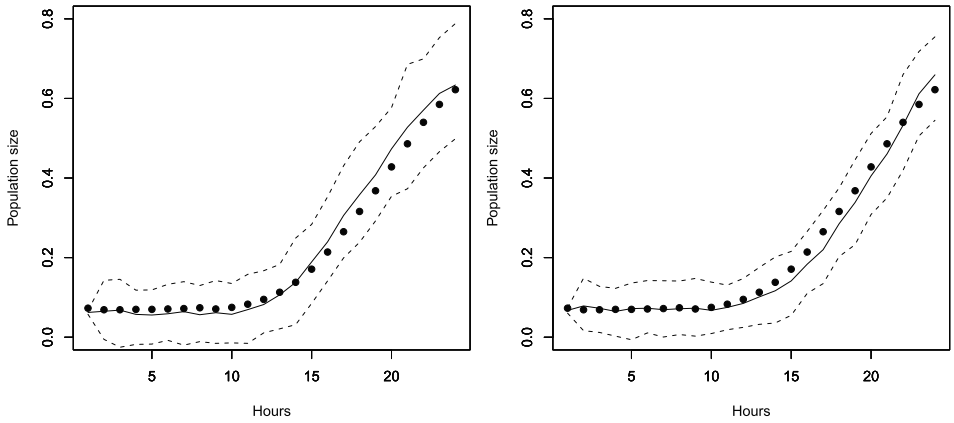


FIG. 5. *Fitting of the GNN model* (left) *and the NN model* (right). *Points represent real data, the solid lines represent the posterior means and the dashed lines represent the* 95% *credible interval.*

seem to be irregular and interacting, which emphasizes the utility of a neural network model which does not impose a rigid functional form on the dependencies. To illustrate these effects, we plot the posterior mean of the growth rate parameter as a function of the environmental factors (see Figure 6).

For example, when pH values range between 4.5 and 5.5, an increase in the temperature values is needed to decelerate the growth rate of *Listeria*. In contrast, when pH is equal to 6.5 or 7.4, the temperature must be decreased to diminish the microorganism growth.

Regarding the percentage of NaCl, we found a decrease in the growth rate when the percentage of NaCl increases. Additionally, the impact grade of the temperature changes for different values of NaCl. When NaCl is equal to 5.5% the differ-

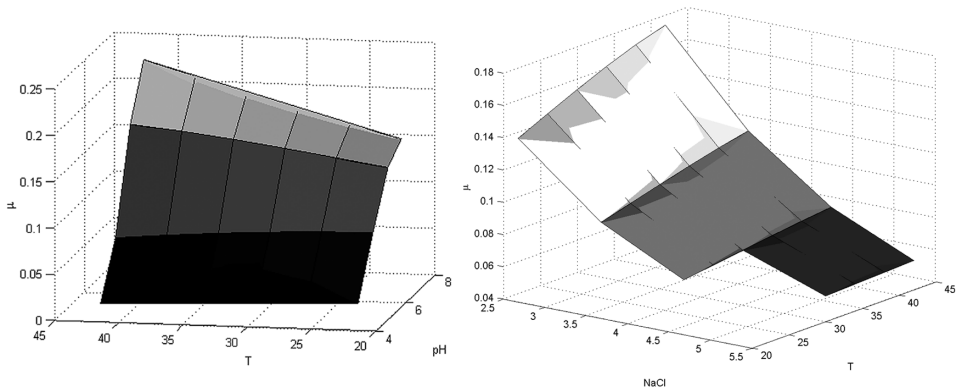


FIG. 6. *Posterior mean of the growth rate parameter* $\mu$ *for the GNN model.* $NaCl = 2.5\%$ (*left*) *and* $pH = 6.5$ (*right*).

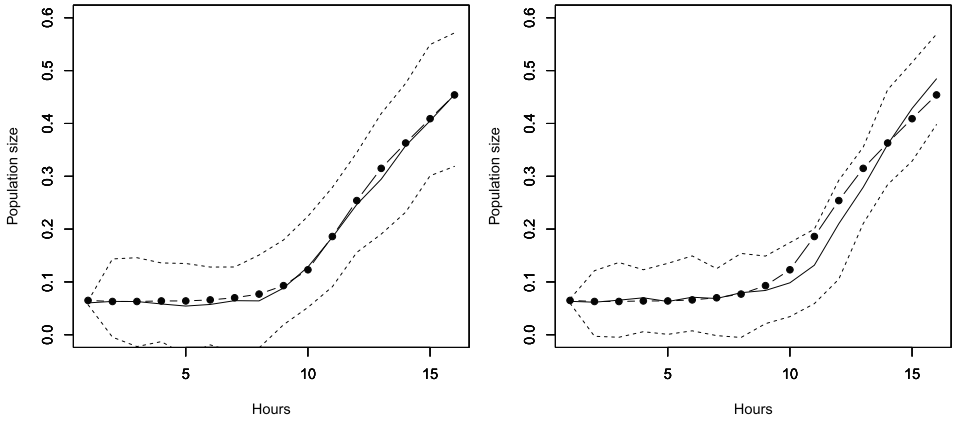


FIG. 7. *One-step-ahead predictions of the GNN model* (left) *and the NN model* (right). *Points represent real data, the solid lines represent the posterior means and the dashed lines represent the* 95% *credible interval.*

ences among the temperature effects are minimal, but those differences increase for lower levels of NaCl.

Now, we consider predictions of future values of a growth curve and predictions of a full curve under an unobserved set of environmental conditions. For the first case, we computed one-step-ahead predictions. That is, for a particular curve we observe data until observation $t$ and predict the population size at $t+1$. In the next step, we observe data until $t+1$ and predict the population size at $t+2$ and so on, until the completion of the predictive curve. Figure 7 shows the one-step-ahead predictive curves for both models for a particular growth curve ($T = 42°\text{C}$, pH $=$ 5.5 and NaCl $= 2.5\%$). In contrast to the fitting results, the Gompertz model shows a slightly better performance regarding the mean prediction. The mean square error of the prediction in the Gompertz model is equal to 0.001, while for the NNs model it is 0.008. But in the second model higher accuracy is reached, as can be seen from the narrower credible interval.

In the context of model checking, several authors, for example, Gelfand (1996) and Vehtari and Lampinen (2003), have proposed the use of cross-validatory predictive densities. Following this approach, the data set is divided in two subsets $(\mathbf{y}_1, \mathbf{y}_2)$. The first subset is used to fit the model and to estimate the posterior distribution of the parameters, while the second set is used to compute the cross-validatory predictive density: $f(\mathbf{y}_1|\mathbf{y}_2) = \int f(\mathbf{y}_2|\boldsymbol{\theta}) f(\boldsymbol{\theta}|\mathbf{y}_1)\, d\boldsymbol{\theta}$. In our case, we computed the predictive density for one of the groups which was not used in the model fitting. Given the hierarchical structure of the models, it is possible to make predictions of a growth curve under an unobserved set of conditions, due to the knowledge learned from the other observed group of conditions. To illustrate, we make predictions for a new unobserved group with $T = 26°\text{C}$, pH $= 6.5$ and

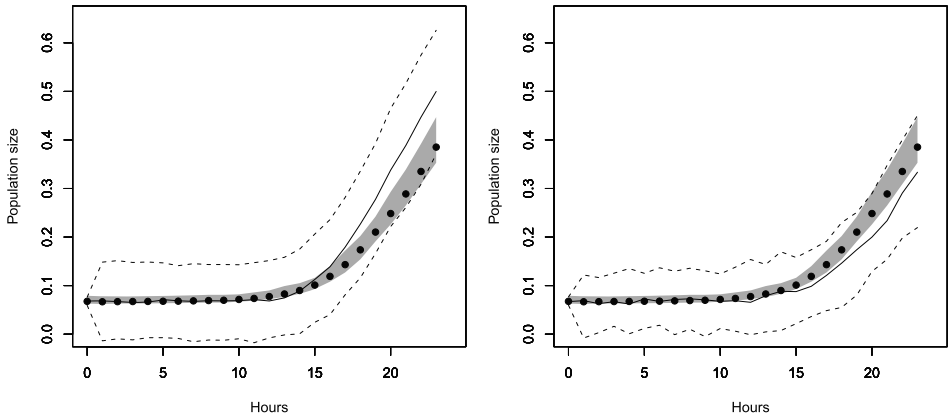


FIG. 8. *Prediction out of sample of the GNN model* (left) *and the NN model* (right). *Shadings represent the area where real data of all replications lie*, *the solid lines represent the posterior means and the dashed lines represent the* 95% *credible interval*.

NaCl = 5.5%. Figure 8 shows the mean prediction (solide line) and the 95% credible interval (dashed line) for both models, GNN on the left and NN on the right. As there are many replications for this group, we plot only the mean curve and shade the area between the minimum value and the maximum value observed for each time $t$ among replications. As an input of the neural network for the NN model we used the mean curve of the replications.

The out-of-sample predictions of both models are fairly good and constitute one of the main contributions of this work. Although the good performance of both models, in the case of the GNN model some observations lie outside the credible interval—a small shaded area lies outside the dashed line. Moreover, comparing the mean prediction with the mean observed curve, the NN model yields more accurate predictions.

**6. Conclusions and extensions.** In this paper we have shown a methodological contribution which can be easily and directly applied for microbiological researchers. Neural networks were used as a secondary model that explains the dependence on environmental factors and also as a primary model which, besides time, includes experimental conditions as explanatory variables. Inference was carried on in a Bayesian approach that avoids the problems for doing inference in two steps. Both models yield accurate estimations and good predictions which show that NNs can be used to model bacterial growth, describing accurately the complex interacting effects of environmental factors without imposing any simplifying assumption. On the other hand, the modified Gompertz equation was used as the base model for the first approach we considered, but other parametric bacteria growth models such as Baranyi or logistic are equally applicable.

Estimations were implemented in `WinBugs` via `R2WinBugs`, showing that `WinBugs` can be a powerful and flexible tool that is able to handle very complex models such as neural networks with great ease. As MacKay (1995) pointed out, the Gibbs sampling method is not the most efficient of MCMC methods, but there may be problems of interest where the convenience of this tool outweighs this drawback.

Previous studies have special interest for the food industry. The conditions inside a food-processing plant (humidity, temperature, food processing techniques, sanitation procedures, etc.) relate to each other in a very complex way, creating microenvironments with adequate conditions for the growth of *Listeria*, such as hard-to-reach areas (drains, etc.). The use of NNs gives more flexibility, as they do not impose restrictions to the hurdle technology effects on microorganisms and can show more freely the variability inherent to any form of life under different environmental conditions. And it is necessary to take into account that variability does not only appear in a laboratory assay, but also and most importantly appears in a food industry production chain, in a foodservice company, in a food distributor or at home before the moment of consumption. Following this reasoning, the application of the NNs to quantitative microbial risk assessment seems a very useful and realistic tool, reflecting with fewer restrictions the behavior of foodborne pathogens. This flexibility in the model has allowed us to get new conclusions, different to previous studies.

Food safety conditions and food handling are part of the foodservice industry, and different conditions of temperature, pH and percentage of NaCl give a new insight in terms of inhibitory effects of those conditions. Montville and Matthews (2001), who studied the effect of temperature with different pH values, concluded that the growth rate increases with temperature, reaching a maximum at 40°C to decay afterward. The behavior of the growth rate is similar for different values of pH. Similar conclusions can be found in the literature, however, in our work the results are fairly different. The effect of temperature on the growth rate is not as it was shown in previous studies. Secondary models show a very continuous line of increasing growth rate values with temperature or pH changes [Montville and Matthews (2001); McKellar and Lu (2004)]. In contrast, in our work, for a fixed pH value the effect of temperature is not so smooth, and the growth rate shows oscillations that have not been described in the literature with any secondary model as far as we know. Moreover, the maximum growth rates are achieved at different levels of temperature when the pH values vary, differing from the CAPM models.

Specifically, when pH is about 7.4, the temperature must be diminished to decrease the growth rate of *Listeria*, but if pH is about 4.5, then the temperature must be increased to decrease growth rates. Therefore, consequences in terms of food conservation vary regarding their respective pH. For example, in fruits and vegetables, which present in general low pH values, it is convenient to increase temperature; on the contrary, in dairy products, biscuits, chocolate and eggs it is convenient to decrease temperatures.

It is generally accepted that predictions of the response within that range can be made by interpolation. Usually, a three-dimensional space could be constructed with the ranges of the three parameters studied. The interpolation region of the combinations tested is called the minimum convex polyhedron [Baranyi et al. (1996)] and it is used to make predictions. However, Baranyi et al. (1996) noted that this is not always a good approach. These authors reported a prediction of an optimal growth for *Salmonella* under approximately 2% NaCl, which is not correct for that microorganism. Additionally, Davey (1989) noted that polynomial equations did not have a consistent form across a range of bacterial growth data and that such models appeared to lack of universality. That is, the coefficient values of polynomial models are very data dependent. In this work, we have implemented two kinds of predictions which were not widely used in the literature but which are of greater relevance in the context of the microbiology. We have shown that predictions out of sample are very accurate, being a good alternative to the use of polynomials of different orders (2nd or 3rd order) and response surfaces for predictive microbiology.

A restriction in the models, as assumed here, is that we suppose that data are equally spaced in time. Although this is typically the case in Petri dish or in optical density experiments, this may not be true with more general populations. In the case of irregularly spaced data, differential equation models with diffusion type approximations with the neural network models for the growth functions may be considered [see Donnet, Foulley and Samson (2010)].

Finally, alternative approximations to the hierarchical neural network models for growth functions may be considered as spline methods from a classical point of view, functional data analysis or Gaussian process approximations.

## SUPPLEMENTARY MATERIAL

**Supplement A: Code for the NN and GNN models** (DOI: 10.1214/14-AOAS720SUPPA; .zip). The file contains two programs, `NN model.odc` for running the neural network model and `GNN model.odc` for running the Gompertz neural network model.

**Supplement B: Data sets** (DOI: 10.1214/14-AOAS720SUPPB; .xls). The file `data.xls` contains 10 replications in 6 groups of bacteria under the environmental conditions outlined in Figure 2.

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