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Quality and shelf-life prediction for retail fresh hake (*Merluccius merluccius*)

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ABSTRACT

Fish quality has a direct impact on market price and its accurate assessment and prediction are of main importance to set prices, increase competitiveness, resolve conflicts of interest and prevent food wastage due to conservative product shelf-life estimations. In this work we present a general methodology to derive predictive models of fish freshness under different storage conditions.

The approach makes use of the theory of optimal experimental design, to maximize data information and in this way reduce the number of experiments. The resulting growth model for specific spoilage microorganisms in hake (*Merluccius merluccius*) is sufficiently informative to estimate quality sensory indexes under time-varying temperature profiles. In addition it incorporates quantitative information of the uncertainty induced by fish variability.

The model has been employed to test the effect of factors such as fishing gear or evisceration, on fish spoilage and therefore fish quality. Results show no significant differences in terms of microbial growth between hake fished by long-line or bottom-set nets, within the implicit uncertainty of the model. Similar conclusions can be drawn for gutted and un-gutted hake along the experiment horizon.

In addition, whenever there is the possibility to carry out the necessary experiments, this approach is sufficiently general to be used in other fish species and under different stress variables.

1. Introduction

The Food and Agricultural Organization of the United Nations (FAO) has estimated that between 30–50% of all fish catches are lost in different links of the food supply chain (Gustavsson et al., 2011), being fundamental the development of new tools to monitor and control fish quality (Dalgaard, 2002; Ólafsdóttir et al., 1997). One of the main factors affecting this attribute is the so-called Specific Spoilage Organisms (SSOs), a fraction of the total fish microbiota that degrades the fish into biochemical components, usually perceived by the consumer with loss of freshness.

Fish quality may be affected by many factors related to the environment (e.g., catching ground and season), fishing practices, storage conditions or handling, including bleeding and gutting procedures (Dowlati et al., 2013). Consequently, its dynamics may present a significant variability from catch to catch, but also from fish to fish inside the same batch.

In the sequel we will adopt the interpretation of variability and uncertainty given in Shorten et al. (2006). In this way we will refer to variability to describe the inherent differences between individuals (fish composition, bacterial load or bacterial composition, etc.). Uncertainty, on the other hand, would be related to the error associated to a measurement (i.e., errors implicit in the analytical method), as well as the uncertainty induced by such error on the estimation of a state. While such distinction is clear in terms of the states (observables) of the system, its effect on parameter estimation is unclear. This is why when we refer to parameter uncertainty it should be understood as the uncertainty due to the analytical method (measurement) and the characteristic variability of the system.

Statistical models, such as the ones developed for haddock (*Melanogrammus aeglefinus*) in Ólafsdóttir et al. (2006) and for seabass (*Dicentrarchus labrax*) in Carrascosa et al. (2014), have been employed to detect factors affecting microbiota and hence shelf-life uncertainty. These models are essentially static correlations that link a pre-determined set of experimental conditions, including a pre-established range of storage temperatures and times, with fish quality or shelf-life. Unfortunately, the own structure of these models prevents its use outside the set of experimental conditions on which they were built. This in turn, limits their application in real scenarios where quality or shelf-life must be estimated under fluctuations in temperature, or in other relevant stress variables.

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Alternatively, food researchers have been exploring in the last two decades the use of predictive (dynamic) models of microbial spoilage to estimate shelf-life under fluctuating temperatures, which is one of the most important stress variables in bacterial growth. Some examples are the models included in the software developed by Dalgaard et al. (2002), or the works by Koutsoumanis (2001) on gilt-head seabream (*Sparus aurata*), Taoukis et al. (1999) on bogue (*Boops boops*) and Nuin et al. (2008) on fresh turbot (*Psetta maxima*). Concerning their application for quality assessment, such models are typically employed only to estimate fish shelf-life in terms of rejection time; the moment where the concentration of SSOs reaches the value that corresponds to sensory rejection. From the authors' best knowledge, only the work by Giuffrida et al. (2013) exploits the potential of dynamic models to estimate different levels of fish freshness, although without any analysis of the uncertainty associated to the model parameters and thus to model predictions.

In this work, we propose a methodology, which includes the design of optimal experiments for parameter estimation, to develop models to predict fish quality and its uncertainty under non-isothermal storage conditions and test its validity in hake (*Merluccius merluccius*). In keeping with this objective, a dynamic model to describe the growth of representative SSOs in hake is proposed. In order to estimate model parameters, we present an optimal experimental protocol which takes advantage of non-isothermal conditions (varying time–temperature profiles) to make data more informative thus reducing the number of experiments, as well as confidence intervals of model parameters (i.e., parameter uncertainty).

Note that previous studies made use of isothermal experiments to estimate model parameters (Koutsoumanis, 2001; Nuin et al., 2008; Taoukis et al., 1999), what may result into parameter estimates unable to reproduce non-isothermal conditions (Van Boekel, 1996; Dolan, 2003; Valdramidis et al., 2008). The resulting model, which incorporates fish-to-fish variability (via parameter uncertainty), will be employed to calculate the so-called *core predictions*; a standard method in systems biology to test predictive capabilities of complex models subject to uncertainty (Brännmark et al., 2010). The capability of the model to do reliable predictions under different temperature profiles, will be tested under an arbitrary storage temperature profile, different from those employed for parameter estimation.

Model building has been based on data from hake captured by bottom-set nets and eviscerated, whereas for validation purposes a number of storage experiments were programmed. These included different storage temperature profiles, as well as different fish handling protocols (e.g., evisceration) and fishing techniques to test whether they may have a significant influence on microbial growth, and therefore on final fish quality, as reported in literature (e.g., Huss, 1995; Dowlati et al., 2013).

Previous studies have reported a significant influence of the catching method on microbial spoilage (or shelf-life) of fish (Özyurt et al., 2007). Colonization dynamics by SSOs can be traced back to stress physiology (e.g., Matos et al., 2010) or physical damage of fish caused by the fishing gear (e.g., Rotabakk et al., 2011). As a result fish muscle may become softer earlier, enhancing bacterial colonization by SSOs. In order to evaluate the effect of fishing gear on microbial spoilage a validation experiment is performed that compares hake caught by bottom-set nets and long-line during storage.

Concerning handling protocols, some authors suggest that spoilage of ungutted hake may follow a different trend (Baixas-Nogueras et al., 2009). In order to provide an answer for this question an experiment was performed that included gutted and ungutted hake stored at the same temperature conditions.

Finally a Quality Sensory Method (QSM) based on freshness ratings established for whitefish by the Council Regulation (EC) No 2406/96 (1996) is related to core predictions allowing us to forecast hake quality and its variability under different storage conditions of fluctuating temperature.

2. Materials and methods

2.1. Experimental methods

2.1.1. Fish handling and storage conditions

Fresh gutted medium-sized hake (*Merluccius merluccius*) (400–500 g) caught in Galician waters either by bottom-set or long-line were purchased from the retail market in Vigo (Spain) during the first 24 h after slaughtering. Hake was transferred to the laboratory within 30 min in expanded polysterene boxes with ice. Once in the lab, three-four specimen were analyzed to assess initial quality. Ice was completely removed and boxes containing hake were sealed and stored under refrigeration conditions during 5–12 days either on an incubator (Model EC-570, Radiber S.A.) for experiments at $T \geq 5$ °C, or on a KIDE universal cold room for experiments at $T < 5$ °C. Four experiments were performed using gutted hake captured by bottom-set nets. In these experiments, the refrigeration temperature was fixed to 1 °C, 3 °C, 5 °C and 7 °C. Samples from experiment at 3 °C were used for validation and the remaining were employed in the parameter estimation procedure. Also, hake captured by long-line gear was stored at 3 °C and used for another validation experiment. A last validation experiment was carried out by using ungutted and gutted hake stored at 2.5 °C. A thermocouple, inserted within the abdominal cavity of one fish, recorded the temperature every 5 min throughout the storage period. Fish (3–4 specimen) were taken out of refrigerated storage on a daily basis (except on weekends). 3–4 samples per specimen were employed for microbiological analyses.

2.1.2. Microbiological analysis

A quantity of 25 ± 1 g of fish dorsal muscle was homogenized in 100 mL of 0.9% NaCl by using a stomacher (ITUL Instruments, 2997). This ratio is in accordance with the recommendations made by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986). These homogenates were, afterwards, ten-fold serially diluted in peptone water. Aliquots (0.1 mL) of adequate dilutions were spread on glutamate starch phenol red agar (GSP) and Iron agar Lyngby (IAL). GSP agar was composed of 10.0 g/L sodium glutamate (Oxoid, England), 20 g/L soluble starch (Panreac, Spain), 2.0 g/L potassium dihydrogen phosphate (VWR, Belgium), 0.50 g/L magnesium sulfate (Panreac, Spain), 0.36 g/L phenol red (Merck, Germany), 100.000 UI/L penicillin G (ERN Labs, Spain) and 12 g/L agar (Panreac, Spain). IAL agar consisted of 20 g/L peptone (Panreac, Spain), 3.0 g/L yeast extract (Panreac, Spain), 3.0 g/L meat extract (Panreac, Spain), 5.0 g/L sodium chloride (VWR, Belgium), 0.32 g/L ferric citrate monohydrate (Sigma Aldrich, Germany), 0.47 g/L sodium thiosulfate pentahydrate (Probus, Barcelona), 0.6 g/L L-cysteine (Sigma Aldrich, Germany), 12 g/L agar (Panreac, Spain). *Pseudomonas* spp. were counted as colonies grown on GSP plates after incubation at 25 °C during 48 h (Druggan and Iversen, 2012). Black colonies formed on IAL were counted after 3–5 days of incubation at 17 °C to enumerate H_2S-producing bacteria (Gram, 1992). The great majority of H_2S-producing bacteria isolated from ice-stored fish have been identified as *Shewanella* spp. (Jørgensen and Huss, 1989; Vogel et al., 2005).

2.1.3. Sensory analysis

Fish freshness was organoleptically assessed following the Quality Index Method (QIM) developed by Baixas-Nogueras et al. (2003b). Sensory quality was also assessed by reference to the freshness ratings for whitefish set out in Annex I of Council Regulation (EC) No 2406/96 (1996), named along the document as Quality Sensory Method (QSM).

2.2. Microbial spoilage model

(Psychrotrophic) *Pseudomonas* and *Shewanella* are considered to be the two main bacterial groups responsible for spoilage of fresh fish aerobically stored in ice (Gram and Dalgaard, 2002; Gram and Huss, 1996). Experiments show that these SSOs exhibit a growth and

Table 1
Bounds on the unknown parameters for the optimization involved in the parameter estimation procedure. The estimated initial conditions, denoted as Ps_0, Sh_0, depend on the experiment and are calculated from the statistics of the measured concentrations at $t = 0$. Model parameters are common to all experiments.

θ	θ_{min}	θ_{max}	Units
Ps^*, Sh^*	5.5	8.0	–
b_{Ps}, b_{Sh}	0.01	1.0	$\sqrt{1/(\mathrm{d}^\circ\mathrm{C}^2)}$
T^*_{Ps}, T^*_{Sh}	−130	1.0	°C
$Ps_0^{exp_i}$, $Sh_0^{exp_i}$	$\tilde{y}_0 - 2\sigma_{y_0}$	$\tilde{y}_0 + 2\sigma_{y_0}$	–

stationary phase following the standard logistic model (Baranyi and Roberts, 1994):

$$\frac{dm}{dt} = \mu(T)\left(1-10^{m-m^*}\right) \tag{1}$$

where m and m^* refer, respectively, to the concentration and maximum concentration (concentration in the stationary phase) of the selected microorganisms in log-10 scale. In this equation, the growth rate denoted as $\mu(T)$ depends on the temperature T according to the standard square-root model (Ratkowsky et al., 1982):

$$\sqrt{\mu(T)} = b(T-T^*) \tag{2}$$

where b is the slope of the regression line and T^* should be understood as a conceptual temperature, being an intrinsic property of the organism, which does not necessarily mean the lowest temperature where growth was observed or would occur (Whiting, 1995; Ratkowsky, 1993). Model (1)–(2) was used to represent the dynamics of both SSOs, being of the form:

$$\frac{dPs}{dt} = b_{Ps}^2\left(T-T^*_{Ps}\right)^2\left(1-10^{Ps-Ps^*}\right) \tag{3}$$

$$\frac{dSh}{dt} = b_{Sh}^2\left(T-T^*_{Sh}\right)^2\left(1-10^{Sh-Sh^*}\right) \tag{4}$$

where *Ps* and *Sh* refer to the concentration of *Pseudomonas* and *Shewanella*, respectively. Along the paper, SSOs are expressed in $\frac{CFU}{g}$ in log-10 scale.

Some authors have reported inhibitory effects of *Pseudomonas* on *Shewanella* growth (Gram and Melchiorsen, 1996). However, according to the same authors, such effects would only occur at cell levels much higher than the ones found in the present study. Nonetheless, most *Pseudomonas* isolated from fish did not manifest such effect in ice-stored conditions (Gram, 1993). Therefore, no interactions between *Pseudomonas* and *Shewanella* were considered in the model.

Table 2
Parameter estimations results using preliminary experiments 1, 2 and 3. Model parameters and initial conditions follow a normal distribution defined by the mean and standard deviation.

	Parameter	$\tilde{\theta} \pm \sigma_\theta$	Units
Model parameters	Ps^*	6.44 ± 0.0384	–
	b_{Ps}	0.0533 ± 0.0139	$\sqrt{1/(\mathrm{d}^\circ\mathrm{C}^2)}$
	T^*_{Ps}	-12 ± 5.9	°C
	Sh^*	6.21 ± 0.0464	–
	b_{Sh}	0.0442 ± 0.0081	$\sqrt{1/(\mathrm{d}^\circ\mathrm{C}^2)}$
	T^*_{Sh}	-16 ± 4.3	°C
Initial conditions	$Ps_0^{exp_1}$	1.31 ± 0.583	–
	$Ps_0^{exp_2}$	1.76 ± 0.245	–
	$Ps_0^{exp_3}$	2.06 ± 0.158	–
	$Sh_0^{exp_1}$	1.51 ± 0.254	–
	$Sh_0^{exp_2}$	1.75 ± 0.214	–
	$Sh_0^{exp_3}$	2.16 ± 0.113	–

2.3. Statistical methods

2.3.1. Parameter estimation

Unknown parameters of model in Eqs. (3)–(4) (namely, Ps^*, Sh^*, b_{Ps}, b_{Sh}, T^*_{Ps}, T^*_{Sh}) are to be estimated by means of data regression, employing

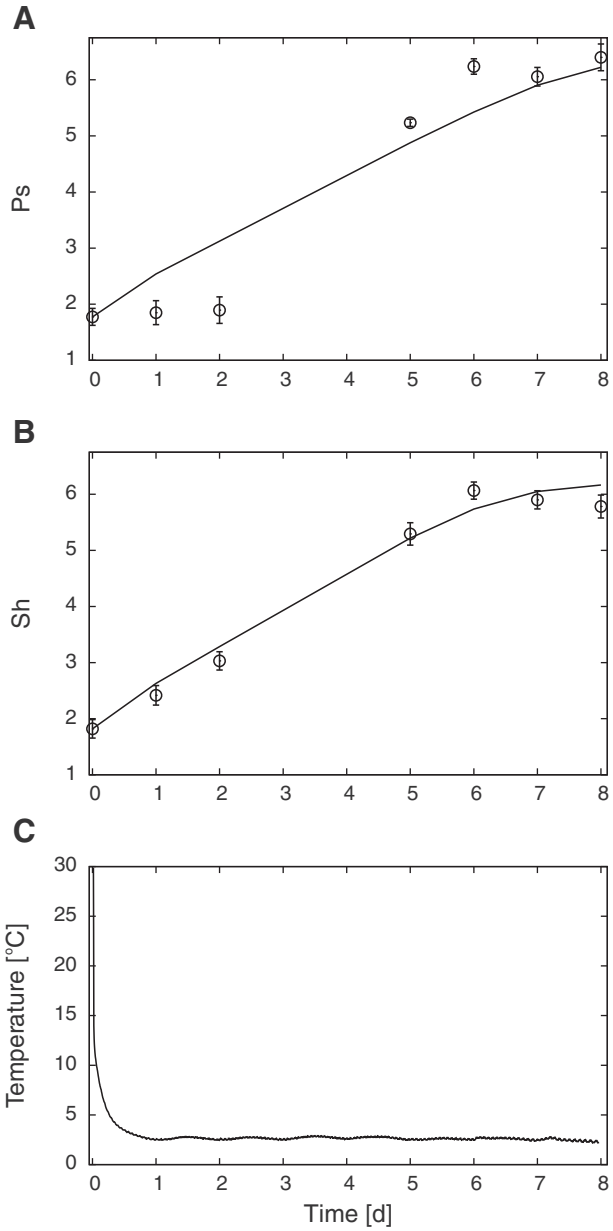

Fig. 1. Validation after the initial parameter estimation (experiments 1, 2 and 3). A and B show the evolution of *Pseudomonas* and *Shewanella* concentrations during the validation experiment. Dots and bars represent, respectively, the mean and standard deviation of the experimental data ($\tilde{y} \pm \sigma_y$) whereas continuous lines depict model predictions. C corresponds with the recorded temperature in the abdominal cavity of fish during the experiment.

a one-step procedure, in order to take advantage of non-isothermal experiments to estimate all relevant parameters at once (Dolan et al., 2007; Rodríguez-Fernández et al., 2007).

This approach has been selected over the classical two-step regression method, where parameters are estimated sequentially on a set of isothermal experiments. On the one hand, because it requires a lower number of experiments. On the other, because according to several authors (Van Boekel, 1996; Dolan, 2003; Valdramidis et al., 2008) the two-step approach may result into larger uncertainty on the parameter estimates which may lead to unreliable predictions under non-isothermal conditions.

The underlying idea of the one-step model regression is to formulate an optimization problem where the objective is to compute those parameter values that minimize a measure of the distance among the model predictions and the experimental data. In this work this measure has been chosen to be the maximum likelihood.

The maximum likelihood method seeks for the parameter values that give the highest likelihood to the experimental data given the considered model (Walter and Pronzato, 1997). The advantage of this approach is that it allows taking into account the available information on the nature of the experimental noise.

For this particular case in which bacterial concentrations follow a log-normal distribution (Busschaert et al., 2010; Crépet et al., 2007), and their measurements are independently and identically distributed, the maximum likelihood approach translates into the minimization of the following weighted least squares function:

$$J(\theta) = \sum_{i}^{nd} \frac{(y_{mi} - y_i(\theta))^2}{\sigma_{y,i}^2} \tag{5}$$

where i indicates the measurement point, being nd the total amount of data; θ corresponds to the set of parameters to be estimated; y_{mi} and $y_i(\theta)$ refer, respectively, to bacterial concentration (either *Pseudomonas* or *Shewanella*) experimentally measured and predicted by the model for a given value of the parameters at the measurement time i; $\sigma_{y,i}$ corresponds to the standard deviation of the experimental noise at measurement time i as estimated from experimental measurements in different fish samples. Note that the objective function to be minimized (J) is constructed so to give more relevance to those data with less variability.

2.3.2. Confidence intervals, core predictions and experimental design

The error of the estimated model parameters is evaluated by constructing the confidence region in the model parameter space. To do so a Monte Carlo sampling method has been used. This technique generates different growth curves following the statistical distributions estimated from experimental data. Each of these realizations is considered as an acceptable set of data and used to estimate the model parameters. This procedure is repeated for a sufficiently large number of times (500 in this work) to obtain a good estimation of the parameters uncertainty. In order to remove possible outliers, we select those parameters in the 0.05–0.95 interquantile range. The calculated parameter confidence is expressed by a mean and a standard deviation as $\tilde{\theta} \pm \sigma_\theta$. Note that for normal distributions, such as the ones found for this problem, this corresponds with a confidence interval of 68.3%.

This technique can be also exploited to calculate the uncertainty in the predictions (an analysis known as core predictions in systems biology). For the validation experiments (i.e., with data not used for parameter estimation) we compute the range of possible solutions corresponding to different realizations of the parameter statistics and initial population of SSOs.

It should be noted that confidence intervals, and thus core predictions, highly depend on the experimental setup and the standard deviation of the experimental noise. In order to minimize confidence

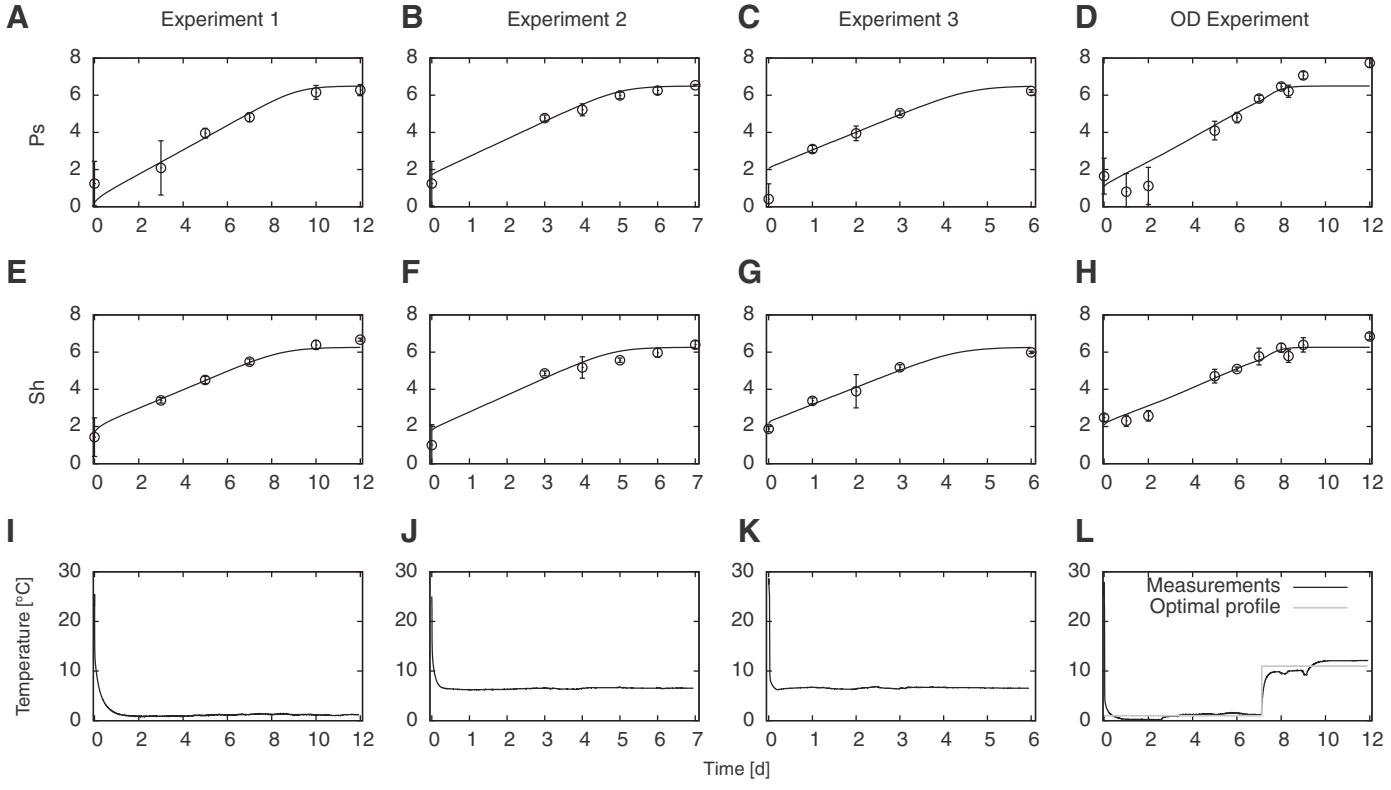

Fig. 2. Model fit vs. data using 1, 2, 3 and the OD experiments. A–D and E–H correspond, respectively, with *Pseudomonas* and *Shewanella* concentrations. Dots and bars represent the mean and standard deviation of data ($\tilde{y} \pm \sigma_y$) whereas continuous lines show the model fit. I–L correspond with the recorded temperature inside the abdominal cavity of fish during the experiments.

Table 3
Parameter estimations using preliminary experiments (1, 2 and 3) in conjunction with the OD experiment.

	Parameter	$\tilde{\theta} \pm \sigma_\theta$	Units
Model parameters	Ps^*	6.50 ± 0.0361	–
	b_{Ps}	0.0301 ± 0.0092	$\sqrt{1/(\mathrm{d}\,^\circ\mathrm{C}^2)}$
	T^*_{Ps}	-26 ± 8.14	°C
	Sh^*	6.26 ± 0.0453	–
	b_{Sh}	0.0458 ± 0.0052	$\sqrt{1/(\mathrm{d}\,^\circ\mathrm{C}^2)}$
	T^*_{Sh}	-14 ± 1.82	°C
Initial conditions	$Ps_0^{exp_1}$	0.238 ± 0.332	–
	$Ps_0^{exp_2}$	1.71 ± 0.257	–
	$Ps_0^{exp_3}$	2.06 ± 0.158	–
	$Ps_0^{exp_{OD}}$	1.10 ± 0.407	–
	$Sh_0^{exp_1}$	1.70 ± 0.134	–
	$Sh_0^{exp_2}$	1.80 ± 0.196	–
	$Sh_0^{exp_3}$	2.19 ± 0.0767	–
	$Sh_0^{exp_{OD}}$	2.08 ± 0.0811	–

intervals in the context of microbiological models, the use of optimal experimental design has been suggested (see, for example, Versyck et al., 1999; Bernaerts et al., 2000; Balsa-Canto et al., 2008). Here, the optimal experimental design problem is formulated as a general dynamic optimization problem whose objective is to find the time-dependent temperature profile so as to maximize the determinant of the so called Fisher Information Matrix (Walter and Pronzato, 1997). It should be noted that the Fisher Information Matrix contains the sensitivities of the microbial concentrations with respect to the model parameters evaluated at each measurement point. Therefore maximizing its determinant maximizes sensitivity and, as a result, confidence intervals are minimized.

2.3.3. Numerical methods

The solution of the parameter estimation and optimal experimental design problems requires the use of advanced numerical techniques. In this work we made use of AMIGO (Advanced Model Identification using Global Optimization), a multi-platform toolbox implemented in Matlab which covers parameter estimation but also sensitivity analysis and experimental design (Balsa-Canto and Banga, 2011). From the set of numerical methods offered in the toolbox, the global optimizer based on scatter search (eSS, Enhanced Scatter Search) method (Egea et al., 2010), was selected due to its efficiency and robustness in finding the best parameter values and experimental designs. In addition, the model simulator CVODES (Hindmarsh et al., 2005) was selected to

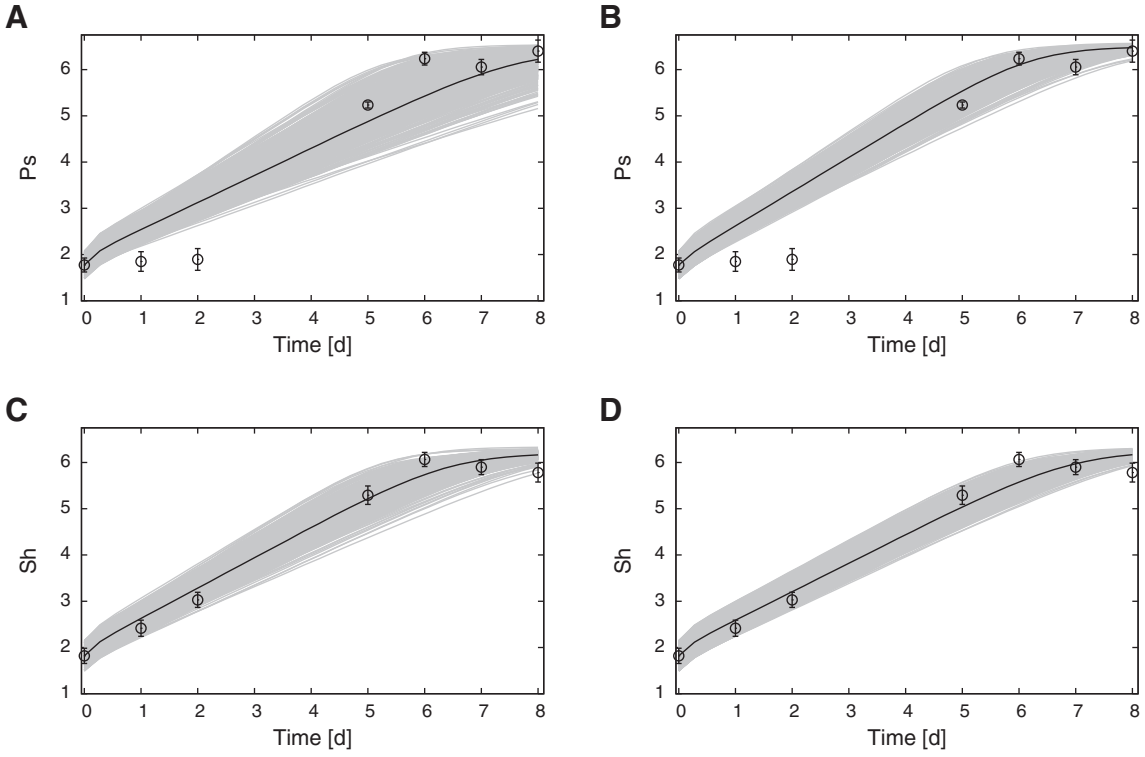

Fig. 3. Comparison of core predictions in validation experiment. A and C correspond with the results obtained using the initial preliminary experiments only, whereas B and D were obtained by also including the information of the OD experiment. Dots and bars represent experimental data ($\bar{y} \pm \sigma_y$), while continuous black lines show the most probable predictions and gray bands their respective uncertainty.

solve the microbial spoilage model (3)–(4) and to evaluate the parametric sensitivities required to compute the Fisher Information Matrix.

3. Results and discussion

3.1. Parameter estimation

To solve the parameter estimation problem as described in Section 2.3, a reasonable range of parameter values (parameter bounds) needs to be supplied to the optimizer. Selected bounds are reported in Table 1. In addition, initial conditions need to be provided to the model simulator to solve Eqs. (3)–(4).

Many factors such as fishing gear, handling, or catching ground and season, affect such conditions (Huss, 1995). A bad choice for the initial conditions may lead to over- or under-estimation of the growth curve. In order to avoid this situation, initial conditions for each experiment will be estimated within the range computed from the experimental data statistics at $t = 0$ ($\tilde{y}_0 \pm 2\sigma_{y_0}$) that corresponds with a confidence interval of 95%.

In the following, hake captured by bottom-set nets will be used unless specified otherwise. As mentioned before, data from samples stored at 1 °C, 5 °C and 7 °C (denoted as Experiments 1, 2 and 3, respectively) are used for parameter estimation whereas data from samples stored at 3 °C are saved for model validation.

Parameter estimates and their respective confidence intervals obtained from experiments 1, 2 and 3 are presented in Table 2. Here, confidence in terms of coefficients of variation ($\sigma_\theta/\tilde{\theta}$) is larger in the initial conditions than in model parameters. This is so because estimation of initial conditions are experiment-dependent in the sense that each estimation uses only the information of one experiment. It should be stressed, however, that only model parameters can be exploited to forecast the growth of SSOs in new experiments. Consequently, the accuracy of the measured initial conditions will be essential to avoid over- or under-estimations of the growth curves.

In order to test the predictive capabilities of the model, the 3 °C experiment has been used. The predictive model requires the use of the estimated model parameters (Table 2), measured initial concentration of SSOs and the storage temperature profile. Comparison between model predictions and experimental data is depicted in Fig. 1A and B. Points and error bars represent the mean and standard deviation of data ($\tilde{y} \pm \sigma_y$), respectively, whereas continuous lines show the model response. Note that predictions reproduce experimental data considerably well, in particular for *Shewanella*. Experimental data are in agreement with the values reported in the literature. In ice-stored fresh hake (*Merluccius merluccius*), maximum levels of *Shewanella* reported by Baixas-Nogueras et al. (2009) were 6.36 and 6.71 in hake stored for 14 and 16 days, respectively (storage was in flake ice (0 °C) inside a refrigerator set at 4 °C). In the same study, *Pseudomonas* counts were 5.03 and 5.45, respectively. Similar results were reported in previous works (Baixas-Nogueras et al., 2003a).

3.2. Optimal experimental design

In order to improve the quality of the estimation, the temperature profile which maximizes the information content of the experiment (described by the determinant of the Fisher Information Matrix) is computed. Due to experimental restrictions, only one measuring time per working day was allowed, except for the day of the temperature jump where two consecutive samples are considered just after the event. Results show that the best experiment found started at 1 °C and jumps to 11 °C on the 8th day of the experiment (see Fig. 2L).

The implementation of the optimally designed experiment (denoted in the following as OD experiment) is presented in Fig. 2D, H, L. It can be observed that the recorded temperatures follow the theoretical profile and never deviate more than two degrees once the temperature reaches 1 °C in the abdominal cavity. Even in case of larger deviations, this experiment would be more informative than the preliminary ones since, as stated in Bernaerts et al. (2000), it introduces sharp changes in the temperature that can be used to feed the model in the parameter estimation.

Fig. 2 shows that the model is able to follow, not only preliminary experiments, but also the OD experiment, which includes a variable time–temperature profile. The OD experiment is used in conjunction with experiments 1, 2 and 3 to estimate the model parameters (3)–(4). As in the previous section, we have exploited the Monte Carlo method to find out the confidence intervals in the parameters given fish-to-fish variability. Results, gathered in Table 3, show how the confidence intervals of most of the parameters are reduced by adding the OD experiment. The exception is the temperature reference of *Pseudomonas* (T^*_{Ps}) where the standard deviation increases by including information of the OD experiment.

This may be reasonable since adding a new experiment requires estimating two new initial conditions. Independently of this effect, however, we can conjecture that the model predictive capabilities are

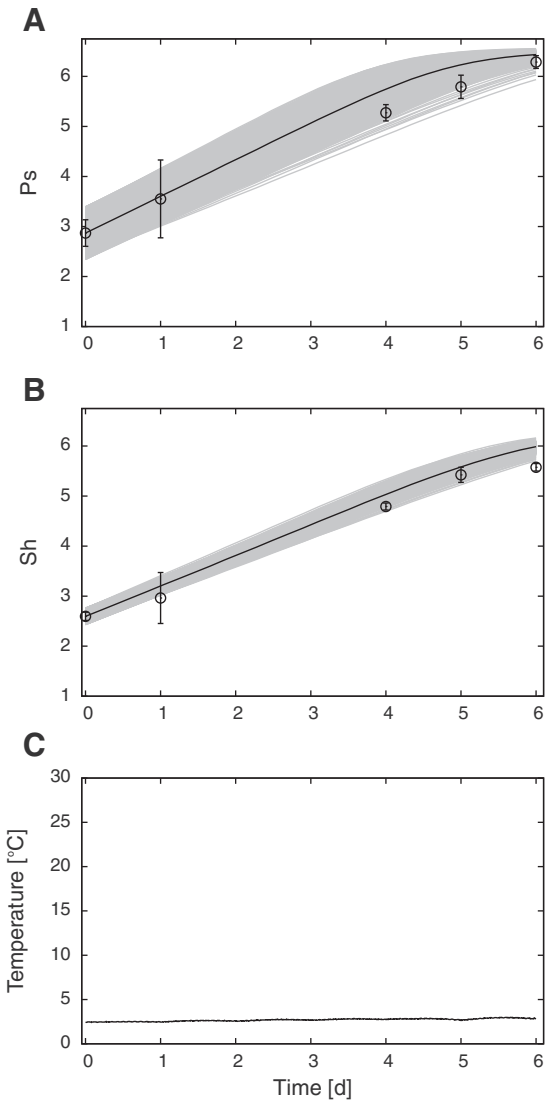

Fig. 4. Core predictions for long-line hake. Fish was caught by long-line and stored, as in the previous validation experiment, at 3 °C. A and B show the evolution of *Pseudomonas* and *Shewanella* concentrations. Dots and bars represent, respectively, the mean and standard deviation of the experimental data ($\tilde{y} \pm \sigma_y$) whereas continuous black lines show the most probable predictions and gray bands their respective uncertainty. C depicts the recorded temperature in the abdominal cavity of fish during the experiment.

mainly affected by errors in Ps^* and b_{Ps}. As a consequence, reducing the confidence intervals for these parameters is critical, even at the expenses of increasing the uncertainty in T^*_{Ps}. We cannot fully test this hypothesis simply by checking the goodness of the prediction as it has been done in Fig. 1, since only the best estimated values of the parameters, but not their confidence intervals, are considered.

It should be noted that Figs. 1A, 2D and H suggest the presence of a lag time at least for low temperatures (in the range of 1–3 °C). This lag time was not observed in experiments performed at higher temperatures. Model in Eqs. (3)–(4) could be modified in different ways to account for the delay in the exponential growth (Swinnen et al., 2004). In this respect, the modification proposed by Baranyi and Roberts (1994) has shown to be the most consistent in the sense that it typically provides the best fit to the data and gives reasonable estimates of the lag time (Baty and Delignette-Muller, 2004), provided that the quantity and quality of data is enough.

However, there are, at least, three reasons not to incorporate this modification in models (3)–(4): first, the number of cells initially present in fish is low and the experimental error is rather important (more than 100 % in many cases) thus preventing the use of those data to estimate the lag time (see also Nuin et al., 2008, which neglects latency for a similar reason); second, the model is intended to predict quality in the growth phase thus the lag will not have an impact on the final value; and third, the use of the modified model for the sake of prediction

Table 4
Correspondences between sensory assessment methods QSM and QIM marks were obtained from sensory analysis in experiments 1, 2, 3 and OD. Indexes E, A, B and NA correspond to Extra-quality, A-quality, B-quality and Not admitted, respectively.

QSM marks	QIM range
E	[0, 3]
A	[3, 6]
A–B	[6, 8]
B	[8, 12]
B–NA	[12, 14]
NA	[14, 19]

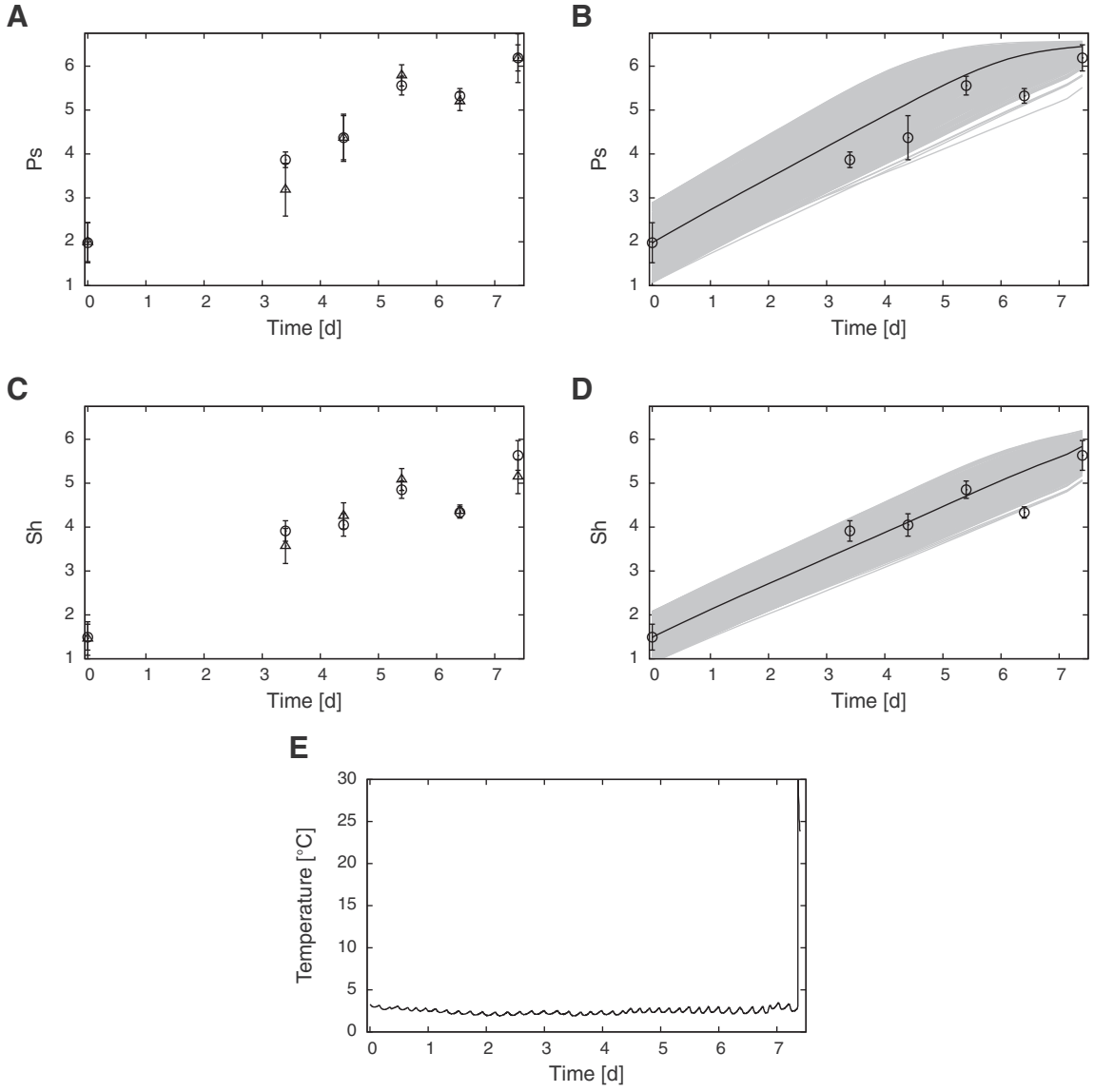

Fig. 5. Microbial growth during storage of gutted and ungutted hake. Experimental data for gutted (triangles) and ungutted (circles) hake are presented in A (*Pseudomonas*) and C (*Shewanella*). The comparison between experiments and model core predictions (gray bands) is presented in B (*Pseudomonas*) and D (*Shewanella*). E depicts the recorded temperature in the abdominal cavity of fish during the experiment.

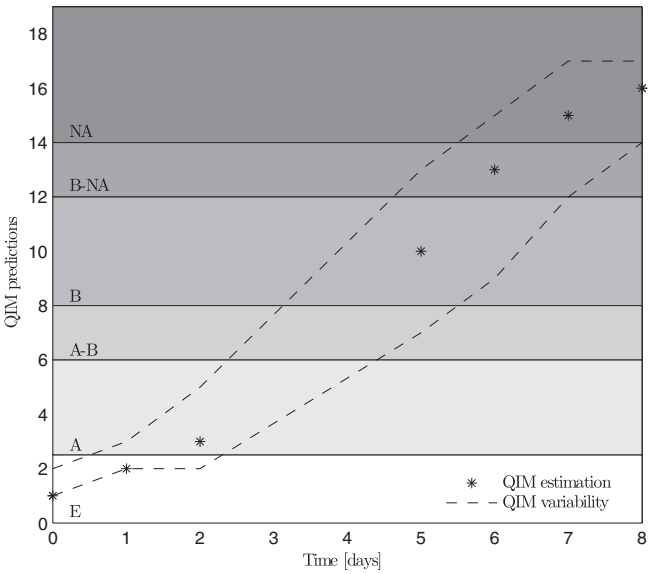

Fig. 6. Minimum and maximum QIM evolution based on the microbial spoilage model. The background of the plot depicts the QSM regions described in Table 4 on a gray scale.

requires the knowledge about the physiological state of the cells at initial time, which is not available and not measurable thus making the model useless.

In the next section, the so-called "core predictions" will be exploited to estimate the uncertainty of microorganism growth curves due to fish-to-fish variability, and to evaluate previous hypothesis.

3.3. Validation using core predictions

Model predictions depend on the uncertainty on both model parameters and initial concentration of SSOs in the considered experiment. Statistics of model parameters were calculated in the previous sections using preliminary experiments (Table 2) and the optimally designed experiment (Table 3). The normal distribution of initial conditions is extracted from experimental replicas of bacterial concentrations in fish samples. The mean values of model parameters and initial condition distributions allow us to calculate the most probable growth curves considering fish-to-fish variability. In order to assess such model uncertainty, the Monte Carlo method will be now employed.

Results are shown in Fig. 3, where continuous black lines indicate the most probable growth curves and gray bands represent their uncertainty. Fig. 3A and C corresponds with the results obtained after the initial parameter estimation (parameters of Table 2), whereas the results obtained after including the OD experiment (parameters of Table 3) are presented in Fig. 3B and D. Model uncertainty is reduced by adding the optimally designed experiment for both microorganism classes. This is the case even if confidence in some of the model parameters is not so high, as it occurs with *Pseudomonas*.

Table 5
Validation of quality prediction Quality Sensory Method (QSM) and its estimation using the microbial spoilage model for the validation experiment.

Time [days]	Estimated QSM (range)	QSM found in fish samples
0	E (E–E)	E
1	E (E–A)	E
2	A (E–A)	A
5	B (B–B)	B
6	B-NA (B–NA)	B
7	NA (B–NA)	NA
8	NA (NA–NA)	NA

Previous studies have reported a significant influence of the method of catching on microbial spoilage (or shelf-life) of fish (Özyurt et al., 2007). In order to test model predictive capabilities for gutted hake caught by a different fishing gear, a new validation experiment has been performed.

The results are presented in Fig. 4, where it can be seen that, despite some slight overestimation, the model is able to reasonably predict bacterial growth. It is difficult to identify the factors behind such overestimation with the available data. Nonetheless, as some authors point out, they might be related with damages induced by the fishing gear on fish tissues that accelerate bacterial growth (Özyurt et al., 2007). In any case these results suggest that the influence if any, is embedded within the implicit uncertainty of the model.

Previous literature reported controversial results on the effectiveness of gutting as a method to increase shelf-life. In particular for hake, it was concluded that gutting would accelerate the proliferation of gram-negative bacteria (Baixas-Nogueras et al., 2009). In order to validate our model under different scenarios, an experiment was programmed that included storage of gutted and ungutted hake. To that purpose, two batches of medium-size hake, one gutted and the other ungutted, were prepared. As in previous experiments, fish was caught by bottom-set nets, in Galician waters, using similar handling procedures from the retailer to the lab except that fish was left ungutted. Once in the lab, half of fish was gutted and both batches were stored at 2.5 °C during 9 days. The same methodology described in Section 2.1.2 has been employed for samplings and microbiological analyses.

The results of this experiment are shown in Fig. 5. Experiments show no significant differences in terms of microbial growth for gutted (triangles) and ungutted (circles) hake along the experiment horizon (Fig. 5A and C). Concerning the predictive capabilities of the proposed model, core predictions (gray bands) are presented in Fig. 5B and D, showing a good agreement between model predictions and experimental data (marks).

3.4. Quality predictions using the microbial spoilage model

As it has been previously reported (Nuin et al., 2008), models as the one proposed are excellent tools to predict shelf-life under different storage conditions. In this section we explore the potential of our predictive model of SSOs to estimate not just shelf-life, but also different freshness categories of whitefish as defined by the QSM (Council Regulation (EC) No, 2406/96, 1996) and the QIM (Bremner, 1985). QSM establishes four fish quality categories that range from extra-quality (E) to not admitted (NA), and includes two intermediate quality grades (A and B). On the other hand, QIM classifies hake quality on a natural number scale which depends on the fish species. In this study, it has been found that SSO concentrations correlate particularly well with QIM via an expression of the form:

$$QIM = nint\left(10^{lQ}\right) - 1 \quad \text{where} \quad lQ = \alpha Ps + \beta Sh \tag{6}$$

where QIM is the corresponding quality index (ranging between 0–19) for hake (Baixas-Nogueras et al., 2003b), and "*nint*" is the function that computes the nearest integer value.

Data from experiments 1, 2, 3 and OD, which included QIM and QSM evaluations from a panel of experts, have been used to estimate parameters in expression (6), resulting in $\alpha = 0.068$ and $\beta = 0.129$ with a regression coefficient $r = 0.89$. Note that such value should be considered reasonably good, specially taking into account that QIM is an integer variable which refers to skin, eyes, gill and flesh quality; while concentrations of SSOs were measured only in flesh. To the authors' best knowledge, only the work by Giuffrida et al. (2013) has tried to connect concentrations of SSOs with QIM directly although for a different species

and with SSO concentrations taken not just in flesh, but also in skin and gill.

Comparison of the QSM and QIM values obtained for the different experiments suggested the equivalences presented in Table 4. Such characterization recognizes the existence of two overlapping categories which we resolve by defining the new grades A–B and B–NA between the A and B and between B and NA fish grades, respectively.

Uncertainty in microorganism concentrations *Sh* and *Ps*, defined as the bandwidth of the core predictions obtained by including the information of the OD experiment (Fig. 3), is employed to compute, by means of expression (6), the corresponding QIM uncertainty. Its evolution, given in terms of a minimum and maximum QIM evolution (slashed lines) as well as its most probable value (stars), are presented in Fig. 6. As it can be seen from the figure, the QSM grades, defined in Table 4 and represented on the background of the plot, can be predicted from the QIM evolution.

To test the validity of the prediction, the panel of experts evaluated the QSM in the validation experiment presented in Fig. 3. The results are summarized in Table 5 showing that the marks obtained by the experts coincide for all times with the ones estimated by the model. It should be stressed that, despite the fact that the model here considered does not include the lag phase, quality predictions at the initial times coincide with the evaluations of the panel of experts. In agreement with previous discussion on lag phase (Section 3.2), this supports the assumption that the effect of this latency time is not relevant to estimate quality.

Shelf-life, understood as the time required to reach a not-admitted (NA) grade, can be obtained in a straightforward manner from the quality prediction model. For the scenario depicted in Fig. 6 (hake stored at the temperature profile depicted in Fig. 1C), a simple inspection gives a shelf-life range between approximately 5 to 8 days with a most probable value of 7 days what coincides with the experimental evidence.

4. Conclusions

A methodology to forecast hake quality during storage at different temperature conditions has been proposed. The method makes use of a dynamic model of microbiological growth that incorporates the uncertainty caused by fish-to-fish variability. An essential step in model development is the application of optimal experimental design to generate sufficient non-isothermal informative experiments to reduce the uncertainty in the parameters and, thus, in model predictions. The estimated growth curves are used to estimate the most probable value of the Quality Sensory Index (Council Regulation (EC) No, 2406/96, 1996) and its associated uncertainty.

Fish quality predictions are estimated from any profile of the storage temperature making use of the dynamic model of the SSOs (Specific Spoilage Organisms) and confidence measurements of their initial conditions. In addition, the model has been employed to test the effect on fish quality of factors such as the fishing gear or evisceration. Results from the microbial growth dynamics show no significant differences, within the inherent model uncertainty, between hake fished by long-line or bottom-set nets. Similar conclusions can be drawn for gutted and un-gutted hake along the experiment horizon.

Finally, it must be remarked that the methodology here proposed is flexible enough to include other stress variables (e.g., atmosphere composition) or to be extended to shelf-life assessment for other fish species.

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References

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, M.T., Vidal-Carou, M.C., 2003a. Amino acid-decarboxylase activity in bacteria associated with Mediterranean hake spoilage. Eur. Food Res. Technol. 217, 164–167.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, T., Nunes, M.L., Vidal-Carou, M.C., 2003b. Development of a quality index method to evaluate freshness in Mediterranean hake (*Merluccius merluccius*). J. Food Sci. 68, 1067–1071.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, T., Vidal-Carou, M.C., 2009. Effect of gutting on microbial loads, sensory properties, and volatile and biogenic amine contents of European hake (*Merluccius merluccius* var. *mediterraneus*) stored in ice. J. Food Prot. 72, 1671–1676.

Balsa-Canto, E., Banga, J.R., 2011. Amigo, a toolbox for advanced model identification in systems biology using global optimization. Bioinformatics 27, 2311–2313.

Balsa-Canto, E., Alonso, A.A., Banga, J.R., 2008. Computing optimal dynamic experiments for model calibration in predictive microbiology. J. Food Process Eng. 31, 186–206.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277–294.

Baty, F., Delignette-Muller, M.L., 2004. Estimating the bacterial lag time: which model, which precision? Int. J. Food Microbiol. 91, 261–277.

Bernaerts, K., Versyck, K.J., Van Impe, J.F., 2000. On the design of optimal dynamic experiments for parameter estimation of a Ratkowsky-type growth kinetics at suboptimal temperatures. Int. J. Food Microbiol. 54, 27–38.

Brännmark, C., Palmér, R., Glad, S.T., Cedersund, G., Strålfors, P., 2010. Mass and information feedbacks through receptor endocytosis govern insulin signaling as revealed using a parameter-free modeling framework. J. Biol. Chem. 285, 20171–20179.

Bremner, H., 1985. A convenient, easy to use system for estimating the quality of chilled seafoods. Fish Process. Bull. 59–70.

Busschaert, P., Geeraerd, A.H., Uyttendaele, M., Van Impe, J.F., 2010. Estimating distributions out of qualitative and (semi) quantitative microbiological contamination data for use in risk assessment. Int. J. Food Microbiol. 138, 260–269.

Carrascosa, C., Millán, R., Saavedra, P., Jaber, J.R., Montenegro, T., Raposo, A., Pérez, E., Sanjuán, E., 2014. Predictive models for bacterial growth in sea bass (*Dicentrarchus labrax*) stored in ice. Int. J. Food Sci. Technol. 49, 354–363.

Council Regulation (EC) No 2406/96, 1996. Laying down common marketing standards for certain fishery products. OJ L 334 pp. 1–15.

Crépet, A., Albert, I., Dervin, C., Carlin, F., 2007. Estimation of microbial contamination of food from prevalence and concentration data: application to *Listeria monocytogenes* in fresh vegetables. Appl. Environ. Microbiol. 73, 250–258.

Dalgaard, P., 2002. Modelling and predicting the shelf-life of seafood. In: Bremner, H. (Ed.), Safety and Quality Issues in Fish Processing. Woodhead Publishing Ltd., pp. 191–219.

Dalgaard, P., Buch, P., Silberg, S., 2002. Seafood spoilage predictor—development and distribution of a product specific application software. Int. J. Food Microbiol. 73, 343–349.

Dolan, K.D., 2003. Estimation of kinetic parameters for nonisothermal food processes. J. Food Sci. 68, 728–741.

Dolan, K.D., Yang, L., Trampel, C.P., 2007. Nonlinear regression technique to estimate kinetic parameters and confidence intervals in unsteady-state conduction-heated foods. J. Food Eng. 80, 581–593.

Dowlati, M., Mohtasebi, S.S., Omid, M., Razavi, S.H., Jamzad, M., de la Guardia, M., 2013. Freshness assessment of gilthead sea bream (*Sparus aurata*) by machine vision based on gill and eye color changes. J. Food Eng. 119, 277–287.

Druggan, P., Iversen, C., 2012. Culture media for food spoilage bacteria of the order pseudomonadales: *Pseudomonas*, *Acinetobacter* and *Psychrobacter* spp. In: Corry, J.E.L., Curtis, G.D.W., Baird, R.M. (Eds.), Handbook of Culture Media for Food and Water Microbiology. Royal Society of Chemistry, Cambridge, U.K.

Egea, J.A., Martí, R., Banga, J.R., 2010. An evolutionary method for complex-process optimization. Comput. Oper. Res. 37, 315–324.

Giuffrida, A., Valenti, D., Giarratana, F., Ziino, G., Panebianco, A., 2013. A new approach to modelling the shelf life of gilthead seabream (*Sparus aurata*). Int. J. Food Sci. Technol. 48, 1235–1242.

Gram, L., 1992. Evaluation of the bacteriological quality of seafood. Int. J. Food Microbiol. 16, 25–39.

Gram, L., 1993. Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. Appl. Environ. Microbiol. 59, 2197–2203.

Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria—problems and solutions. Curr. Opin. Biotechnol. 13, 262–266.

Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33, 121–137.

Gram, L., Melchiorsen, J., 1996. Interaction between fish spoilage bacteria *Pseudomonas* sp. and *Shewanella putrefaciens* in fish extracts and on fish tissue. J. Appl. Bacteriol. 80, 589–595.

Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R., Meybeck, A., 2011. Global Food Losses and Food Waste: Extent, Causes and Prevention. FAO, Rome.

Hindmarsh, A.C., Brown, P.N., Grant, K.E., Lee, S.L., Serban, R., Shumaker, D.E., Woodward, C.S., 2005. Sundials: suite of nonlinear and differential/algebraic equation solvers. ACM Trans. Math. Softw. 31, 363–396.

Huss, H.H., 1995. Quality and Quality Changes in Fresh Fish 348. Food and Agriculture Organization (FAO) (URL: http://www.fao.org/docrep/v7180e/v7180e00.htm).

ICMSF, 1986. Microorganisms in foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications 2nd ed. Blackwell Scientific Publications.

Jørgensen, B.R., Huss, H.H., 1989. Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. Int. J. Food Microbiol. 9, 51–62.

Koutsoumanis, K., 2001. Predictive modeling of the shelf life of fish under nonisothermal conditions. Appl. Environ. Microbiol. 67, 1821–1829.
Matos, E., Goncalves, A., Nunes, M.L., Dinis, M.T., Dias, J., 2010. Effect of harvesting stress and slaughter conditions on selected flesh quality criteria of gilthead seabream (*Sparus aurata*). Aquaculture 305, 66–72.
Nuin, M., Alfaro, B., Cruz, Z., Argarate, N., George, S., Le Marc, Y., Olley, J., Pin, C., 2008. Modelling spoilage of fresh turbot and evaluation of a time–temperature integrator (TTI) label under fluctuating temperature. Int. J. Food Microbiol. 127, 193–199.
Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J., Nilsen, H., 1997. Methods to evaluate fish freshness in research and industry. Trends Food Sci. Technol. 8, 258–265.
Ólafsdóttir, G., Lauzon, H., Martinsdóttir, E., Kristbergsson, K., 2006. Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. Int. J. Food Microbiol. 111, 112–125.
Özyurt, G., Özogul, Y., Özyurt, C.E., Polat, A., Özogul, F., Gökbulut, C., Ersoy, B., Küley, E., 2007. Determination of the quality parameters of pike perch *Sander lucioperca* caught by gillnet, longline and harpoon in turkey. Fish. Sci. 73, 412–420.
Ratkowsky, D.A., 1993. Principles of nonlinear regression modelling. J. Ind. Microbiol. 12, 195–199.
Ratkowsky, D., Olley, J., McMeekin, T., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149, 1–5.
Rodríguez-Fernández, M., Balsa-Canto, E., Egea, J.A., Banga, J.R., 2007. Identifiability and robust parameter estimation in food process modeling: Application to a drying model. J. Food Eng. 83, 374–383.
Rotabakk, B.T., Skipnes, D., Akse, L., Birkeland, S., 2011. Quality assessment of Atlantic cod (*Gadus morhua*) caught by longlining and trawling at the same time and location. Fish. Res. 112, 44–51.
Shorten, P.R., Soboleva, T.K., Pleasants, A.B., Membre, J.M., 2006. A risk assessment approach applied to the growth of *Erwinia carotovora* in vegetable juice for variable temperature conditions. Int. J. Food Microbiol. 109, 60–70.
Swinnen, I.A.M., Bernaerts, K., Dens, E.J.J., Geeraerd, A.H., Van Impe, J.F., 2004. Predictive modelling of the microbial lag phase: a review. Int. J. Food Microbiol. 94, 137–159.
Taoukis, P., Koutsoumanis, K., Nychas, G., 1999. Use of time–temperature integrators and predictive modelling for shelf life control of chilled fish under dynamic storage conditions. Int. J. Food Microbiol. 53, 21–31.
Valdramidis, V.P., Geeraerd, A.H., Bernaerts, K., Van Impe, J.F.M., 2008. Identification of non-linear microbial inactivation kinetics under dynamic conditions. Int. J. Food Microbiol. 128, 146–152.
Van Boekel, M.A.J.S., 1996. Statistical aspects of kinetic modeling for food science problems. J. Food Sci. 61, 477–485.
Versyck, K.J., Bernaerts, K., Geeraerd, A.H., Van Impe, J.F., 1999. Introducing optimal experimental design in predictive modeling: a motivating example. Int. J. Food Microbiol. 51, 39–51.
Vogel, B.F., Venkateswaran, K., Satomi, M., Gram, L., 2005. Identification of *Shewanella baltica* as the most important H_2S-producing species during iced storage of danish marine fish. Appl. Environ. Microbiol. 71, 6689–6697.
Walter, E., Pronzato, L., 1997. Identification of Parametric Models from Experimental Data. Springer.
Whiting, R.C., 1995. Microbial modeling in foods. Crit. Rev. Food Sci. Nutr. 35, 467–494.