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Performance assessment of a predictive microbiology framework for *Listeria monocytogenes* growth in dry-cured fish

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ABSTRACT

The consumption of processed seafood in the European Union has significantly grown, raising food safety concerns, especially for ready to eat products that are consumed without cooking. This growing market interest has driven innovations, including patented fish dry-curing cabinets. Among biological hazards, *Listeria monocytogenes* is of particular concern in seafood, prompting regulatory scrutiny and the need for predictive modelling to assess its potential growth. This study aims to assess the performance of a model predicting the behavior of *L. monocytogenes* in vacuum-packed dry-cured fish under dynamic temperature conditions. The fish tested included salmon, swordfish, and tuna, processed following standardized curing and processing methods. A mixture of three *L. monocytogenes* strains was used for the challenge tests, ensuring a controlled level of contamination. Microbiological and physicochemical parameters, as well as growth dynamics of *L. monocytogenes,* were measured throughout the shelf life of the products. A dynamic Baranyi model coupled with a secondary cardinal model was employed to simulate the bacterial growth under varying temperature profiles, utilizing established equations to predict microbial behavior. Initial water activity (a_w) and pH values showed stability throughout the study period. *L. monocytogenes* exhibited growth in salmon and swordfish, while it declined in tuna, probably due to unfavorable a_w conditions (0.904 ± 0.010). The model demonstrated good predictive accuracy, with the majority of predictions (88.89 % for salmon and 77.22 % for swordfish) falling within an acceptable prediction zone. This study provides valuable insights into the growth dynamics of *L. monocytogenes* in dry-cured fish products. The evaluated predictive model can be applied by seafood producers to assess *L. monocytogenes* risk and make informed decisions about dry-cured fish products shelf life and storage conditions.

1. Introduction

In 2022, processed fish and seafood consumption in the EU reached nearly 2.2 million tons, driven by demand in the food service and retail sectors. The majority of this consumption was concentrated in Germany, Spain, Italy, and France, which together account for 75 % of the total (European Commission & EUMOFA, 2023). As global seafood demand continues to rise, consumers increasingly seek ready-to-eat (RTE) products with extended shelf life, bold flavors, and convenience. This growing market interest has led to innovations, such as patented fish dry-curing cabinets, which, similarly to meat dry-agers, entered the market to capture new more demanding customers. These cabinets, by monitoring and managing on continuous temperature, relative humidity (RH) and ventilation, transform raw fish into RTE products through techniques like maturation, smoking, fermentation, and drying. The RTE products obtained with these cabinets, which have similar dimensions to standard refrigerators, have become popular among hotels, restaurants, and catering sectors. The cabinets are easy to use and the organoleptic properties as well as freshness of the RTE products obtained are very unique (Rebezov et al., 2022).

The rise in consumption of RTE seafood products results in increasing concerns about food safety, because these food commodities

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are usually consumed without prior cooking. One of the most relevant biological hazards in these products is *Listeria monocytogenes*, which was the fifth most common zoonotic disease in the EU in 2023 (EFSA & ECDC, 2024) and the second most reported hazard in seafood-related notifications through the Rapid Alert System for Food and Feed (RASFF) over the period 1996–2020. Within the latter, the RTE food category is a significant source of listeriosis infection, and fish and fishery products account for the highest *L. monocytogenes* occurrence, with rates between 2.3 and 2.6 % (Pigłowski, 2023). Even more worrying is the fact that, according to a risk assessment model, the growth of *L. monocytogenes* in RTE products during storage and handling by consumers is potentially contributing to one-third of listeriosis cases in the EU, posing a significant food safety risk (Ricci et al., 2018).

To address these concerns, Commission Regulation EC 2073/2005 (Commission Regulation (EC), 2005) establishes strict food safety criteria for *L. monocytogenes* in RTE foods. Recently, an amendment (Commission Regulation (EU), 2024) to this Regulation was published, stating that for RTE foods able to support the growth of *L. monocytogenes*, the pathogen should either not be detected in 25 g of RTE product or should be present at a maximum concentration of 100 CFU g^{-1} for products placed on the market during their shelf life. Ensuring compliance with this regulation requires substantial time and resources, often involving challenge tests. However, the regulation also permits the use of predictive mathematical modelling to assess microbial growth under various conditions, such as temperature, pH, and water activity (a_w), enabling more efficient safety evaluations. Most published studies on the predictive modelling of *L. monocytogenes* have focused on RTE smoked, salted, or gravad fish products. While these products are important due to their widespread consumption and potential to support the growth of the pathogen, there is a notable gap in research regarding the behavior of *L. monocytogenes* in dry-cured fish matrices. Dry-cured fish undergoes different processing conditions, such as prolonged curing and low a_w, which can significantly influence the pathogen's survival and growth dynamics. Understanding the fate of *L. monocytogenes* in these matrices is essential to develop accurate predictive models, ensure food safety, and comply with regulatory requirements. Whenever predictive models for matrices similar to those under study have already been developed, they can be validated with new data, eliminating the need to create new models from scratch. In this study, the cardinal model developed by Mejlholm et al. (2015) has been applied using newly generated data to assess its applicability and performance in the specific matrixes under study, i.e., vacuum packed dry-cured fish, including salmon, swordfish, and tuna, during their shelf life. This model can support food business operators to assess the *L. monocytogenes* growth during refrigerated storage and temperature abuse scenarios. The findings of this study will help the fish industry mitigating *L. monocytogenes* related risks, while supporting the development of innovative RTE products that meet both consumer expectations and regulatory standards.

2. Materials and methods

2.1. Fish products

In this study, fillets of *Salmo salar* (salmon), *Xiphias gladius* (swordfish), and *Thunnus albacares* (yellowfin tuna) were supplied by an Italian seafood processor. The salmon and tuna were sourced from the Atlantic Ocean (FAO zone 34), while the swordfish came from the Pacific Ocean (FAO zone 87). Four fillets from each species were provided, with one served as a control, while the other three were deliberately contaminated with *L. monocytogenes*. All the fillets belonged to different batches.

The production process started with thawing the fillets, followed by salting in false-bottomed boxes with a salting mixture (20 % w/w) containing salt (37 %), sucrose, dextrose along with a mix of spices and antioxidants to enhance stability through osmotic dehydration and improve flavor. The salting and drying steps as well as the subsequent fish processing have been performed following description given in a previous study (Savini et al., 2024) following manufacturer's procedures. Afterwards, the fillets underwent a dry curing process, utilizing a "climatic recipe" tailored to each species' characteristics to prevent over-drying while enhancing quality. The curing process was carried out in a patented Stagionello® Fish Curing Device (Crotone, Italy), allowing precise control of temperature, humidity, and ventilation. The entire production process followed the method detailed in Savini et al. (2024), where further specifications of the curing process are available. The fillets were vacuum-packaged and shipped on ice within 24 h from production. For this study, three fish fillets for each species were used, each of them coming from a different production batch to account for the inter-batch variability.

2.2. Bacterial strains, inoculation and sampling

A mixture of three *L. monocytogenes* strains was used to inoculate the fish fillets, specifically ATCC 15313 and two wild strains from ANSES, 12MOB099LM and 12MOB102LM, which were isolated from fishery products. Inoculum preparation for each stock culture followed the procedures outlined in ISO 20976-1 (ISO, 2019) and ISO 20976-2 (ISO, 2022b).

All strains were stored at −80 °C for long-term preservation, and prior to use in the study each strain was cultured in a 9 mL tube of Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h, after which two successive subcultures of each strain were prepared and used to inoculate the fish fillets.

Before inoculation, the fillets were tested for the presence of *L. monocytogenes* in accordance with ISO 11290-1 (ISO, 2017c). The target inoculum concentration of approximately 3 log CFU g^{-1} was achieved on the spiked fillets by inoculating a volume of 50 μL to each sample, covering a 10 cm^2 area. Control samples were inoculated with 50 μL of sterile saline and used for physicochemical analysis. The 10 cm^2 area, corresponding to 10 g of the cured fish samples, were then placed in sterile, filtered polyethylene stomacher bags (VWR, Radnor, PA), vacuum packaged, and immediately stored in incubators, programmed for the dynamic time-temperature profiles provided in the EURL *Listeria monocytogenes* technical guidance document (34 days at 7 °C and 27 days at 10 °C) (Bergis et al., 2021), as no specific temperature data were available, until sampling, with appropriate sampling points (i.e., at 10, 19, 32, 45 and 61 days after inoculation) determined based on data obtained during a pilot study (data not shown).

2.3. Microbiological analysis

At each sampling point, the samples were processed by firstly diluting 1:10 in 0.1 % sterile peptone water (Thermofisher, Milan, Italy), after which the fish samples were homogenized for 60 s using a Stomacher (LB 400, VWR, Radnor, PA, USA) to ensure thorough and uniform blending, and the growth of *L. monocytogenes* was evaluated by plate count method following ISO 11290-2 (ISO, 2017b), with tenfold serial dilutions plated onto Brilliance Listeria agar (Oxoid, Basingstoke, UK).

The total aerobic colony count (TBC), Enterobacteriaceae (ENT), and lactic acid bacteria (LAB) were enumerated from 10 g samples using the international standard protocols ISO 4833-2 (ISO, 2022a), ISO 21528-2 (ISO, 2017d), and ISO 15214 (ISO, 1998), respectively.

Water activity (a_w) was measured in triplicate from samples inoculated with sterile water using an a_w-meter (Aqualab CX 4-TE, Decagon Devices Inc., Pullman, WA, USA), following ISO 18787 (ISO, 2017a). In addition to a_w, pH was also measured in triplicate from non-inoculated samples at each sampling point using a portable pH meter (Mettler-Toledo, OH, USA) equipped with a penetration probe, in accordance with ISO 2917 (ISO, 1999).

Statistical analysis was performed using Microsoft® Excel® (version 16.88 for Mac OS) and JASP (Version 0.19.1). Means were calculated from duplicate samples per time point for each fish, with each experiment performed in triplicate. Differences in enumeration results among

products and sampling points were investigated using ANOVA to determine whether there were statistically significant variations across groups. When significant differences were identified, a Tukey post-hoc test was applied to pinpoint specific pairwise differences between means. All analyses were conducted assuming a significance level of $p < 0.05$.

2.4. Mathematical modelling

In this study, the impact of dynamic temperatures on the fate of *L. monocytogenes* in dry-cured fish was investigated. Bacterial growth during dynamic temperature storage was described using the dynamic Baranyi model (Baranyi & Roberts, 1994), expressed as two coupled differential equations:

$$\frac{dY}{dt} = \mu\,(t)\,\frac{q}{1+q}\,[1 - exp\,(Y - Y_{max})] \quad (1)$$

$$\frac{dq}{dt} = \mu(t)\,q \quad (2)$$

where Y is the bacterial concentration at time t (ln CFU g^{-1}), Y_{max} is the logarithm of the maximum population density (MPD) of the bacteria (ln CFU g^{-1}), $q(t)$ is the physiological state of the inoculum, $\mu(t)$ is the instantaneous maximum specific growth rate (h^{-1}) determined by the secondary (cardinal) model described below, which was transformed to log units for model predictions. Initial and final concentrations have been experimentally determined; $Y_0 = 8.06$ ln CFU g^{-1}; $Y_{max} = 17.27$ ln CFU g^{-1}.

The initial physiological state, q_0, determines the adaptation period.

Temperature dependence of the instantaneous growth rate was modelled using a cardinal parameter expression, previously validated for *L. monocytogenes* (Mejlholm & Dalgaard, 2009):

$$\mu(t) = \mu_{ref}\,\gamma_T(T(t))\,\gamma_{aw}(aw)\,\gamma_{pH}(pH) \quad (3)$$

with:

$$\gamma_T = \left(\frac{T(t) - T_{min}}{T_{ref} - T_{min}}\right)^2, \gamma_{aw} = max\left[0, \frac{aw - aw_{min}}{aw_{opt} - aw_{min}}\right], \gamma_{pH} = max\left[0, 1 - 10^{(pH_{min} - pH)}\right] \quad (4)$$

Water activity (a_w) and pH were constant for each fish species and therefore treated as fixed inputs. The estimated cardinal parameter values from the model of Mejlholm and Dalgaard (2009) have been used: $T_{min} = -2.83$ °C, $a_{w\ min} = 0.923$, $a_{w\ opt} = 1.000$, $pH_{min} = 4.97$. Finally, μ_{ref} is the reference specific growth rate with a value of 0.419 h^{-1} for μ_{max} at the reference temperature (T_{ref}) of 25 °C.

The system of differential equations was integrated using the LSODA solver from the *deSolve* R package. Parameter estimation was carried out using the Levenberg–Marquardt algorithm (function nls.lm() from the *minpack.lm* package), by minimizing residuals between observed and predicted log concentrations. Standard errors (SE) and 95 % Wald confidence intervals (CI) were obtained for the estimated q_0 values.

This model was used to simulate changes in *L. monocytogenes* concentration in the different studied products, based on the default dynamic time-temperature profiles provided in the EURL Lm technical guidance document (7–10 °C) (Bergis et al., 2021), as abovementioned.

To assess the goodness of fit, the root mean square error (RMSE) (Chai & Draxler, 2014) was used, as represented by Equation (6).

$$Root\ Mean\ Square\ Error\ (RMSE) = \sqrt{\frac{\Sigma(predicted - observed)^2}{n}} \quad (5)$$

Residual distribution plots have been performed to show the potential bias across the full dynamic range.

In addition, the predicted *L. monocytogenes* concentrations were evaluated against the observed data using the Acceptable Prediction Zone (APZ) method. Prediction errors (PE) were calculated by subtracting the observed values from the predicted ones (Oscar, 2005). The APZ's acceptable limits were defined between −1.0 and 0.5 log CFU g-1 (Argyri et al., 2013; Mishra et al., 2017). Model performance was evaluated using the percentage of PE (% PE) in the APZ as an overall measure:

$$\%PE = \left(\frac{PE_{in}}{PE_{tot}}\right)\cdot 100 \quad (6)$$

The model was considered to provide acceptable predictions for the test data set when % PE was >70 %.

3. Results and discussion

3.1. Physicochemical characterization

Dry-cured salmon, swordfish, and tuna exhibited an initial a_w of 0.936 (±0.002), 0.942 (±0.001), and 0.914 (±0.002) respectively. No relevant variations were noted during the challenge test, with a mean a_w of 0.943 (±0.018), 0.941 (±0.006), and 0.904 (±0.010) for salmon swordfish and tuna respectively (Table 1).

Values of pH and a_w were in line with those reported in previous works (Savini et al., 2024), even though some variability can be highlighted, which can be attributed to inter-batch variability often characterizing of artisanal products (Bonilla-Luque et al., 2024; Pasquali et al., 2023).

A similar stable trend was observed for pH values, with initial readings of 6.00 (±0.06), 5.65 (±0.01), and 5.90 (±0.01) for salmon, swordfish, and tuna, respectively. The mean pH values remained steady throughout the experiment at 5.91 (±0.01), 5.83 (±0.11), and 5.86 (±0.04), respectively (Table 1). These results are in accordance with those reported by Savini et al. (2024) for the same products at the end of the production process (starting point of this study). Furthermore, these physicochemical parameters, both a_w and pH, are in line with those of other similar RTE fish products such as smoked, gravad and salted fish (Giménez & Dalgaard, 2004; Lakshmanan & Dalgaard, 2004; Mejlholm & Dalgaard, 2007; Roseiro et al., 2017). Because of the near-neutral pH and relatively high a_w, pathogens such as *L. monocytogenes* can potentially grow on these RTE products, although tuna showed mean a_w values below the minimum growth for *L. monocytogenes* ($a_w < 0.92$) established in Commission Regulation 2073/2005 (Commission Regulation (EC), 2005).

3.2. Microbial behavior

Regarding the background microbiota, the TBC at the experimental starting point ranged from 2.36 ± 0.21 (log CFU g^{-1}) of salmon, to 4.50 0.17 and 3.85 ± 0.07 (log CFU g^{-1}) of swordfish and tuna respectively. At the end of the experiment TBC had increased up to 9.09 ± 0.09 (log CFU g^{-1}) in salmon with an exponential growth already from the first

Table 1
Mean counts (± standard deviation) of LAB, TBC and ENT and measured a_w and pH at each sampling time for the three tested products.

	Timepoints (days)	LAB (log CFU g^{-1})	TBC (log CFU g^{-1})	ENT (log CFU g^{-1})	aw	pH
Salmon	0	<1	2.36 (±0.21)	1.85 (±0.38)	0.936 (±0.025)	6.09 (±0.06)
	10	<1	5.98 (±0.13)	2.58 (±0.20)	0.949 (±0.012)	6.10 (±0.05)
	19	<1	6.00 (±0.00)	4.39 (±0.02)	0.939 (±0.019)	6.07 (±0.04)
	32	<1	7.91 (±0.15)	3.24 (±0.34)	0.942 (±0.023)	6.08 (±0.09)
	45	<1	8.81 (±0.09)	4.09 (±0.03)	0.946 (±0.010)	5.69 (±0.16)
	61	<1	9.09 (±0.09)	2.02 (±0.03)	0.944 (±0.008)	5.43 (±0.11)
Swordfish	0	2.82 (±0.24)	4.50 (±0.17)	<1	0.942 (±0.001)	5.65 (±0.01)
	10	3.07 (±0.10)	3.57 (±0.38)	<1	0.939 (±0.000)	5.84 (±0.05)
	19	5.11 (±0.01)	5.42 (±0.07)	<1	0.934 (±0.005)	5.83 (±0.05)
	32	3.50 (±0.12)	4.51 (±0.14)	<1	0.938 (±0.002)	5.92 (±0.02)
	45	5.52 (±0.08)	4.83 (±0.09)	<1	0.951 (±0.002)	5.98 (±0.02)
	61	5.52 (±0.08)	5.88 (±0.12)	<1	0.942 (±0.048)	5.78 (±0.01)
Tuna	0	<1	3.85 (±0.07)	3.52 (±0.12)	0.914 (±0.003)	5.90 (±0.01)
	10	<1	3.56 (±0.35)	3.24 (±0.09)	0.913 (±0.003)	5.86 (±0.01)
	19	<1	3.56 (±0.12)	3.14 (±0.13)	0.888 (±0.000)	5.86 (±0.05)
	32	<1	3.09 (±0.12)	2.81 (±0.12)	0.905 (±0.003)	5.92 (±0.01)
	45	<1	3.24 (±0.25)	2.76 (±0.01)	0.909 (±0.003)	5.84 (±0.00)
	61	<1	3.06 (±0.03)	2.49 (±0.20)	0.906 (±0.008)	5.81 (±0.01)

sampling points. At the final sampling point, for swordfish a slight growth was observed, with 5.88 ± 0.12 (log CFU g^{-1}), while in tuna the counts decreased to 3.06 ± 0.03 (log CFU g^{-1}) (Table 1). ENT were 1.85 (±0.38) and 3.52 (±0.12) log CFU g^{-1} for salmon and tuna respectively and remained stable for the whole shelf life, on the contrary were below enumeration limit (10 CFU g^{-1}) for swordfish (Table 1). These results differ from those reported by Indio et al. (2024), which described ENT below the detection limit in all three species and higher counts (4.80–5.90 log CFU g^{-1}) for TBC. This difference may be due to variability in the quality of raw material and to the handling of the products before or during processing, which can influence the microbiota of the final products. LAB counts were below the enumeration limit in all the analyzed samples, apart from swordfish samples in which they had an initial concentration of 2.82 ± 0.24 (log CFU g^{-1}) that grew up to 5.52 ± 0.08 (log CFU g^{-1}) by the end of the challenge test (Table 1). These LAB results are also coherent with the little to null modification in pH values during the storage.

L. monocytogenes behavior in the studied products is presented in Table 2. The initial concentration (t = 0 days) of the pathogen in all fish products was around 3.00–3.50 (log CFU g^{-1}). In salmon and swordfish, the pathogen was able to grow up to a maximum population density (MPD) of 8.02 and 7.69 (log CFU g^{-1}) respectively. Additionally, in swordfish, no significant growth was observed until day 19, whereas in salmon, significant growth was already observed by day 10. In general, the strains showed relatively slow growth in the products at the studied conditions, with a relatively long lag phase. In contrast, the pathogen did not grow in tuna, where instead gradually declined to a minimum of 1.39 (log CFU g^{-1}).

Because of the near-neutral pH and relatively high a_w, *L. monocytogenes* can potentially grow on salmon and swordfish, although the growth observed was relatively slow, with a long lag phase.

Table 2
Mean counts (± standard deviation) of *Listeria monocytogenes* at each sampling time for the three tested products. Lowercase superscript letters indicate statistically significant differences within the same product across different time points, while uppercase superscript letters indicate differences at the same time point across different products.

Timepoints (days)	Salmon (log CFU g^{-1})	Swordfish (log CFU g^{-1})	Tuna (log CFU g^{-1})
0	3.59 ^{aA} (0.06)	3.36 ^{aA} (0.03)	3.09 ^{bA} (0.06)
10	3.95 ^{aA} (0.24)	3.43 ^{aA} (0.58)	2.59 ^{abA} (0.37)
19	5.83 ^{bA} (0.29)	3.55 ^{aB} (0.16)	2.25 ^{abB} (0.31)
32	6.62 ^{bcA} (0.24)	5.98 ^{bA} (0.25)	2.40 ^{abB} (0.03)
45	7.50 ^{cA} (0.26)	7.00 ^{bA} (0.48)	1.97 ^{aB} (0.32)
61	7.45 ^{cA} (0.72)	6.58 ^{bA} (0.98)	1.79 ^{aB} (0.32)

This is consistent with the borderline physicochemical conditions: the pH values are near the lower limit for optimal *L. monocytogenes* growth, and the a_w values, while above the minimum growth threshold for salmon and swordfish (<0.92), are close enough to this limit to partially inhibit growth. In contrast, tuna exhibited a mean a_w of 0.904, below the minimum required for *L. monocytogenes* growth, which likely explains the observed decline in the pathogen population. These findings illustrate how small differences in a_w and pH around critical limits can substantially influence *L. monocytogenes* kinetics, including lag duration, growth rate, and maximum population density. Furthermore, inter-batch variability inherent to artisanal products may create localized microenvironments that either favor or inhibit pathogen proliferation, highlighting the importance of consistent control of physicochemical parameters. Overall, the results support the use of a_w and pH as key hurdles in ensuring the safety of ready-to-eat dry-cured fish products, complementing regulatory limits and predictive modeling tools for risk assessment.

3.3. Mathematical modelling

In this study, the growth data obtained under the dynamic temperature profile were used to fit and assess the goodness of fitting of the model. Fig. 1 shows the comparison between the predicted and observed growth of *L. monocytogenes* in dry-cured salmon obtained under the

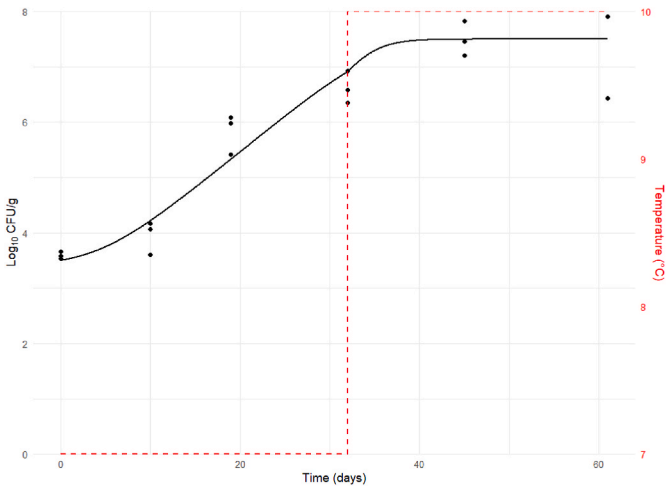

Fig. 1. Predicted and observed growth of *L. monocytogenes* in dry-cured salmon under the dynamic temperature profile.

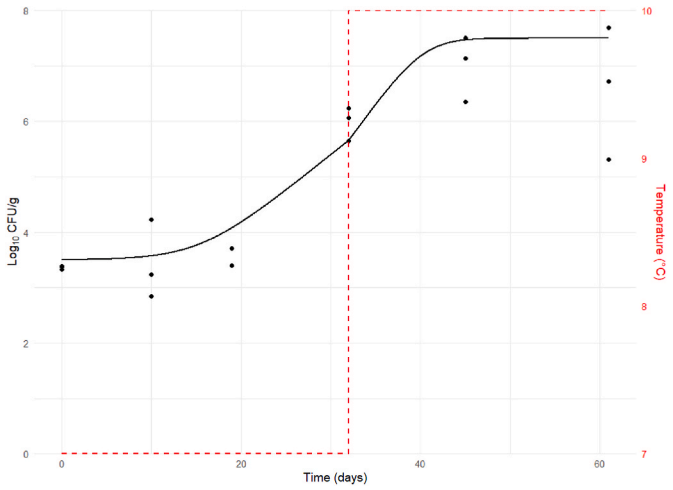

Fig. 2. Predicted and observed growth of *L. monocytogenes* in dry-cured swordfish under the dynamic temperature profile.

Table 3
Estimation of the initial physiological state (q_0) with standard errors (S.E), 95 % confidence intervals (CI (95 %)) and Root Mean Square Error (RMSE) from the dynamic Baranyi primary model coupled with a cardinal secondary model for dry-cured salmon and swordfish.

	Salmon			Swordfish		
	Mean	*S.E.*	*CI (95 %)*	*Mean*	*S.E.*	*CI (95 %)*
q_0	0.402	0.226	0.001–0.845	0.031	0.027	0–0.084
RMSE	0.448			0.729		

dynamic profile. For dry-cured swordfish, the comparison between the predictions and observed data is shown in Fig. 2. Since in dry-cured tuna no growth was observed the growth model could not be used to predict the fate of the pathogen.

The dynamic Baranyi–cardinal model provided estimates of the initial physiological state (q_0), of *L. monocytogenes* in both dry-cured salmon and swordfish (Table 3). The fitted q_0 values differed substantially between the two products, reflecting differences in the initial physiological readiness of the inoculated cells and the distinct environmental constraints imposed by each matrix. Based on the secondary (cardinal) growth model, the maximum specific growth rates were estimated as 0.012 h^{-1} at 7 °C and 0.020 h^{-1} at 10 °C. These growth rates were calculated using the mean pH and a_w values specific to dry-cured salmon and swordfish, thereby accounting for product-specific environmental conditions. The higher growth rate at 10 °C compared with 7 °C is consistent with the expected temperature dependence of *L. monocytogenes* growth.

To further assess the predictive performance of the dynamic Baranyi–cardinal model, Fig. 3 presents the residuals plotted against the model-predicted values for both salmon and swordfish, together with the Acceptable Prediction Zone (APZ) defined by the limits of −1.0 to +0.5 log CFU/g. In dry-cured salmon (Fig. 3A), residuals were symmetrically distributed around zero with no clear trend across the prediction space, indicating absence of systematic over- or under-prediction and confirming that the Baranyi–cardinal formulation captured the growth kinetics effectively. Swordfish exhibited a slightly wider spread of residuals (Fig. 3B), consistent with the larger variability observed in the experimental data and the longer adaptation period; however, residuals remained largely contained within the APZ. Regarding the estimation of the percentage prediction error (%PE), for salmon, the results were highly satisfactory, with 83.3 % of the predictions falling within the APZ, while for swordfish, the model slightly underperformed, with 70.6 % of the predictions falling within the APZ, just above the acceptable limit of 70 %. Overall, these results demonstrate the model's ability to predict the behavior of *L. monocytogenes* in these two products under dynamic storage temperature conditions.

To complement this graphical analysis, the distribution of residuals was evaluated (Fig. 4). The histograms for salmon and swordfish exhibited a unimodal shape centered near zero, and no strong skewness was observed, indicating an overall unbiased model behaviour. The dispersion of residuals was narrow for salmon (Fig. 4A), reflecting the close agreement between predicted and observed counts. For swordfish (Fig. 4B), residual variability was slightly higher, in line with the longer adaptation period and the reduced physiological adaptability inferred from the q_0 estimate. Importantly, neither dataset displayed multimodality or heavy tails, which would have suggested structural deficiencies in the model. These findings provide support for the robustness of the dynamic Baranyi–cardinal model across both dry-cured matrices.

After model assessment, the calculated times for a 2-log increase of *L. monocytogenes* under thermal abuse conditions, 20.30 days for salmon and 32.03 days for swordfish, highlight potential safety risks associated with different ready-to-eat seafood products. While the predicted growth of *L. monocytogenes* is inherently determined by the temperature profile, the results provide meaningful insights into product-specific risk factors and potential safety concerns. Differences in a_w, fat content, and the presence of competing microflora between salmon and swordfish can modulate pathogen growth under identical temperature conditions. Moreover, variability in these intrinsic factors, combined with differences in initial contamination levels and storage conditions, introduces uncertainty in actual pathogen growth, emphasizing the need for conservative safety margins in shelf-life determinations and the importance of proper product characterization to ensure that models accurately reflect the specific properties and microbiological behavior of each product. These insights go beyond the temperature history itself, providing a basis for risk-based decisions related to shelf-life determination, storage management, and the selection of appropriate control measures. This is particularly relevant within the regulatory framework governing ready-to-eat seafood. Under Commission Regulation (EC) No 2073/2005, products capable of supporting the growth of *L. monocytogenes*, such as smoked fish, must comply with a legal limit of 100 CFU g^{-1} at the end of shelf life. To demonstrate compliance, food business operators are required to assess the potential for pathogen growth through challenge studies or predictive modelling. The model-derived growth estimates presented here therefore serve not only as a scientific evaluation of product-specific risk but also as a practical tool to support regulatory decision-making.

For salmon, the relatively short time to reach a 2-log increase indicates a higher likelihood of exceeding safe levels if storage conditions are not strictly maintained. Swordfish, with a longer time to reach the same increase, presents a comparatively lower risk under similar conditions. Nevertheless, both products could pose safety concerns in cases of high initial contamination. The higher susceptibility of salmon is particularly relevant given that *Salmo salar* is frequently reported with high *L. monocytogenes* contamination levels (Zakrzewski et al., 2024) and has been linked to several listeriosis outbreaks associated with cold-smoked and sushi-grade products (Eicher et al., 2020).

From a practical standpoint, controlling a_w through formulation or drying protocols, along with pH and careful temperature management, can slow microbial growth. Integrating predictive models into food safety management allows producers to make informed decisions regarding shelf-life, storage, and additional control measures, such as optimizing packaging conditions, or incorporating antimicrobial treatments. Additionally, non-thermal inactivation models may provide insights into natural die-off, as observed in certain products like dry-cured tuna, where slight reductions in *L. monocytogenes* were noted over time.

In this study, the growth model considered only the effects of storage temperature and a few intrinsic product parameters on the growth dynamics of *L. monocytogenes*. A more comprehensive compositional

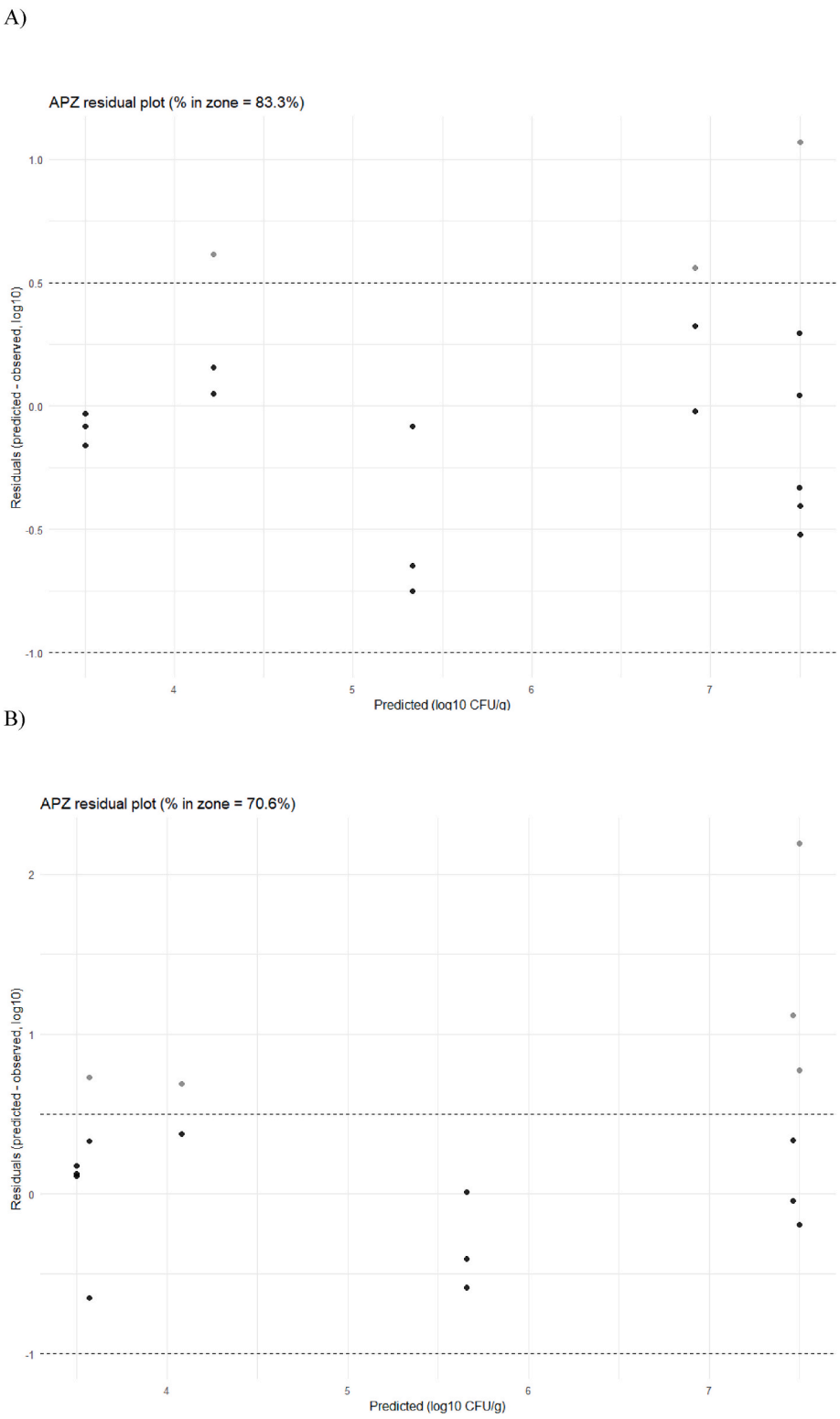

Fig. 3. Residuals vs. predicted values of *L. monocytogenes* as estimated from the model: A) salmon, B) swordfish. The dashed horizontal lines represent the acceptable prediction zone (APZ).

analysis of the products, along with a detailed investigation of the microbiota present, could lead to more accurate models by taking into account a wider range of factors that influence the behavior of this microorganism.

Several studies have assessed the impact of storage temperature and product characteristics on the growth kinetics of *L. monocytogenes* in fish products; however, to the authors' knowledge, no data are available in the literature for dry-cured fish products.

4. Conclusions

This study is among the first to quantify and model the growth of *L. monocytogenes* in dry-cured fish products under storage conditions. The results demonstrated the pathogen's ability to grow in the studied

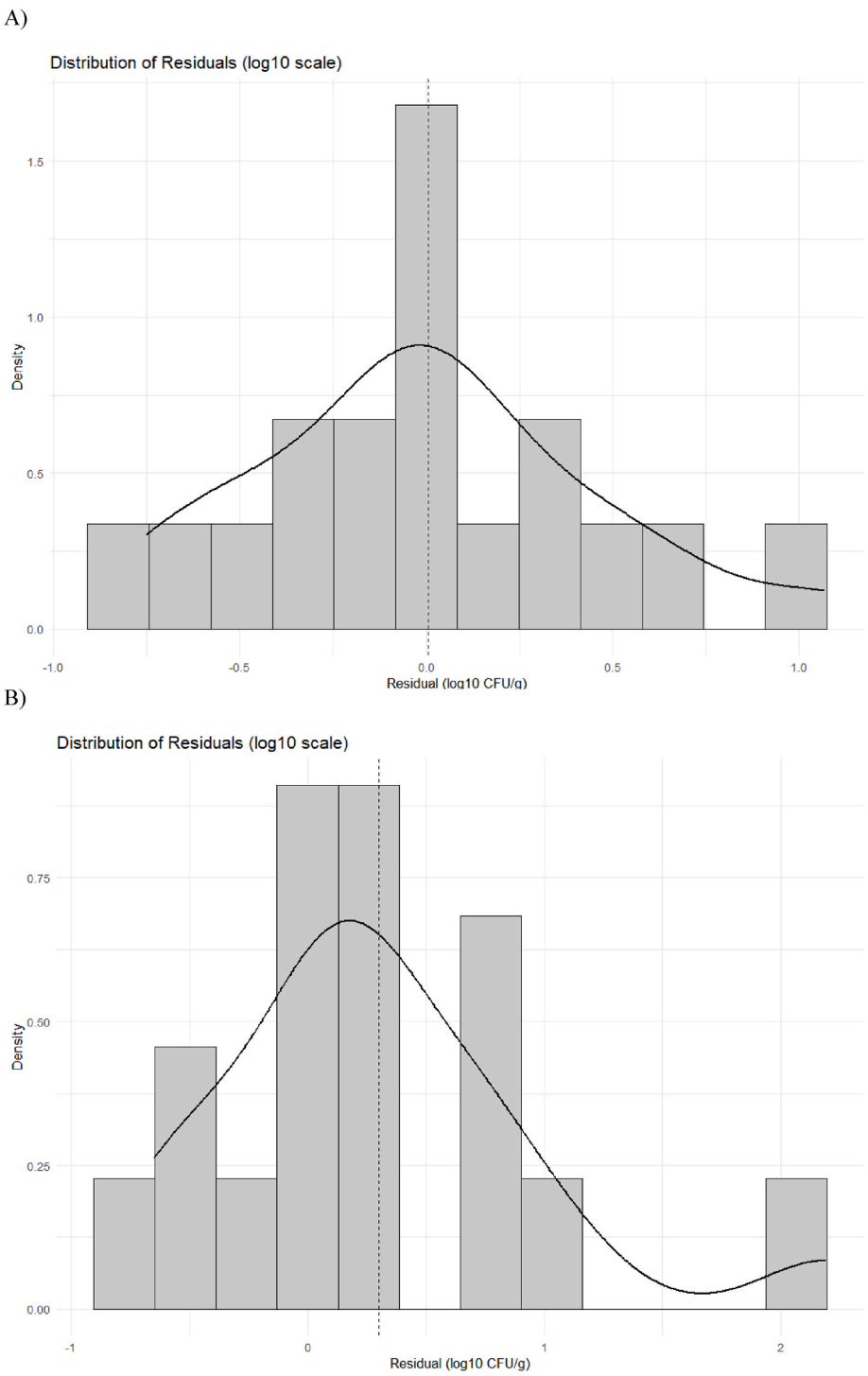

Fig. 4. Distribution of the model residuals for A) salmon; and B) swordfish.

salmon and swordfish products, while it decayed in dry-cured tuna across a dynamic storage temperature range representative of the products' storage conditions along the shelf life. The study successfully assessed an accurate predictive model for *L. monocytogenes* growth, which can be applied in both shelf life and quantitative risk assessment studies. Furthermore, the model presented in this work can serve as a valuable tool for the fish industry and end-users, helping to establish control measures aimed at limiting the growth of *L. monocytogenes* in dry-cured fish products, thereby ensuring the safety of a product that is gaining popularity.

CRediT authorship contribution statement

Federico Tomasello: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Valentina Indio:** Writing – review & editing, Writing – original draft. **Laura Prandini:** Methodology, Investigation, Data curation. **Antonio Valero Diaz:** Writing – review & editing, Methodology. **Andrea Serraino:** Writing – review & editing, Project administration. **Federica Giacometti:** Writing – review & editing, Writing – original draft. **Alessandra De Cesare:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Federica Savini:** Writing – review & editing, Writing –

original draft, Project administration, Methodology, Investigation, Data curation.

Declaration of competing interest

The authors have nothing to declare.

Data availability

Data will be made available on request.

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