Modelling the interaction of the sakacin-producing *Lactobacillus sakei* CTC494 and *Listeria monocytogenes* in filleted gilthead sea bream (*Sparus aurata*) under modified atmosphere packaging at isothermal and non-isothermal conditions

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Highlights

- *L. sakei* CTC494 inhibited *L. monocytogenes* growth in sea bream fillets during chilled and moderate abuse temperature storage.
- *L. sakei* CTC494 did not increase deterioration of filleted sea bream at an initial level of ≤ 4 log cfu/g.
- *L. sakei* CTC494 showed potential as bioprotective culture for fish products.
- An approach from broth to food was developed for modelling microbial interaction.
- Interaction models simulated *L. monocytogenes* inhibition by the bioprotective *L. sakei* in filleted sea bream under static and dynamic temperature.

Abstract

The objective of this work was to quantitatively evaluate the effect of *Lactobacillus sakei* CTC494 (sakacin-producing bioprotective strain) against *Listeria monocytogenes* in fish juice and to apply and validate three microbial interaction models (Jameson, modified Jameson and Lotka Volterra models) through challenge tests with gilthead sea bream *(Sparus aurata)* fillets under modified atmosphere packaging stored at isothermal and non-isothermal conditions. *L. sakei* CTC494 inhibited *L. monocytogenes* growth when simultaneously present in the matrix (fish juice and fish fillets) at different inoculation ratios pathogen:bioprotector (i.e. 1:1, 1:2 and 1:3). The higher the inoculation ratio, the stronger the inhibition of *L. monocytogenes* growth, with the ratio 1:3 yielding no growth of the pathogen. The maximum population density (N_{max}) was the most affected parameter for *L. monocytogenes* at all inoculation ratios. According to the microbiological and sensory analysis outcomes, an initial inoculation level of 4 log cfu/g for *L. sakei* CTC494 would be a suitable bioprotective strategy without compromising the sensory quality of the fish product. The performance of the tested interaction models was evaluated using the Acceptable Simulation Zone approach. The Lotka Volterra model showed slightly better fit than the Jameson-based models with 75-92 % out of the observed counts falling into the Acceptable Simulation Zone, indicating a satisfactory model performance. The evaluated interaction models could be used as predictive modelling tool to simulate the simultaneous behaviour of bacteriocin-producing *Lactobacillus* strains and *L. monocytogenes*; thus, supporting the design and optimization of bioprotective culture-based strategies against *L. monocytogenes* in minimally processed fish products.

Keywords: biopreservation, food-borne pathogen, lactic acid bacteria, competition model, minimally processed fish, predictive microbiology

1. Introduction

Global consumption of fresh and minimally processed fish has grown rapidly in recent decades. In this regard, aquaculture has been responsible for the extraordinary growth in the supply of fish for human consumption, which resulted in a record-high per capita consumption of 20.3 kg in 2016 (FAO, 2018). The combination of chemical oxidation of lipids, autolytic biochemical reactions and physico-chemical characteristics make fish a highly perishable product, but also an ideal environment for growth of spoilage microorganisms and food-borne pathogens (Dalgaard et al., 2006; Parlapani et al., 2014). Among the pathogenic bacteria, *Listeria monocytogenes* stands out because of its ability to tolerate salty environments and multiply in refrigerated foods, coupled with the high mortality rates in humans (CDC, 2017). The pathogen has been isolated from a variety of raw fish and processed fish products (Abdollahzadeh et al., 2016; Lennox et al., 2017; Rožman et al., 2016), and according to the last report of the European Food Safety Authority (EFSA), "fish and fishery products" showed the highest levels of non-compliance with the food safety microbiological criteria for *L. monocytogenes* laid down by Regulation (CE) 2073/2005 (EFSA, 2017).

Lactic acid bacteria (LAB), and lactobacillus in particular, constitute the dominant microbiota in several types of foods and many LAB species are used as microbial food cultures (MFC) in food production. In the EU, there is no specific regulation regarding MFC; but with a long history of safe use, they are considered traditional food ingredients and are legally permitted without premarket approval. Thus, MFC defined as characteristic food ingredients must be listed on the ingredient labels of the final food in agreement with the Regulation (EU) 1169/2011. In addition, when added to a food, MFC must comply with the requirements established in the General Food Law

(Regulation (EC) 178/2002), i.e. they must be safe for their intended use (Herody et al., 2010; Laulund et al., 2017). Many LAB genera and species are generally recognized as safe (GRAS) by the FDA (2018) and have the qualified presumption of safety (QPS) status established by EFSA. Among LAB, *Lactobacillus* is the genus including a high number of GRAS species, and particularly, *Lactobacillus sakei* is included in the QPS list (EFSA BIOHAZ, 2017), thus not requiring the full safety assessment (antibioresistance, virulence, and biogenic amine characterization) for its market authorisation in the EU. The application of selected LAB strains as bioprotective cultures has demonstrated a high potential to inhibit undesirable spoilage and pathogenic bacteria in fresh fish and RTE fish products, including *L. monocytogenes* (Anacarso et al., 2014; Brillet et al., 2005). The inhibitory mechanism of LAB includes microbial growth competition as well as microbial antagonism associated with the production of antimicrobial metabolites such as organic acids (lactic acid, acetic acid, etc.), hydrogen peroxide and more specifically, bacteriocins active against specific bacteria such as *L. monocytogenes* (Gómez-Sala et al., 2016). In relation to the latter, sakacins, being produced by certain *L. sakei* strains, belong to subclass IIa of bacteriocins which are generally known to have a strong antilisterial activity (Leroy and De Vuyst, 2000). The lethal action of these bacteriocins results from membrane pore formation of the target cell causing depletion of vital components as well as dissipation of the proton motive force (Héchard and Sahl, 2002).

Microbial interaction has been addressed in the predictive microbiology field mainly focused on the inhibitory effect of endogenous LAB on *L. monocytogenes* behavior (Mejlholm and Dalgaard, 2007). Interaction models are usually intended to quantify how much the growth of one population is reduced by the growth of other populations

(Cornu et al., 2011; Pérez-Rodríguez and Valero, 2013). Thus, two model approaches are generally used to describe the interaction of LAB and *L. monocytogenes:* i) those based on the Jameson effect phenomenon (Jameson, 1962) that describes the simultaneous stop of growth of all bacterial populations at the time when the dominant bacteria population reaches its stationary phase (Giménez and Dalgaard, 2004; Mellefont et al., 2008; Møller et al., 2013) and ii) the predator-prey models based on the Lotka Volterra equation, which allow to describe the dynamics of two competing bacterial populations by incorporating an additional term describing the reduction of the growth rate of a given population, this being proportional to the population density of other competing population (Powell et al., 2004; Valenti et al., 2013; Vereecken et al., 2000).

Predictive models dealing with the interaction between the pathogen *Listeria* and bacteriocin-producing LAB strains in foods other than fermented meat products (Drosinos et al., 2006; Leroy et al., 2005) are, to the best knowledge of the authors, not available in literature. Their development would provide the food industry with valuable tools to evaluate the effect of potential bioprotective cultures against *L. monocytogenes* in specific food matrices, thereby enhancing food safety. In this respect, minimally processed and RTE fish products made of raw fish, which are consumed without applying any lethal treatment, could pose a serious risk in relation to *L. monocytogenes* (Jami et al., 2014; Miettinen and Wirtanen, 2005; Rožman et al., 2016). Sea bream, considered a valuable fish species in Mediterranean EU countries, has been included over the last years as main ingredient in popular non-heated RTE fish products, such as sushi, carpaccio and other products (Bolívar et al., 2018). This fish species is mostly

commercialized fresh as whole fish and in several supermarket chains as filleted fish under modified atmosphere packaging (MAP).

Therefore, the objective of this work was i) to quantitatively evaluate the effect of the sakacin-producing bioprotective strain *Lactobacillus sakei* CTC494 against *L. monocytogenes* in a fish model system and ii) to apply and validate microbial interaction models to simulate the simultaneous growth of both microorganisms in gilthead sea bream *(Sparus aurata)* fillets under MAP at isothermal and non-isothermal conditions.

2. Material and Methods

2.1. A step-wise approach for interaction model development

A step-wise approach was followed to develop interaction models simulating the growth of the bioprotective *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in fish fillets under MAP during isothermal and dynamic storage temperature. A schematic overview of the step-wise method is shown in Figure 1.

In the first step, the primary kinetic parameters lag time (λ), maximum specific growth rate (μ_{max}) and maximum population density (N_{max}) were obtained for each microorganism from experimental data in mono-culture in fish (sea bream) juice at different temperature conditions (section *2.3.1*) and based on those, secondary models were generated (section *2.7.1* and *2.7.2*). Secondly, experimental data obtained in fish juice in co-culture were used (section *2.3.2*) to estimate competition parameters in interaction models by means of a regression process (section *2.7.4*). In a third step, the parameters from the secondary models and estimated interaction parameters for the model showing the best performance were used to simulate microbial interaction on fish

158 fillets stored under MAP at isothermal and non-isothermal conditions (section *2.5*). The
159 values for interaction parameters were assumed to be constant in the tested ratios for
160 both microorganisms, hence the average from all assayed temperatures was used to
161 define these parameters. Since an effect of the fish matrix and MAP conditions on
162 kinetic parameters was expected, the specific growth rate obtained in fish juice was
163 adjusted to consider such effects. To determine the adjustment factor, data from
164 experiments made with fish fillets (section *2.5*) were used, in which both
165 microorganisms were inoculated at the same level and monitored under the same
166 temperature conditions used in the fish juice experiments. The adjustment factor for μ_{max}
167 of each microorganism was calculated as the ratio between the μ_{max} values obtained in
168 fish product and in fish juice and were assumed to be constant for the range of
169 temperatures tested. Therefore, the same adjustment factor was applied to simulate the
170 microbial interaction on fresh fish fillets at isothermal and dynamic temperature
171 conditions.

172 ***2.2. Bacterial strains and inoculum preparation***

173 The bacteriocin-producing *L. sakei* CTC494 strain was selected as bioprotective culture
174 in this study. This strain is a producer of bacteriocin, sakacin K, being able to inhibit the
175 growth of spoilage bacteria and *Listeria* (Hugas et al., 1993). The strain *L.*
176 *monocytogenes* CTC1034 previously used as indicator to study the antagonism the LAB
177 produced bacteriocins (Garriga et al., 2002) was used in the present study as target
178 pathogen. This strain has the same serotype (i.e. 4b) as the clinical isolate Scott A.
179 Stock cultures were stored at -80 °C in de Man Rogosa and Sharpe (MRS, Oxoid, UK)
180 broth for the LAB strain and in Brain Heart Infusion (BHI, Oxoid) for the pathogen,
181 both with 20% glycerol as cryoprotectant.

Before experiments, *L. sakei* CTC494 and *L. monocytogenes* CTC1034 were pre-cultured separately at static conditions in MRS (Oxoid, UK) at 33 ºC with 10 % CO_2 and BHI broth (BHI, Oxoid) at 37 °C, respectively. Two consecutive 24 h-subcultures were made for each microorganism by transferring 0.1 mL to tubes containing 9 mL of fresh respective media and incubating at the same above-mentioned temperatures. Then, a third subculture was prepared, and tubes were incubated for 18-20 h at the appropriate temperature resulting in early stationary phase cultures, with a cell density of *ca.* 10^8 cfu/mL and 10^9 cfu/mL for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, respectively.

2.3. Experiments with L. sakei *CTC494 and* L. monocytogenes *CTC1034 in mono- and co-culture in fish juice*

Sterile fish juice was prepared from fresh muscle of gilthead sea bream following the protocol described by Bolívar et al. (2018). The prepared cultures (Section *2.2*) were twice-washed in phosphate buffered saline solution (PBS) (Medicago AB, Uppsala, Sweden) by centrifugation at 4100 rpm (Jouan C4i, Thermo Electron Corporation, France) for 10 min and cells were re-suspended in fish juice. The suspensions of *L. monocytogenes* and *L. sakei* were serially diluted ten-fold in fish juice to obtain the desired concentration to be inoculated to fish juice at 1 % (v/v).

Growth experiments were carried out at static conditions in sterile 250-mL Schott bottles containing fish juice. In the mono-culture experiments, the inoculum concentration of each microorganism was set to *ca.* 10^2 cfu/mL. For the co-culture experiments, the inoculum concentration of *L. monocytogenes* was always 10^2 cfu/mL, while for *L. sakei* CTC494, three different concentrations were investigated, (10^2, 10^4 and 10^6 cfu/mL), thus generating three (initial) inoculation ratios *L. monocytogenes*: *L.*

206 *sakei* that corresponded to 1:1, 1:2 and 1:3 when bacterial concentrations were
207 expressed in logarithmic scale. After inoculation, flasks were stored at four constant
208 temperatures targeted at 2, 5, 8 and 12 °C during a period from 5 to 46 days. Storage
209 temperature was recorded at regular time intervals using data loggers (Fourtec,
210 MiniLitE5032L, USA) and the mean of registered temperatures (i.e. 2.2, 5.0, 8.1 and
211 12.1 °C) was used for modelling purposes. Each experiment was performed in
212 duplicate.

213 ***2.4. Quality deterioration assessment of fresh sea bream fillets under MAP***

214 *2.4.1. Fish fillet product description*

215 Individual plastic trays containing two fresh gilthead sea bream fillets packed under
216 MAP were supplied by a private company (Zaragoza, Spain). Fish trays were received
217 at the laboratory 18-24 h after processing in expanded polystyrene boxes with flake ice.
218 The average weight of the fish fillets was 332.2 ± 12.1 g with an initial pH of 6.11 ±
219 0.05 (Hanna Edge, HI2020, USA). The initial headspace gas composition in the trays
220 was measured using a O_2/CO_2 gas analyser (Gaspace 2, Systech Instruments, U.K.) and
221 the obtained values corresponded to 37.4 % ± 0.7 for O_2 and 27.0 % ± 1.0 for CO_2.

222 *2.4.2. Inoculation of fish fillets*

223 Bacterial suspensions prepared as described in Section *2.2* were serially diluted ten-fold
224 with physiological saline water (PSW, 0.85 % w/v NaCl). For inoculation, aliquots of
225 0.01 mL were taken from the appropriate decimal dilution and deposited on the caudal
226 region of the fish fillet. Inoculation was performed using a 1-mL syringe with needle
227 (BD Plastipak, Spain) inserted through an adhesive septum (ø 15 mm, PBI Dansensor,
228 Denmark) which was previously placed on the laminate film of the plastic tray.

229 *2.4.3. Sensory analysis*

230 A preliminary sensory analysis was conducted to assess the effect of the initial level of
231 *L. sakei* CTC494 on fish quality deterioration. In that aim, fish fillets were inoculated
232 with *L. sakei* CTC494 as described in the previous section at three initial concentrations
233 of 10^2, 10^4 and 10^6 cfu/g (n = 14, 14 and 10, respectively). A control batch was prepared
234 without added bacteria (n = 14). All trays were stored at 5.0±0.12 ºC.

235 A semi-trained sensory panel made up of five members from the Faculty of Veterinary
236 (University of Cordoba, Spain) was required in order to evaluate the quality changes of
237 the fish fillets using the Quality Index Method (QIM) (Bremner et al., 1985). This
238 method is based on the use of significant sensory parameters and characteristic
239 attributes for raw fish with a scoring system of demerit points (≤ 3), which is in direct
240 proportion to their importance in the deterioration pattern of the species (Huidobro et
241 al., 2000). The scores for all the characteristics are summed-up to give an overall
242 sensory score, the so-called Quality Index (QI) (Botta 1995). A QI of 0 indicates a very
243 fresh fish and score increases as the freshness's characteristics lapses (Campus et al.,
244 2011).

245 In our study, the QIM was adapted from the scheme proposed by Lougovois et al.
246 (2003) and Campus et al. (2011) to evaluate freshness in gilthead sea bream fillets under
247 MAP. The attributes scored by the sensory panel are shown in Supplementary Table S1.
248 A linear correlation was established for each experimental condition (i.e. control and
249 inoculated batches) between the freshness expressed by the QI and storage time
250 (Microsoft Excel, Redmond, USA). The QI scores obtained by the five panellists in
251 each evaluation day for inoculated and control fillets were statistically compared by a t-

252 Student test (p=0.05) using the statistical software package SPSS 24.0 (Chicago,
253 Illinois, USA).

254 Sensory results demonstrated that the rate of freshness loss was similar for fillets
255 inoculated with 10^2 and 10^4 cfu/g of *L. sakei* compared to control fillets (data not
256 shown). Hence, a level of 10^2 and 10^4 cfu/g of *L. monocytogenes* and *L. sakei*,
257 respectively (ratio 1:2 in log scale) was defined for co-inoculation experiments in fish
258 fillets (Section *2.5.2*).

259 The application of *L. sakei* CTC494 as protective culture was sensory validated on fish
260 fillets inoculated with both microorganisms at a ratio 1:2, which corresponded to, in
261 arithmetic scale, *ca.* 10^2 cfu/g *L. monocytogenes* CTC1034 and *ca.* 10^4 cfu/g *L. sakei*
262 CTC494. Inoculated fish and control (i.e. non-inoculated) fillets were stored at 5 ºC for
263 8 days. Sensory assessment was performed on days 0, 4, 6 and 8.

264 ***2.5. Experiments with* L. sakei *CTC494 and* L. monocytogenes *CTC1034 on fresh***
265 ***gilthead sea bream fillets***

266 *2.5.1. Mono-culture experiments*

267 The effect of food matrix on the growth of *L. sakei* CTC494 and *L. monocytogenes*
268 CTC1034 was evaluated by inoculating both microorganisms independently in fresh
269 fish fillets. For that, fish fillets were acquired and inoculated (n = 36) as described in
270 Sections *2.4.1* and *2.4.2*. An additional control batch (n = 22) with non-inoculated fish
271 fillets was prepared. Experiments were carried out at a target temperature of 5 °C
272 (measured mean temperature of 4.8 ºC) for 25 days until microorganisms reached the
273 stationary phase.

274 *2.5.2. Co-culture experiments*

275 The interaction between *L. sakei* and *L. monocytogenes* on fish fillets was evaluated by
276 co-inoculation at the selected 1:2 ratio (i.e. 2 log cfu/g *L. monocytogenes* and 4 log
277 cfu/g *L. sakei*), which was previously defined according to results from Section *2.4.3*.
278 Before inoculation, bacterial suspensions were serially diluted ten-fold in PSW to obtain
279 the desired concentration and mixed at equal volumes. Control (n = 56) and inoculated
280 (n = 106) fillets were stored at two isothermal conditions with a mean of 4.8±0.14 and
281 8.2±0.10 °C for to 14 and 10 days, respectively. For the experiments at non-isothermal
282 conditions, fillets were stored at two dynamic temperature profiles, ranging from 4 to 8
283 ºC (profile 1) and from 2.5 to 12 °C (profile 2), for a total period of 12 days. The storage
284 temperature was recorded at regular time intervals using data loggers (Fourtec,
285 MiniLitE5032L, USA).

286 ***2.6. Microbiological analyses***

287 For experiments in fish juice, at each sampling point, 1 mL sample was aseptically
288 taken from each flask and serially diluted ten-fold in PSW. For experiments with fish
289 product, a 25-g portion of the (inoculated) fish fillet's caudal region, considered as the
290 analytical sample, was taken aseptically and transferred to a stomacher bag containing
291 225 mL PSW. Samples were homogenized for 60 s (1500 rpm) in a stomacher
292 (Masticator, IUL Instruments, Spain).

293 MRS agar supplemented with bromocresol purple (BP, 0.12 g/L, Sigma-Aldrich, USA)
294 and *Listeria* selective agar base (Oxoid) containing selective supplement (SR140E;
295 Oxoid) were used for the enumeration of *L. sakei* and *L. monocytogenes*, respectively.
296 BP is a pH indicator used for the enumeration of LAB in foods that indicates the

297 production of lactic acid by changing the MRS colour from purple to yellow (Sobrun et
298 al., 2012). Plates were incubated for approx. 48 h at 33 ºC under 10 % CO_2 for *L. sakei*
299 and at 37 ºC for *L. monocytogenes*.

300 ***2.7. Development of predictive models***

301 *2.7.1. Primary model fitting to mono-culture data*

302 Plate counts for *L. sakei* and *L. monocytogenes* were transformed into decimal
303 logarithmic values (i.e. log cfu/g or mL). The growth parameters λ, μ_{max} and N_{max}
304 obtained from each storage temperature for mono and co-culture experiments were
305 estimated by fitting the Baranyi and Roberts model (1994) defined by Eqs. (1) and (2)
306 to the observed data (mean of duplicates at each sampling point) using DMFit Excel
307 Add-in v. 3.5.

308 $$log\ N_t = log\ N_0 + \frac{\mu_{max}}{ln(10)} \cdot F(t) - \frac{1}{m \cdot ln(10)} \cdot ln\left(1 + \frac{e^{m \cdot \mu_{max} \cdot F(t)} - 1}{10^{m(log\ N_{max} - log\ N_0)}}\right) \quad (1)$$

309 $$F(t) = t - \lambda + \frac{1}{\mu_{max}} \cdot ln\left(1 - e^{-\mu_{max} \cdot t} + e^{-\mu_{max} \cdot (t-\lambda)}\right) \quad (2)$$

310 where N_t is the cellular concentration (cfu/g or mL) at time *t*, N_0 is the initial
311 concentration (cfu/g or mL), μ_{max} is the specific maximum growth rate (h^{-1}), λ is the lag
312 time (h), N_{max} is the maximum population density (cfu/g or mL), *m* is a curvature factor,
313 *F(t)* represents an adjustment function for the model.

314 *2.7.2. Secondary models for mono-culture experiments*

315 The influence of temperature on the primary growth parameters of *L. sakei* and *L.*
316 *monocytogenes* in fish juice was estimated using the square-root model (Eq. 3)
317 (Ratkowsky et al., 1982) which was fitted in MS-Excel (Microsoft, Redmond, USA).

318 $$\sqrt{p} = b \cdot (T - T_{min}) \quad (3)$$

319 where p is the kinetic parameter (i.e. λ and μ_{max}), b is a constant, T (°C) is temperature
320 and T_{min} is the theoretical minimum temperature for growth.

321 *2.7.3. Effect of microbial interaction on kinetics parameters*

322 To quantify the reduction on *L. monocytogenes* growth by the bioprotective *L. sakei*
323 CTC494 in fish juice, a reduction ratio (α) was calculated based on the fraction between
324 the parameters obtained in co-culture (p_{co}) and mono-culture (p_{mono}) as shown by Eq.
325 (4). To that aim, the parameters from co-culture experiments were also obtained by the
326 Baranyi model (see section *2.7.1*).

327 $$\alpha = 1 - \left(\frac{p_{co}}{p_{mono}} \right) \quad (4)$$

328 where α is the reduction ratio and p_{co} and p_{mono} the kinetic parameters (i.e. λ and μ_{max}) in
329 co-culture and mono-culture, respectively.

330 *2.7.4. Modelling microbial interaction between* L. sakei *CTC494 and* L. monocytogenes
331 *CTC1034*

332 To predict the simultaneous growth between the bioprotective *L. sakei* strain (at
333 different initial concentrations) and *L. monocytogenes* in fish juice stored at 2.2±0.08,
334 5.0±0.33, 8.1±0.33 and 12.1±0.12 °C, three different microbial interactions models were
335 tested.

Firstly, the Jameson effect model based on Eqs. (5) and (6), which assumes that the growth of the pathogen halts when the dominant microbial population reaches its N_{max} (Cornu et al., 2011; Jameson, 1962).

$$\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right) \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right) \cdot \left(\frac{Q_{Ls}}{1+Q_{Ls}}\right) \quad (5)$$

$$\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right) \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right) \cdot \left(\frac{Q_{Lm}}{1+Q_{Lm}}\right) \quad (6)$$

$$\frac{dQ_{Ls}}{dt} = Q_{Ls\,t-1} \cdot \mu_{maxLs} \quad (7)$$

$$\frac{dQ_{Lm}}{dt} = Q_{Lm\,t-1} \cdot \mu_{maxLm} \quad (8)$$

where N is the cell concentration (cfu/mL) at time t, μ_{max} is the maximum specific growth rate (h^{-1}), N_{max} is the maximum population density (cfu/mL) and Q is a measure of the physiological state of cells at time t, for *L. sakei* (Ls) or *L. monocytogenes* (Lm).

The value of Q at t=0 (Q_0) was calculated for both microorganisms as follows:

$$Q_0 = \frac{1}{e^{(\mu_{max} \cdot \lambda)} - 1} \quad (9)$$

In our study, a modification of the Jameson effect model was also used, represented by Eqs. (10) and (11). This modification includes the parameters $Ncri_{Ls}$ and $Ncri_{Lm}$ that describe the maximum critical concentration that a population should reach to inhibit the growth of the other population (Jameson, 1962; Le Marc et al., 2009; Vasilopoulos et al., 2010).

353 $$\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right) \cdot \left(1 - \frac{N_{Lm}}{N_{criLm}}\right) \cdot \left(\frac{Q_{Ls}}{1 + Q_{Ls}}\right) \quad (10)$$

354 $$\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right) \cdot \left(1 - \frac{N_{Ls}}{N_{criLs}}\right) \cdot \left(\frac{Q_{Lm}}{1 + Q_{Lm}}\right) \quad (11)$$

355 $$\frac{dQ_{Ls}}{dt} = Q_{Ls\,t-1} \cdot \mu_{maxLs} \quad (12)$$

356 $$\frac{dQ_{Lm}}{dt} = Q_{Lm\,t-1} \cdot \mu_{maxLm} \quad (13)$$

357 where N_{cri} is the maximum critical concentration (cfu/mL) of *L. sakei* (Ls) on *L.*
358 *monocytogenes* (Lm) and vice-versa. The rest of model parameters are described in Eqs.
359 (5) to (9).

360 Finally, the traditional Lotka Volterra model, also referred to as predator-prey model,
361 was used according to Eqs. (14) and (15). This model includes two empirical parameters
362 reflecting the degree of interaction between microbial species (F_{LsLm} and F_{LmLs}) (Cornu
363 et al., 2011; Fujikawa et al., 2014; Giuffrida et al., 2008). Depending on the empirical
364 parameter value for *L. sakei* (F_{LsLm}), the growth of *L. monocytogenes* can be affected in
365 three different ways:

366 1) If $0 < F_{LsLm} < 1$, *L. monocytogenes* grows with reduced μ_{max} after *L. sakei* reaches
367 N_{max}.

368 2) If $F_{LsLm} = 1$, *L. monocytogenes* stops growing when *L. sakei* reaches its N_{max}.

369 3) If $F_{LsLm} > 1$, *L. monocytogenes* population declines when *L. sakei* reaches its N_{max}.

370 $$\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls} + F_{LsLm} \cdot N_{Lm}}{N_{maxLs}}\right) \cdot \left(\frac{Q_{Ls}}{1 + Q_{Ls}}\right) \quad (14)$$

$$\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm} + F_{LmLs} \cdot N_{Ls}}{N_{maxLm}}\right) \cdot \left(\frac{Q_{Lm}}{1 + Q_{Lm}}\right) \quad (15)$$

$$\frac{dQ_{Ls}}{dt} = Q_{Ls\,t-1} \cdot \mu_{maxLs} \quad (16)$$

$$\frac{dQ_{Lm}}{dt} = Q_{Lm\,t-1} \cdot \mu_{maxLm} \quad (17)$$

where F_{LsLm} and F_{LmLs} are, respectively, the competition factor parameters of *L. sakei* CTC494 on *L. monocytogenes* CTC1034 and vice-versa. The other parameters are as indicated in Eqs. (5-9).

The interaction parameters N_{cri} (maximum critical concentration of one population) and F_{LsLm} and F_{LmLs} (competition factors of one species on the other) were estimated by regression using kinetic parameters derived from mono-culture data (see sections *2.7.1* and *2.7.2*). To estimate the best-fit values of interaction parameters, an optimization procedure was implemented in MATLAB version R2015b using the functions *fmincon* and *ode45* (The MathWorksInc®, Natick, USA).

2.7.5. Goodness-of-fit indexes and predictive model performance

The goodness-of-fit of the primary and secondary models was assessed by root mean square error (RMSE) and coefficient of determination (R^2).

The performance of the developed interaction models to predict the behaviour of the bioprotective *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in MAP-fish fillets under isothermal and dynamic temperature conditions was evaluated by the acceptable simulation zone (ASZ) approach. Model performance is considered acceptable when at least 70 % of the observed log counts values are within the ASZ, defined as ± 0.5 log-

391 units from the simulated concentration in log units (Mejlholm and Dalgaard, 2015;
392 Møller et al., 2013).

393 ***3. Results***

394 *3.1. Primary growth parameters of* L. sakei *CTC494 and* L. monocytogenes *CTC1034*
395 *in mono-culture on fish juice and fish fillets*

396 The two studied microorganisms were able to grow in sterile fish juice when stored at
397 2.2±0.08, 5.0±0.33, 8.1±0.33 and 12.1±0.12 °C and on fish fillets at 4.8±0.14 °C. The
398 growth curves obtained from the fit of the Baranyi and Roberts model provided a good
399 description of the observed data (Supplementary Figure S1). The parameters λ and μ_{max}
400 varied with temperature, while N_{max} was not affected, with average values of 7.92 and
401 8.74 log cfu/mL for *L. sakei* CTC494 and *L. monocytogenes*, respectively. The
402 parameters estimated by the Baranyi and Roberts model are shown in Table 1. For both
403 fish matrices (juice and fillets) the model showed good fit to data (R^2 > 0.98)
404 (Supplementary Table S2). A minimum of 7 and a maximum of 23 sampling points
405 were taken for each microorganism depending on the storage temperature. In summary,
406 results in mono-culture confirmed that the bioprotective strain *L. sakei* CTC494
407 presented better ability to grow in fish juice at low temperatures, which was also
408 observed on fish fillets.

409 *3.2. Secondary growth models*

410 The parameters λ and μ_{max} obtained from the Baranyi and Roberts model were used to
411 fit a square-root model (Eq. 3). The ability of the secondary models to describe the
412 influence of temperature on the growth parameters was proven to be satisfactory
413 according to the values from RMSE and R^2, whose values were in the ranges 0.064-

414 0.086 and 0.874-0.999, respectively. A summary of results from the fitting of the
415 square-root model for both microorganisms is shown in Table 2.

416 *3.3. Interaction of* L. sakei *CTC494 and* L. monocytogenes *CTC1034 in fish juice at*
417 *different temperatures and inoculation ratios*

418 The influence of storage temperature and the inoculation ratio (1:1, 1:2 and 1:3) on the
419 interaction of *L. sakei* CTC494 and *L. monocytogenes* was assessed. To allow a
420 comparison with kinetic parameters in mono-culture, the Baranyi and Roberts model
421 without considering interaction was fitted to experimental data in co-culture (Table 3).
422 The statistical indexes for the fitted model presented satisfactory RMSE and R^2 values
423 (Supplementary Table 3). The most evident outcome from these experiments was that
424 higher ratios produced stronger inhibition of *L. monocytogenes* growth, with the ratio
425 1:3 yielding no apparent growth for the pathogen. The parameter μ_{max} was little
426 influenced, even though values obtained in co-culture were generally lower than those
427 obtained in mono-culture. Figure 2 represents, through a bar diagram, a comparison of λ
428 and N_{max} obtained from mono-culture and co-culture at the different conditions by using
429 the reduction ratio (α) calculated according to Eq. (4). From this figure, it can be
430 observed that α for λ varied for *L. monocytogenes* among the different inoculation
431 ratios, but in all co-culture experiments, λ presented a reduction with respect to that
432 observed in mono-culture. However, further analysis of data confirmed that differences
433 were rather produced by the fitting process (i.e. prediction error) affected by the
434 relatively λ short duration ($\geq$ 5 ºC; $\lambda \leq$ 36 h) than a hypothetical interaction between
435 microorganisms.

436 On the other hand, N_{max} was the most affected parameter for *L. monocytogenes* at all
437 concentration ratios. For instance, in mono-culture experiments at 5.0 °C (Table 1), log

438 N_{max} was 8.65 log cfu/mL while for co-culture experiments, the parameter was gradually
439 decreasing to 5.94 (α = 31 %), 4.22 (α = 51 %) and 1.37 (α = 84 %) log cfu/mL for
440 inoculation ratios 1:1, 1:2 and 1:3, respectively. For the latter, the putative "N_{max}" was
441 taken from observations since the Baranyi and Roberts model could not be fitted to data
442 at ratio 1:3 as no growth was observed. Similar inhibition patterns were observed for the
443 other assayed temperatures (Fig. 2).

444 *3.4. Sensory analysis*

445 The sensory evaluation results obtained for sea bream fillets under MAP conditions
446 stored at 5 ºC are presented in Table 4. The QI scores obtained for fish samples
447 inoculated at a ratio 1:2 (*L. monocytogenes: L. sakei*) were compared to control samples
448 (i.e. non-inoculated). In general, QI scores increased linearly during storage with a
449 correlation coefficient (R^2) of 0.82 and 0.67 for control and inoculated batches,
450 respectively. The statistical analysis of QI scores showed that *L. sakei* CTC494 did not
451 significantly affect the sensory properties of fish fillets ($p > 0.05$) during the evaluated
452 storage time (8 days). Though the deterioration rate was slightly lower for control (slope
453 = 0.47) than for inoculated samples (slope = 0.55), the differences were not statistically
454 significant ($p > 0.05$). Therefore, from the sensory perspective, the addition level of 10^4
455 cfu/g of *L. sakei* CTC494 would be suitable as bioprotective strategy without modifying
456 the spoilage rate in comparison with a control (non-bioprotected) product.

457 *3.5. Modelling interaction of* L. sakei CTC494 *and* L. monocytogenes CTC1034 *in fish*
458 *juice*

459 The three interaction models (Fig. 3) were tested using the kinetic parameters (λ, μ_{max}
460 and N_{max}) obtained from the Baranyi and Roberts model fitted to mono-culture

experiment data and estimating the respective interaction factors by regression analysis. The statistical performance of the models was evaluated by RMSE whose values are shown in Table 5 together with the estimated parameters.

The Jameson effect model presented the worst fitting to data, showing the highest RMSE values. This result suggests that interaction between both microorganisms could not be exclusively explained by the Jameson effect, where growth inhibition is the result from a depletion in nutrient bioavailability and toxicity increase when the dominant population reaches N_{max}. The modified Jameson effect model including the parameter N_{cri} showed better performance, with RMSE lower values. For both microorganisms, N_{cri} remained in the same order of magnitude for the different temperatures and inoculation ratios, with average values, in log scale, of 7.7 and 8 log cfu/mL for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, respectively (Table 5).

The Lotka Volterra interaction model showed slightly better fit to data than the above models according to RMSE (Table 5) and visual analysis of growth curves (Fig. 3). In the case of the ratio 1:3, a poor fitting was observed for *L. monocytogenes* although this condition also yielded unsatisfactory fitting results for the Jameson effect-based models. This could be due to the difficulty of the models to suitably describe the large decline of *L. monocytogenes* population at this ratio.

As regards the inhibitory effect of *L. sakei* CTC494 on *L. monocytogenes* growth, competition factors (F_{LsLm}) at the lowest temperature (2.2 ºC) were below 1 for inoculation ratios 1:1 and 1:2, with values of 0.84 and 0.94, respectively. However, for the same temperature at ratio 1:3, the competition factor was equal to 2.67. This higher value reflected the noticeable decline of *L. monocytogenes* population (down to 0.70 log cfu/mL). For inoculation ratios 1:2 and 1:3, at 5 °C, the competition factors increased up

485 to 1.54 and 1.46, respectively. For higher temperatures (8-12 ºC), this increasing trend
486 in the competition factor was minimized showing a rather variable pattern, and
487 therefore, no mathematical model could be derived for such a relationship. Thus, for
488 modelling purposes, this parameter was fixed to the average value observed at different
489 temperatures for the corresponding inoculation ratio.

490 *L. monocytogenes* did not exert any inhibitory effect on *L. sakei* CTC494 as
491 demonstrated by the competition factor (F_{LmLs}) being equal to 0 (Table 5).

492 *3.6. Simulation of growth interaction of* L. sakei *CTC494 and* L. monocytogenes
493 *CTC1034 on fish fillets*

494 The simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* was evaluated in
495 sea bream fillets under MAP at two isothermal (4.8 and 8.2 °C) as well as at two non-
496 isothermal conditions (profile 1: 4-8 °C and profile 2: 2.5-12 °C) at an initial inoculation
497 ratio 1:2 based on the sensory analysis outcome (Fig. 4).

498 For isothermal conditions, *L. sakei* CTC494 reached the stationary phase with 10 and 5
499 days of storage at 4.8 °C and 8.2 °C, respectively, with an average log N_{max} of 7.91 log
500 cfu/g, while for *L. monocytogenes* under the same conditions, log N_{max} was 2.23 and
501 1.87 log cfu/g respectively. The value obtained at 4.8 ºC represented for a reduction of
502 67 % compared with log N_{max} estimated in mono-culture in fish fillets (5.68 log cfu/g).

503 The average pH values for fish fillets remained constant throughout the storage time
504 (6.15 ± 0.02) and the gas concentration in the packaging at the end of storage was 31.2
505 % ± 0.85 and 29.0 % ± 0.19 for O_2 and CO_2, respectively.

506 The kinetic parameters, in the model, for both microorganisms were estimated by using
507 the secondary models (λ and μ_{max}) derived from mono-culture experiments except for

508 log N_{max} which was not temperature dependent and therefore, the average value was
509 used instead (i.e., in log scale, 7.92 and 8.74 log cfu/mL for *L. sakei* and *L.*
510 *monocytogenes,* respectively). To consider the effect of food matrix on μ_{max} (h^{-1}), the
511 reduction of this parameter on the fresh fish product (4.8 ºC) in relation to that observed
512 in fish juice in mono-culture was estimated (5.0 ºC), which corresponded to 0.68. Thus,
513 for simulating growth, the specific growth rate for *L. monocytogenes* was adjusted
514 applying the above reduction rate in the Lotka Volterra model. Due to the difficulty to
515 set an equation describing the temperature effect on the competition factor, this was
516 fixed to the average of the values obtained at the different temperatures at the ratio 1:2,
517 which corresponded to 1.54. It was deemed that the value was representative for the
518 assayed temperatures, considering that most temperatures in challenge tests were in the
519 range 4-12 ºC, where the competition factor was similar. The same reduction rate and
520 competition factor were used for the experiments at isothermal conditions (4.8 and 8.2
521 °C) as well as for the two dynamic time-temperature profiles.

522 Table 6 shows RMSE and ASZ values for the growth interaction of *L. sakei* CTC494
523 and *L. monocytogenes* CTC1034 on fish fillets predicted by Lotka Volterra model. The
524 RMSE values for experiments under isothermal conditions varied between 0.378-0.555
525 and 0.452-0.593 for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, respectively.
526 The visual inspection of the simulated line also confirmed that good performance of
527 models, demonstrating that the model was able to simulate the observed slight *Listeria*
528 increase and subsequent decline, though at 8.2 ºC, observations showed a more
529 prominent decline than the one predicted by the model simulation (i.e. fail-safe
530 prediction). Furthermore, values for ASZ considering as criterion ± 0.5 log.units showed

that models can mostly accounted for the counts recorded during the interaction experiments, with values of 79 % (Table 6).

For non-isothermal temperature conditions, RMSE values ranged from 0.645-0.894 and 0.309-0.615 for *L. sakei* and *L. monocytogenes*, respectively. Lotka Volterra model showed closer predictions to experimental data in fish fillets for *L. monocytogenes* under profile 1. The percentages for the ASZ corresponded to 92 % (11/12) and 77 % (10/13) for profile 1 and 2, respectively. For *L. sakei*, ASZ values varied between 75 (9/12) and 77 % (10/13) for both profiles. Lotka Volterra model overestimated the exponential phase of *L. sakei* CTC494, while for *L. monocytogenes* the same was observed only for profile 2. The overestimation in profiles for *L. monocytogenes* could be considered as a "fail-safe" prediction since growth was predicted when no-growth was actually observed (i.e. profile 2).

4. *Discussion*

4.1. L. monocytogenes *growth in mono-culture*

For *L. monocytogenes* in mono-culture at 5º C, μ_{max} values obtained in our study were 16 % higher than those found by Verheyen et al. (2018) for in fish-protein based emulsions at 4 ºC used as food model system for fish. On the contrary, the μ_{max} observed by Bolívar et al. (2018) in fish juice within the interval 5-11 ºC were higher (30-57%) than those found in our study in the range 4-12 ºC. Differences in growth rates could be mainly attributed to strain variability and experimental conditions. By the contrary, the predictions provided by Combase Predictor (https://www.combase.cc/index.php/en/) considering the same physico-chemical characteristics as those obtained for fish juice (pH = 6.66; a_w = 0.997) were similar in all temperatures studied. Furthermore, the obtained μ_{max} for *L. monocytogenes* in our fish

555 juice were in the range of values reported for other fish matrices (i.e. 0.0329-0.2075 at
556 4-12 °C) such as smoked salmon, raw tuna, vacuum-packed rainbow trout fillets and sea
557 bream fillets under MAP conditions (Faber, 1991; Hisar et al., 2005; Hwang, 2007; Liu
558 et al., 2016; Provincial et al., 2013).

559 *4.2. Growth interaction of* L. sakei CTC494 *and* L. monocytogenes CTC1034

560 In general, observations in our study showed that the suppression of *Listeria* growth
561 occurred when the dominant population, i.e. *L. sakei* CTC494 reached their N_{max}. This
562 result would signal a potential Jameson effect between populations. Several studies have
563 considered the Jameson effect in the simultaneous growth of microorganisms and *L.*
564 *monocytogenes* on fish products (Beaufort et al., 2007; Giménez and Dalgaard, 2004;
565 Koseki et al., 2011; Mejlholm and Dalgaard, 2007).

566 According to results, the inhibitory effect was influenced by the inoculation ratio and
567 temperature, which has been also reported in other works (Quinto et al., 2016;
568 Yamazaki et al., 2003). Differences in inoculum level is key to determine the dominant
569 microorganism in the microbial interaction and thus, the level of inhibition between
570 microbial populations (Mellefont et al., 2008). Despite this fact, we observed that *L.*
571 *sakei* CTC494 exerted a slight inhibition on N_{max} of *L. monocytogenes* even when both
572 microorganisms were inoculated the same level (ratio 1:1). This inhibition at equal
573 inoculum level could be associated with production of bacteriocin since *L. sakei*
574 CTC494 produces sakacin K (Hugas et al., 1995; De Vuyst and Leroy, 2007; Leroy et
575 al., 2005; Ravyts et al., 2008) and the influence of other metabolites such as organic
576 acids was discarded as potential inhibitors because of no relevant changes in pH were
577 detected during growth experiments in fish juice and fish samples.

In summary, the interaction between *L. sakei* CTC494 and *L. monocytogenes* presented in our study could be understood by a combination of two mechanisms: i) a non-specific interaction involving the Jameson effect on the inhibition of *L. monocytogenes*, occurring when *L. sakei* CTC494 is present at an initial concentration higher than *L. monocytogenes* together with the fact that the bioprotective strain grows faster than the pathogen (Mellefont et al., 2008; Jameson, 1962) and ii) specific interaction caused by modification of the medium where both microorganisms coexist, resulting in an specific antagonistic effect on the growth of *L. monocytogenes* due to bacteriocin production (i.e. sakacin K) by the bioprotective strain (Aguilar and Klotz, 2010; Vescovo et al., 2006). However, the production of bacteriocin was not quantified in our study, thus no conclusion can be drawn about which interaction phenomenon was more relevant. Nor could mechanistic models be applied due to the lack of biological insight into the metabolic and genetic phenomena arising from the simultaneous growth of two microbial populations.

4.3. Lotka Volterra´s competition factor

The competition factors for *L. monocytogenes* (F_{LsLm}) in fish juice were slightly temperature dependent for all ratios (Table 5). The largest increase in the competition factor took place at low temperatures for ratios 1:2 and 1:3 (i.e. 2.2-5 ºC) while for ratio 1:1, higher temperatures (8-12 ºC) were responsible for a higher rise of this factor. No mathematical expression could be derived from data because of the limited number of observations, reduced temperature range and the lack of a clear pattern in data. Møller et al. (2013) estimated the competition factors for natural microbiota on growth of *Salmonella* spp. in fresh pork using the Lotka Volterra model and expanded Jameson effect model and found dependency on range of storage temperature assayed. By the

602 contrary, Mejholm and Dalgaard (2015) using the model proposed by Giménez and
603 Dalgaard (2004) did not find that the competition factor was temperature dependent.
604 Furthermore, the traditional Jameson effect model or its modification suggested by Le
605 Marc et al. (2009) have been used to predict growth of microorganisms in food at
606 different storage temperature (Giménez and Dalgaard, 2004; Le Marc et al., 2009;
607 Mejholm and Dalgaard, 2007; Vermeulen et al., 2011). In those studies, however, the
608 effect of microbial interaction on growth patterns was independent of the studied
609 storage temperatures. The divergence between studies to correlate interaction factors
610 with temperature can be related to the different conditions used in experiments (i.e. type
611 of microorganism, food matrix and inoculum concentration).

612 Competition factors, in our study, were also under the influence of the inoculation ratio.
613 Thus, the lowest values were obtained for ratios 1:1 and 1:2 (Table 5). Baka et al.
614 (2014) estimated low competition factors for the interaction between *Leuconostoc*
615 *carnosum* and *L. monocytogenes* in vacuum packed Frankfurter sausages stored at 4 °C
616 for the ratio 1:1. At intermediate temperatures (8.1-12.1 °C), the competition factors
617 decreased with the initial increase of concentration of *Leuconostoc carnosum*. Fujikawa
618 et al. (2014) found that the values for the competition factors did not vary with the
619 combinations of the initial populations of *Staphylococcus aureus*, *Escherichia coli* and
620 *Salmonella* spp. at 28 °C.

621 According to Baka et al. (2014), differences in values of the competition factor can be
622 attributed to the combination of different variables, such as temperature, nutrient
623 depletion, pH, bacteriocin production, organic acid, MAP conditions, etc., which can be
624 considered as part of the hurdle concept (Leistner, 1995). When these variables are
625 identified, the Lotka Volterra model can be modified for more realistic microbial

626 interaction descriptions, for instance, the effect of environmental conditions (i.e.
627 temperature), the influence of inhibitory substances on lag phase duration of pathogenic
628 organisms or whether bacteriocin production is dependent quorum sensing (Dens et
629 al.,1999, Powell et al., 2004).

630 *4.4. Simulating growth inhibition and bioprotective activity of* L. sakei CTC494 *on* L.
631 monocytogenes CTC1034 *on fish fillets*

632 A challenge test on gilthead sea bream fillets under MAP inoculated with *L.*
633 *monocytogenes* and *L. sakei* CTC494 in co-culture at ratio 1:2 was carried out under
634 isothermal and non-isothermal conditions. The Lotka Volterra model slightly
635 overestimated the experimental observations of *L. sakei* in the exponential phase for the
636 profiles 1 and 2. These discrepancies can be partly explained by the fact that the
637 performance of a dynamic model depends on the performance of the primary and
638 secondary models, and the sudden temperature changes can cause an intermediate lag
639 time that cannot be predicted by the models (Longhi et al., 2013). Nevertheless, this fact
640 did not affect predictions for *Listeria* growth, providing a reliable estimate for this
641 pathogen for two dynamic conditions, according to the ASZ approach.

642 The control of pathogenic bacteria using LAB as bioprotective cultures in fish products
643 is widely reported in literature (Bernardi et al., 2011; Chowdhury et al., 2012; Ghanbari
644 et al., 2013; Hisar et al., 2005; Matamoros et al., 2009; Nath et al., 2014; Tahiri et al.,
645 2009; Tomé et al., 2008; Weiss and Hammes, 2006), thus showing that live
646 microbiological cultures can be a more effective alternative to the use of bacteriocins
647 (Pilet and Leroy, 2011), which in addition are not permitted by most of the food
648 additive regulations. However, the selection of candidates as bioprotective cultures to
649 improve food quality and extend shelf-life has been attributed to the capacity not to

produce undesirable organoleptic changes in foods. In this sense, *L. sakei* CTC494 is reported in literature as a starter culture providing good organoleptic and sensory properties in fermented meat products and as bioprotective (not spoiling) culture in cooked ham (Aymerich et al., 2002; Bover-Cid et al., 2001; Hugas et al., 1995; Hugas et al., 1998; Hugas et al., 2002). Our study proposed the extension of the use the bacteriocinogenic *L. sakei* CTC494 in raw fish and other minimally processed fish products demonstrating that its availability to grow in a different food matrix and its application as a suitable approach for controlling *L. monocytogenes* growth in packaged sea bream fillets stored under isothermal and non-isothermal conditions including moderate abuse.

5. Conclusion

Results demonstrated that the use of the bacteriocinogenic strain *L. sakei* CTC494, as bioprotective culture is a suitable strategy for controlling *L. monocytogenes* growth in minimally processed fresh fish products (i.e. filleted gilthead sea bream) under refrigerated storage. Furthermore, the modelling approach, developed herein, based on a step-wise scheme from mono-culture experiments in fish juice under isothermal conditions to experiments performed in co-culture in actual fish product under dynamic temperature profiles was proved to be effective to derive reliable microbial interaction models. These mathematical models could be used as a predictive tool to simulate the simultaneous behaviour of bioprotective lactobacillus strain and *L. monocytogenes*. Thus, these tools can support the design and optimization of bioprotective culture based strategies against *L. monocytogenes* in minimally processed fish products.

Supplementary data to this article can be found online at.

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680 **Declarations of interest**

681 The authors declare that there is no conflict of interest in the publication of this paper.

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Supplementary Table S1. Quality Index Method (QIM) scheme applied to the sensory analysis of filleted sea bream under modified atmosphere packaging adapted from Lougovois et al. (2003) and Campus et al. (2011).

	Parameter	Attributes	Demerit points
Appearance	Skin	Bright, shining, iridescent	0
		Less bright, some loss of iridescence	1
		Pale, dull	2
	Slime/Mucus	Clear-transparent	0
		Slightly cloudy/cloudy	1
Flesh	Colour	Fresh, translucent	0
		Waxy, milky	1
		Dull, slightly discoloured, yellowish	2
	Stiffness	Firm	0
		Some softening	1
		Soft	2
Odour	Odour	Fresh	0
		Neutral	1
		Slight off-odours	2
		Spoiled	3
Quality Index (QI, as the sum of assigned demerit points)			0-10

Supplementary Table S2. Goodness-of-fit indexes for the fit of the Baranyi and Roberts model to the growth data of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in mono-culture in sterile fish juice of sea bream and sea bream fillets under modified atmosphere packaging at different temperatures.

Matrix	Temp. (°C)	n (Ls)[c]	n (Lm)[d]	*Lactobacillus sakei* CTC494		*Listeria monocytogenes* CTC1034	
				RMSE[e]	R^2	RMSE	R^2
Fish juice[a]	2.2	12	19	0.061	0.9991[f]	0.154	0.9955
	5.0	18	23	0.107	0.9972	0.091	0.9985
	8.1	14	14	0.120	0.9968	0.122	0.9965
	12.1	14	17	0.126	0.9965	0.105	0.9977
Fresh fish fillets[b]	4.8	7	11	0.191	0.9930	0.151	0.9840

[a] Experiments in sterile fish juice of gilthead sea bream inoculated with *ca.* 10^2 cfu/mL of *L. sakei* or *L. monocytogenes*.
[b] Experiments on gilthead sea bream fillets under modified atmosphere packing inoculated with *ca.* 10^2 cfu/g of *L. sakei* or *L. monocytogenes*.
[c] n (Ls), number of data (sampling points) for *L. sakei* CTC494.
[d] n (Lm), number of data (sampling points) for *L. monocytogenes* CTC1034.
[e] RMSE, Root mean square error.
[f] R^2, Coefficient of determination.

Supplementary Table S3. Goodness-of-fit indexes for the Baranyi and Roberts model without interaction fitted to co-culture growth data of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in sterile fish juice of sea bream and sea bream fillets under modified atmosphere packaging at different temperature.

Ratio[a]	Temp. (°C)	n (Ls)[b]	n (Lm)[c]	*Lactobacillus sakei* CTC494		*Listeria monocytogenes* CTC1034	
				RMSE[d]	R^2	RMSE	R^2
1:1	2.2	17	17	0.100	0.9979[e]	0.092	0.9954
1:2		14	14	0.081	0.9963	0.037	0.9976
1:3		11	11	0.071	0.9818	NF[f]	
1:1	5.0	14	14	0.113	0.9968	0.074	0.9974
1:2		13	7	0.117	0.9924	0.157	0.9432
1:3		10	10	0.023	0.9969	NF	
1:1	8.1	14	14	0.135	0.9965	0.472	0.9090
1:2		11	7	0.060	0.9986	0.180	0.9684
1:3		11	11	0.092	0.9800	NF	
1:1	12.1	16	11	0.128	0.9965	0.122	0.9970
1:2		11	7	0.081	0.9970	0.143	0.9823
1:3		11	11	0.100	0.9761	NF	

[a] Ratio of inoculation of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in sterile fish juice of gilthead sea bream where the ratios 1:1, 1:2 or 1:3 represent the initial concentrations of 2 log cfu/mL for the *L. monocytogenes* strain and 2, 4, 6 log cfu/mL for the *L. sakei* strain, respectively.
[b] n (Ls), number of data (sampling points) for *L. sakei* CTC494.
[c] n (Lm), number of data (sampling points) for *L. monocytogenes* CTC1034.
[d] RMSE, Root mean square error.
[e] R^2, Coefficient of determination.
[f] NF, no fit as no growth was observed.

Tables

Table 1. Estimated lag time (λ), maximum specific growth rate (μ_{max}) and N_{max} (maximum population density) and associated standard errors for *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in mono-culture obtained from the Baranyi and Roberts model in sterile fish juice of sea bream and sea bream fillets under modified atmosphere packaging.

Matrix	Temp. (°C)	*Lactobacillus sakei* CTC494				*Listeria monocytogenes* CTC1034			
		log N_0 (Ls) (log cfu/mL or g)	λ (h)	μ_{max} (h^{-1})	*log* N_{max} (log cfu/mL or g)	log N_0 (Lm) (log cfu/mL or g)	λ (h)	μ_{max} (h^{-1})	*log* N_{max} (log cfu/mL or g)
Fish juice[a]	2.2	2.36	92.4 ± 7.55	0.0351 ± 0.0004	7.70 ± 0.03	2.59	166.7 ± 23.20	0.0226 ± 0.0004	8.92 ± 0.10
	5.0	2.04	43.1 ± 6.72	0.0697 ± 0.0005	7.85 ± 0.05	1.53	36.1 ± 7.13	0.0477 ± 0.0005	8.65 ± 0.05
	8.1	2.67	18.7 ± 5.04	0.1273 ± 0.0039	7.94 ± 0.05	2.29	15.1 ± 6.26	0.0892 ± 0.0019	8.68 ± 0.07
	12.1	2.48	5.3 ± 2.68	0.2140 ± 0.0052	8.17 ± 0.07	2.39	2.0 ± 2.01	0.1685 ± 0.0020	8.70 ± 0.06
Fresh fish fillets[b]	4.8	1.49	33.8 ± 11.39	0.0806 ± 0.0036	7.08 ± 0.13	2.71	56.1 ± 35.23	0.0154 ± 0.0006	5.68 ± 0.13

[a] Experiments in sterile fish juice of gilthead sea bream inoculated with *ca.* 10^2 cfu/mL of *L. sakei* or *L. monocytogenes*.

[b] Experiments on gilthead sea bream fillets under modified atmosphere packing inoculated with *ca.* 10^2 cfu/g of *L. sakei* or *L. monocytogenes*.

Table 2. Coefficients of the square-root model describing the effect of temperature on lag time (λ) and maximum specific growth rate (μ_{max}) of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in sterile fish juice of sea bream.

Parameters	Microorganisms	*b*	T_{min} (°C)	RMSE[a]	R^2
λ	*L. sakei* CTC494	-0.7269	14.69	7.365	0.9695[b]
	L. monocytogenes CTC1034	-1.0868	12.42	30.332	0.8737
μ_{max}	*L. sakei* CTC494	0.0280	-4.50	0.086	0.9994
	L. monocytogenes CTC1034	0.0263	-3.40	0.064	0.9990

[a] RMSE, Root mean square error.
[b] R^2, Coefficient of determination.

Table 3. Estimated lag time (λ), maximum specific growth rate (μ_{max}), N_{max} (maximum population density) and associated standard error from the Baranyi and Roberts model without interaction fitted to the growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in co-culture in sterile fish juice of sea bream.

Ratio[a]	Temp. (°C)	*Lactobacillus sakei* CTC494				*Listeria monocytogenes* CTC1034			
		log N_0 (Ls) log cfu/mL	λ (h)	μ_{max} (h^{-1})	*log* N_{max} (log cfu/mL)	log N_0 (Lm) log cfu/mL	λ (h)	μ_{max} (h^{-1})	*log* N_{max} (log cfu/mL)
1:1	2.2	2.37	53.3 ± 11.72	0.0350 ± 0.0007	7.74 ± 0.05	2.52	75.4 ± 16.32	0.0223 ± 0.0006	5.77 ± 0.04
1:2		4.58	59.5 ± 8.08	0.0355 ± 0.0097	7.63 ± 0.04	2.60	106.1 ± 6.45	0.0268 ± 0.0008	4.28 ± 0.01
1:3		6.77	39.4 ± 14.39	0.0284 ± 0.0027	7.99 ± 0.03	2.57		NG[b]	
1:1	5.0	2.84	30.0 ± 4.72	0.0675 ± 0.0012	7.94 ± 0.07	2.42	36.9 ± 6.87	0.0490 ± 0.0012	5.94 ± 0.03
1:2		4.59	23.0 ± 7.52	0.0625 ± 0.0026	7.86 ± 0.05	2.49	14.5 ± 18.80	0.0334 ± 0.0053	4.22 ± 7.35
1:3		6.62	11.8 ± 3.13	0.0294 ± 0.0007	7.68 ± 0.01	2.46*		NG	
1:1	8.1	2.42	20.3 ± 3.50	0.1373 ± 0.0029	8.11 ± 0.06	2.27	12.3 ± 19.73	0.1085 ± 0.0131	6.07 ± 0.18
1:2		4.34	15.2 ± 2.15	0.1266 ± 0.0023	8.14 ± 0.03	1.92	0.0 ± 0.00	0.0977 ± 0.0058	4.03 ± 0.11
1:3		6.37	0.0 ± 0.00	0.0703 ± 0.0027	7.96 ± 0.03	2.24*		NG	
1:1	12.1	2.49	6.4 ± 1.62	0.2292 ± 0.0035	8.17 ± 0.06	2.33	1.3 ± 1.86	0.1959 ± 0.0035	6.81 ± 0.08
1:2		4.40	5.1 ± 1.16	0.2273 ± 0.0046	8.20 ± 0.03	2.47	0.0 ± 0.00	0.1733 ± 0.0065	4.86 ± 0.12
1:3		6.37	0.0 ± 0.00	0.1716 ± 0.0073	8.06 ± 0.03	2.47*		NG	

[a] Ratio of inoculation of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in fish juice of sea bream where the ratios 1:1, 1:2 or 1:3 represent the initial concentrations of 2 log cfu/mL for the *L. monocytogenes* strain and 2, 4, 6 log cfu/mL for the *L. sakei* strain, respectively.

[b] NG, no growth.

[*] Observed initial concentration of *L. monocytogenes*.

Table 4. Quality Index values obtained from the sensory analysis of sea bream fillets packaged under modified atmosphere and stored under refrigerated conditions (5 ºC, 8 days) for samples inoculated at a ratio 1:2 (*Listeria monocytogenes*: *Lactobacillus sakei*) (i.e., 2 log cfu/g and 4 log cfu/g, respectively) and control fillets (non-inoculated).

Storage time (days)[a]	Quality Index	
	Inoculated fillets	Control fillets
0	0.3 ± 0.5[b]	0.0 ± 0.0
4	0.6 ± 1.3	1.2 ± 1.6
6	2.0 ± 1.7	2.0 ± 2.0
8	6.3 ± 0.5	4.8 ± 1.9

[a] Storage under modified atmosphere packaging at 5 ºC.

[b] Mean ± standard deviation (n = 5 panellists).

Table 5. Estimated maximum critical concentration (N_{cri}) of the modified Jameson effect model and competition factors (F_{LsLm} and F_{LmLs}) of the Lotka Volterra model and goodness-of-fit index (RMSE) for *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in co-colture in fish juice of sea bream.

Ratios[a]	Temp. (°C)	n[b]	Jameson model		Modified Jameson model				Lotka Volterra model			
			Lactobacillus sakei CTC494	*Listeria monocytogenes* CTC1034	*Lactobacillus sakei* CTC494		*Listeria monocytogenes* CTC1034		*Lactobacillus sakei* CTC494		*Listeria monocytogenes* CTC1034	
			RMSE[c]	RMSE	RMSE	Lm_{cri}[d] (cfu/mL)	RMSE	Ls_{cri}[e] (cfu/mL)	RMSE	F_{LmLs}[f]	RMSE	F_{LsLm}[g]
1:1	2.2	17	0.314	0.423	0.349	$1.00*10^8$	0.469	$5.00*10^7$	0.314	0.00	0.370	0.84
1:2		14	0.293	0.189	0.335	$1.00*10^8$	0.215	$5.00*10^7$	0.293	0.00	0.181	0.94
1:3		11	0.224	1.214	0.350	$1.00*10^8$	0.369	$1.90*10^7$	0.294	0.00	0.308	2.67
1:1	5.0	14	0.370	0.387	0.371	$1.00*10^8$	0.316	$7.49*10^7$	0.371	0.00	0.316	0.90
1:2		13	0.279	1.058	0.322	$1.00*10^8$	0.687	$5.00*10^7$	0.274	0.00	0.481	1.54
1:3		10	0.139	1.240	0.139	$1.00*10^8$	0.612	$4.62*10^7$	0.139	0.00	0.612	1.46
1:1	8.1	14	0.216	0.666	0.217	$1.00*10^8$	0.592	$7.16*10^7$	0.216	0.00	0.530	1.20
1:2		11	0.300	0.930	0.300	$1.00*10^8$	0.609	$5.00*10^7$	0.301	0.00	0.606	1.87
1:3		11	0.190	1.796	0.190	$1.00*10^8$	0.978	$5.00*10^7$	0.190	0.00	0.973	1.86
1:1	12.1	16	0.213	1.138	0.210	$9.99*10^7$	0.328	$8.92*10^7$	0.214	0.00	0.312	1.63
1:2		14	0.108	1.424	0.108	$1.00*10^8$	0.382	$7.57*10^7$	0.124	0.00	0.341	1.81
1:3		11	0.105	2.050	0.105	$1.00*10^8$	1.089	$7.51*10^7$	0.105	0.00	1.089	1.95

[a] Ratio of inoculation of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in sterile fish juice where the ratios 1:1, 1:2 or 1:3 represent the initial concentrations of 2 log cfu/mL for the *L. monocytogenes* strain and 2, 4, 6 log cfu/mL for the *L. sakei* strain, respectively.

[b] n, number of data (sampling points) for *L. sakei* CTC494 and *L. monocytogenes*.

[c] RMSE, Root mean square error.
[d] Lm_{cri} maximum critical concentration for *L. monocytogenes* CTC1034 obtained from the Jameson´s modified model.
[e] Ls_{cri} maximum critical concentration for *L. sakei* CTC494 obtained from the Jameson´s modified model
[f] F_{LmLs} competition factor of *L. monoctytogenes* CTC1034 in *L. sakei* CTC494 obtained from the Lotka Volterra model.
[g] F_{LsLm} competition factor of *L. sakei* CTC494 in *L. monoctytogenes* CTC1034 obtained from the Lotka Volterra model

Table 6. Predictive performance of the Lotka Volterra model when applied to simulate the simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in sea bream fillets under modified atmosphere packaging stored under isothermal and non-isothermal conditions.

Temp. (°C)	n[a]	*Lactobacillus sakei*			*Listeria monocytogenes*		
		N_0 (LAB) cfu/g	RMSE[b]	ASZ[c]	N_0 (Lm) cfu/g	RMSE	ASZ
4.8	14	3.36	0.555	79 %	1.83	0.593	79 %
8.2	14	3.55	0.378	79 %	1.65	0.452	79 %
Profile 1 (4-8)	12	3.71	0.894	75 %	1.66	0.309	92 %
Profile 2 (2.5-12)	13	3.96	0.645	77 %	1.65	0.615	77 %

[a] n, number of data (sampling points) for *L. sakei* CTC494 and *L. monocytogenes* CTC1034.
[b] RMSE, Root mean square error
[c] ASZ, acceptable simulation zone defined as ± 0.5 log-units from the simulated log cfu/g values (Møller et al., 2013).

Figure captions

Figure 1. A schematic overview of the modelling approach used in this study. Solid lines represent the experiments carried out for data generation, while dotted and dashed lines stand for the model building process and interaction model simulation, respectively.

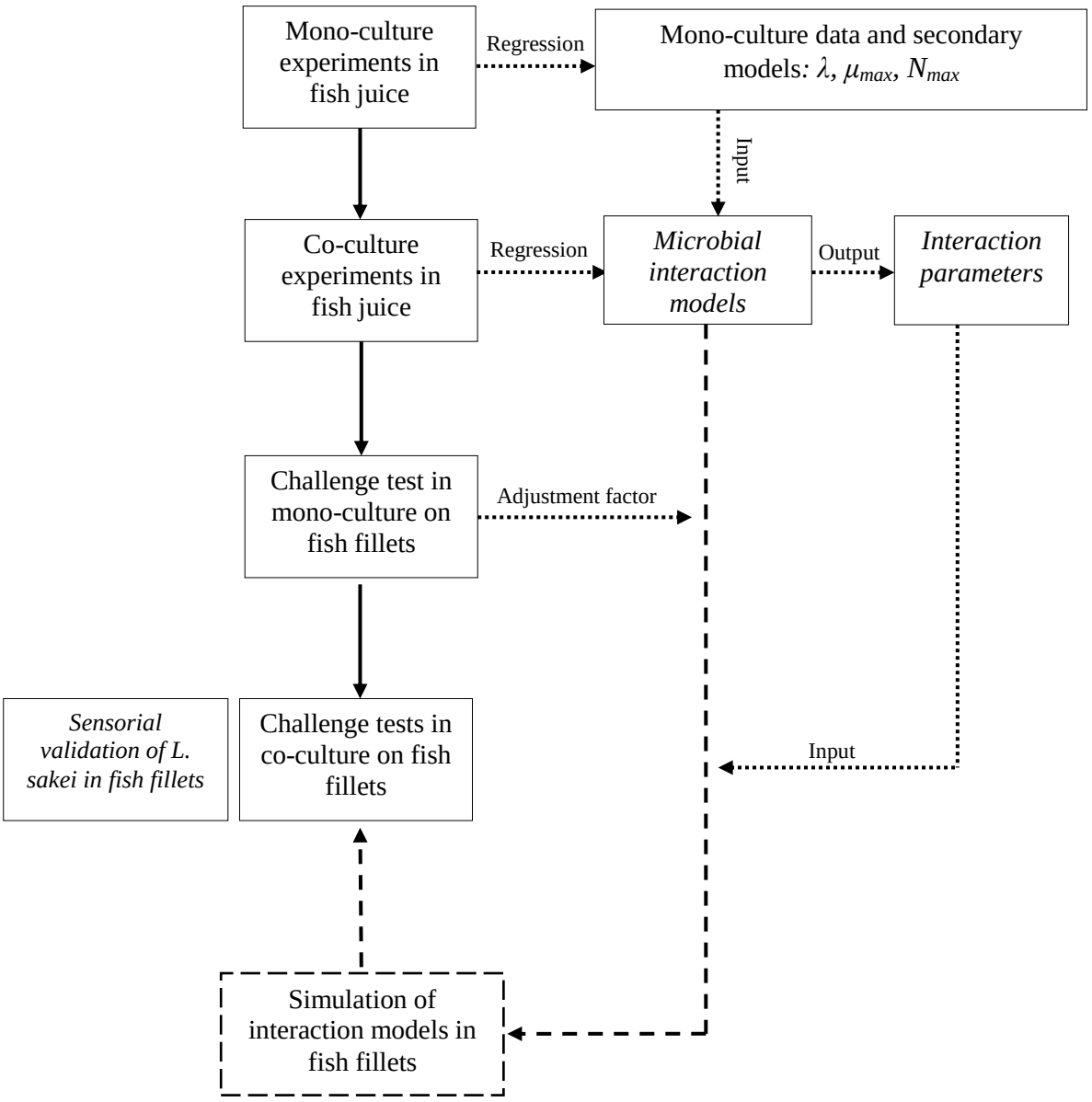
Figure 2. Reduction ratio (α) of the parameters (a) lag time (λ) and (b) N_{max} for *Listeria monocytogenes* CTC1034 (black bars) and *Lactobacillus sakei* CTC494 (greys bars) in co-culture on sterile juice fish of sea bream at different storage temperatures with three inoculation ratios of *L monocytogenes*: *L. sakei*. The negative bars represent an increase in co-culture for the specific parameter. No growth of *L. monocytogenes* was observed at the ratio 1:3 (NG).

Figure 3. Experimental observed data and fitted Jameson (dotted line), modified Jameson (dashed line) and Lotka Volterra (solid line) models for *Lactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (♦) in sterile fish juice of sea bream stored at (a, b, c) 2.2, (d, e, f) 5.0, (g, h, i) 8.1 and (j, k, l) 12.1 °C for the inoculation ratios of *L. monocytogenes*: *L. sakei*, 1:1, 1:2 and 1:3, respectively. The grey dotted line stands for the storage temperature recorded.

Figure 4. Experimental observed data (mean and standard deviation of 3 replicates) and simulations provided by the predictive model based on the Lotka Volterra equation for *Latobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (♦) on sea bream fillets under modified atmosphere packaging at isothermal conditions: (a) 4.8 °C, (b) 8.2 °C; and dynamic temperature conditions (c) profile 1 (4-8 °C) and (d) profile 2 (2.5-12.0 °C). Dashed and solid line represent the simulations for *L. sakei* and *L. monocytogenes* strains, respectively. Dotted lines show the acceptable simulation zone (ASZ) used to compare observations versus predictions of the interaction between *L. sakei* CTC494 and *L. monocytogenes* CTC1034. Grey dashed line stands for the storage temperature recorded.

Figures

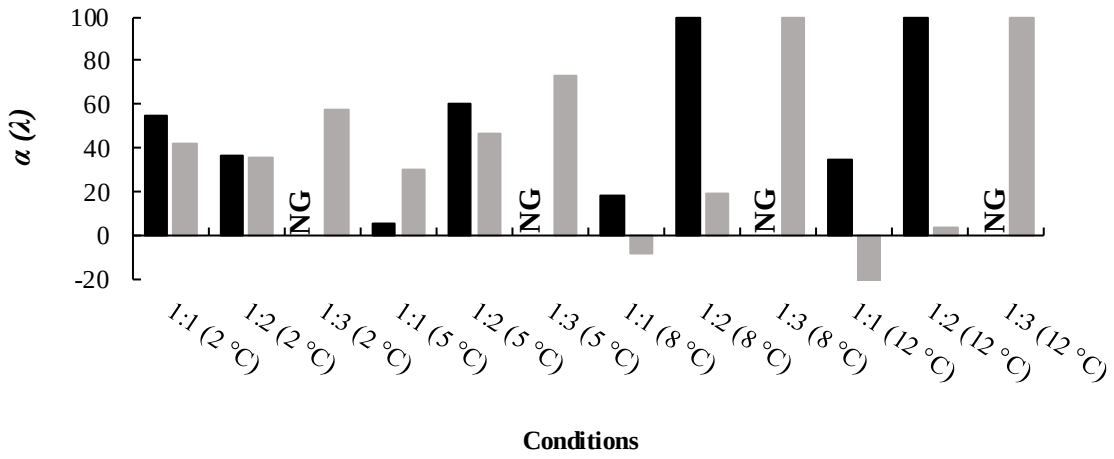
Figure 1



Lag phase duration: λ, maximum specific growth rate: μ_{max} and maximum population density: N_{max}

Figure 2

(a)


α (λ)
100
80
60
40
20
0
-20
NG
NG
NG
1:1 (2 °C)
1:2 (2 °C)
1:3 (2 °C)
1:1 (5 °C)
1:2 (5 °C)
1:3 (5 °C)
1:1 (8 °C)
1:2 (8 °C)
1:3 (8 °C)
1:1 (12 °C)
1:2 (12 °C)
1:3 (12 °C)
Conditions

(b)

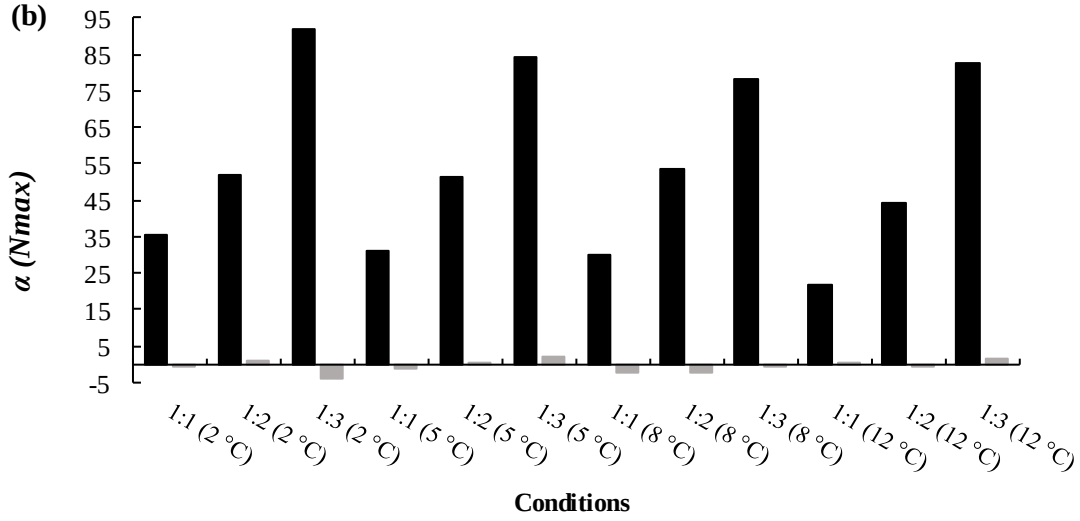

α (Nmax)
95
85
75
65
55
45
35
25
15
5
-5
1:1 (2 °C)
1:2 (2 °C)
1:3 (2 °C)
1:1 (5 °C)
1:2 (5 °C)
1:3 (5 °C)
1:1 (8 °C)
1:2 (8 °C)
1:3 (8 °C)
1:1 (12 °C)
1:2 (12 °C)
1:3 (12 °C)
Conditions

Figure 3

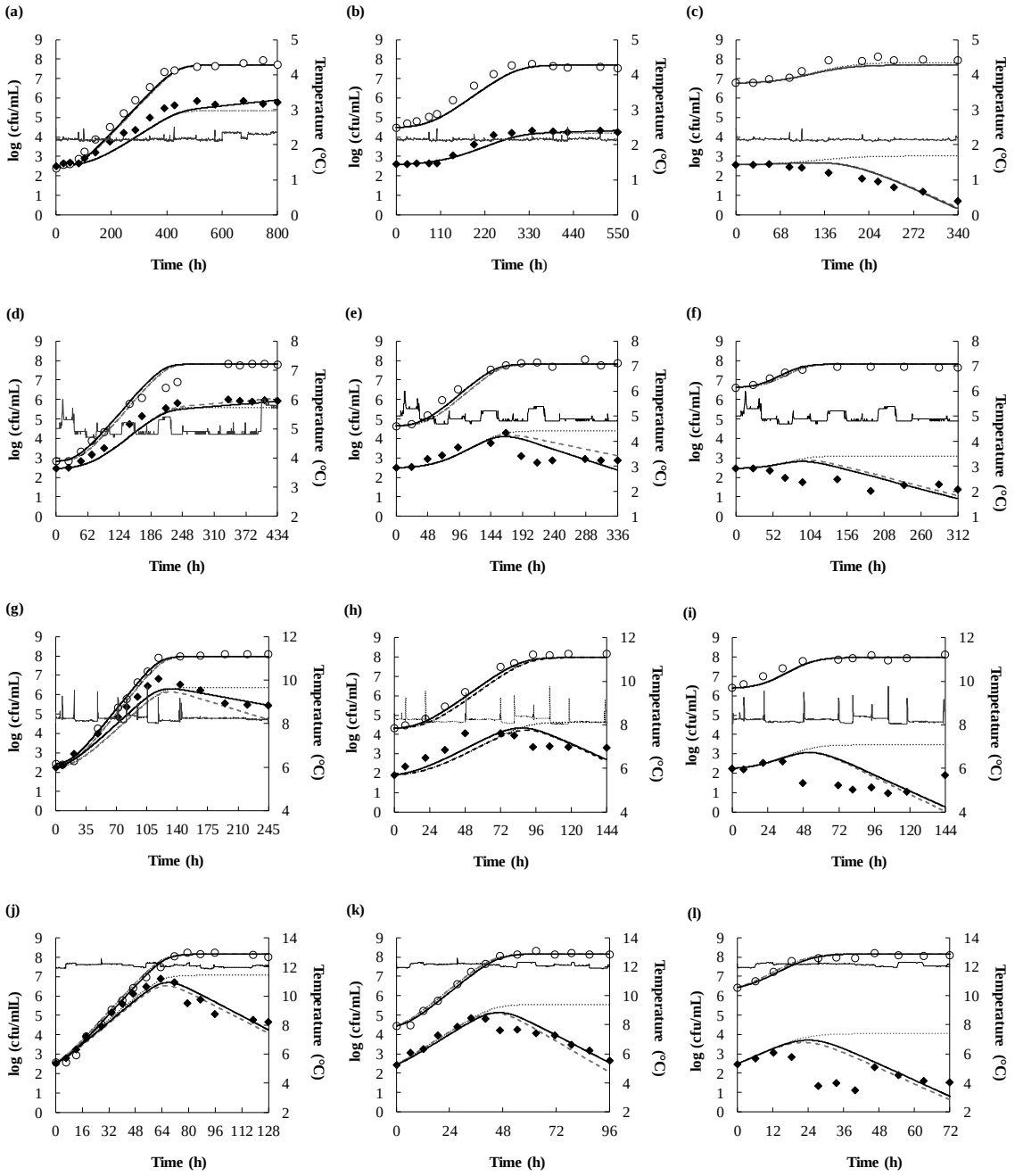
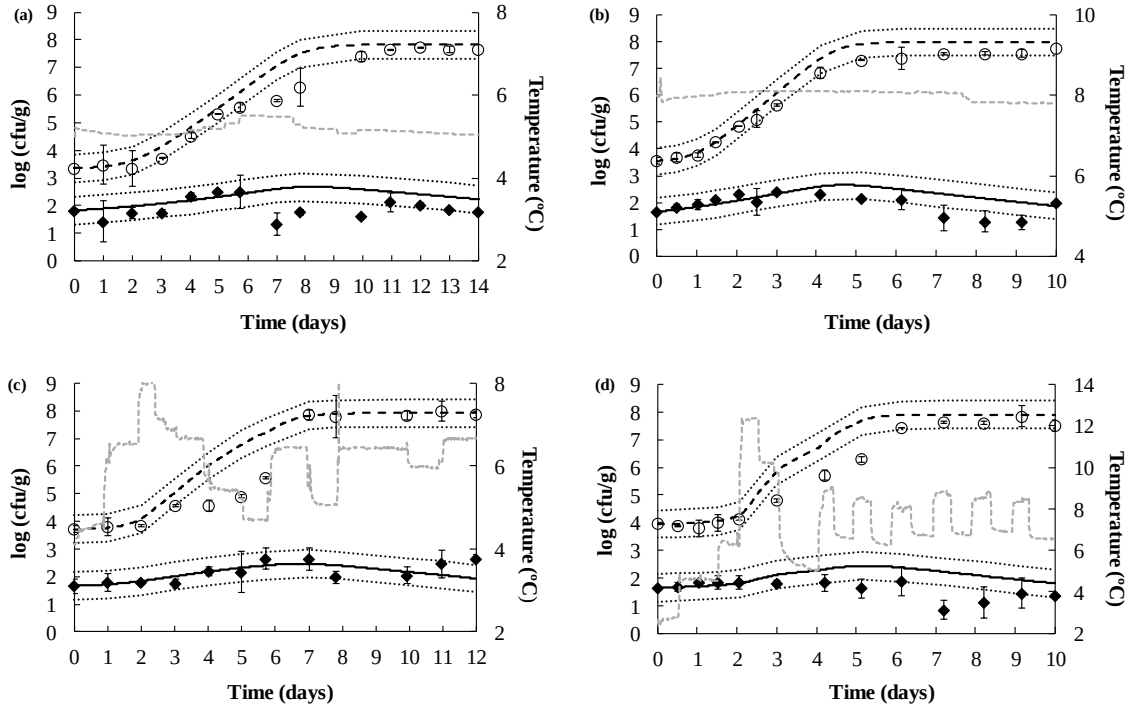
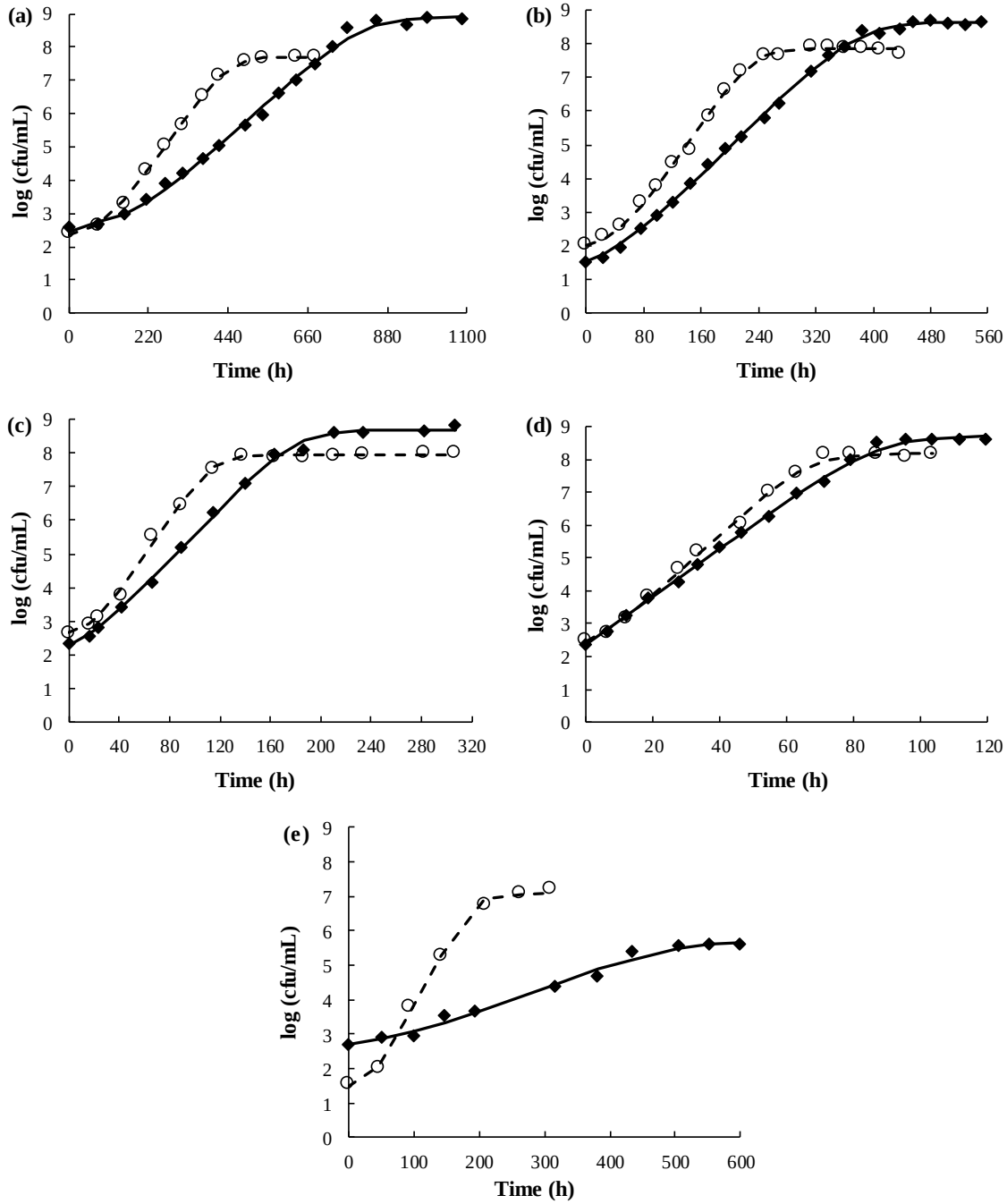


Figure 4


(a)
log (cfu/g)
Temperature (°C)
Time (days)
(b)
log (cfu/g)
Temperature (°C)
Time (days)
(c)
log (cfu/g)
Temperature (°C)
Time (days)
(d)
log (cfu/g)
Temperature (°C)
Time (days)



Supplementary Figure S1. Growth curves of *Lactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (♦) in mono-culture obtained in sterile fish juice of gilthead sea bream at (a) 2.2, (b) 5.0, (c) 8.1 and (d) 12.1 °C and (e) on sea bream fillets at 4.8 °C. Dashed line and solid line represent the fittings for the *L. sakei* and *L. monocytogenes* strains, respectively, obtained with the Baranyi and Roberts model.