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Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions

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Abstract

A systematic experimental procedure for fish shelf-life modelling was used to develop a model for predicting the quality of fish in the chill chain. For this, the growth of the naturally occurring bacteria pseudomonads, *Shewanella putrefaciens*, Enterobacteriaceae, lactic acid bacteria and yeasts, on gilt-head seabream (*Sparus aurata*), was studied at temperatures from 0 to 15°C. The results from the microbiological, organoleptical and chemical analysis conducted on naturally contaminated fish as well as on inoculated sterile fish blocks identified pseudomonads as a good spoilage index. Growth of pseudomonads was modelled as a function of storage temperature and correlated to organoleptical shelf life. To reduce the time required for the enumeration of the initial pseudomonads number, which is crucial information for shelf life prediction, a conductance assay was established. Compared with the conventional microbiological tests, this method gave results in one-fourth of the time.

Keywords: Fish spoilage; Mathematical modelling; Impedance technique

1. Introduction

Spoilage of fish and fish products is a result of the production of off-odours and -flavours mainly caused by bacterial metabolites (Gram and Huss, 1996). The constantly increasing awareness of fish quality assurance has focused the attention on mathematical modelling to quantify and predict microbial behavior. Microbial growth models can be used to predict the effect of various time–temperature combinations on fish shelf life in a production and distribution chain. However, the use of a model which accurately predicts growth over a range of conditions may often lead to misleading shelf life predictions. This can be explained by the fact that depending on intrinsic and extrinsic factors, each product has its own specific spoilage microflora and as a consequence, a microbial growth model is applicable within a limited range defined by the origin of the species, the type of product and the storage conditions. Thus, in order to achieve accurate predictions of shelf life, it is essential to choose and apply a microbial model based on the spoilage process of the product of concern. Dalgaard (1995a) considered that the main requirements for shelf life predictions were to collect information about (i) the

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specific spoilage organisms (SSO) i.e. the microorganisms responsible for spoilage; (ii) the spoilage domain (S.D.) i.e. the range of environmental conditions over which a particular SSO is responsible for spoilage and (iii) the spoilage level (SL) i.e. the population level of SSO at which spoilage occurs. If SSOs and SL for a fish product have been identified, shelf life could be easily predicted using one of the existing growth models for the SSOs within their spoilage domain (Dalgaard et al., 1997b).

Mathematical models have been published for the growth of a number of spoilage bacteria such as *Photobacterium phosphoreum* (Dalgaard, 1995a; Dalgaard et al., 1997b), pseudomonads (Ratkowsky et al., 1982, 1983; Neumeyer et al., 1997; Pin and Baranyi, 1998), *Shewanella putrefaciens* (Dalgaard, 1995a) and *Brochothrix thermosphacta* (McClure et al., 1993). Most of these models have been developed using data obtained from experiments conducted in liquid media. The main reason for this is that in laboratory media, different factors can be controlled more easily than in actual food and a large number of data can be collected within a short time. However, it has often been shown that such models overestimate the growth actually occurring in the real food as they do not take into account significant factors for microbial growth such as structure and microbial interaction (Robins and Wilson, 1994; Brocklehurst et al., 1995; Gram and Melchiorsen, 1996). Another approach on predictive models development is using data from experiments conducted directly on natural contaminated products. Despite the practical difficulties of product experiments the use of this approach minimizes the prediction error since significant errors due to growth substrate and microbial diversity are eliminated (Pin et al., 1999).

Besides mathematical models, prediction of remaining shelf life at any point of the chill chain requires reliable estimates of the initial population of SSO since this can vary significantly from batch to batch, depending on a number of factors such as season, feeding, catching method and handling after catch. Since traditional microbiological methods for bacterial counts are laborious and have long time required to obtain results, growth models have to be combined with rapid methods for microbial enumeration. Among the microbiological methods which can be used to determine bacterial counts within a short period of time the impedance is the most promising (McMeekin et al., 1993; Koutsoumanis et al., 1999; Gibson, 1985). A combination of mathematical models and impedance technique could provide information about the remaining shelf life within less than 24 h and significantly improve distribution and marketing in the fish industry.

The objective of the present study was to establish and apply a systematic, standard experimental procedure based on spoilage (microbiological, sensory, chemical) studies, mathematical modelling and studies on impedance technique in order to develop a microbial spoilage model for rapid predictions of shelf life of gilt-head seabream (*Sparus aurata*), a species of great importance in South Europe.

2. Material and methods

2.1. Description of the experimental procedure

A graphical representation of the experimental approach used in the present study is shown in Fig. 1. The following stages were applied: Stage 1: Microbiological, sensory and chemical studies were conducted with naturally contaminated gilt-head seabream stored aerobically at different temperatures from 0 to 15°C; Stage 2: Strain mixtures of organisms isolated from gilt-head seabream at the time of organoleptical rejection were inoculated in sterile fish blocks and microbiological, sensory and chemical analysis were carried out. The results from these two stages were used to define the SSO and their spoilage level; Stage 3. The growth data derived from stage 1 were used to model the effect of temperature on the growth of SSO; Stage 4: An impedance technique was established for the rapid enumeration of the initial SSO level in gilt-head seabream; Stage 5: A shelf life model was developed based on the growth of SSO; Stage 6: Individual experiments with gilt-head seabream were conducted at various temperature conditions within the range tested in stage 1 (0–15°). Observed SSO growth and shelf lives were compared to the predicted ones in order to validate the model.

2.2. Studies with naturally contaminated fish

Gilt-head seabream (*Sparus aurata*), a Mediterranean fish of high consumption and hence commercial

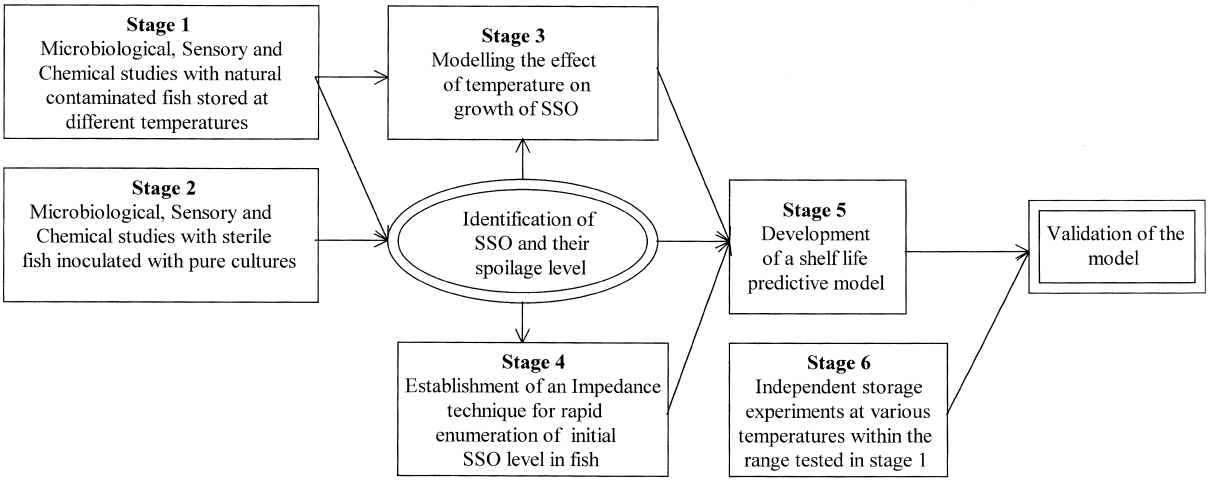

Fig. 1. Experimental procedure followed in the present study in order to develop a microbial model for rapid predictions of shelf life of gilt-head seabream (*Sparus aurata*). SSO: specific spoilage organisms.

interest in Greece, was studied. Four replicated storage experiments were carried out with ungutted fresh gilt-head seabream transported to the laboratory in ice within 6 h after catching. The fish were stored in individual pouches (not sealed), at controlled temperature conditions (0, 5, 10 and 15°C) in high precision low temperature incubators (MIR Sanyo). Samples were taken at appropriate time intervals to allow for an efficient kinetic analysis of sensory quality, chemical changes and microbial growth.

For the validation of the model (stage 5) four individual storage experiments were carried out with fresh gilt-head seabream stored at 0, 3, 8 and 15°C.

2.3. Sample preparation

Fish (25 g) was transferred to a stomacher bag (Seward, London, UK), 225 ml of 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added and homogenized for 60 s with a stomacher (Lab Blender 400, Seward Medical, London, UK).

2.4. Microbiological media and enumeration

Samples (0.1 ml) of ten-fold serial dilutions of either treated (inoculated with the isolates) or naturally contaminated fish homogenates were spread on the surface of the appropriate media in Petri dishes for enumeration of: (i) total aerobic viable count on a modified Long and Hammer's agar (mLHA) (Van Spreekens, 1974) and incubated at 10°C for 7 days. The medium was composed of ($g\,l^{-1}$ distilled water): proteose peptone (Sigma, P 0431), 20; gelatine (Merck, 4070), 40; K_2HPO_4, 1; NaCl, 10; agar (Oxoid, L11), 15; ammonium ferric citrate, 0.25; (ii) pseudomonads on cetrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103) and incubated at 20°C for 2 days (Mead and Adams, 1977); (iii) Yeasts on rose bengal chloramphenicol agar, incubated at 25°C for 7 days and wrapped in aluminium foil to avoid any adverse effects of light; For Enterobacteriaceae and hydrogen sulphide-producing bacteria, 1.0 ml was inoculated into 10 ml of molten (45°C) violet red bile glucose agar (VRBGA, Oxoid code CM 485) and Iron Agar (IA, Oxoid code CM 867), respectively. After setting, a 10 ml overlay of molten medium was added. For the former, incubation was at 30°C for 24 h. The large colonies with purple halos were counted (Mossel et al., 1979). Iron agar plates were incubated at 20°C for 4 days (Gram et al., 1987). Black colonies formed by the production of H_2S were enumerated after 2–3 days (Gennari and Campanini, 1991). Three replicates of at least three appropriate dilutions (Anonymous, 1978) were enumerated.

2.5. Bacterial strains

Pure cultures of *Pseudomonas* spp. and *S. putrefaciens*, were screened in sterile muscle blocks of gilt-head seabream for their spoilage potential, i.e., their ability to produce chemical changes typical of the spoiling product. All strains of each group were selected at the time of organoleptical rejection of gilt-head seabream stored aerobically at 5°C. Colonies were isolated from iron agar and *Pseudomonas* agar supplemented with CFC (Mead and Adams, 1977). They were characterized further as, *S. putrefaciens* and *Pseudomonas* spp. respectively. These isolates were examined for the shape of colony and pigmentation (King et al., 1954), Gram reaction (Gregersen, 1978), cell morphology (phase contrast microscopy), flagella arrangement (Mayfield and Inniss, 1977), oxidase reaction (Kovacs, 1956), aerobic–anaerobic breakdown of glucose (Hugh and Leifson, 1953) and TMA, H_2S production (Gram et al., 1987). Gram negative, motile rods with positive catalase and oxidase reactions, an oxidative glucose metabolism, and arginine dihydrolase activity were identified as pseudomonads if they do not reduce TMAO or produce H_2S. The identity of *S. putrefaciens* was confirmed by salmon pink pigment on nutrient agar and H_2S, TMA production in test tubes with TMAO-medium (Gram et al., 1987).

The inocula were prepared as follows: each bacterial strain was maintained on slopes of an appropriate agar medium at 4°C. A loopful of a fresh working subculture (ca. 10^6 cfu) was used for inoculation of the corresponding broth (100 ml in 250-ml conical flasks). The flask was incubated aerobically without agitation at 25°C for 18 h. Cells harvested by centrifugation and washed with sterile saline and an appropriate dilution in saline (NaCl, 0.85%, w/v) were used for inoculation.

2.6. Studies with sterile fish blocks

Fish tissue blocks were prepared after Herbert et al. (1971). Sterile tissue cut into 40–50 g portions and ten of these were inoculated immediately with the mixture of pseudomonads strains and ten portions with *S. putrefaciens*. The inoculum target was 10^4 cfu/g. The inoculated tissues were placed in sterile 17-cm plastic Petri dishes and stored at 5°C. All inoculations were done in duplicate and two independent trials were carried out. When sampling, one piece from each case was taken for microbiological, physicochemical and sensory analysis.

2.7. Chemical analysis

Total volatile nitrogen (TVN) and trimethylamine (TMA) were determined according to the modified Conway's micro-diffusion method (Conway and Byrne, 1933). Extracts were prepared by mixing 25 g of homogenized flesh with 75 ml of water. After lowering the pH to 5.2 and warming to 70–80°C, the mixture was filtered and the filtrate frozen at −20°C.

2.8. Evaluation of organoleptical shelf life

Whole fish was examined by a trained sensory panel of six persons who evaluated the odour of raw and the taste and odour of cooked fish. Fish were scaled, gutted and gilled before cooking. Fish were cooked whole, individually wrapped steam tightly in aluminum foil, at 180°C for 30 min. An adaptation of a simple three-point scoring system (Dalgaard et al., 1993; Taoukis et al., 1999a,b) was used. Taste and odour was judged and recorded in appropriate forms with descriptive terms reflecting the organoleptic evolution of quality deterioration. Rating was assigned on a continuous 0 to 3 hedonic scale (0 being the highest quality score and 2 the limit of acceptance). In the case of sterile fish blocks the same methods was used but the organoleptic evaluation analysis was carried out only in raw material.

2.9. Impedance measurements for the enumeration of pseudomonads

The Rapid Automated Bacterial Impedance Technique (RABIT) from Don Whitley Scientific (Shipley, UK) was used for the impedance measurements. A test lasted 48 h with a measurement every 6 min; the TTD (time to detection) signal appeared when there were three consecutive measurements of 5 μS minimum. Metronidazole, carbenicilline, cetrimide, cycloheximide, diamide (MCCCD) (Salvat et al., 1997) was used as conductance medium.

One hundred and four fish samples of different microbiological quality were tested. Fish (25 g) was transferred to a stomacher bag (Seward, London,

UK), 225 ml of 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added and homogenized for 60 s with a stomacher (Lab Blender 400, Seward Medical, London, UK). A volume (500 μl) of this dilution was used to inoculate the impedance tube and 100 μl were used to inoculate cetrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103) (Mead and Adams, 1977). Each colony growing on CFC agar was confirmed as pseudomonads by an oxidase test in which a solution of 1% *N,N,N′,N′*-tetramethylphenylene-1, 4-diamine dichlorohydrate, mixed in equal parts with 1% α-naphydratethol alcoholic solution was poured onto the surface of the agar. The oxidase-positive colonies appeared dark blue.

2.10. Data analysis

The growth data from the enumeration of different groups of microbial association were modelled as a function of time in order to estimate the maximum specific growth rate and the Lag phase. The log-transformed form of the four-parameter logistic model was used (Dalgaard, 1995a):

$$\mathrm{Log}\,N(t) = \mathrm{Log}\left\{N_{\min} + \frac{N_{\max} - N_{\min}}{1 + \exp[-\mu_{\max}{}^{*}(t - t_i)]}\right\} \quad (1)$$

In Eq. (1) t is the time (h), $N(t)$ is the number of microorganisms at time t (cfu/g), $N_{\min}$ and $N_{\max}$ are the minimum and maximum asymptotic cell concentration (cfu/g), $\mu_{\max}$ is the maximum specific growth rate (h^{-1}) and t_i the time (h) when half of the maximum cell concentration is reached. The duration of the lag phase (Lag) was calculated as described by Dalgaard (1995a).

The obtained estimates of $\mu_{\max}$ (h^{-1}) and lag phase (h) were further expressed as a function of temperature. For comparison two different models were used:

(a) The Arrhenius model:

$$\ln(\mu_{\max}) = \ln(\mu_{\mathrm{ref}}) - \left[\frac{E_{A_\mu}}{R}\right]{}^{*}\left[\frac{1}{T} - \frac{1}{T_{\mathrm{ref}}}\right] \quad (2)$$

$$\ln\left(\frac{1}{\mathrm{Lag}}\right) = -\ln(\mathrm{Lag}_{\mathrm{ref}}) - \left[\frac{E_{A_{\mathrm{Lag}}}}{R}\right]{}^{*}\left[\frac{1}{T} - \frac{1}{T_{\mathrm{ref}}}\right] \quad (3)$$

where T (K) is the absolute temperature, T_{ref} is the reference temperature (273 K), μ_{ref} (h^{-1}) and $\mathrm{Lag}_{\mathrm{ref}}$ are the maximum specific growth rate and lag phase respectively at reference storage conditions (T_{ref}), $E_{A\mu}$ and $E_{A\mathrm{Lag}}$ (kJ/mole) are the activation energies referring to $\mu_{\max}$ and the lag phase respectively and R is the universal gas constant.

(b) The square root model (Ratkowsky et al., 1982):

$$\sqrt{\mu_{\max}} = b_\mu{}^{*}(T - T_{\min_\mu}) \quad (4)$$

$$\sqrt{\frac{1}{\mathrm{Lag}}} = b_{\mathrm{Lag}}{}^{*}(T - T_{\min_{\mathrm{Lag}}}) \quad (5)$$

where b is a constant, T (°C) is temperature and $T_{\min}$ is the respective estimated by extrapolation of the regression line to $\sqrt{\mu_{\max}} = 0$, theoretical minimum temperature for growth of the organism.

All data were fitted using non-linear regression with the FIG. P version 2.5 software (Anonymous, 1995).

3. Results and discussion

3.1. Identification of SSO and determination of their spoilage level

The experimental data for the growth of the different measured components of the natural microflora of gilt-head seabream are shown in Fig. 2 along with the fitted logistic growth curves for two representative isothermal conditions. Pseudomonads were the dominant bacteria at all temperatures tested followed by *S. putrefaciens* while the rest of the members of the microflora i.e. Enterobacteriaceae, lactic acid bacteria and yeasts remained at significantly lower levels. This is in agreement with other results reported for Mediterranean fish (Kakouri et al., 1997; Koutsoumanis and Nychas, 1999; Taoukis et al., 1999a,b). At all temperatures, growth of

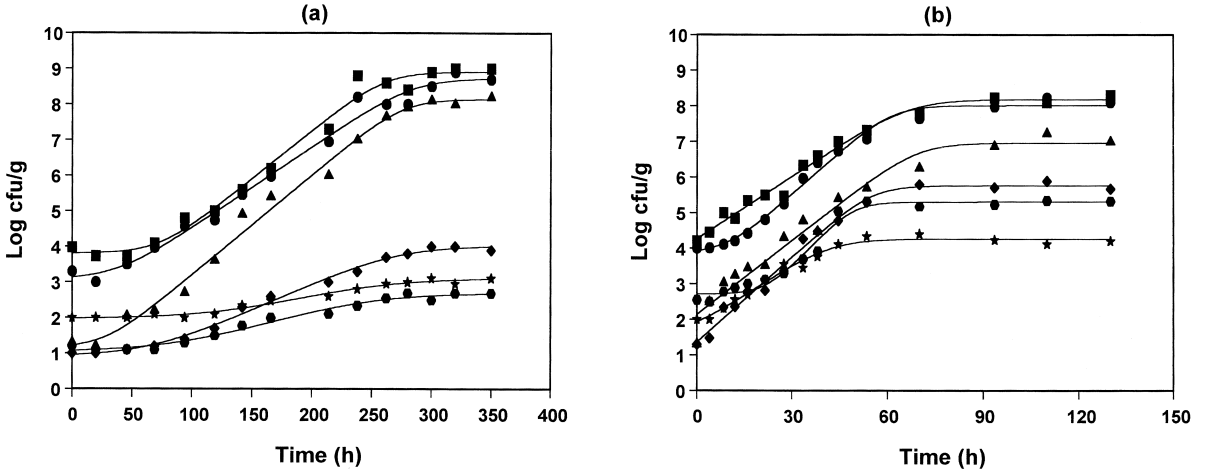

Fig. 2. Development of the microbial flora of Mediterranean gilt-head seabream (*Sparus aurata*) stored aerobically at 0 (a) and 10 (b) °C (■: total viable count, ●: pseudomonads, ▲: H_2S-producing bacteria, ✦: Enterobacteriaceae, ★: yeasts, ♦: lactic acid bacteria).

Pseudomonas spp. and *S. putrefaciens* followed closely the decrease of sensory quality and the end of shelf life coincided with an average level of $10^{7.0}$ and $10^{6.1}$ cfu/g respectively (Table 1).

Since fish spoilage is the result of off-odours and -flavours caused by metabolic products of bacteria, the qualitative and quantitative determination of the compound(s) responsible for spoilage is essential for the identification of the SSOs and their spoilage level (SL). Total volatile basic nitrogen (TVBN) and trimethylamine (TMA) have been reported as spoilage compounds and proposed as fish spoilage indicators in many studies (Ehrenberg and Shewan, 1955; Shewan and Jones, 1957; Burmet, 1965; Lannelongue et al., 1982; Malle and Poumeyrol, 1989; Dalgaard et al., 1993). In the present study, TMA was found at very low concentrations even after a long storage period of natural contaminated seabream (Fig. 3a). Similar results with other Mediterranean fresh fish and possible explanations are given in the literature (Drosinos and Nychas, 1996; Dalgaard et al., 1997a; Drosinos et al., 1997; Koutsoumanis and Nychas, 1999).

In contrast to TMA production, the total volatile basic nitrogen (TVBN), which includes the measurements of TMA, DMA (dimethylamine), ammonia and other volatile basic nitrogen compounds (Huss, 1995; Oehlenschlager, 1997), increased exponentially during storage and reached relatively high levels (50–100 mg/100 g) at the end of storage period depending on the storage temperature (Fig. 3b). Similar results for gilt-head seabream stored in ice

Table 1
Microbial (pseudomonads and *S. putrefaciens*) populations at the beginning of storage time and at the time of organoleptical rejection of Mediterranean gilt-head seabream (*Sparus aurata*) stored aerobically at 0, 5, 10 and 15°C

T (°C)	Pseudomonads (Log_{10} cfu/g)		*S. putrefaciens* (Log_{10} cfu/g)		Shelf life (h)
	N_0 [b]	N_S [c]	N_0	N_S	
0	3.18±0.02[a]	7.11[d]±0.02	1.57±0.39	6.49±0.11	212±4.2
5	3.24±0.01	7.11±0.09	2.20±0.04	6.14±0.16	104±8.5
10	3.93±0.10	6.88±0.37	1.39±0.12	5.97±0.20	48.0±4.2
15	4.20±0.07	6.99±0.26	1.23±0.33	5.90±0.30	29.5±0.7

[a] Average±standard deviation.
[b] Initial population (Log_{10} cfu/g).
[c] Population (Log_{10} cfu/g) at the time of organoleptical rejection.
[d] Value estimated from the logistic equation by setting time equal to shelf life.

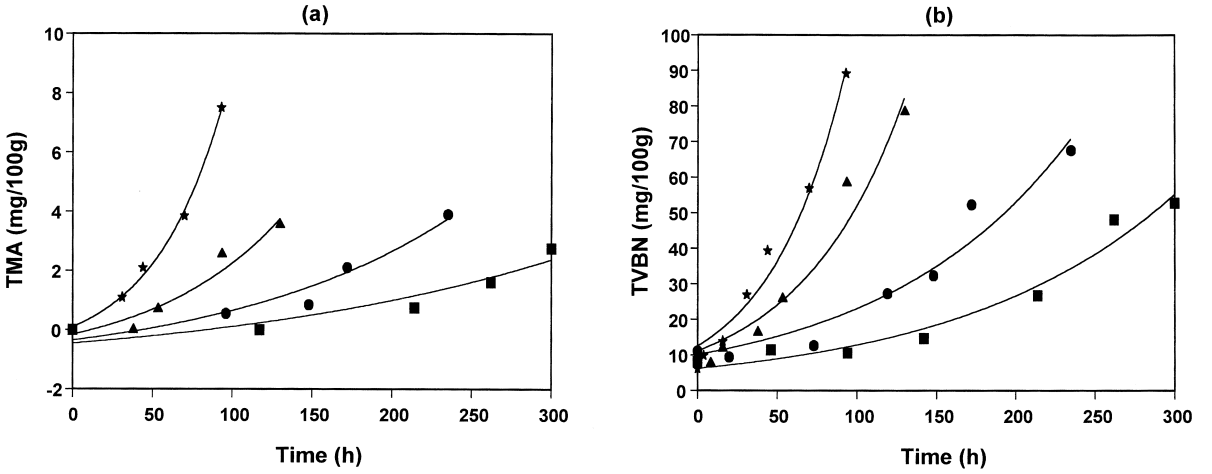

Fig. 3. Changes in concentration of trimethylamine (a) and total volatile basic nitrogen (b) during aerobic storage of Mediterranean gilt-head seabream (*Sparus aurata)* at 0 (■), 5 (●), 10 (▲) and 15 (★) °C. Each point is an average of two measurements.

have been reported previously (Kyrana et al., 1997) At all temperatures tested, the time of sensory rejection coincided with an average TVBN concentration of 26 mg/100 g (value estimated from the regression line of Fig. 3b). Similar amounts of TVBN at the end of shelf life have been reported for other fish species (Oehlenschlager, 1997). Ammonia, the other component of TVBN, has been successfully used as a spoilage indicator of fresh fish quality (Crooks and Ritchie, 1946; Hughes, 1959; Field et al., 1986). In our study, the low formation of TMA (Fig. 3a) as well as the insignificant amount of DMA measurements during storage of fresh fish found in other studies (Miller et al., 1973; Dalgaard et al., 1993; Guldager et al., 1998) could possibly account for the significant contribution of ammonia to increase of TVBN. This was also supported by the ammonia-like odours of the spoiled fish samples observed by the panel during the organoleptical analysis. Similar odours were observed in the sterile fish blocks inoculated with *Pseudomonas* spp. when the bacterial counts reached the level of 10^7 cfu/g. At that time the level of TVBN was very close to that found at the organoleptical rejection of natural contaminated gilt-head seabream (Fig. 4a). On the other hand, in the case in sterile fish blocks inoculated with *S. putrefaciens*, TVBN started to increase when the population of the organism exceeded 10^8 cfu/g (Fig. 4b) and only after that time did the panel observe off-odours which were similar to these detected at the advanced spoilage stage of the natural contaminated fish. The information derived from the first part of the experimental procedure followed in the present study led to the conclusion that a spoilage model for aerobically stored gilt-head seabream can be based on pseudomonad growth while a pseudomonad level of 10^7 cfu/g is a good determinant for the end of shelf life (Table 2).

3.2. Establishment of an impedance method for the rapid enumeration of the initial pseudomonads count in gilt-head seabream

The main advantage of kinetic spoilage models is that they allow initial microbiological quality to be taken into account. However, the variability of initial microbial flora on Mediterranean fish (Lambropoulou, 1999) as well as the fact that conventional microbiological methods needs about 48–72 h to provide results limits their potential use in shelf life prediction. Among the microbiological methods which can be used to detect microbial counts within a relative short time, impedance technique is one of the most promising. Impedance instruments have been used for the determination of microbial count in different products (Salvat et al., 1997; Gibson, 1985; Gibson and Hobbs, 1987; Gibson and Ogden, 1987; Gibson et al., 1984). In the present study, an impedance method enumerating pseudomonads associated with gilt-head seabream was established using

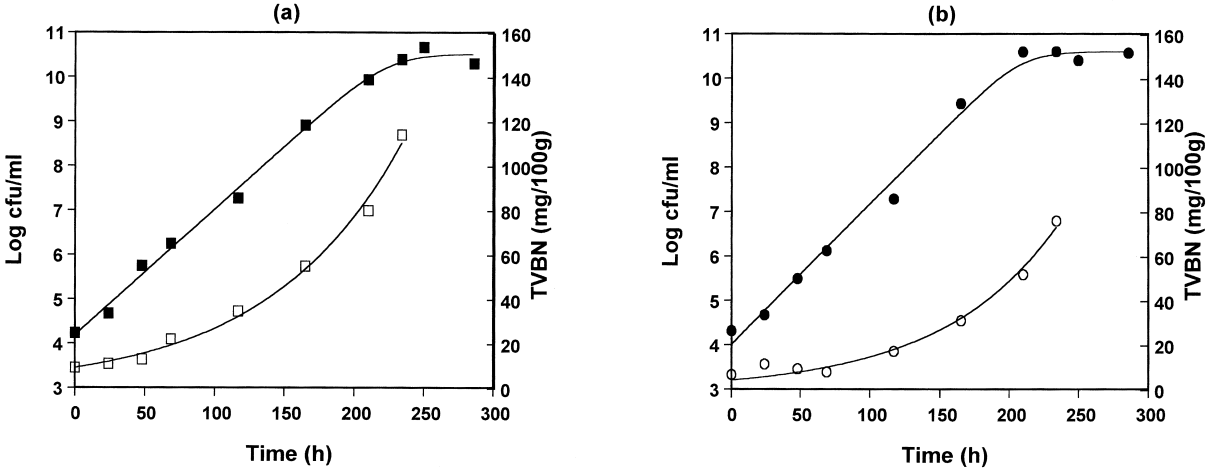

Fig. 4. Growth (solid symbols) of pseudomonads (a) and *S. putrefaciens* (b) on blocks of fish stored at 5°C and changes in concentration of total volatile basic nitrogen (open symbols).

Table 2
Maximum specific growth rate (μ_{max}), lag phase (LP) and maximum cell concentration (N_{max}) of pseudomonads grown on Mediterranean gilt-head seabream (*Sparus aurata*) stored aerobically at 0, 5, 10 and 15°C

Temperature °C	μ_{max} (h^{-1})	LP (h)	N_{max} (Log_{10} cfu/g)
0	0.055[a]±0.0002	38.8±3.7	8.61±0.17
5	0.105±0.008	18.3±0.8	8.17±0.02
10	0.191±0.009	11.8±1.2	7.94±0.09
15	0.296±0.013	7.56±0.08	8.25±0.27

[a] Average±standard deviation.

a selective conductance medium previously used for the detection of pseudomonads in poultry meat (Salvat et al., 1997). One hundred and four fish samples of different microbiological quality (from fresh to spoiled) were tested. A satisfactory agreement ($r = -0.950$) between time to detection (TTD) and pseudomonads count (Log cfu/g) was found (Fig. 5). The equation (parameters±95% confidence limits) of the regression line was:

$$\text{Log}_{10}\,\text{cfu/g} = -0.341(\pm 0.022)^{*}\text{TTD} + 9.63(\pm 0.30) \quad (6)$$

The time to detect (TTD) pseudomonads in samples with initial counts 10^3 cfu/g were achieved within 20 h.

3.3. Modelling the growth of SSO — shelf life prediction

The pseudomonads kinetic parameters (μ_{max}, lag phase and N_{max}) from four individual replicated experiments with gilt-head seabream are shown in Table 2. At all temperatures tested, the maximum concentration (N_{max}) of pseudomonads was found to be constant with an average value of 8.24±0.27 Log_{10} cfu/g (average±S.D.). Further, the temperature dependence of pseudomonads kinetic parameters was modelled using the Arrhenius and the square root equations (Fig. 6). The estimated values and statistics of the two model parameters are shown in Table 3. The correlation coefficient of the regression for the Arrhenius and the square root model (Table 3) showed that the latter better described the temperature dependence of pseudomonads. This has been previously reported for other microorganisms (Dalgaard, 1996; McMeekin et al., 1993). Although several growth models for pseudomonads have been reported (Pin and Baranyi, 1998; Neumeyer et al., 1997), they have been developed in systems simulating the real food (laboratory media). It has often been shown, that such models overestimate the

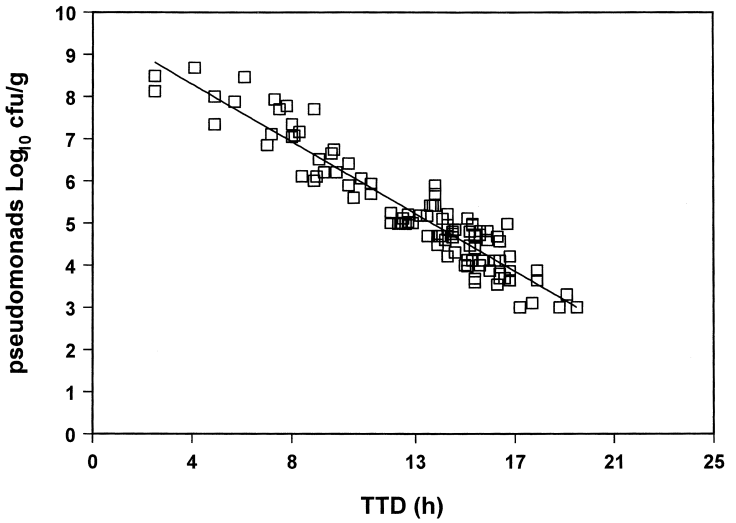

Fig. 5. Linear regression between time to detection (TTD) (h) derived from the impedance instrument and pseudomonad population (Log_{10} cfu/g) of Mediterranean gilt-head seabream (*Sparus aurata*).

Table 3
Predicted (prd) and observed (obs) kinetic parameters, maximum specific growth rate (μ_{max}), lag phase (LP), and initial population (N_0) of pseudomonads, grown on Mediterranean gilt-head seabream (*Sparus aurata)* stored aerobically at 0, 3, 8 and 15°C

Temperature °C	N_0 [a] (VC) Log_{10} cfu/g	N_0 [b] (TTD) Log_{10} cfu/g	μ_{max} obs (h^{-1})	μ_{max} prd[c] (h^{-1})	LP obs (h)	LP prd[c] (h)
0	4.65	4.41	0.052	0.050	39.7	38.0
3	4.45	4.10	0.0957	0.083	30.3	24.5
8	3.10	3.32	0.158	0.155	24.3	13.9
15	2.95	3.66	0.313	0.292	8.90	7.64

[a] Initial pseudomonads level estimated with the viable count method.
[b] Initial pseudomonads level estimated with the impedance method [Eq. (4)].
[c] Predicted values estimated with the square root models [Eqs. (4) and (5)].

growth of microorganisms actually occurring in the real food. For example, Pin et al. (1999) applied the pseudomonads model of Pin and Baranyi (1998) to real food and reported a prediction error over 40% due to growth substrate and microbial diversity. In the present study, possible effects of structure and microbial interaction on the growth of pseudomonads are 'included' in the model since all the growth data were obtained from experiments conducted in naturally contaminated, real fish.

As the maximum growth rate (μ_{max}) and the lag phase of pseudomonads can be estimated from the square root models (Tables 3,4) and the N_{max} was found to be constant (8,24) the aerobic growth of pseudomonas on gilt-head seabream can be predicted for any storage temperature from 0 to 15°C using the logistic equation as following:

$$t \leq \text{lag phase} \quad N(t) = N_0$$

$$t > \text{lag phase} \quad N(t) = \frac{N_{max}}{1 + \left[\frac{N_{max}}{N_0} - 1\right] * \exp[-\mu_{max} * (t - t_{Lag})]} \tag{7}$$

By replacing N_0 (initial pseudomonads level, cfu/g) with time to detection (TTD) taken from the impe-

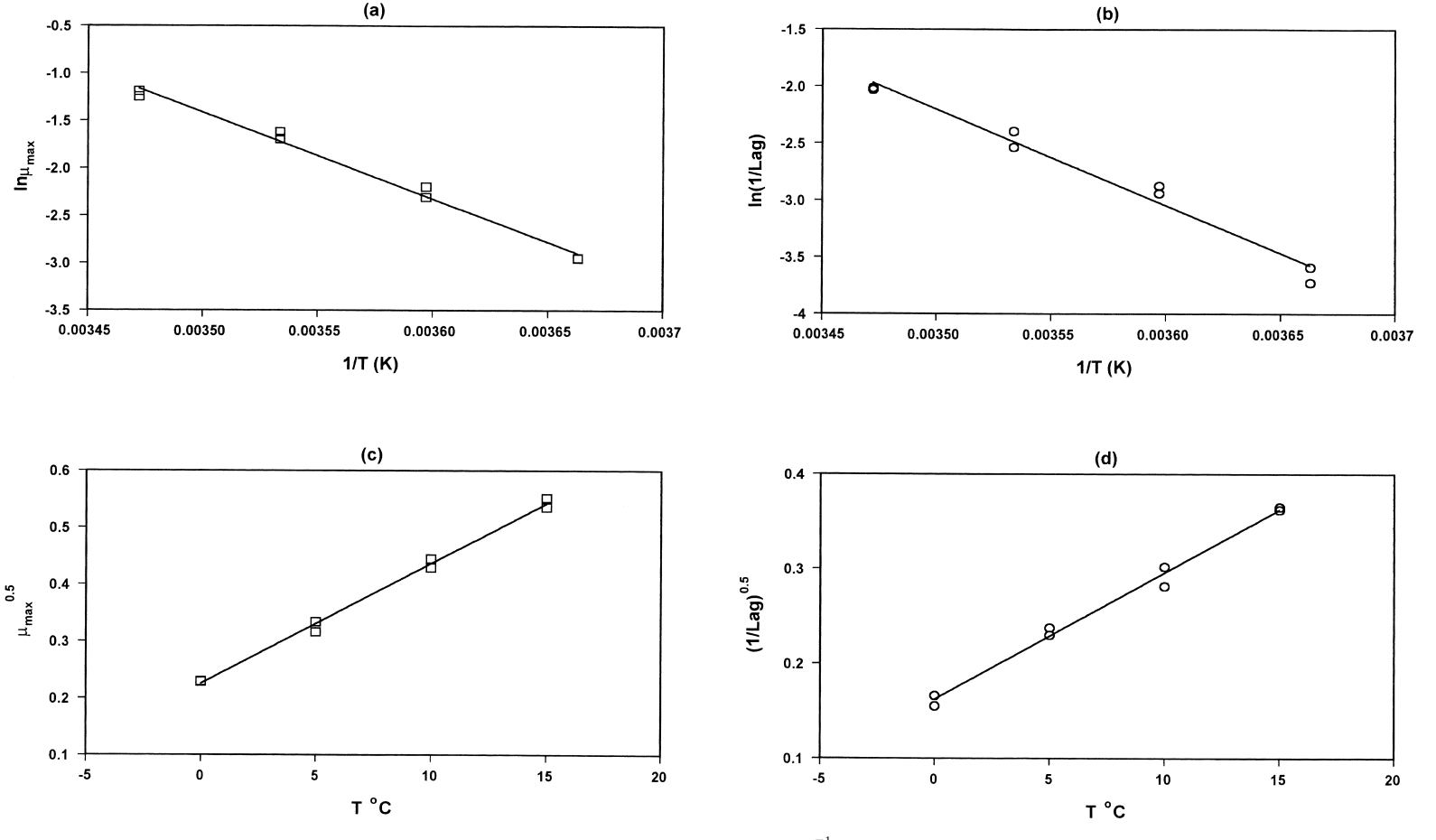

Fig. 6. Arrhenius (a,b) and square root (c,d) plots showing the temperature dependence of μ_{max} (h^{-1}) and lag phase (h) of pseudomonads growth on Mediterranean gilt-head seabream (*Sparus aurata*).

Table 4
Parameters and statistics of the Arrhenius and square root models [Eqs. (2), (3), (4) and (5)] for the maximum specific growth rate (μ_{max}) and lag phase (LP) of pseudomonads, grown on Mediterranean gilt-head seabream (*Sparus aurata*) stored aerobically at 0, 5, 10 and 15°C

Parameter	Value	±95% C.I.	r^2
Arrhenius			
μ_{ref}	0.0552	0.0025	0.989
E_{A_μ}	75.7	7.6	
Lag_{ref}	35.7	5.5	0.977
$E_{A_{Lag}}$	70.0	10.7	
Square root			
b_μ	0.0211	0.0014	0.995
T_{min_μ}	−10.65	1.29	
b_{Lag}	0.0133	0.00124	0.991
$T_{min_{Lag}}$	−12.19	1.91	

Table 5
Predicted (prd) and observed (obs) shelf lives (SL) of Mediterranean gilt-head seabream (*Sparus aurata*) stored aerobically at 0, 3, 8 and 15°C

Temperature °C	SL_{obs} [a] (h)	SL_{prd} (VC) [b] (h)	SL_{prd} (TTD) [c] (h)
0	145	147	158
3	100	97	106
8	80	72	69
15	48	40	34

[a] Shelf life observed from the organoleptical analysis.
[b] Shelf life predicted from Eq. (9) (N_0 estimated with the viable count method).
[c] Shelf life predicted from Eq. (10) (N_0 estimated with the impedance method).

dance method [Eq. (6)] Eq. (7) changes to:

$$t \le \text{lag phase} \quad N(t) = \exp(-0.341*\text{TTD} + 9.66)$$

$$t > \text{lag phase} \quad N(t) = \frac{N_{max}}{1 + [\frac{N_{max}}{(-0.341*\text{TTD} + 9.66)} - 1]*\exp[-\mu_{max}*(t - t_{Lag})]} \quad (8)$$

Shelf life of aerobically stored gilt-head seabream can be predicted as the time required by pseudomonads (SSO) to multiply from the initial level to $10^{7.02}$ (spoilage level). This can be easily estimated form Eq. (7):

$$\text{Shelf life } (h) = t_{Lag} + \frac{1}{\mu_{max}}*\ln\left\{\frac{10^{8.24} - 10^{N_0}}{15.6*10^{N_0}}\right\} \quad (9)$$

By replacing N_0 with TTD taken from the impedance method [Eq. (6)]

$$\text{Shelf life } (h) = t_{Lag} + \frac{1}{\mu_{max}} *\ln\left\{\frac{10^{8.24} - 10^{(-0.341*\text{TTD}+9.66)}}{15.6*10^{(-0.341*\text{TTD}+9.66)}}\right\} \quad (10)$$

Using Eq. (10) shelf life prediction of a gilt-head seabream is available in less than one day (time required by the impedance method to give response) while if the classical microbiological methods were used [Eq. (9)] the prediction would be available after 3 days.

3.4. Validation of the model

Four independent experiments with gilt-head seabream at different temperature conditions (0, 3, 8 and 15°C) were carried out in order to evaluate the applicability of the developed model. Predicted values such as pseudomonads kinetic parameters (μ_{max}, lag phase) estimated with the square root model were compared to the observed ones (Table 4). In addition initial pseudomonads populations derived from the impedance method [Eq. (4)] were compared to these estimated with the viable count method (Table 4). Both predicted growths of pseudomonads calculated with Eq. (7) (N_0 estimated with the viable count method) and Eq. (8) (N_0 estimated with the impedance method) were very close to the observed growth data (Fig. 7). The difference between observed and predicted shelf life was less than 20% in all cases (Table 5). The results derived from the validation showed that the model developed in this study gave rapid and realistic predictions of Mediterranean gilt-head seabream shelf life. The present study shows a systematic experimental procedure to be followed for chilled fish shelf life modelling in order to achieve an effective tool for quality monitoring and prediction. However, further studies on the kinetic behavior of spoilage should verify the developed model under dynamic storage conditions.

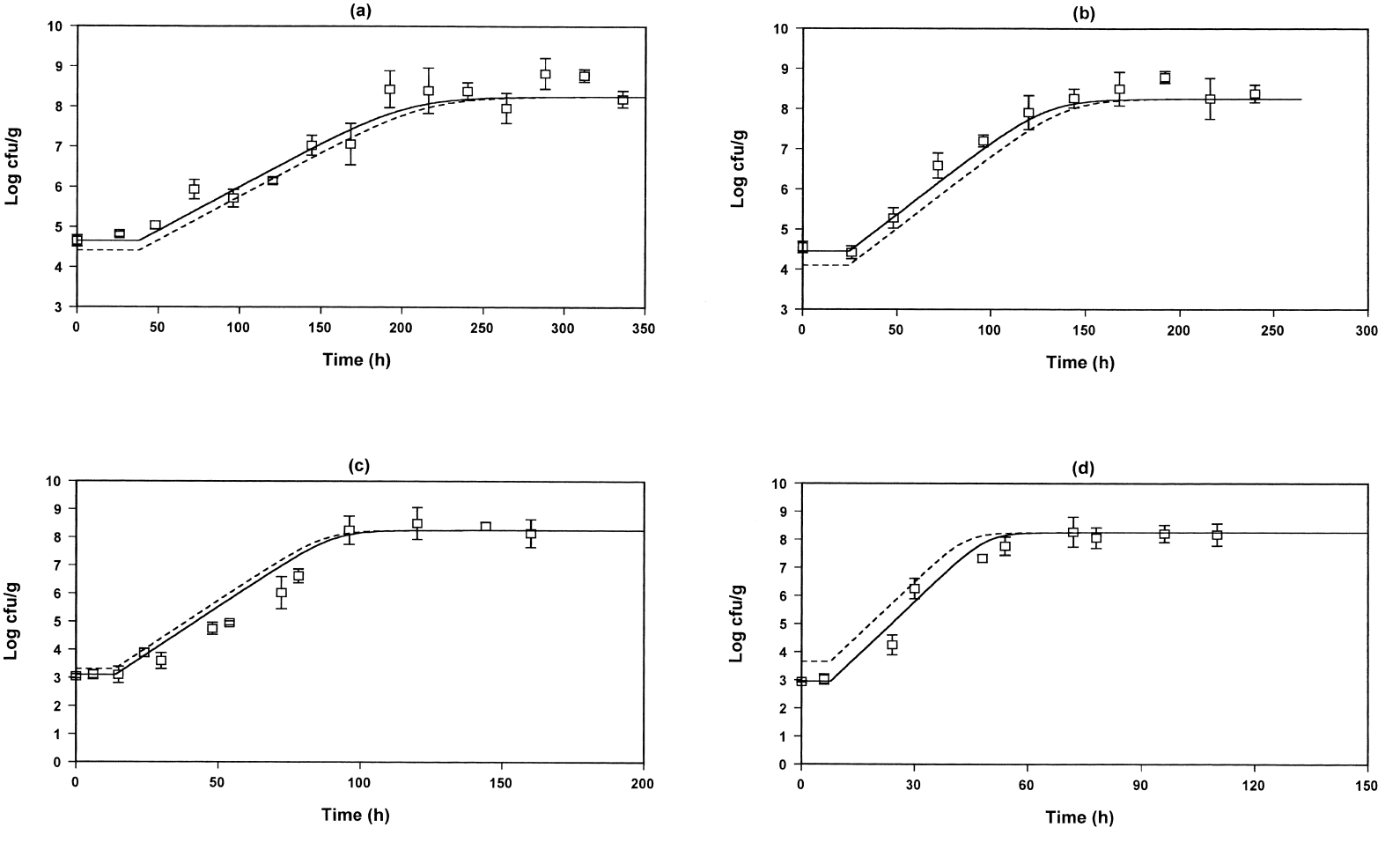

Fig. 7. Comparison between predicted growth (lines) and observed growth (points) of pseudomonads on Mediterranean gilt-head seabream (*Sparus aurata*) stored aerobically at 0 (a), 3 (b), 8 (c) and 15 (d) °C. The solid line represent the prediction derived from Eq. (9) (N_0 estimated with the viable count method) and the dotted line represents the prediction derived from Eq. (10) (N_0 estimated with the impedance method).

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