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Original article

A new approach to modelling the shelf life of Gilthead seabream (*Sparus aurata*)

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Summary A total of 217 Gilthead seabreams were subdivided in four groups, according to four different storage conditions. All fish were evaluated by both Quality Index Method (QIM) and microbiological analysis, sampling skin, gills and flesh, separately. A QIM score predictive system was set by modelling the growth of microflora of skin, gills and flesh and coupling these predictions to each related partial QIM score (QIM_{Skin}, QIM_{Gills}, QIM_{Flesh}). The expression of QIM score as a function of bacterial behaviour was carried out by the employment of two coefficients. The predicted mean bacterial concentrations corresponding to the QIM score at 14 days were always near to Log 8 CFU g^{-1} in the case of 'S' (skin) and 'G' (gills) series. Moreover, predicted QIM scores were in a good agreement with observed data, reproducing the observed mean time of rejection as well as the bacterial spoilage level (Log 8 CFU g^{-1}), for all kinds of storage condition.

Keywords Predictive model, quality index method, *Sparus aurata*, spoilage bacteria.

Introduction

Loss of freshness of fish is the consequence of post-mortem biochemical, physicochemical and microbiological processes as well as of several extrinsic factors such as the handling on board and on land, and technological processing. These changes are appreciable in sensory terms and can be evaluated by sight, touch, smell and taste.

The Quality Index Method (QIM) is a scoring system for freshness and quality sensorial estimation of fishery products, developed by the Tasmanian Food Research Unit (Bremner, 1985) and, in the last decade, has been developed for several species and products (Jonsdottir, 1992, 1992; Larsen *et al.*, 1992; Martinsdóttir & Arnason, 1992; Andrade *et al.*, 1997; Warm *et al.*, 1998; Huidobro *et al.*, 2000; Luten, 2000; Sveinsdottir *et al.*, 2003; Barbosa & Vaz-Pires, 2004; Sykes *et al.*, 2009). A range of demerit points is assigned to the set of characteristic attributes of each parameter; the scores are summed to give an overall sensory score, the Quality Index (QI).

Several authors (Hyldig & Green-Petersen, 2004; Huidobro *et al.*, 2000; Larsen *et al.*, 1992) have obtained a linear correlation between the QI score and the time of storage in ice, providing a prediction of freshness for a given fishery product. This kind of prediction, however, is not directly related to the behaviour of spoilage agents and it applies only to the storage in ice. In this context, mathematical models for the seafood shelf life have been proposed predicting the spoilage agents' behaviour or obtaining the expected spoilage compound production (Ratkowsky *et al.*, 1983; Mcclure *et al.*, 1993; Dalgaard, 1995; Dalgaard *et al.*, 1997; Neumeyer *et al.*, 1997; Pin & Baranyi, 1998). In the case of bacterial prediction, the shelf life is obtained by calculating the time of the spoilage microflora takes to reach the Minimum Spoilage Level (MSL), as proposed for *Sparus aurata* by Koutsoumanis & Nychas (2000) or, according to Dalgaard *et al.* (1997), by explicating the 'spoilage criterion'. The latter approach allows to model the trimethylamine (TMA) production as a function of specific spoilage bacteria (SSB) growth by the calculation of a 'yield factor' that links the bacterial load to the TMA production (Dalgaard, 1995).

Some of the above studies have related the SSB behaviour to the product sensorial rejection, which was determined, however, on the basis of different kinds of approach. These discrepancies in the shelf life evaluating techniques have reduced the reliability of

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the evaluation, producing different results for the same product stored under equal conditions. This is the case of Gilthead seabream (*Sparus aurata*). Koutsoumanis & Nychas (2000), for example, have found a shelf life of 8/9 days at 0 °C, showing that the end of Gilthead seabream shelf life coincides with a pseudomonads average level of Log 7 CFU g^{-1}; these observed results were simulated by using a predictive model for pseudomonas. On the other hand, Huidobro *et al.* (2000), applying the QIM methods to a large sample of *Sparus aurata* specimens, have found a shelf life of 14/15 days. Lougovois *et al.* (2003) have studied the shelf life of *Sparus aurata* stored in ice by sensorial, chemical and microbiological parameters (QIM evolution, k_1 value, GR Torrymeter, and bacterial counts of flesh), finding a rejection time of 16 days. However, they used a different QIM scheme compared with Huidobro *et al.* (2000) as well as a different sampling site (only the flesh) for microbiological analysis compared with Koutsoumanis & Nychas (2000), finding a MSL of Log 5.9 CFU g^{-1} for sulphide-producing bacteria (SPB). Moreover, in fish as Gilthead seabream, the bacterial penetration through the skin can result very slowly because of the skin/scales thickness and aspecific immunological defences of the cutaneous mucus (Giuffrida *et al.*, 2005). For these reasons, the sensorial modifications involving Gilthead seabream stored in ice could be mainly due to the growth of spoilage bacteria on skin and gills. These sites, in fact, are those mainly taken into account by the most important sensorial evaluation schemes such as QIM. Therefore, a precise prediction of whole-fish freshness as well as of the QIM score would be carried out by modelling separately the behaviour of SSB on skin, gills and flesh.

To verify this hypothesis, the aim of this study was to evaluate the SSB load of skin, gills and flesh during different storage conditions of *Sparus aurata* specimens and to model the behaviour of these bacterial populations obtaining, at the same time, a related QIM score using a cause-effect approach.

Materials and methods

Fish, storage conditions and sampling plan

Ten batches of Gilthead seabream (*Sparus aurata*) (300–500 g), fasted for 48 h, were obtained from three Italian fish farms (farm 1, 2 and 3) and killed by immersion in ice-water slurry. After death, fish were packed in expanded polystyrene boxes with perforated bottoms, covered by a plastic film with ice flakes on top and freighted to the laboratory within 2 h. At the laboratory, the fish were subdivided in four groups. The first group (Group 1) consisted of 147 fish subdivided in seven batches, each containing twenty-one specimens. All samples were covered by ice and stored at 2 ± 1 °C for 21 days and ice was added to the boxes as required. This group was employed to carry out seven replicated trials (twenty-one fish for each trial; three trials from farm 1, two from farm 2 and two from farm 3) to characterise the variability of fish shelf life and to obtain a better parameterisation for Eqn 1 with particular regard to coefficients Beta 1 and Beta 2. The second group (Group 2) consisted of one batch of twenty-eight fish from farm 1, which were stored without ice at 4 ± 1 °C for 21 days, while Group 3 and 4 (one batch of twenty-one fish for each group, always from farm 1) were stored under an experimental fluctuating temperature (T) regime. For group 3, a temperature fluctuation of ±2 °C around a mean T of 4 °C was set while, for group 4, the same fluctuation was set around a mean T of 6 °C. In all groups, T was monitored by four data loggers (FT 800; Econorma, Vendemmiano, Italy) for each batch, placed in opercula, recording the T value every 5.45 min.

Microbiological and sensorial evaluations were carried out after 0, 72, 168, 216, 336, 408 and 504 h from the beginning of storage, by sampling three fish for each time interval. Microbiological assays were performed by sampling, with sterile instruments, 10 g of dorsal skin, 5 g of gills and 20 g of dorsal flesh; this last kind of sample was obtained from the opposite side where skin was sampled, rinsing the skin with 70% ethanol and removing the flesh aseptically.

Microbiological analyses

Each sample was transferred to a stomacher bag and 0.1% peptone water was added with a ratio of 1:9 (w/v); samples were homogenised for 60 s at 230 rpm, with a stomacher (Stomacher® 400 Circulator; International PBI s.p.a., Milan, Italy) and tenfold dilutions in 0.1 peptone water were prepared. 1-mL aliquots were plated, in duplicate, in Iron Agar (Gram *et al.*, 1987). Total viable counts, selective counts of hydrogen sulphide-producing and hydrogen sulphide non-producing bacteria were enumerated after 3 days incubation at 20 °C. Black colonies were recorded as sulphide-producers, whereas white colonies were counted as sulphide non-producers. A representative percentage (about 10%) of white and black colonies was picked and purified by restreaking onto tubes of Trypticase soy agar (Oxoid, Basingstoke, UK). These isolates were examined for cell morphology, Gram reaction, biochemical tests (API 20E system; BioMérieux, Marcy l'Etoile, France) and oxidase test (identification sticks, Oxidase BR64, Oxoid).

Sensorial evaluation

For sensory evaluation, the QIM scheme developed by Huidobro *et al.* (2000) for raw whole Gilthead

seabream was used. The method considers parameters relating to surface and eyes appearance, odour, elasticity of the muscle and gills, and takes into account a maximum of 15 demerit points. The QIM scheme was applied by an expert panel of three persons.

Mathematical predictive model

To construct the predictive model, QIM parameters were grouped in three main categories: QIM_S, including scores for surface/eyes appearance and odour (0–10 demerit points); QIM_G related to gills scores (0–4 demerit points); QIM_F that considers the score assigned to the flesh evaluation (0–1 demerit points). QIM_S, QIM_G and QIM_F were associated with the bacterial counts on Iron Agar of skin, gills and flesh, respectively; therefore, for the dynamical prediction of each QIM category as function of the time was used the following general differential equation:

$$\frac{dQIM_{(S-G-F)}}{dt} = \frac{dNw_{(S-G-F)}}{dt}\ \beta_{1(S-G-F)} + \frac{dNb_{(S-G-F)}}{dt}\ \beta_{2(S-G-F)} \quad (1)$$

where *Nw* and *Nb* are the concentration (Log CFU g^{-1}) of sulphide non-producers (white colonies in Iron agar) and sulphide-producers (black colonies in Iron agar) bacteria, respectively, at time *t;* β_1 and β_2 are two coefficients that translate bacterial concentration into demerit points.

According to the Baranyi & Roberts (1994), the bacterial concentration *N* (*Nw* or *Nb*) at time *t* is generically expressed as follows:

$$\frac{dN}{dt} = \mu_{max}\ N\ \frac{Q}{1+Q}\left(1-\frac{N}{N_{max}}\right) \quad (2)$$

Here, μ_{max} is the maximum specific growth rate and N_{max} the theoretical maximum population density of both species under monospecific growth conditions; *Q* represents the physiological state of the species and, as expressed in Eqn (3a-b) (Baranyi & Roberts, 1994), allows to calculate the Lag-time (λ) duration (hours).

$$\lambda(t) = \frac{-\ln \alpha(t)}{\mu_{max}(t)} \quad (3a)$$

$$\alpha(t) = \frac{Q(t)}{1+Q(t)} \quad (3b)$$

The model of Baranyi & Roberts (1994) was used to calculate the main growth parameters (μ_{max}, Q, N_{max}) of all growth curves obtained from group 1 (seven batches) on Iron Agar (black and white colonies from skin, gills and flesh).

The obtained μ_{max}, *Q* and N_{max} values for series 'S', 'G', 'F' and for '*w*' and '*b*' populations, from each batch of group 1, were introduced into Eqns (1-2), whereas coefficients β_1 and β_2 for the above three series were calculated by fitting the predicted values of QIM_S, QIM_G and QIM_F to the observed QIM scores of each batch belonging to group 1; the comparison between observed and predicted QIM scores was evaluated by Root Mean Squared Error (RMSE). The Eqn (1) was numerically solved, for each batch and for all series (*S*, *G* and *F*), by the Runge and Kutta method.

A second kind of resolution of the model (Eqns 1-2) was achieved introducing two secondary predictive models for the calculation of μ_{max} for '*w*' and '*b*' population. The former model, used for $\mu_{max}(w)$, was the modified square root equation (Ratkowsky *et al.*, 1983), refined by Neumeyer *et al.* (1997) for pseudomonads; the latter, employed for $\mu_{max}(b)$, referred to *Shewanella* spp., according to Dalgaard (1995). Concerning the other parameters, N_{max} and the initial value of *Q* were obtained from the observed data, according to Bovill *et al.* (2000), whereas for the coefficients β_1 and β_2, the mean of the obtained values was used. Using these settings and considering as initial bacterial concentration, the mean value recorded for each group, the QIM trend for Groups 1, 2, 3 and 4 was predicted by applying the recorded T mean profile to express $\mu_{max}(w)$ and $\mu_{max}(b)$ values at each *t* interval. The predicted QIM values were compared with the mean scores of each group using the RMSE test.

Results and discussion

Microbiological analysis

The growth of sulphide non-producer and sulphide-producer bacteria for all groups, as shown in Figs 1–4, was very similar. The average initial count of white and black colonies, both on the skin and gills, was rather low (always <Log 3 CFU g^{-1}); the flesh had always a bacterial load <Log 1 CFU g^{-1} until the 72nd hour. In all groups, bacterial populations on skin and gills trended to increase, about in the same manner, but in groups 2, 3 and 4, this increase was quicker, reaching a concentration >Log 8 CFU g^{-1} after 168–216 h. On the contrary, the bacterial load of the flesh has never exceeded Log 4.5 CFU g^{-1}. As Figs 1–4 show, when the bacterial concentration of series '*S*' and '*G*' reaches a value $\geq$ Log 8 CFU g^{-1} and QIM_S or QIM_G scores are near to the maximum value (10 and 4, respectively), the bacterial load of flesh is always very low (<Log 4 CFU g^{-1}).

These data agree with the studies of Huss (1995) and demonstrate that main quality changes of whole refrigerated Gilthead seabream are especially explained

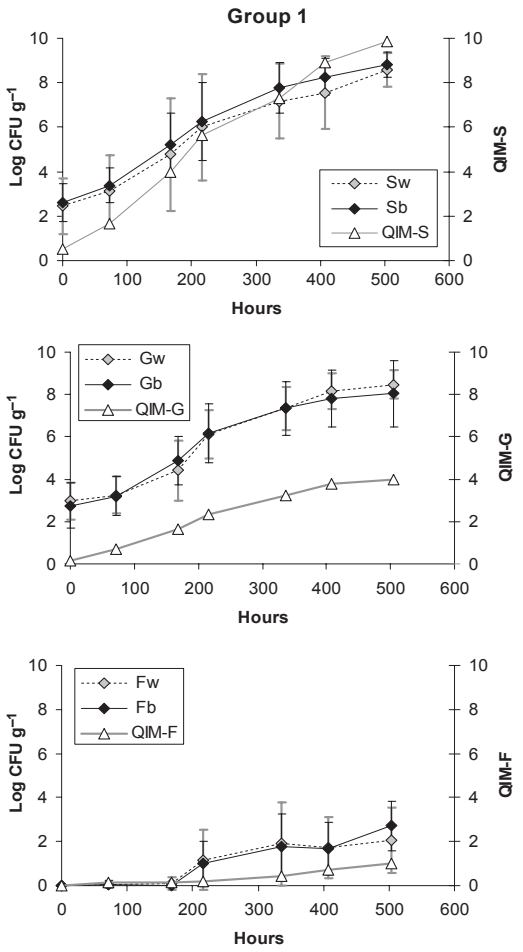

Figure 1 Group 1: growth of sulphide non-producer (w) and sulphide-producer (b) bacteria for skin (S), gills (G) and flesh (F) together with the related QIM partial score. QIMS: surface/eyes appearance and odour (0 – 10 demerit points); QIMG: gills (0 – 4 demerit points); QIMF: flesh (0 – 1 demerit points).Values are averages of twenty-one determinations; error bars indicate ±1SD.

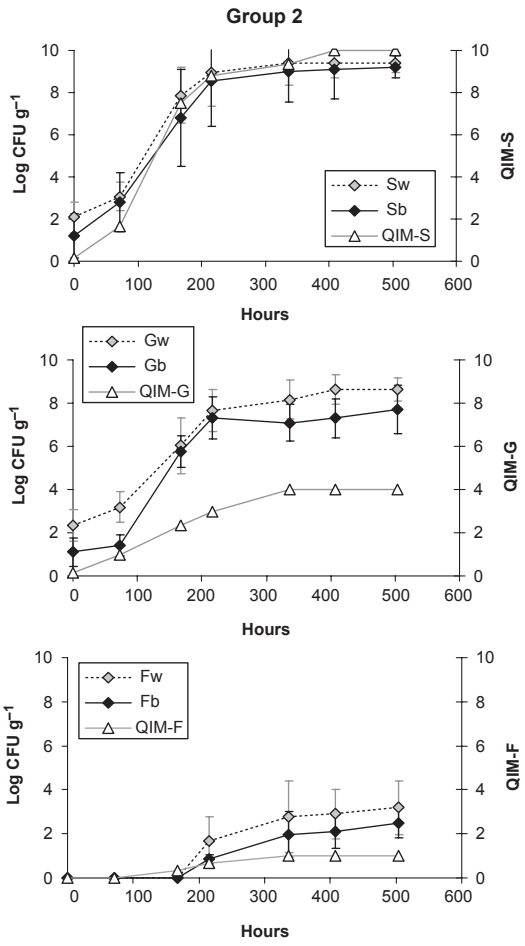

Figure 2 Group 2: growth of sulphide non-producer (w) and sulphide-producer (b) bacteria for skin (S), gills (G) and flesh (F) together with the related QIM partial score. QIMS: surface/eyes appearance and odour (0 – 10 demerit points); QIMG: gills (0 – 4 demerit points); QIMF: flesh (0 – 1 demerit points).Values are averages of twenty-one determinations; error bars indicate ±1SD.

by the bacterial growth on skin and gills, which are always, as well known, more contaminated than flesh. Moreover, the mucus of these sites is rich in proteins, glucides and lipids as well as in several ammonia excreta (Wood, 1993), which can be metabolised by bacteria in off-flavour substances by bacteria.

Concerning these bacterial populations, the morphological and biochemical tests allow to identify the 86.56% of strains as belonging to genera *Pseudomonas* and *Shewanella*. In particular, the 100% of white colonies were pseudomonads, whereas the 66.67% of black colonies belonged to *S. putrefaciens* and *S. baltica*. The remaining percentage of black colonies belonged to genera *Citrobacter* and *Proteus*. These isolations are in agreement with several results (Gram & Huss, 1996; Koutsoumanis & Nychas, 1999, 1999) concerning fish caught or harvested in temperate waters and explain the kind of spoilage of the Gilthead seabream, which is characterised by development of offensive fishy, rotten, H_2S-off-odours and -flavours. This sensory impression is distinctly different for some tropical fish and freshwater fish, where fruity, sulphydryl off-odours and -flavours are more typical (Gram & Huss, 1996).

Freshness assessment by QIM

As Fig. 5 shows, group 1 reached the mean maximum value (fifteen demerit points) after 21 days, whereas this value was registered after 17 days for groups 2–3

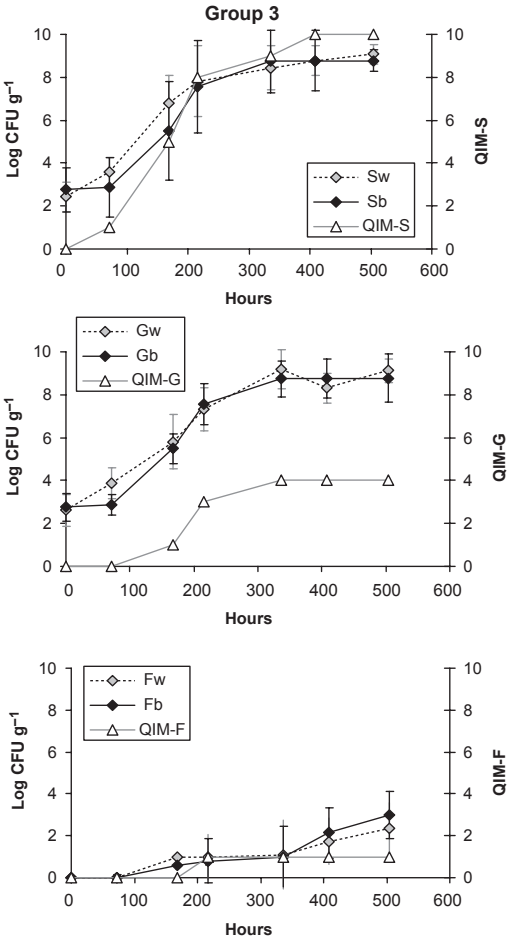

Figure 3 Group 3: growth of sulphide non-producer (w) and sulphide-producer (b) bacteria for skin (S), gills (G) and flesh (F) together with the related QIM partial score. QIMS: surface/eyes appearance and odour (0 – 10 demerit points); QIMG: gills (0 – 4 demerit points); QIMF: flesh (0 – 1 demerit points).Values are averages of twenty-one determinations; error bars indicate ±1SD.

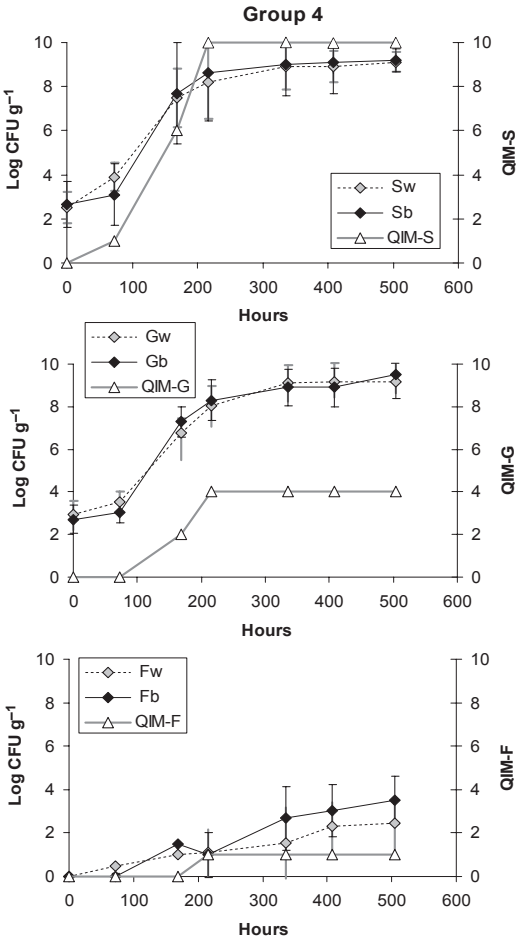

Figure 4 Group 4: growth of sulphide non-producer (w) and sulphide-producer (b) bacteria for skin (S), gills (G) and flesh (F) together with the related QIM partial score. QIMS: surface/eyes appearance and odour (0 – 10 demerit points); QIMG: gills (0 – 4 demerit points); QIMF: flesh (0 – 1 demerit points).Values are averages of 21 determinations; error bars indicate ±1SD.

and after 14 days for group 4. In all cases, the QIM score was linearly correlated to the time with a coefficient of determination (R^2) of 0.984, 0.798, 0.919 and 0.801, respectively, for groups 1, 2, 3 and 4. However, the QIM rate increase in each group, which can be represented by the slope value '*m*' in the equation (QIM = *m* * hours + *b*), did not appear related to the mean temperature value recorded for each group (group 1 = 2.49 ± 0.152 °C; group 2 = 4.49 ± 0.274 °C; group 3 = 4.40 ± 1.174 °C; group 4 = 5.40 ± 1.280 °C), since the linear regression between slope values '*m*' and mean temperatures showed a low value of the coefficient of determination (R^2 = 0.556). This suggests that the linear relation between QIM scores and time is strongly affected by the bacterial spoiling activity, which can be characterised by a large intrinsic and extrinsic variability.

QIM score prediction by mathematical model

The mean values of the coefficient β_1 and β_2 for the series '*S*', '*G*' and '*F*', calculated by fitting the predicted values of QIM_S, QIM_G and QIM_F to the observed QIM scores of each batch belonging to group 1, are reported in Table 1 together with the predicted bacterial mean concentrations at time in which QIM score is 14. In particular, according to Huidobro *et al.* (2000), this score was used since it coincides with the rejection point for cooked products. Coefficients $\beta_{1(S\text{-}G\text{-}F)}$ (related to 'white' colonies, mainly belonging

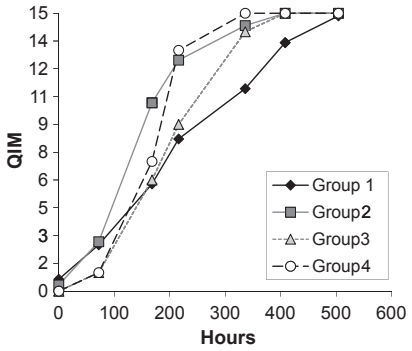

Figure 5 QIM score trends for each group. Values are averages of twenty-one determinations for group 1, four determination for group 2 and three determinations for groups 3 and 4; error bars indicate ±1SD.

to *Pseudomonas* genus) had always values higher than $\beta_{2(S\text{-}G\text{-}F)}$ ones (related to 'black' colonies, mainly belonging to *Shewanella* genus) and this confirms that pseudomonads have a greater influence on Gilthead seabream spoilage with respect to the *Shewanella* spp. population (Koutsoumanis & Nychas, 2000). The wide standard deviation of the β values indicates the high variability, which characterises the spoilage capacity of the bacterial populations of each batch. This variability is presumably due to the heterogeneity of bacterial populations of each batch and to the related intrinsic differences in the metabolic activity with respect to nutrients (especially nitrogenous compounds) present in cutaneous and branchial mucus.

As Table 1 shows, the predicted mean bacterial concentrations, corresponding to a QIM score of 14, were always near to Log 8 CFU g^{-1}, in the case of '*S*' and '*G*' series. These data are in agreement with the observed ones (Figs 1–4) as similar bacterial concentrations have been recorded when QIM_S and QIM_G are in proximity of their maximum values (10 and 4 respectively).

In Fig. 6a–d are reported the predicted and observed mean QIM scores for all groups together with the related mean temperature profiles. These predictions were obtained by introducing into Eqns (1) and (2), secondary predictive models for pseudomoads (Ratkowsky *et al*., 1983; Neumeyer *et al*., 1997) and *Shewanella* spp. (Dalgaard, 1995), to calculate μ_{max} for '*Nw*' and '*Nb*' population, respectively, as function of temperature profiles. As Figs 6a–d show, there was a good agreement with the observed mean data also considering the RMSE values for group 1, 2, 3 and 4, respectively, equal to 0.610, 0.614, 1.398 and 1.208, which appeared lower than those obtained by the linear regression model (0.666, 1.458, 1.515 and 1.328 respectively) and showed in Table 2. In this regard, the model fits the observed QIM scores both when the trend is almost linear (group 1) and when it is basically sigmoidal (groups 2, 3 and 4), allowing to predict the time of rejection as well as the entire QIM score evolution.

Table 1 Mean β coefficients, predicted mean time of sensorial rejection (TSR) for iced Gilthead seabreams and bacterial concentration at time of sensorial rejection

Parameter	Mean ± SD
β_{1S}	1.23 ± 0.561
β_{2S}	0.40 ± 0.576
β_{1G}	0.47 ± 0.336
β_{2G}	0.21 ± 0.308
β_{1F}	0.32 ± 0.287
β_{2F}	0.27 ± 0.286
TSR (hours)	413.33 ± 35.651
$N_{14}w_S$ (Log CFU g^{-1})	7.95 ± 1.195
$N_{14}w_G$ (Log CFU g^{-1})	8.43 ± 0.709
$N_{14}w_F$ (Log CFU g^{-1})	2.53 ± 1.803
$N_{14}b_S$ (Log CFU g^{-1})	8.59 ± 0.665
$N_{14}b_G$ (Log CFU g^{-1})	8.35 ± 1.294
$N_{14}b_F$ (Log CFU g^{-1})	2.90 ± 1.596

β is the coefficient, which expresses QIM score as a function of bacterial concentration; β_1 and β_2 refer, respectively, to '*w*' and '*b*' bacterial populations.
'*w*' and '*b*' represent, respectively, the predicted concentration of white and black colonies on Iron Agar.
'*S*', '*G*' and '*F*' indicate the sampling series (skin, gills and flesh respectively).
N_{14} is the predicted bacterial concentration at Time of Sensorial Rejection.

Table 2 Root Mean Squared Error (RMSE) values calculated for predictive model and linear regression (Observed vs. Predicted QIM score)

	RMSE	
	Predictive model	**Linear regression**
Group 1	0.610	0.666
Group 2	0.614	1.458
Group 3	1.398	1.515
Group 4	1.208	1.328

Conclusion

This study allows to conclude that the prediction of whole-fish freshness obtained by modelling the QIM score trend as a function of the specific spoilage bacteria behaviour, in a good agreement with the observed data. The β coefficients allow to translate the bacterial behaviour into QIM scores using a cause-effect approach in which the β values remain constant and SSB concentration changes, varying the environmental

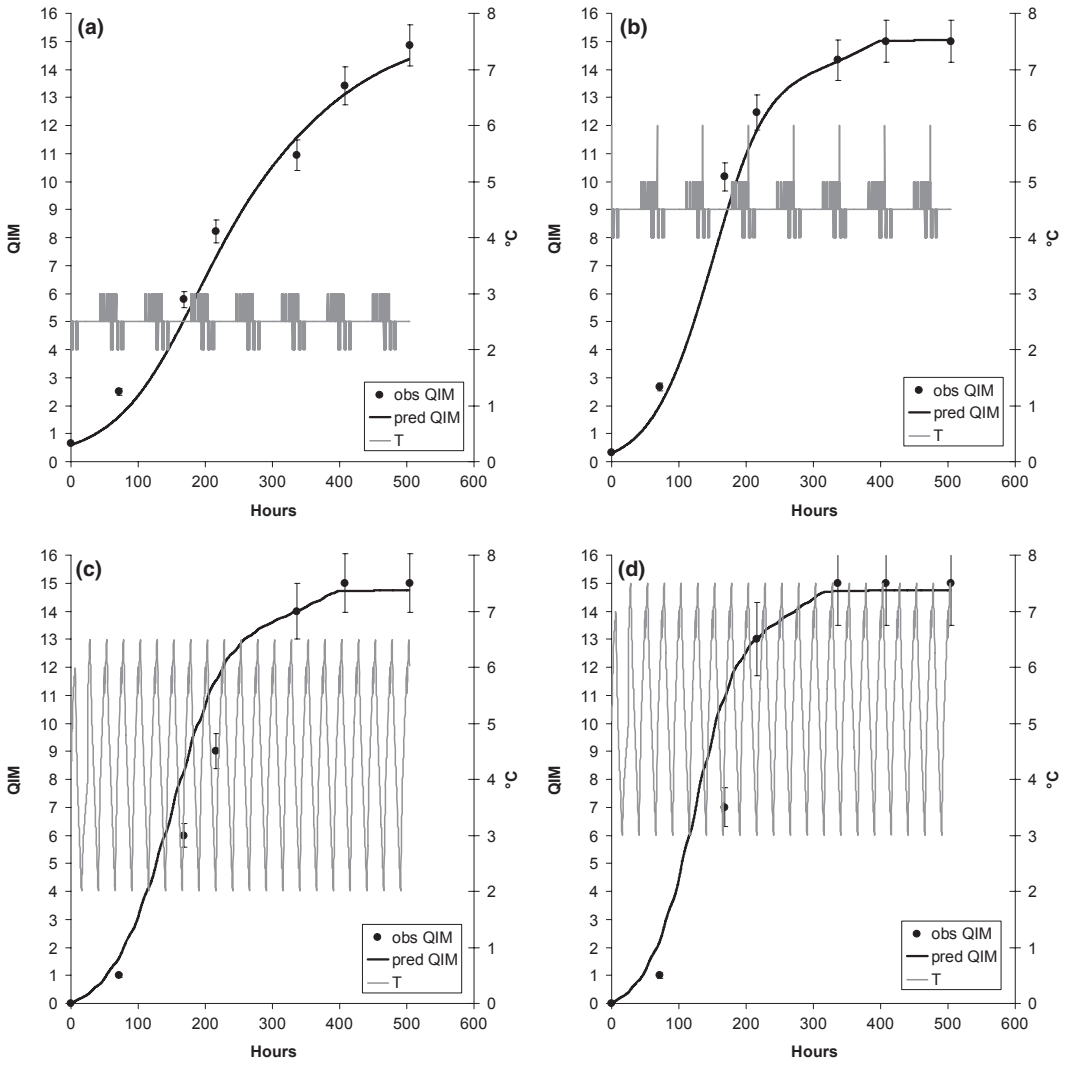

Figure 6 Observed QIM mean values (obs QIM) and related predictions (pred QIM) as function of temperature mean profiles for Group 1 (a), Group 2 (b), Group 3 (c) and Group 4 (d). Values are averages of twenty-one determinations for group 1, four determinations for group 2 and three determinations for groups 3 and 4; error bars indicate ±1SD.

conditions. In this way, the modelling of whole-fish freshness can be potentially applied to several storage conditions as demonstrated in this study.

They showed strict connection between bacterial load and freshness degree that allows to indicate a Bacterial Spoilage Level for pseudomonads and *Shewanella* spp. of Log 8 CFU g^{-1} on skin and gills. Concerning the time of rejection for the whole fish under refrigerated storage (Group 1), our observed and predicted results agree with those of other authors (Kyrana *et al.*, 1997; Huidobro *et al.*, 2000; Alasalvar *et al.*, 2001).

Finally, to reproduce the variability of the bacterial spoilage activity according to the standard deviation of β coefficients (Table 1), the present model can be modified by the employment of stochastic dynamic equations, as already proposed by other authors (Caruso *et al.*, 2005; Valenti *et al.*, 2006; Giuffrida *et al.*, 2009) for other purposes. This approach could allow to express the probability distribution of QIM scores at a given time.

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