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Influence of ice-to-fish ratio on spoilage kinetics, microbiological quality, and shelf life of refrigerated farmed gilthead sea bream (*Sparus aurata*)

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Abstract

The preservation of fish quality during post-harvest storage remains a major challenge for the aquaculture industry, particularly in Mediterranean countries where chilled storage is the most commonly used preservation method. This study evaluated, for the first time in Algeria, the effect of different ice-to-fish ratios on the quality and shelf life of farmed gilthead sea bream (*Sparus aurata*) during refrigerated storage. Fish were stored at three ice-to-fish ratios (1:2, 1:3, and 1:4) and monitored over 16 days. Sensory quality, total viable counts (TVC), H₂S-producing bacterial counts, pH, total volatile basic nitrogen (TVB-N), and trimethylamine (TMA) were determined at regular intervals. Storage time significantly affected all quality parameters ($p < 0.05$), with progressive sensory deterioration, microbial growth, and accumulation of spoilage metabolites observed in all treatments. However, the extent of quality loss depended on the ice-to-fish ratio. Fish stored at a ratio of 1:2 exhibited significantly lower TVC, H₂S-producing bacterial counts, TVB-N, TMA, and pH values than fish stored at ratios of 1:3 and 1:4 throughout storage. Pearson correlation analysis revealed strong positive relationships among all spoilage indicators, with the highest correlation observed between TVC and H₂S-producing bacterial counts ($r = 0.997$). Principal component analysis explained 98.9% of the total variance and clearly associated spoilage progression with increasing microbiological and biochemical deterioration. Shelf-life estimation based on the microbiological rejection limit (7 log CFU g⁻¹ TVC) indicated storage lives of 9.9, 7.6, and 5.6 days for fish stored at ice-to-fish ratios of 1:2, 1:3, and 1:4, respectively. Based on a TVB-N limit of 35 mg N 100 g⁻¹, estimated shelf life reached 17.3, 12.2, and 9.4 days, respectively. These findings demonstrate the importance of adequate icing for maintaining fish quality and provide the first scientific basis for optimising cold-chain management of farmed gilthead sea bream in Algeria.

Keywords

Sparus aurata, Ice-to-fish ratio, Shelf life, TVB-N, Trimethylamine, Aquaculture, Food quality

Introduction

The gilthead seabream (*Sparus aurata*) occupies a strategic position in Mediterranean aquaculture together with the European seabass (*Dicentrarchus labrax*), these being the two dominant marine finfish species produced in the region (FAO & GFCM, 2025). Over the last two decades, seabream production has increased steadily, driven largely by the expansion of offshore cage farming systems across Mediterranean and Black Sea countries. According to the latest assessments by the Food and Agriculture Organization and the General Fisheries Commission for the Mediterranean, gilthead seabream and European seabass account for a substantial proportion of Mediterranean marine aquaculture production, with Turkey, Greece, and Spain remaining the leading producing countries (FAO & GFCM, 2025). Marine cage farming continues to represent the predominant production system for these species (European Commission,

2025). Furthermore, the commercial success of gilthead seabream is attributed to its favourable growth performance under intensive farming conditions, high adaptability to aquaculture systems, and strong consumer acceptance associated with its white flesh, firm texture, and desirable sensory qualities (Haberle et al., 2024).

However, farmed gilthead seabream is primarily marketed and consumed in fresh form immediately after harvest, making strict post-harvest handling practices essential. Continuous maintenance of the cold chain is critical to preserve microbiological safety, physicochemical stability, and sensory quality throughout storage and distribution. Inadequate temperature control accelerates microbial growth, lipid oxidation, and quality deterioration, thereby reducing shelf life and increasing economic losses associated with spoilage (Parlapani et al., 2015; FAO, 2023).

Fish is a highly perishable food product, and its post-mortem deterioration is governed by complex interactions between spoilage microbiota, endogenous enzymatic activity, and oxidative reactions. During refrigerated storage, psychrotrophic bacteria, particularly *Pseudomonas* spp., *Shewanella* spp., and *Photobacterium* spp., contribute significantly to quality degradation through the production of volatile metabolites. These processes, together with autolysis and lipid oxidation, lead to the accumulation of total volatile basic nitrogen (TVB-N) and trimethylamine (TMA), which are widely recognised indicators of fish spoilage and are responsible for characteristic off-odours (Parlapani et al., 2014; Anagnostopoulos et al., 2022). Concurrently, muscle pH generally increases during storage as a consequence of the accumulation of alkaline compounds produced by microbial and enzymatic degradation of nitrogenous substrates (Nie et al., 2025).

Fish freshness assessment relies on complementary approaches. Sensory analysis, based on odour, texture, external appearance (eyes, gills, and skin), and muscle firmness, remains the reference method in both industry and research. Among sensory techniques, the Quality Index Method (QIM) is the most widely applied and standardised approach for evaluating fish freshness. It is based on the systematic scoring of sensory attributes that change during storage and has been shown to correlate strongly with microbiological, physicochemical, and shelf-life indicators, making it particularly useful for commercial quality assessment and freshness monitoring (Prabhakar et al., 2020; Esteves & Aníbal, 2021).

Studies on iced gilthead seabream (*Sparus aurata*) indicate that sensory shelf life generally ranges between 10 and 14 days under optimal refrigeration conditions, with gradual deterioration of sensory attributes during storage (Alasalvar et al., 2001; Lougovois et al., 2003). Lougovois et al. (2003) specifically reported that sensory rejection limits are reached at approximately 12 days depending on the evaluation method used.

Chemical indicators such as total volatile basic nitrogen (TVB-N) are widely used for assessing fish freshness and spoilage. Within the European Union, official analytical methods and regulatory TVB-N limits for specific categories of fishery products are currently established under Commission Implementing Regulation (EU) 2019/627, which superseded the relevant parts of Regulation (EC) No 2074/2005. Although this legislation sets maximum acceptable TVB-N levels (ranging from 25 to 35 mg N/100 g of flesh) for certain fish species, no legal TVB-N limit is specifically established for gilthead seabream (*Sparus aurata*). Therefore, a widely accepted scientific threshold of 35 mg N/100 g is commonly used in the literature to assess the spoilage of this species. In addition, total viable count (TVC) is extensively used as a microbiological indicator of fish freshness and spoilage level, and is often combined with sensory and chemical assessments to determine product shelf life (European Commission, 2005; Prabhakar et al., 2020).

Ice storage remains the most widely used traditional method for preserving fresh fish. By maintaining the product at temperatures close to the melting point of ice (0 °C), icing effectively slows microbial growth, enzymatic activity, and biochemical spoilage reactions, thereby extending shelf life and preserving sensory quality during storage and transport (Aubourg, 2021; Tavares et al., 2021). Fish is commonly stored in alternating layers of fish and ice to ensure efficient heat transfer and temperature control throughout storage. The amount of ice required depends on storage duration and environmental conditions, and higher ice-to-fish ratios are generally recommended for prolonged storage or warm climates (Aubourg, 2021). In the present study, lower ratios (1:2, 1:3, and 1:4) are evaluated to simulate suboptimal icing conditions.

Alternative preservation strategies, such as slurry ice systems and mild antimicrobial treatments based on organic acids (e.g., lactic and citric acids), have been extensively investigated as complementary approaches to improve fish preservation. Slurry ice has been shown to enhance cooling efficiency and delay microbial and biochemical spoilage, resulting in shelf-life extensions of several days compared with conventional flake ice. Similarly, organic acid treatments can inhibit microbial growth and reduce the formation of spoilage metabolites, thereby contributing to quality preservation during chilled storage (Amaral et al., 2021; Ntzimani et al., 2023). Nevertheless, strict maintenance of the cold chain remains the primary factor determining fish quality and shelf life throughout storage and

distribution.

Although the spoilage dynamics of gilthead seabream during iced storage have been previously investigated, most available studies have relied on a single, standardised ice-to-fish ratio (typically 1:1 or 1:2) without systematically evaluating how varying icing conditions influence the kinetics of quality deterioration (Lougovois et al., 2003; Papadopoulos et al., 2003; Erkan, 2007). Consequently, the quantitative relationship between ice-to-fish ratio and shelf-life extension remains poorly characterised, and there is limited experimental evidence on the spoilage progression under suboptimal icing conditions that are frequently encountered in practice. Furthermore, while individual spoilage indicators such as TVB-N, TMA, pH, and total viable counts have been widely used to monitor freshness loss, few studies have simultaneously modelled their interrelationships and their combined response to different icing intensities using multivariate approaches such as principal component analysis.

In addition, information on the post-harvest preservation of farmed gilthead seabream remains scarce in emerging Mediterranean aquaculture sectors, including Algeria, where local environmental conditions (high ambient temperatures and potential delays in cold-chain operations) may accelerate fish spoilage and compromise product quality. Therefore, optimizing preservation practices under these specific conditions is essential to maintain freshness and extend shelf life.

The present study was therefore designed to (i) systematically evaluate the effect of three different ice-to-fish ratios (1:2, 1:3, and 1:4) on the sensory, microbiological, and physicochemical quality of farmed gilthead seabream during refrigerated storage; (ii) quantify the impact of icing intensity on microbiological and chemical shelf-life; and (iii) investigate the multivariate relationships among spoilage indicators using correlation analysis and principal component analysis. By addressing these objectives, this study provides quantitative evidence to support the optimisation of icing practices and cold-chain management in both regional and broader Mediterranean aquaculture contexts.

Materials and methods

Sampling

This study was conducted on market-size gilthead seabream (*Sparus aurata*), raised at SARL Boumerdes Fish Company, an aquaculture farm specialising in the intensive farming of gilthead seabream, located in Zemmouri commune, Boumerdes province, Algeria.

Fish were harvested using standard commercial aquaculture practices. Upon harvest, the fish were rapidly transferred and immersed in an ice-water slurry to induce immediate thermal shock and asphyxiation, which is the standard commercial practice to minimise stress and ensure immediate quality preservation.

The study focused on the ice preservation of gilthead seabream, using a total of 120 fish divided into three experimental groups of 40 individuals each, corresponding to the three ice-to-fish ratios tested (1:2, 1:3, and 1:4, corresponding to 1 kg of ice per 2, 3, and 4 kg of fish, respectively). To avoid pseudoreplication and ensure statistical independence, separate storage boxes were used for each sampling day. Specifically, for each ice-to-fish ratio, the fish were distributed into 9 independent polystyrene boxes, with each box dedicated to a single sampling day (Days 0, 2, 4, 6, 8, 10, 12, 14, and 16). Each box contained the exact number of fish required for that day's destructive analyses (8 fish for Day 0, and 4 fish for each subsequent sampling day), resulting in a total of 40 fish per ratio.

The seabream were layered alternately with ice in the polystyrene boxes following ice-to-fish ratios of 1:2, 1:3, and 1:4. The ice was made from satisfactory-quality municipal water. The time between capture, icing, and arrival at the laboratory ranged from 45 minutes to 1 hour. The first analyses were performed immediately upon arrival at the laboratory (Day 0). The boxes were then placed in a large refrigerator at a controlled ambient temperature of 2–3 °C. To ensure that the target storage conditions were met, the internal temperature of the fish mass was monitored throughout the 16-day storage period using temperature probes placed in the centre of a representative box for each treatment, confirming that the core temperature of the fish remained consistently between 0 °C and 2 °C.

To prevent waterlogging and the rapid proliferation of spoilage bacteria in standing water, the polystyrene boxes were equipped with perforations at the bottom, allowing meltwater to drain freely. Meltwater was drained and removed daily. Furthermore, to compensate for ice melting and strictly maintain the target ice-to-fish weight ratios (1:2, 1:3, and 1:4), fresh flake ice was inspected and replenished daily. The added ice was distributed evenly to ensure the fish remained completely surrounded by ice, thereby preserving the experimental conditions throughout the entire storage period.

Because sensory, physicochemical, and bacteriological analyses are destructive, different fish were analysed at each sampling time point, and each fish came from an independent storage box. This design ensured that the individual fish was the true experimental unit ($n = 4$ to 8 fish per treatment per day), with complete statistical independence between sampling days.

For each fish, sensory evaluation was performed first. Immediately afterwards, the fish was filleted aseptically. The muscle tissue from the same individual fish was then homogenised and subdivided to perform all microbiological and physicochemical analyses, ensuring that all parameters (pH, TVB-N, TMA, TVC, and H_2S -producing bacteria) were evaluated from the same biological subsample, thereby minimising inter-individual variability. For microbiological and chemical analyses, each homogenised subsample was analysed in triplicate (analytical/technical replicates, $n = 3$), and the mean values were used for statistical analysis.

Sensory analysis

The sensory examination used in this work was based on the characteristics retained by the official veterinary services in Algeria for fish inspection, as well as on the organoleptic criteria established by the European Community methods for the assessment of fish freshness. These criteria, originally defined in the now-repealed Council Regulation (EEC) No. 103/76 and currently governed by Council Regulation (EC) No. 2406/96 laying down common marketing standards for certain fishery products, include the evaluation of external appearance, condition, eyes, gills, odour, skin, and flesh texture (Council of the European Communities, 1976; Council of the European Union, 1996).

Sensory evaluation was performed by a panel of three trained assessors in a dedicated sensory evaluation room maintained at a controlled temperature of 20–22 °C under standardised lighting conditions. Prior to the study, the panel underwent training and calibration sessions using reference samples to ensure consistency and reliability in scoring. Evaluations were based on the organoleptic criteria established by Council Regulation (EC) No. 2406/96 and focused on four main attributes: eyes, gills (including odour), flesh texture, and skin/general appearance. Each attribute was scored on a structured scale from 0 to 3 (0 = excellent/fresh, 1 = very good, 2 = acceptable/slight alteration, and 3 = unacceptable/spoiled). The total sensory score was calculated as the sum of the individual attribute scores, with a maximum possible score of 12. The sensory rejection threshold, corresponding to the end of sensory shelf life and the limit of preservation or rejection, was defined as the day when the total sensory score reached ≥ 9 or when any single critical attribute, particularly gills/odour, reached the maximum score of 3. Thus, the preservation or rejection time corresponded to the moment just before the appearance of obvious signs of spoilage.

To ensure blinding and independence of assessment, whole fish samples were presented to the assessors on coded trays bearing randomly generated three-digit labels, and evaluations were performed independently. In cases of disagreement between assessors, defined as a difference of >1 point for a specific attribute, a consensus was reached through discussion, or the median score was retained.

Sensory data were expressed as mean total sensory scores $\pm$ standard deviation (SD). Statistical differences in sensory scores among the three ice-to-fish ratios at each sampling time were analysed using the Kruskal–Wallis test followed by Dunn's post-hoc test. Sensory shelf life was determined as the storage time at which the predefined rejection threshold was reached.

Bacteriological analysis

Total viable counts (TVC) of aerobic psychrotrophic bacteria were determined using the pour-plate method with Plate Count Agar (PCA; BioMérieux, France), in accordance with the ISO 4833-1:2013 standard. Aseptically, 25 g of fish muscle was homogenised in 225 mL of buffered peptone water (BPW) in a sterile stomacher bag to obtain an initial 1:10 dilution. Serial decimal dilutions were prepared in BPW. For each dilution, 1 mL was transferred into sterile Petri dishes, and 10–15 mL of previously melted and tempered (45 °C) PCA was poured into each dish. After mixing and solidification, plates were incubated at 20–22 °C for 5 to 7 days. All morphologically distinct colonies developing on the plates were counted, and results were expressed as log CFU per gram of fish muscle. Plates containing between 15 and 300 colonies were selected for counting.

The enumeration of hydrogen sulphide (H_2S)-producing bacteria was performed using the spread-plate method on Iron Agar (IA; BioMérieux, France), a selective medium containing sodium thiosulphate as a sulphur source and ferric ammonium citrate as an indicator. Serial dilutions of fish muscle homogenates were prepared in BPW, and aliquots of 0.1 mL from appropriate dilutions were spread onto the surface of Iron Agar plates in duplicate. Plates were incubated at 25 °C for 72 to 96 hours, conditions selected to favour the growth of psychrotrophic spoilage bacteria commonly

associated with iced fish. H₂S-producing bacteria were identified based on the formation of colonies with a black or dark brown centre, resulting from the reaction of hydrogen sulphide produced by the bacteria with the iron salts to form insoluble iron sulphide. The detection limit of the method was 100 CFU/g of fish muscle, based on the inoculum volume (0.1 mL) and the initial 1:10 dilution factor. For each sample, two to three appropriate dilutions were plated in duplicate, and plates yielding between 15 and 150 colonies were selected for counting, in accordance with standard microbiological practices. Results were expressed as log CFU per gram of fish muscle.

Chemical analysis

For all chemical analyses, muscle samples were collected from a standardised anatomical site to minimise inter-individual variability. Specifically, skinless dorsal white muscle was excised from the region just below the lateral line on the left side of each fish, immediately posterior to the dorsal fin. Samples were homogenised using a laboratory blender, and the resulting homogenate was used for all subsequent analyses.

The pH of fish muscle was measured using a portable pH meter (HI98163; Hanna Instruments, Inc., Woonsocket, RI, USA) equipped with a penetration probe (FC2023). Prior to each measurement session, the pH meter was calibrated using standard buffer solutions at pH 4.01 and 7.00 (Hanna Instruments, Inc., Woonsocket, RI, USA). For each sample, approximately 5 g of homogenised muscle was placed in a beaker, and the penetration probe was inserted to a depth of approximately 1 cm into the muscle tissue. Three independent measurements were performed per sample, and the mean value was recorded. The pH electrode was rinsed with distilled water and gently dried between successive measurements.

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined by steam distillation of a deproteinised sample, as recommended by current EU legislation (Commission Implementing Regulation (EU) 2019/627). Briefly, 100 g of muscle homogenate was mixed with 200 mL of 7.5% trichloroacetic acid (TCA; Sigma-Aldrich, St. Louis, MO, USA) to obtain a protein-free extract. The mixture was centrifuged, and the supernatant was filtered through Whatman® filter paper (grade 3; Cytiva, Little Chalfont, UK). For TVB-N analysis, 25 mL of the filtered extract (or TCA alone as a blank) was transferred to the distillation tube, and 6 mL of 10% sodium hydroxide was added. The steam distillation was performed using a Kjeldahl apparatus (VAPODEST®; C. Gerhardt, Königswinter, Germany), and the distillate was collected in 10 mL of 4% aqueous boric acid solution containing 0.4% methyl red and bromocresol green (Merck, Darmstadt, Germany) as indicators. The boric acid solution, which turned green upon alkalisation by the collected TVB-N, was titrated with 0.1 N sulphuric acid until complete discolouration. TVB-N values were calculated and expressed as mg N/100 g of sample.

For TMA determination, an additional step was included between deproteinisation and distillation: 20 mL of formaldehyde was added to the sample filtrate to block primary and secondary amine groups (Malle & Tao, 1987). TMA values were calculated and expressed as mg TMA/100 g of sample.

To ensure the accuracy and reliability of the analytical results, several quality control measures were implemented throughout the study. All chemical and microbiological analyses were performed in analytical triplicate for each individual fish (technical replicates, $n = 3$). The mean of these three technical replicates was calculated for each fish to yield a single value per biological sample. These biological means were then used for all subsequent statistical analyses. Analytical blanks (reagents without a sample) were included in each batch of analyses to verify the absence of contamination. The repeatability of the TVB-N method was verified according to the requirements of Commission Implementing Regulation (EU) 2019/627: the absolute difference between the results of the two duplicate analyses performed on the same sample was required to be no greater than 2 mg N/100 g; otherwise, the analysis was repeated. All chemical reagents used were of analytical grade, and solutions were prepared using distilled water.

Shelf-life estimation

The shelf life of gilthead seabream was estimated by integrating microbiological, chemical, and sensory rejection criteria, as these parameters are intrinsically linked during the spoilage process.

Microbiological shelf life was defined as the storage time required for total viable counts (TVC) to reach the rejection limit of 7 log CFU/g. This threshold is widely recognised in international literature as the critical point at which specific spoilage organisms proliferate sufficiently to cause noticeable sensory deterioration and render chilled marine fish unacceptable for consumption (Gram & Dalgaard, 2002).

Chemical shelf life was estimated using a TVB-N limit of 35 mg N/100 g. While Commission Implementing Regulation (EU) 2019/627 establishes maximum permissible TVB-N levels for various fishery products, it does not specify a legal

limit for gilthead seabream (*Sparus aurata*). However, numerous studies focused specifically on this species have empirically demonstrated that a TVB-N value in the range of 30–35 mg N/100 g consistently coincides with the onset of obvious sensory rejection, characterised by strong off-odours and gill discolouration (Lougovois et al., 2003; Papadopoulos et al., 2003; Erkan, 2007).

The relationship between these criteria is causal and sequential: the proliferation of spoilage microbiota (reaching ~7 log CFU/g) drives the enzymatic and bacterial degradation of nitrogenous compounds, leading to the accumulation of volatile bases (TVB-N reaching ~35 mg N/100 g). This biochemical accumulation directly manifests as the sensory defects (putrid odours, slime, loss of texture) that define the sensory rejection limit. Therefore, the microbiological and chemical thresholds serve as objective, quantifiable proxies for the subjective sensory rejection point, ensuring a comprehensive and robust estimation of the product's marketable life.

The exact time at which each threshold was reached was determined by linear interpolation between two consecutive sampling points surrounding the selected rejection limit.

Statistical analysis

All statistical analyses were performed using R software (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) via RStudio (version 1.1.383). Sensory scores were expressed as mean $\pm$ standard deviation (SD). Differences in sensory scores among the three ice-to-fish ratios at each sampling time were assessed using the Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction for multiple comparisons. Sensory shelf life was defined as the storage time at which the predefined rejection threshold was reached, i.e., a total sensory score ≥ 9 or a score of 3 for a critical attribute, as described in the Sensory analysis subsection.

Differences among the three ice-to-fish ratios at each sampling time for TVC, H₂S-producing bacterial counts, TVB-N, TMA, and pH were evaluated using the Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction. Changes across storage time within each treatment were assessed using the Friedman test followed by Wilcoxon signed-rank tests with Bonferroni correction. Results were summarised as mean $\pm$ SD for figures and mean $\pm$ standard error (SE) for tables.

Pearson correlation coefficients were calculated to assess the relationships among TVB-N, TMA, pH, TVC, and H₂S-producing bacterial counts. Correlation coefficients were visualised using a heatmap.

Principal component analysis (PCA) was performed on standardised data to investigate multivariate relationships among spoilage indicators and to identify patterns associated with storage time and ice-to-fish ratio. The variables included TVB-N, TMA, pH, TVC, and H₂S-producing bacterial counts. Results were presented as a PCA biplot showing sample distribution and variable loadings.

Microbiological shelf life was estimated as the storage time required for TVC to reach the rejection limit of 7 log CFU/g. Chemical shelf life was estimated using a TVB-N threshold of 35 mg N/100 g. These thresholds were selected based on the criteria described in the Shelf-life estimation subsection, where the TVC limit is supported by Gram and Dalgaard (2002), while the TVB-N threshold is supported by Lougovois et al. (2003), Erkan (2007), and Papadopoulos et al. (2003). Shelf-life values were estimated by linear interpolation between two consecutive sampling points surrounding the respective rejection threshold.

For all statistical tests, differences were considered statistically significant at $p < 0.05$.

Results

Storage time and ice-to-fish ratio significantly affected all measured quality parameters, including TVB-N, TMA, pH, total viable counts (TVC), and H₂S-producing bacterial counts during iced storage of gilthead seabream. Statistical comparisons at each sampling day revealed significant differences among the three ice-to-fish ratios from day 2 onwards for most parameters ($p < 0.05$, Kruskal–Wallis test).

Sensory evaluation

Sensory quality declined progressively during storage, with the rate of deterioration strongly influenced by the ice-to-fish ratio (Table I). Fish stored at the 1:4 ratio exhibited the most rapid spoilage, reaching sensory rejection by day 8, characterised by sulphur odours and blood-stained gills. Fish at the 1:3 ratio reached rejection at day 10, while fish at the 1:2 ratio maintained acceptable sensory characteristics until day 10, reaching the rejection threshold on day 12. Statistical analysis confirmed significant differences between treatments from day 4 onwards ($p < 0.05$).

Storage time (Days)	Ice-to-fish ratio 1:2 (Score / 12)	Ice-to-fish ratio 1:3 (Score / 12)	Ice-to-fish ratio 1:4 (Score / 12)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0.7 ± 0.5 ^a	1.3 ± 0.5 ^b	2.3 ± 0.5 ^c
4	1.7 ± 0.5 ^a	3.0 ± 0.6 ^b	4.7 ± 0.8 ^c
6	3.0 ± 0.6 ^a	5.0 ± 0.8 ^b	7.0 ± 0.9 ^c
8	4.7 ± 0.8 ^a	7.3 ± 0.9 ^b	9.3 ± 0.8 ^{c *}
10	6.7 ± 0.9 ^a	9.3 ± 0.8 ^{b *}	11.0 ± 0.7 ^c
12	9.0 ± 0.7 ^{a *}	11.0 ± 0.6 ^b	11.7 ± 0.5 ^c

Table I. Evolution of the total sensory score of gilthead seabream (*Sparus aurata*) stored under different ice-to-fish ratios during refrigerated storage. Values are presented as the mean ± SD of the total sensory score (maximum score = 12), calculated as the sum of the score for the four attributes (eyes, gills, texture, and skin), each scored from 0 to 3. Within each storage day, means with different superscript letters (a–c) differ significantly ($p < 0.05$; Kruskal–Wallis test followed by Dunn’s post-hoc test). Bold values indicate the day on which the sensory rejection threshold was reached (total score ≥ 9 or critical attribute score = 3). The sample size was $n = 4$ fish per treatment per day, except on Day 0 ($n = 8$).

Chemical changes

TVB-N and TMA increased significantly throughout storage in all treatments (Tables II and III, Figure 1). Initial values were similar among treatments (15.1–15.2 mg N/100 g for TVB-N; 0.36 mg/100 g for TMA). By day 16, TVB-N reached 34.3, 39.3, and 49.5 mg N/100 g for the 1:2, 1:3, and 1:4 ratios, respectively, while TMA reached 10.78, 15.11, and 17.06 mg/100 g. The accumulation of volatile nitrogen compounds was significantly slower in fish stored with higher ice quantities ($p < 0.05$ from day 2 onwards, Kruskal–Wallis test).

Time (day)		0	2	4	6	8	10	12	14	16
Ice: Fish ratio	1:2	15.1±0.3a	16.3±0.4a	18.5±0.5a	21.8±0.6a	23.9±0.7a	25.8±0.8a	28.0±0.9a	32.1±1.0a	34.3±1.1a
	1:3	15.2±0.3a	17.0±0.4b	22.8±0.6b	26.0±0.7b	28.0±0.8b	30.9±0.9b	34.8±1.0b	36.9±1.1b	39.3±1.2b
	1:4	15.2±0.3a	18.7±0.5c	23.5±0.7c	27.6±0.8c	32.5±0.9c	36.0±1.0c	39.5±1.1c	44.1±1.2c	49.5±1.3c

Table II. Effect of ice-to-fish ratio and storage time on total volatile basic nitrogen (TVB-N) values (mg N/100 g) in gilthead seabream (*Sparus aurata*) stored in ice. Values are presented as the mean ± SE ($n = 4$ fish per treatment per day). Means within the same storage day bearing different superscript letters (a–c) differ significantly ($p < 0.05$, Kruskal–Wallis test followed by Dunn’s post-hoc test with Bonferroni correction).

Time (day)		0	2	4	6	8	10	12	14	16
Ice: Fish ratio	1:2	0.36±0.02a	0.66±0.04a	0.99±0.06a	2.19±0.12a	3.53±0.18a	4.60±0.23a	5.75±0.29a	8.83±0.44a	10.78±0.54a
	1:3	0.36±0.02a	1.02±0.05b	2.01±0.10b	3.54±0.18b	5.68±0.28b	7.86±0.39b	9.03±0.45b	12.20±0.61b	15.11±0.76b
	1:4	0.36±0.02a	2.04±0.10c	3.59±0.18c	5.82±0.29c	7.01±0.35c	9.09±0.45c	12.11±0.61c	14.53±0.73c	17.06±0.85c

Table III. Effect of ice-to-fish ratio and storage time on trimethylamine (TMA) content (mg/100 g) in gilthead seabream (*Sparus aurata*) stored in ice. Values are presented as the mean ± SE ($n = 4$ fish per treatment per day). Means within the same storage day bearing different superscript letters (a–c) differ significantly ($p < 0.05$, Kruskal–Wallis test followed by Dunn’s post-hoc test with Bonferroni correction).

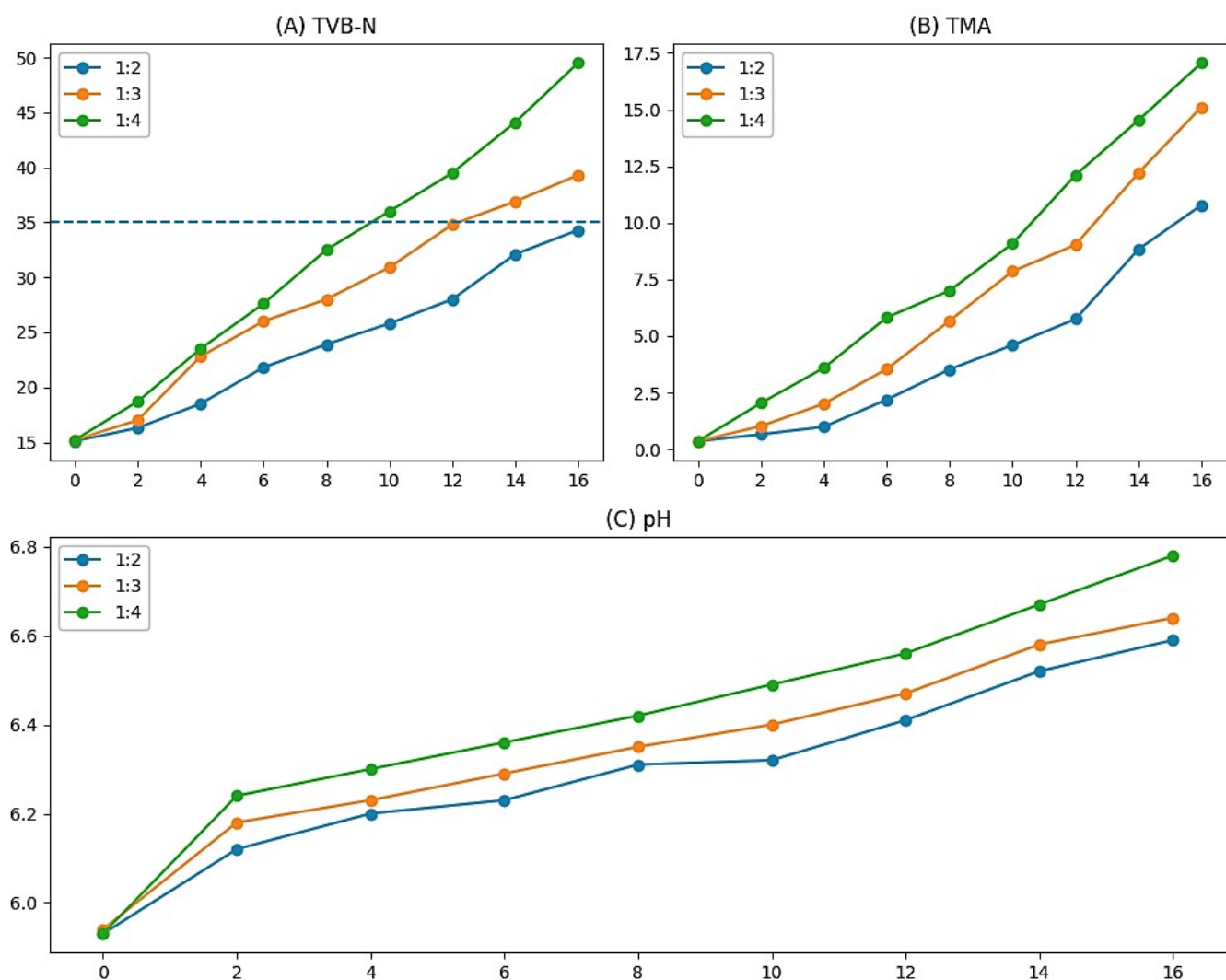


Figure 1. Physicochemical quality changes of farmed gilthead seabream (*Sparus aurata*) stored under different ice-to-fish ratios during refrigerated storage. Values are expressed as the mean $\pm$ SD for individual fish per treatment per day ((n = 4 fish per treatment per day, except on Day 0, when n = 8). The dashed horizontal line in panel A indicates the commonly accepted TVB-N rejection limit (35 mg N/100 g).

Muscle pH increased progressively during storage, from initial values of 5.93–5.94 to 6.59, 6.64, and 6.78 on day 16 for the 1:2, 1:3, and 1:4 ratios, respectively (Table IV, Figure 1). Fish stored with lower ice quantities consistently exhibited significantly higher pH values from day 2 onwards ($p < 0.05$, Kruskal–Wallis test).

Time (day)		0	2	4	6	8	10	12	14	16
Ice: Fish ratio	1:2	5.93 $\pm$ 0.01a	6.12 $\pm$ 0.02a	6.20 $\pm$ 0.02a	6.23 $\pm$ 0.02a	6.31 $\pm$ 0.02a	6.32 $\pm$ 0.02a	6.41 $\pm$ 0.02a	6.52 $\pm$ 0.02a	6.59 $\pm$ 0.02a
	1:3	5.94 $\pm$ 0.01a	6.18 $\pm$ 0.02b	6.23 $\pm$ 0.02b	6.29 $\pm$ 0.02b	6.35 $\pm$ 0.02b	6.40 $\pm$ 0.02b	6.47 $\pm$ 0.02b	6.58 $\pm$ 0.02b	6.64 $\pm$ 0.02b
	1:4	5.93 $\pm$ 0.01a	6.24 $\pm$ 0.02c	6.30 $\pm$ 0.02c	6.36 $\pm$ 0.02c	6.42 $\pm$ 0.02c	6.49 $\pm$ 0.02c	6.56 $\pm$ 0.02c	6.67 $\pm$ 0.02c	6.78 $\pm$ 0.02c

Table IV. Effect of ice-to-fish ratio and storage time on muscle pH of gilthead seabream (*Sparus aurata*) during iced storage. Values are presented as the mean $\pm$ SE (n = 4 fish per treatment per day). Means within the same storage day bearing different superscript letters (a–c) differ significantly ($p < 0.05$, Kruskal–Wallis test followed by Dunn’s post-hoc test with Bonferroni correction).

Microbiological changes

Total viable counts (TVC) increased significantly during storage (Table V, Figure 2). Initial loads were low (2.58–2.61 log CFU/g) and reached 8.59, 9.47, and 9.84 log CFU/g at day 16 for the 1:2, 1:3, and 1:4 ratios, respectively. The 1:2 ratio consistently resulted in significantly lower bacterial counts throughout storage ($p < 0.05$ from day 2 onwards, Kruskal–Wallis test).

H₂S-producing bacteria followed a similar pattern, increasing from 2.07–2.10 log CFU/g on day 0 to 7.21, 8.15, and 8.36 log CFU/g on day 16 for the 1:2, 1:3, and 1:4 ratios, respectively (Table VI, Figure 2). Significant differences among treatments were observed from day 2 onwards ($p < 0.05$, Kruskal–Wallis test).

Time (day)		0	2	4	6	8	10	12	14	16
Ice: Fish ratio	1:2	2.58±0.04a	3.09±0.06a	4.42±0.09a	5.30±0.11a	6.11±0.12a	7.05±0.14a	7.78±0.16a	8.14±0.16a	8.59±0.17a
	1:3	2.60±0.04a	4.17±0.08b	5.15±0.10b	6.10±0.12b	7.22±0.14b	8.16±0.16b	8.58±0.17b	9.18±0.18b	9.47±0.19b
	1:4	2.61±0.04a	5.10±0.10c	6.14±0.12c	7.21±0.14c	8.12±0.16c	8.36±0.17c	9.02±0.18c	9.32±0.19c	9.84±0.20c

Table V. Effect of ice-to-fish ratio and storage time on total viable counts (TVC) (log CFU/g) in gilthead seabream (*Sparus aurata*) stored in ice. Values are presented as the mean ± SE (n = 4 fish per treatment per day). Means within the same storage day bearing different superscript letters (a–c) differ significantly ($p < 0.05$, Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction).

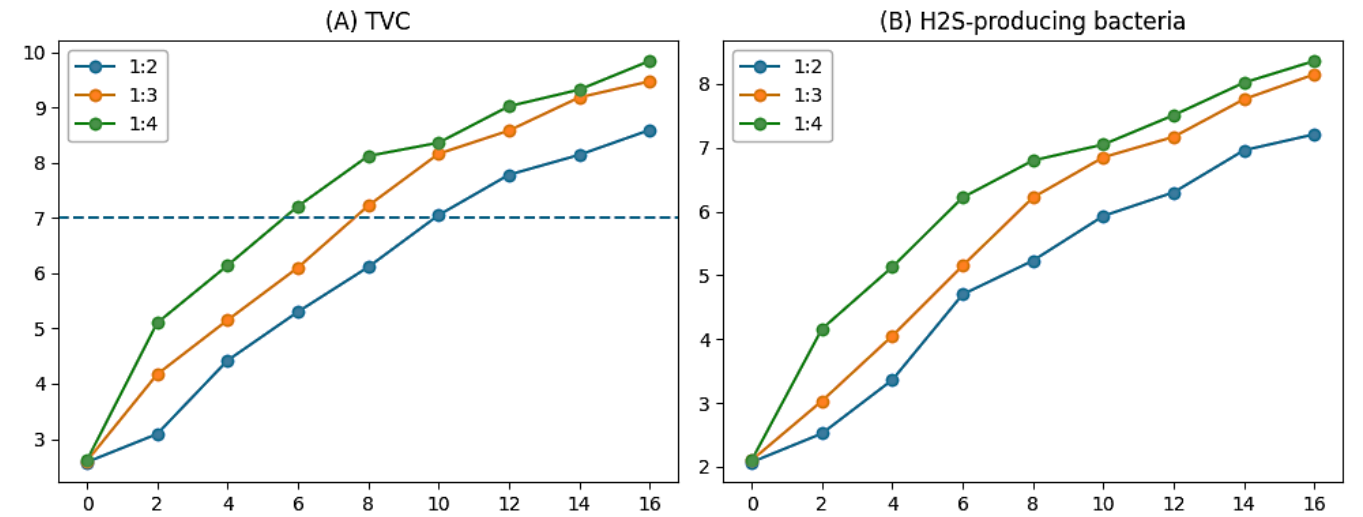


Figure 3. Microbiological quality changes of farmed gilthead seabream (*Sparus aurata*) stored under different ice-to-fish ratios during refrigerated storage. Values are expressed as the mean ± SD for individual fish per treatment per day (n = 4 fish per treatment per day (n = 4 fish per treatment per day, except on Day 0, when n = 8). The dashed horizontal line in panel A indicates the microbiological acceptability limit of 7 log CFU g⁻¹.

Time (day)		0	2	4	6	8	10	12	14	16
Ice: Fish ratio	1:2	2.07±0.04a	2.52±0.05a	3.36±0.07a	4.70±0.09a	5.23±0.10a	5.93±0.12a	6.30±0.13a	6.96±0.14a	7.21±0.14a
	1:3	2.10±0.04a	3.03±0.06b	4.05±0.08b	5.15±0.10b	6.22±0.12b	6.85±0.14b	7.17±0.14b	7.76±0.16b	8.15±0.16b
	1:4	2.10±0.04a	4.16±0.08c	5.13±0.10c	6.22±0.12c	6.80±0.14c	7.05±0.14c	7.51±0.15c	8.02±0.16c	8.36±0.17c

Table VI. Effect of ice-to-fish ratio and storage time on H₂S-producing bacterial counts (log CFU/g) in gilthead seabream (*Sparus aurata*) stored in ice. Values are presented as the mean ± SE (n = 4 fish per treatment per day). Means within the same storage day bearing different superscript letters (a–c) differ significantly ($p < 0.05$, Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction).

Correlation and multivariate analysis

Pearson correlation analysis revealed strong positive relationships among all spoilage indicators (Figure 3). The highest correlation was observed between TVC and H₂S-producing bacteria ($r = 0.997$), followed by that between TVB-N and TMA ($r = 0.979$). Principal component analysis (Figure 4) clearly separated samples according to storage time and ice-to-fish ratio, with the first two components explaining 98.9% of the total variance. All spoilage indicators were positively associated with PC1, representing the overall spoilage gradient.

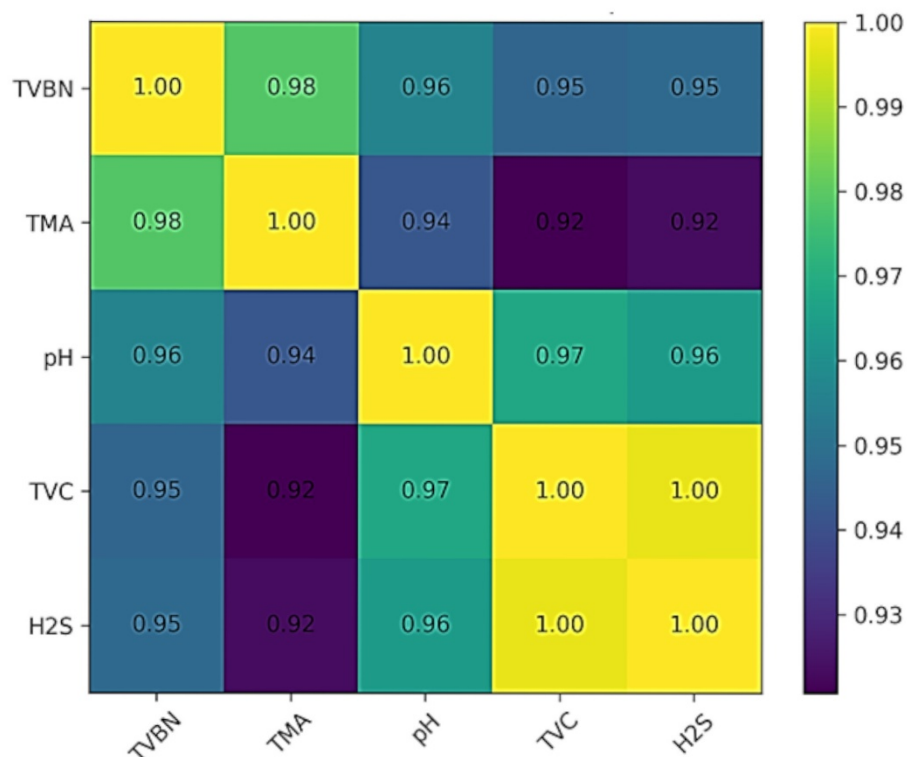


Figure 4. Pearson correlation heatmap illustrating the associations between microbial growth and biochemical spoilage indicators in gilthead seabream (*Sparus aurata*) during chilled storage.

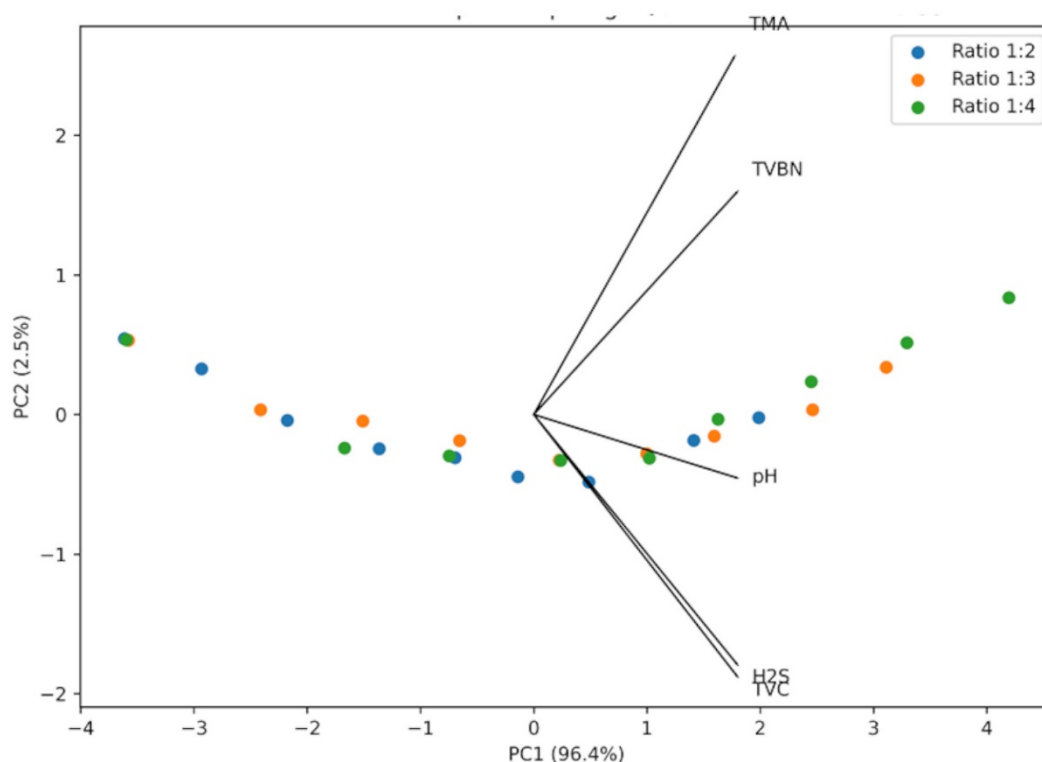


Figure 5. Multivariate discrimination of refrigerated gilthead seabream (*Sparus aurata*) samples according to ice-to-fish ratio and storage time using principal component analysis (PCA)

Shelf-life estimation

Based on the microbiological rejection limit (7 log CFU/g TVC), shelf life was estimated at 9.9, 7.6, and 5.6 days for the 1:2, 1:3, and 1:4 ratios, respectively (Table VII). Using the TVB-N threshold (35 mg N/100 g), shelf life was estimated at 16.6, 12.2, and 9.4 days for the same treatments.

Ice-to-fish ratio	Microbiological Shelf-life (TVC = 7 log CFU/g)	Chemical Shelf-life (TVB-N = 35 mg N/100 g)
1:2	9.9 days	16.6 days
1:3	7.6 days	12.2 days
1:4	5.6 days	9.4 days

Table VII. Effect of ice-to-fish ratio on the estimated microbiological and chemical shelf life of refrigerated gilthead seabream (*Sparus aurata*). Shelf-life values were estimated by linear interpolation between two consecutive sampling points. Microbiological shelf life was calculated using a rejection limit of 7 log CFU/g for total viable counts (TVC), whereas chemical shelf life was estimated using a TVB-N threshold of 35 mg N/100 g.

Discussion

The present study provides robust evidence that the amount of ice available during refrigerated storage substantially modifies the trajectory of quality deterioration in farmed gilthead seabream (*Sparus aurata*). Rather than simply delaying the appearance of spoilage, the higher ice-to-fish ratio (1:2) was associated with a significantly slower progression of sensory, microbiological, and biochemical changes, whereas the lower ratio (1:4) was associated with the most rapid deterioration. The consistency of this pattern across independent quality indicators, combined with the use of separate and independent storage boxes for each sampling day, provides strong evidence that the principal effect of icing was to slow the overall spoilage process, most likely by maintaining fish temperature closer to the melting point of ice and thereby reducing the rate of microbial and enzymatic reactions.

The progressive deterioration of sensory quality was characterised by changes in eye appearance, gill coloration and odour, flesh texture, and general appearance. Importantly, the differences among treatments became evident during storage rather than at the initial sampling point, which is consistent with the expectation that the effect of icing intensity would become increasingly important as storage progressed.

Fish maintained at the 1:2 ratio retained acceptable sensory characteristics for longer than those stored at 1:3 and 1:4, suggesting that maintaining a greater amount of ice was sufficient to delay the physiological and microbial processes responsible for visible and odorous spoilage.

This interpretation is consistent with previous studies of iced gilthead seabream, in which sensory rejection has been associated with off-odours, gill discolouration, loss of elasticity, and deterioration of surface appearance (Kyranas et al., 1997; Lougovois et al., 2003). Alasalvar et al. (2001) similarly demonstrated that sensory deterioration in cultured seabream is closely related to microbiological and biochemical changes during chilled storage. The present findings therefore support the view that sensory assessment remains particularly valuable because it integrates several deterioration processes into a measure directly relevant to consumer acceptability. The sensory shelf life observed in the present study is also broadly compatible with the range reported for iced gilthead seabream under favourable refrigeration conditions, generally around 10–14 days, although the exact rejection point depends on the sensory scoring system and storage conditions (Alasalvar et al., 2001; Lougovois et al., 2003).

The metabolomic evidence reported by Mallouchos et al. (2020) further supports this interpretation by showing that sensory deterioration is associated with coordinated biochemical changes and accumulation of volatile compounds. Thus, the longer sensory acceptability observed under the 1:2 ratio should not be viewed simply as a slower change in appearance; rather, it likely reflects a broader reduction in the rate of biochemical and microbial processes underlying freshness loss.

The increase in total viable counts (TVC) during storage is consistent with the expected development of psychrotrophic spoilage microorganisms under chilled conditions. Although icing strongly restricts bacterial growth by maintaining low temperatures, it does not eliminate microbial activity. This distinction is important because the

practical objective of icing is therefore not sterilisation, but rather the reduction of the rate at which spoilage microorganisms multiply. Recent studies have emphasised that maintaining seafood as close as possible to 0 °C remains one of the most effective approaches for controlling psychrotrophic microorganisms and extending chilled shelf life (Syropoulou et al., 2021; Anagnostopoulos et al., 2022).

The significantly lower bacterial loads observed under the 1:2 ratio are therefore mechanistically plausible. A greater ice-to-fish ratio provides a larger thermal reserve and is expected to improve the maintenance of low product temperature, particularly as heat enters the storage system and ice melts. This interpretation is consistent with the findings of Erkan (2007) and Lougovois et al. (2003), who reported progressive microbial growth during ice storage of gilthead seabream. Gram and Dalgaard (2002) emphasised that temperature is a major determinant of the growth kinetics of specific spoilage organisms in chilled marine fish, meaning that relatively small differences in temperature control can translate into substantial differences in spoilage progression over several days.

The parallel increase in H₂S-producing bacteria is particularly relevant because these organisms are associated with the production of sulphur-containing volatile compounds that contribute directly to undesirable odours. The close association between H₂S-producing bacteria and sensory deterioration observed here therefore provides a biological explanation for why microbiological changes were accompanied by progressive loss of sensory acceptability. However, the present study measured H₂S-producing bacteria as a functional group rather than identifying the specific bacterial species responsible. Consequently, the observed association should not be interpreted as evidence that a particular bacterial taxon was responsible for the spoilage process.

The progressive accumulation of TVB-N and TMA provides complementary evidence that microbial and endogenous biochemical processes accompanied the microbiological deterioration. TVB-N incorporates several volatile basic compounds generated during the degradation of nitrogen-containing substrates, including ammonia and amines, and is therefore widely used as a chemical indicator of fish spoilage (Duarte et al., 2020; Anagnostopoulos et al., 2022). The significantly slower accumulation of TVB-N under the 1:2 ratio suggests that stronger temperature control delayed the processes responsible for the formation of these compounds. Comparable increases during iced storage of gilthead seabream have been reported by Papadopoulos et al. (2003), Lougovois et al. (2003), and Erkan (2007).

TMA showed a similar pattern, which is biologically consistent with its origin from the microbial reduction of trimethylamine oxide (TMAO), a naturally occurring osmolyte in marine fish. Because TMA formation depends substantially on microbial activity, its accumulation provides a more mechanistically specific indication of bacterial spoilage than a general measure of viable bacterial counts. The significantly lower TMA concentrations observed under the 1:2 treatment are therefore consistent with reduced microbial activity under better temperature control. Previous studies have similarly linked increasing TMA concentrations with bacterial growth and sensory rejection during chilled storage (Lougovois et al., 2003; Erkan, 2007), while more recent work has confirmed the usefulness of TMA as part of a broader set of indicators of seafood spoilage (Duarte et al., 2020; Mallouchos et al., 2020).

The parallel behaviour of TVB-N and TMA should nevertheless be interpreted as complementary rather than redundant information. TVB-N represents a broader pool of volatile basic compounds, whereas TMA provides information specifically related to microbial transformation of TMAO. Their simultaneous increase in the present study therefore strengthens the interpretation that the observed deterioration involved both microbial proliferation and biochemical degradation.

Muscle pH also increased during storage, particularly under the lower icing conditions. This pattern is consistent with the accumulation of alkaline compounds, including ammonia and volatile amines, resulting from microbial metabolism and degradation of nitrogenous substrates. Similar increases in pH have been reported during refrigerated storage of gilthead seabream and other marine fish species (Alasalvar et al., 2001; Papadopoulos et al., 2003). Importantly, pH should not be interpreted as an isolated indicator of spoilage, because post-mortem muscle pH can be influenced by several physiological and biochemical factors. In the present study, its strong association with bacterial counts and volatile spoilage compounds supports its interpretation as one component of a broader spoilage pattern rather than as a standalone measure of freshness.

The correlation analysis provides an important interpretative dimension beyond the individual changes in each quality parameter. The very strong association between TVC and H₂S-producing bacteria ($r = 0.997$), together with the high correlations of TVB-N, TMA, and pH with bacterial indicators, suggests that the different measurements captured a common underlying spoilage process. In particular, the strong relationship between TVC and H₂S-producing bacteria indicates that increasing total bacterial abundance was accompanied by an increase in microorganisms associated with characteristic spoilage metabolites.

These relationships are consistent with previous observations in marine fish, in which microbial proliferation is closely

linked to the accumulation of volatile nitrogenous compounds and sensory rejection (Duarte et al., 2020; Mallouchos et al., 2020). Nevertheless, correlation does not establish causation. The high correlation coefficients observed here should therefore be interpreted as evidence that these indicators evolved together during storage, rather than as proof that an increase in one parameter directly caused the increase in another.

The PCA provides complementary evidence by reducing the multidimensional quality data to a dominant deterioration gradient. The fact that the first two components explained 98.9% of the total variance, with PC1 accounting for 96.4%, indicates that most of the measured variation could be described by a common progression from lower to higher spoilage. The association of TVB-N, TMA, pH, TVC, and H₂S-producing bacteria with this gradient supports their combined use for monitoring freshness loss. The displacement of samples stored under lower icing conditions towards higher PC1 values further suggests that inadequate icing accelerated movement along the same general spoilage trajectory rather than generating a fundamentally different spoilage pathway.

The estimated shelf-life differences provide perhaps the most practically relevant interpretation of the study. Using the TVC threshold of 7 log CFU/g, the estimated microbiological shelf life was approximately 9.9 days under the 1:2 ratio, compared with 7.6 days under 1:3 and 5.6 days under 1:4. Thus, the additional ice associated with the 1:2 treatment was associated with a significant extension of approximately 2.3 days relative to 1:3 and more than 4 days relative to 1:4. Similar trends were observed when the TVB-N criterion was applied, although the estimated chemical shelf lives were longer than the microbiological estimates.

The difference between microbiological and chemical shelf-life estimates is itself informative. It indicates that the time required for TVB-N to reach the selected rejection threshold was longer than the time required for TVC to reach 7 log CFU/g. This suggests that microbial proliferation may become unacceptable before the accumulation of volatile basic nitrogen reaches the selected chemical threshold under the conditions tested. Consequently, no single indicator should be regarded as sufficient for defining fish shelf life. The combination of sensory, microbiological, and chemical measurements provides a more comprehensive assessment of product quality, consistent with the complementary role of these indicators described in previous seafood studies (Gram & Dalgaard, 2002; Anagnostopoulos et al., 2022).

The practical importance of these differences is particularly relevant for post-harvest handling. Adequate icing may provide a relatively simple means of gaining additional marketable time without introducing chemical preservatives or more complex preservation technologies. This is consistent with the broader literature showing that maintaining fish temperatures close to 0 °C delays microbial growth and biochemical deterioration (Gram & Dalgaard, 2002; Anagnostopoulos et al., 2022). However, the present results should not be interpreted as establishing a universal shelf life of 9.9 days for fish stored at a 1:2 ratio. Shelf life is also influenced by initial microbial load, handling hygiene, fish size and physiological condition, ambient temperature, drainage, and cold-chain continuity. The estimates obtained here therefore apply primarily to the experimental conditions used in this study, although the use of independent storage boxes for each sampling day strengthens the reliability of the observed treatment effects.

To the best of our knowledge, this study provides the first comprehensive evaluation of the influence of ice-to-fish ratio on sensory quality, microbial growth, biochemical spoilage indicators, and shelf life of farmed gilthead seabream in Algeria. Although the spoilage patterns observed were generally consistent with those reported in other Mediterranean countries, the present findings provide valuable baseline data under Algerian production and distribution conditions. Such information is particularly relevant for the development of evidence-based recommendations aimed at improving post-harvest handling practices and strengthening cold-chain management in the emerging Algerian aquaculture sector.

Limitations of the study

While this study provides valuable insights into the effect of ice-to-fish ratio on gilthead seabream quality, some limitations should be acknowledged. First, this study focused on a single fish species, and the findings may not be directly generalisable to other marine fish species with different physiological characteristics or spoilage microbiota. Second, the study was conducted under controlled laboratory conditions, and further research under more representative field conditions with continuous temperature monitoring throughout the cold chain would be valuable. Third, the relatively small number of fish analysed per treatment and sampling time may limit the robustness and broader generalisability of the findings. Future studies involving a larger number of fish would provide additional evidence and further strengthen the conclusions of this work. Despite these limitations, the study provides a solid scientific basis for optimising icing practices in Algerian aquaculture. The use of independent storage boxes for each sampling day helped ensure statistical independence and strengthened the experimental design.

Conclusion

This study indicates that, under the specific experimental conditions investigated, the ice-to-fish ratio significantly influences the quality deterioration of farmed gilthead seabream (*Sparus aurata*) during refrigerated storage. The 1:2 ratio provided superior preservation performance across all measured indicators compared to the 1:3 and 1:4 ratios. Microbiological shelf life (TVC = 7 log CFU/g) was estimated at 9.9 days for the 1:2 ratio, compared to 7.6 days for 1:3 and 5.6 days for 1:4. Chemical shelf life (TVB-N = 35 mg N/100 g) was estimated at 16.6, 12.2, and 9.4 days, respectively. Under these controlled conditions, increasing the ice-to-fish ratio from 1:4 to 1:2 extended microbiological shelf life by approximately 4.3 days. Strong correlations among all spoilage indicators and principal component analysis confirmed that inadequate icing accelerates progression along a common spoilage trajectory.

While these findings provide quantitative evidence that may support the optimisation of icing practices in Mediterranean aquaculture, particularly in emerging sectors such as Algeria, these recommendations are strictly limited to the conditions investigated in the present study. Within this specific context, the 1:2 ice-to-fish ratio represents a simple, cost-effective strategy to delay spoilage without requiring chemical preservatives. However, these results should not be interpreted as establishing a universal shelf life. Further research under representative commercial field conditions, with continuous temperature monitoring and across different fish species, is necessary to validate these findings and fully assess their broader practical applicability.

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Ethical approval

Ethical review and approval were not required for this study, as it exclusively utilised post-mortem samples from commercially harvested fish. No experimental procedures or manipulations were performed on live animals. The harvesting and slaughter were conducted by trained farm personnel following standard commercial aquaculture practices and the general animal welfare guidelines for the slaughter of fish established by the World Organisation for Animal Health (WOAH, 2021).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Author Contributions

Conceptualisation: DM, NAKT, NO; Methodology: DM, HD, AM; Formal analysis: DM, AM, NAKT, NO; Investigation: DM, NO; Writing—original draft preparation: DM, NAKT, NO; Writing—review and editing: DM, NAKT, NO; Supervision: DM; Project administration: DM; All authors have read and agreed to the published version of the manuscript.

Data availability

Data are available upon request from the corresponding author.

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