

Possible application of non-invasive tools to characterise European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) from two different farming systems

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ABSTRACT

Rearing conditions can affect fish morphology, physiology, and welfare in several manners, for instance given the different hydrodynamic conditions or stocking densities. This preliminary study aimed to evaluate non-invasive tools to understand if and how the farming system can influence external shape, skin pigmentation, and freshness evolution during refrigerated storage of European sea bass and gilthead sea bream. A total of 100 individuals (50 sea bass and 50 sea bream) were obtained from a fish farm (located in Piombino, Italy) equipped with floating cages and inland tanks. Fishes were photographed in lateral aspect to analyse shape, while skin colour was analysed with a colorimeter. Freshness and *Rigor Index* were observed during 7 days of refrigerated storage. Results showed that shape and colour analyses can be useful tools to discriminate fish farmed under different conditions, *i.e.*, marine sea cages and inland tanks. Moreover, farming conditions significantly affected *rigor* resolution and fish freshness, especially in European sea bass.

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1. INTRODUCTION

Aquaculture is an animal husbandry activity concerning the rearing of aquatic organisms, such as fishes, molluscs, crustaceans and algae. Of the 178 million tons of aquatic animals traded in 2020, 49 % derived from aquaculture, an activity projected to grow further in the coming years [1]. Despite this forecast at a global level, the European seafood sector is still strongly characterised by the presence of products derived from fisheries, while aquaculture still remains confined to a few species, including molluscs (*Mytilus galloprovincialis*, *Mytilus edulis*, *Magallana gigas*, *Ruditapes philippinarum*), rainbow trout (*Oncorhynchus mykiss*), gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). With more than 4000 tons of sea bass and sea bream, Tuscany, a region located in central Italy, produces about 2 % of the entire European production of these two species, corresponding to more than

30 % of the Italian one [2]. Unable to compete in volume, Italian aquaculture is primarily focused on premium quality products; indeed, while buying sea bass or sea bream at the supermarket, consumers can pay from 7 to 12 euros per kg for sea bass or sea bream farmed in Italy against 5-7 euro/kg for the same species produced abroad, mainly in Greece or Croatia [3].

It is well established that rearing conditions, such as rearing density, feeding, handling, and environment (water parameters and type and structure of rearing tanks) can affect in several different manners fish morphology, physiology, and welfare, sometimes triggering growth performance, body integrity and fillet quality [4], [5]. In Italy, European sea bass and gilthead sea bream are commonly farmed in salt water, in sea cages or in inland tank systems. These two farming systems are very different in terms of hydrodynamic conditions, greatly affecting fish swimming behaviour and, sometimes, in terms of stocking density. This latter could lead to a variety of consequences, since

stocking density is highly correlated with animal welfare [6]. Considering the increasing consumers' attention to fish welfare and the influence that products' visual appearance (shape and colour) has on the decision-making to purchase or not a whole fresh fish and on the willingness to pay a price premium for certain products, this preliminary study aimed to evaluate the possible application of non-invasive tools to characterise sea bass and sea bream quality attributes and understand if and how the farming system (sea cages *versus* inland tank) can influence external shape, skin pigmentation, and freshness evolution during refrigerated storage (+2 °C).

2. MATERIAL AND METHODS

A total of 100 individuals (50 sea bass and 50 sea bream) was purchased from a fish farm (located in Piombino, Italy) equipped with floating marine cages (CAGE, $n = 25$ for each species) and inland tanks (TANK, $n = 25$ for each species). Fishes (sea bass mean weight = (433 ± 91) g; sea bream mean weight = (474 ± 86) g) were transferred to the laboratory of Animal Science of the Department of Agriculture, Food, Environment and Forestry (University of Firenze, Italy) by courier, in full compliance with the cold chain, and according to the company protocols for transferring the product from the production area to distribution (within 24 hours from harvest). Once they arrived, all fishes were photographed in the left side (Panasonic Lumix® DMC-FZ300, 12.1 M-pixels; Panasonic Italia, Milano, Italy) for shape analysis. Briefly, fishes were placed in a lightbox equipped with a photographic lamp (Correlated Colour Temperature: 6000 K) and covered with a light diffuser at a focal distance of 40 cm. A total of 14 and 16 landmarks were digitised, respectively for sea bream and sea bass (Figure 1), using TpsDig2.0 software [7].

The landmark coordinates for each specimen were aligned and superimposed by the generalised least-squares Procrustes superimposition method (GLS) [8]. Residuals from the superimposition were analysed with the thin-plate spline (TPS) interpolating function [9]. As an ordination method and to display the major features of shape variation, a principal component analysis (PCA) was performed on the covariance matrix. A canonical variate analysis (CVA) was utilised to separate known groups in the data, providing an ordination that maximised the separation of the group means relative to the variation within groups. Individuals of the two species were pooled in two categories: farmed in cages (CAGE) and farmed in tanks (TANK). All the analyses were performed using the software MorphoJ, available at www.flywings.org.uk/MorphoJ_page.htm.

Then, $n. 5$ fish were assigned to evaluate *rigor mortis*, by applying the *Rigor Index* (RI) method, proposed by Bito et al. [10], with

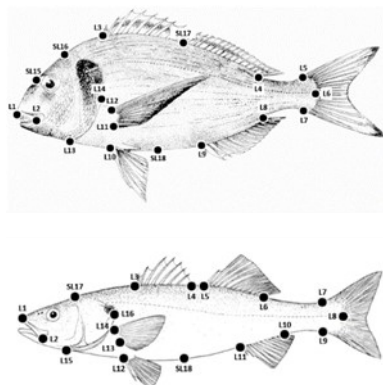


Figure 1. Landmarks selected for sea bream (above) and sea bass (below).

observation of the vertical distance between the caudal extremity of the caudal peduncle and the support plane of the cranial half of the muscular body, with the following sampling times: immediately upon arrival of the fishes (T0) and after 1, 3, 5 and 7 days (T1, T3, T5 and T7, respectively). Throughout the observation period, fishes were stored in polystyrene boxes, covered with ice, and placed in a refrigerated room (+2 °C). The other 15 fishes were stored in the same way and sampled at the same times to evaluate fish freshness through the Quality Index Methods (QIM) specific for sea bass [11] and sea bream [12]. Skin colour parameter values were also analysed in triplicate positions (cranial, medial, and caudal) of the dorsal part of the right side, using a CHROMA METER CR-200 colorimeter (Konica Minolta, Chiyoda, Japan). The colour was expressed as lightness (L^*), redness index (a^*), and yellowness index (b^*) [13]. Based on these values, the colour distance ΔE at the beginning of the trial was calculated as:

$$\Delta E_{\beta-\alpha} = \left[(L_{\beta}^* - L_{\alpha}^*)^2 + (a_{\beta}^* - a_{\alpha}^*)^2 + (b_{\beta}^* - b_{\alpha}^*)^2 \right]^{0.5}, \quad (1)$$

where α and β were the farming systems, CAGE or TANK.

Data from freshness evaluation and colour analysis were processed by a two-way ANOVA (two fixed factors: Farming systems, F, with two levels TANK and CAGE; Storage, S, with 5 sampling times set at 0, 1, 3, 5, and 7 days) while the *Rigor Index* was analysed by a one-way ANOVA, using the PROC GLM of SAS [14].

3. RESULTS AND DISCUSSION

3.1. Freshness and Rigor Index

QIM schemes are recognised to be a suitable method to evaluate freshness evolution during storage. Both farming system and storage affected the QIM scores of the considered species (Figure 2), although the farming effect was more pronounced in sea bass than in sea bream. Not surprisingly, the demerit score increased from the beginning of the storage until the end; irrespective of the day of the evaluation, the TANK sea bass always obtained a higher demerit score than the CAGE ones. This amplified effect of the farming system on sea bass freshness evolution might be due to the high susceptibility of this species

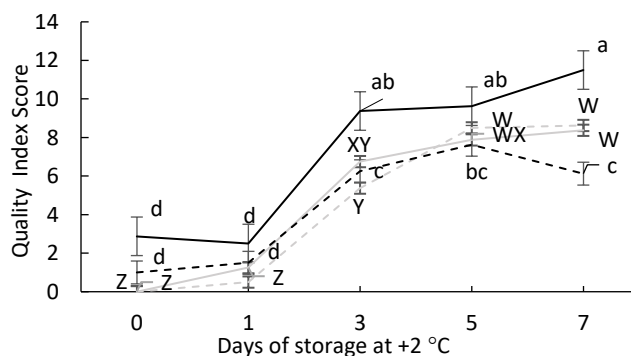


Figure 2. Freshness evolution of sea bass and sea bream during the refrigerated storage, according to the Quality Index Method. Grey lines: gilthead sea bream; Black lines: European sea bass. Full lines: inland tank; Dashed lines: sea cages. a, b, c, d: means with different letters are significantly different ($p < 0.05$) according to the significant interaction Farming system \times Storage obtained for E. sea bass; W, X, Y, Z: means with different letters are significantly different ($p < 0.05$) according to the significant interaction Farming system \times Storage obtained for gilthead sea bream.

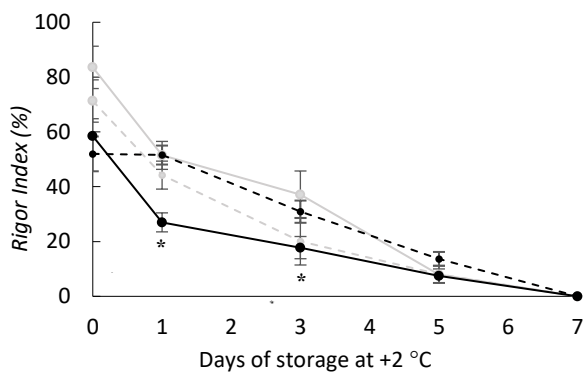


Figure 3. *Rigor Index* (%) evolution of sea bass and sea bream during the refrigerated storage as affected by farming system. Grey lines: gilthead sea bream; Black lines: European sea bass. Full lines: inland tank; Dashed lines: sea cages. *: *Rigor Index* of E. sea bass farmed in different systems was significantly different ($p < 0.05$) within the day of analysis.

to stressful events, as netting and stunning [15]. However, the overall scores calculated after 7 days of storage highlighted that the fish were still acceptable as they were lower than 14.3 (spoiled fish) [16].

In sea bream, *Rigor Index*, shown in Figure 3, was not significantly affected by the farming system. Fish arrived at the laboratory during the phase of a partial *rigor mortis*, that linearly resolved during the 7 days of observation. In contrast, European sea bass arrived with a RI of about 50 %, but the animals farmed in tanks showed a faster ($p < 0.05$) drop of their RI than the fish coming from cages at T1 and T3.

Because cadaveric rigidity develops from the first *post-mortem* hours, the presence of a complete or partial state of *rigor mortis* is generally associated with a high level of freshness. In this regard, both QIM schemes for E. sea bass and gilthead sea bream attribute a demerit score equal to 0 when *rigor* is observed. Hence, the results of QIM and *Rigor Index* here obtained for E. sea bass are consistent and highlighted a different freshness evolution of this species depending on the farming system.

3.2. Shape and colour traits

The farming system had an impact both on shape and skin colour. In both species, the first two PCA axes accounted for about 40 % of the variance explained (data not shown). Specimens were partially overlapped according to farming conditions, but a tendency of shape differentiation between the two farming typologies was evident, in particular for sea bream. Results of the splines relative to the extremes of the discriminant axis, depicting shape maximum variation according to the farming method, are shown in Figure 4. In the case of sea bass, shape variation was related to the height of the dorsal profile, higher in TANK group (Figure 4 A, blue line), and the mouth orientation, upper in CAGE group (Figure 4 A, pink line). Accordingly, the fish profile appeared to be more streamlined, and the caudal peduncle orientation was uppermost. In the case

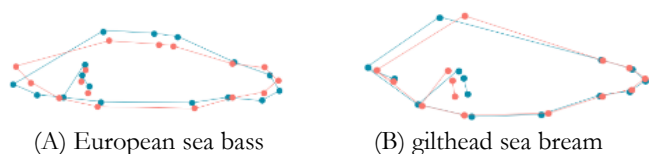


Figure 4. Splines of discriminant scores of fish specimens according to farming system (CAGE in pink and TANK in blue) in (A) European sea bass and (B) gilthead sea bream.

of sea bream (Figure 4 B), shape differences were concentrated in the head region, shorter in fish farmed in cages (Figure 4 B, pink line). The lateral fin was closer to the operculum in fish farmed in cages and the anal fin was slightly shorter. Such results could be interpreted as a consequence of the farming conditions. Morphological variations have been shown to be a valuable tool for describing changes in fish shape features [17] and Geometric Morphometrics tools have been frequently used in the last decades to quantify and describe morphological differences in fish according to ecology [18], [19], swimming behaviour, diet [20], rearing environment [21] and domestication status [22]. This agreed with previous findings on the effects of the rearing system on shape traits [23], [24], which were generally associated to the different hydrodynamic conditions. For sea bass and sea bream, previous studies mainly compared shape in wild and farmed fish, instead of in fish farmed under different conditions [17].

Under the hypothesis that hydrodynamic conditions experienced in floating sea cages are more similar to the wild, consequently fish reared in cages should be morphologically similar to their wild relatives. The available literature confirms this evidence: fish farmed in sea cages are more streamlined, the body profile is shorter, the caudal peduncle is slightly longer. Studies comparing body shape of rainbow trout (*Oncorhynchus mykiss*) farmed under different hydrodynamic regimes showed that a more streamlined body is associated to active swimming, typical of farming conditions as far as possible similar to the natural environment [21]. This is more evident in sea bass, adopting a sub-carangiform swimming, typically involving the posterior half of the body and the caudal fin. In sea bream, the swimming type is typically labriform, thus shape differences are more evident in the pectoral fin position, due to its role in abrupt changes of direction. A higher number of specimens and from different farms should be analysed to find a consistent and general pattern of variation attributable to farming conditions.

Skin colour modification occurred mainly due to physiological changes, as well as hormone-related ones, and to morphological ones, by means the variation of pigment concentration and density and/or distribution of chromatophore cells in skin [25]. Rearing conditions directly impact the physiological response of fish, thus suggesting a possible effect on skin pigmentation. On the one hand, how stress or rearing environment can act on visual appearance is not clear yet. Studies have revealed that acute stress involves a cascade of events generated from catecholamines released that can result in pale fish skin [26]. Moreover, chronic stress might act as a darkening or paling agent depending on the fish species. On the other hand, the link between feed and colour is widely established [27], [28]. Colour modifications can be established in fish flesh according to the pigments fish ingest, whether they are produced by microalgae living in the rearing environment [29] or by pigments directly added to or present in aquafeeds. However, complex interactions between the rearing conditions and skin colour have been recently reviewed; among the evidence, researchers reported that euryhaline fish farmed in tanks had a different skin pigmentation compared to fish reared in the marine environment and fed with the same feed [26]. This could be due to fish ability called “background adaptation”, which refers to their ability to change body colour in response to environmental luminosity, such as dark or bright backgrounds. In this regard, a recent study [30] found out that goldfish (*Carassius auratus*) reared in tanks with white backgrounds were characterised by a lower content of carotenoids in their skin than fish farmed in tanks with black

Table 1. Effect of the farming system and refrigerated storage on skin pigmentation.

		Farming system, F		Storage (days), S					P-value			RMSE
		TANK	CAGE	0	1	3	5	7	F	S	F × S	
European sea bass	L*	52.98	54.09	56.56 ^a	53.68 ^{ab}	54.53 ^a	49.36 ^b	53.54 ^{ab}	ns	0.002	0.011	3.224
	a*	-0.99	-0.76	-0.65	-0.96	-0.82	-0.90	-1.06	ns	ns	ns	0.417
	b*	5.28	0.37	3.46 ^a	2.87 ^{ab}	3.55 ^a	1.83 ^b	2.42 ^{ab}	< 0.0001	0.011	ns	1.029
Gilthead sea bream	L*	65.05	60.74	58.91 ^b	63.12 ^a	65.44 ^a	63.96 ^a	63.04 ^a	< 0.0001	< 0.0001	< 0.0001	2.234
	a*	-0.42	-1.10	-0.35	-0.68	-0.81	-0.89	-1.05	ns	ns	ns	0.533
	b*	0.77	-1.04	1.27 ^a	0.10 ^{ab}	-0.68 ^b	-0.56 ^b	-0.82 ^b	< 0.0001	0.008	ns	1.182

a, b: means with different letters are significantly different ($p < 0.05$); ns: not significant ($p > 0.05$). RMSE: Root Mean Square Error.

backgrounds. In addition, microalgae can develop in the water of tanks, thus potentially providing a good source of pigments for fish. Indeed, it is known that microalgae contain xanthophylls, a group of carotenoids having a yellowish colour; once ingested, these pigments could give a more intense yellow-green colour to the skin of the animals, as found in [27]. The results of the present trial, shown in Table 1, highlighted that E. sea bass farmed in tanks was characterised by a more yellow colour (b^*) compared to the CAGE group, according to the previously mentioned studies.

Consumer perception of fish colour plays a pivotal role on the choice of a product. The colorimetric distance between fish farmed in sea cages and in inland tanks was calculated for both species, using the T0 observations. The results were $\Delta E_{\text{sea bass}} = 5.04$ and $\Delta E_{\text{sea bream}} = 4.37$. As previously published [31], when $3.5 < \Delta E < 5$ a standard observer perceives a clear difference in colour, whereas when $\Delta E > 5$ the observer notices two different colours. Standing on this, it seems that the farming system affected the appearance of E. sea bass more than the appearance of sea bream. Finally, morphological modifications of chromatophores and pigments are generally correlated with skin colour changes during storage [25], explaining the present results. Indeed, increased skin L^* and decreased b^* values were previously observed during the refrigerated storage, in euryhaline species [32], [33].

4. CONCLUSIONS

Consumers should be aware of the rearing conditions in order to make an informed choice, as aquaculture production and seafood trade are expected to grow. However, when an information is added, a tool to verify the given statements should be developed. The present preliminary study showed that an easy-to-use system, as image analysis, could be a useful tool to discriminate among E. sea bass coming from different rearing systems, *i.e.*, from marine sea cages or inland tanks. In fact, it was evident that visual aspects of the fish, such as colour and shape, were significantly affected by the farming conditions that, in addition, induced a different *rigor* resolution and quality loss during refrigerated storage, especially in E. sea bass.

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