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Effect of rearing system on body traits and fillet quality of meagre (*Argyrosomus regius*, Asso 1801) chilled for a short time

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Abstract

The purpose of this study was to evaluate qualitative traits of meagre (*Argyrosomus regius*) from two different rearing systems (land-based tank filled with geothermal water *vs* offshore sea cage) and after short-term storage at chilling temperature (1, 2, or 3 days). Fish originated from the same batch were fed the same diet. Morpho-biometric traits, L*, a*, and b* colour parameters, texture, free water, proximate composition, total lipids, fatty acids, iron, and selenium contents were analyzed in the fillets. Most parameters were affected by rearing system. Compared to tank-reared fish, caged fish were shorter, poorer in visceral fat, and had higher incidence in cavity content and liver, lower incidence in gonads and head. Caged fish also had softer fillets in the epaxial site, which showed a higher tendency towards greenish colour. Caged fish also showed higher lipid content but lower Fe and Se content. Tank-reared fish fillets were more abundant in PUFAn-3, mainly due to DHA (18.54 *vs* 12.95%; P<0.001) and consequently showed the best healthiness indexes. Minimal changes, mostly involving colour and texture, were detected during the first three days of refrigerated storage. During storage, no significant modification of the parameters investigated could be ascribed to the rearing system.

Introduction

Farmed fish are known to grow in more stable conditions than wild fish, and different rearing techniques affect fish flesh quality in different ways (Orban *et al.*, 2000). Several studies have recently addressed the effect of rearing systems on quality characteristics, and especially marketable traits, nutrients, texture, and colour (Orban *et al.*, 1997, 2000; Mairesse *et al.*, 2006; Hallier *et al.*, 2007; Jankowska *et al.*, 2007; Roncarati *et al.*, 2010; Valente *et al.*, 2011). Farming time, rearing temperature, stocking density, water current, difference in nutrient availability, and hydrographic and hydrodynamics conditions in off-shore sites proved to be the main factors linked to the rearing system that affected fillet quality.

Lipids, fatty acids, and mineral profile are among the most important nutrients in fish. Seafood is particularly appreciated by consumers as an important source of n-3 polyunsaturated fatty acids (PUFAs) and mineral components, such as selenium and iron, which are essential in preventing disorders, oxidative stress, and cardiovascular disease (Beard *et al.*, 1996; Watanabe *et al.*, 1997; Rayman, 2000; Ruxton *et al.*, 2004). The levels of such nutrients may differ by rearing system because environmental conditions and diet also vary significantly from one system to another (Orban *et al.*, 2000). Similarly, texture and colour, which have gained increasing importance in quality assurance as sensory attributes, can also be affected by rearing system, and in particular, by rearing temperature, which affects the number and size of muscle fibres, lipid deposition, and physical activity, and has been shown to be the factor that influences rheological properties and colourimetric attributes most (Hyldig and Nielsen, 2001; Ginés *et al.*, 2004; Roth *et al.*, 2010).

It might also be presumed that rearing techniques also affect fish quality changes during storage and shelf life due to the above-mentioned documented effects on fillet physical-chemical properties (Orban *et al.*, 1997, 2000; Mairesse *et al.*, 2006; Hallier *et al.*, 2007; Jankowska *et al.*, 2007; Roncarati *et al.*, 2010; Valente *et al.*, 2011). Rearing techniques might, in fact, also affect at the start of the storage the microbiological quality of fish, which is closely linked to the quality of the water from which the fish are harvested. Scientific literature has provided very little information on this topic until now. It has been recently demonstrated that fish origin (wild or farmed) and rearing techniques both affect consumer perceptions of fillet quality. According to Verbeke *et al.* (2007), a large majority of consumers believes there are no major differences between farmed and wild fish, even if taste perception is mostly in favour of wild fish. With respect to aquacultured products, the type of farming could be relevant in consumer choices. Comparing fish farmed in marine cages to those raised in ponds, for example, mariculture production is perceived more positively than pond production, and this consumer preference is linked to the environmental aspects of fish farming (Stefani *et al.*, 2012).

No studies on how different rearing systems affect the nutrients, colour, and texture of farmed meagre (*Argyrosomus regius*, Asso 1801) fillets have yet been made. Meagre is an emerging species in Mediterranean aquaculture with leanness as its most valuable trait (Poli *et al.*, 2003; Hernandez *et al.*, 2009) that distinguishes it from other marketable farmed fish (*i.e.,* sea bream, sea bass, *etc.*) (Lanari *et al.*, 1999; Poli *et al.*, 2001). Less muscle fat than the amounts present in other aquacultured species permits refrigerated storage for longer periods of time. Poli *et al.* (2003) and Hernandez *et al.* (2009) reported a similar shelf life (9 days) for whole fish stored at 1°C and for fillet wrapped in thin polyethylene film stored at 4°C. Increasing interest in meagre processing has now been documented (Monfort, 2010), whereas the production of innovative and practical meagre-based seafood

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products has recently been reported by Ribeiro *et al.* (2012). In Italy, meagre is intensively reared in land-based tanks or in seawater cages. Cage-rearing in particular has provided excellent results at existing commercial hatcheries, which are in the position to reproduce massive quantities of the species (Cardia and Lovatelli, 2007).

Considering the current status of meagre culture in Italy and the potential for its expansion in Mediterranean area, this study aimed at evaluating any possible differences in the qualitative traits of meagre reared by different techniques (land-based tank *vs* sea cage) and identifying which technique provides fillets of the highest quality. Another aim was to evaluate the differences in fillet quality properties induced by short refrigerated storage of whole fish reared with these two systems.

Materials and methods

Fish were collected from the farm Il Vigneto, located near Ansedonia (Grosseto province, Italy). Meagre juveniles originated from the same batch were raised during the grow-out phase in land-based tanks (Tank) and in seawater cages (Cage). In land-based circular tanks (500 m^3 volume), the density was 60 fish/m^3; water temperature ranged from 19°C to 22°C (geothermal water), and salinity was approximately 16 ppt. In marine circular cages (2000 m^3 volume), the density was 10 fish/m^3, water temperature ranged from 13°C to 24°C, and salinity was approximately 37 ppt. Fish were fed the same commercial extruded feed (crude protein 44%; crude fat 22%; fatty acids (FAs) expressed as a percentage of total FA: SFA 24.2%, MUFA 27.6%, PUFAn-6 20.4%, PUFAn-3 25.0%; Se: 0.85 mg/kg; Fe: 183.9 mg/kg). After reaching marketable size (average weight of 951.1±259.8 g), 18 fish were sampled from both Tank and Cage in two subsequent times (May and July) with 36 fish collected from each rearing system. After slaughtering, the fish arrived at the Laboratory the day after the catch and were kept on melting ice during the entire experiment in a refrigerated room at 1°C. Fish were analysed at three different post-mortem times, *i.e.* 1st, 2nd and 3rd day (6 fish/day/rearing system/sampling time). Attention was focused on the period of commercial life considered most important in the fish trade as currently organized in Italy by the large-scale distribution retail market.

Whole body weight (BW) and total length (TL) were recorded at each sampling. Fish were dissected, and the head, axial skeletal bones, total cavity content, liver, gonads (when evident and separable), perivisceral fat (the fat stored inside the abdominal cavity), and the right and left fillets were weighed. The condition factor (CF) was calculated according to the following formula:

$$CF = 100 \times BW\ (g) / TL^3\ (cm).$$

Total cavity content, liver, gonads and perivisceral fat weights were referred as percentage of total BW, obtaining the viscerosomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI), and fat somatic index (FSI), respectively. Moreover, perivisceral fat was expressed also as a percentage of the total cavity content to obtain the fat visceral index (FVI). Carcass was calculated as (BW – total cavity content weight), whereas dressing yield (DY) and fillet yield (FY) were determined as the percentages of the carcass and fillets on BW, respectively. Physical characteristics were analysed directly on the left fillets, and chemical composition analyses were carried out on samples taken from the same fillet previously skinned, homogenised, and freeze-dried.

Texture, free water and colour

Texture measurements were performed using a Zwick Roell® texturometer (software: Text Expert II) equipped with a 200 N load cell. One cycle compression test was done using a 10 mm diameter cylindrical probe at a constant speed of 30 mm/min to 50% of total deformation. Among textural attributes, hardness was measured on the epaxial, ventral and caudal parts of the fillet. Fillet thickness at the three locations was measured directly by the texturometer at the same time as hardness measurement. The shear test was performed on the central part of the fillet using a straight blade that moved at a constant speed of 30 mm/min to 50% of the total deformation. A Spectrocolour® colourimeter (using Spectral qc 3.6 software) was utilised for colourimetric measurement carried out according to the CIELab system (CIE, 1976). In this system, lightness (L*) is expressed on a 0-100% scale from black to white, redness index (a*) ranges from red (+60) to green (-60) while yellowness index (b*) ranges from yellow (+60) to blue (-60). Colour was measured in duplicate on the epaxial, ventral, and caudal fillet positions. Free water measurement was performed by applying the compression test on filter paper using the Grau and Hamm (1953) method. It was expressed in terms of diffused area (cm^2) of the liquid exuded onto the filter paper.

Proximate composition and total lipid content

Moisture, crude protein (Nx6.25), ether extract, and ash content were determined using AOAC (2000) 950.46, 976.05, 991.36, and 920.153 methods, respectively.

Total lipid extraction was performed by a modified Folch *et al.* (1956) method. Freeze-dried samples, reconstituted fresh by adding distilled water, were homogenised with a 2:1 chloroform-methanol (v/v) solution and filtered. The filter was washed several times, and distilled water with 0.88% KCl was added to the filtrate until the [Choloroform:Methanol] water ratio was 4:1. The tubes were stirred, and a biphasic system was obtained by standing overnight. The lower phase containing lipids dissolved in chloroform was siphoned and recovered. Total lipid content was determined gravimetrically after removal of the solvent (chloroform) by evaporation under vacuum and lipid resuspension in a known volume of chloroform (5 mL). Lipid content was weighed in a crucible after complete chloroform evaporation. The extracted lipids were used for the FA profile analysis.

Fatty acid analysis

Fatty acid methyl esters (FAME) analysis was performed using the modified method of Morrison and Smith (1964). Lipids were saponified with 0.5 M KOH in methanol, and FAs were hydrolysed by adding 2 N HCl. Methyl esters were prepared by transmethylation, using boron fluoride-methanol at a 14% concentration. Methylated FA were dissolved in petroleum ether, dried, and finally resuspended in 1 mL of hexane.

The FA composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary column (30 m × 0.32 mm i.d., 0.25 μm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA) was utilised. The oven temperature was held at 100°C for 2 min, increased to 160°C over 4 min at the rate of 12°C/min, and then increased to 220°C over 14 min at the rate of 3°C/min and kept at 220°C for 25 min. The injector and the detector temperatures were set at 220°C and 300°C, respectively. One microlitre of sample in hexane was injected into the column with the carrier gas (helium) kept at a constant flow of 1.5 mL/min. The split ratio was 1:20.

Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Agilent) computing integrator

software. FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco). FAs were quantified through calibration curves using tricosanoic acid (C23:0) (Supelco) as an internal standard. FAs were expressed as a percentage of total FAME.

Computation of fat quality indexes

The following fat quality indexes were calculated:

- n-6/n-3 ratio;
- LA/ALA, as linoleic acid (LA; C18:2n-6)/alpha-linolenic acid (ALA; C18:3n-3) ratio;
- atherogenic index (AI), according to the formula [C12:0 + (4 × C14:0) + C16:0] / (ΣPUFAn-3 + ΣPUFAn-6 + ΣMUFA) (Ulbricht and Southgate, 1991);
- thrombogenic index (TI) according to the formula [C14:0 + C16:0 + C18:0]/[0.5 × ΣMUFA) + (0.5 × ΣPUFAn-6) + (3 × ΣPUFAn-3) + (ΣPUFAn-3/ΣPUFAn-6)] (Ulbricht and Southgate, 1991);
- hypocholesterolaemic/hypercholesterolaemic FA ratio (HH), calculated as (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0) (Santos-Silva *et al.*, 2002).

Selenium and iron content

In order to determine these trace minerals, solutions were prepared for an ICP optical read using the wet microwave digestion method (999.10) (AOAC, 2000). Dry samples were weighed, put into teflon tubes, and then 5 mL of super pure nitric acid and 1-1.5 mL of hydrogen peroxide were added. The teflon tubes were put suitably sealed into the Ethos 900 Microwave Labstation microwave oven (12-position rotor with teflon liner, Milestone Microwave Laboratory Systems, Sorisole, BG, Italy) at about 175°C for 30 min. Samples underwent different time-microwave power combinations: 5 min at 250W, 5 min at 450W, 6 min at 650W, 2 min at 250W, and then 5 min of ventilation in order to cool. At the end of digestion, teflon boxes were left to cool in a water bath and opened under fume, but only after their temperature had reached 40°C. The walls of the containers were washed with deionized water and the rinse water was poured into 25 cm^3 volumetric flasks. The digested samples were poured in numbered polyethylene bottles and their Fe and Se contents were determined using the MIN 1 method with a (ICP-OES) SPECTRO Ciros Vision EOP spectrometer, a spectrometer with induced coupled plasma source and simultaneous optical detection of emissions in the range of 125 to 770 nm. The instrument had a SPECTRO ADS 500 autosampler and a SPECTRO Smart Analyzer Vision 1.50.534 management software that read Fe and Se levels at absorption lines of 259.940 and 196.090 nm, respectively, with a minimum detection of 0.002 and 0.03 mg/L and a maximum calibrated quantity of 120 and 24 mg/L, respectively.

All analytical methodologies were submitted to validation procedures.

Statistical analysis

Data were analysed by ANCOVA (Analysis of CoVariance) with the SAS® (SAS, 2007) GLM procedure using rearing system (Tank, Cage), storage time (1, 2 and 3 days), and sampling month (May, July) as the discrete effects, and body weight as the continuous effect. Interactions between rearing system and storage time and between rearing system and sampling month were tested in a preliminary model and were excluded from the final model because they never attained significance. The differences between least squares means were statistically tested using the Student's t-test.

Results

Morpho-biometric parameters and indexes

Fish reared in cages showed a similar body weight to those reared in tanks (Table 1). Nevertheless, all subsequent parameters were covaried on BW with the aim of reducing variability and obtaining estimates at the same average BW (951.5 g). After this adjustment, fish reared in cages had significantly ($P<0.001$) lower length and higher CF. Although perivisceral fat content was negligible in both groups and showed no difference between rearing systems when considered as percentage of BW (FSI), it was higher in tank-reared fish when incidence was referred to cavity content (FVI). VSI and HSI were higher

Table 1. Morphological traits of meagre. Means are estimated at average body weight of 951.5 g.

	Rearing system		Storage			Significance				RSD
	Tank	Cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Fish, n	36	36	24	24	24					
Body weight, g	994.61	913.05	958.56	1005.17	897.77	ns	ns	***	/	187.57
Length, cm	44.85^b	43.74^a	43.93	44.20	44.75	***	ns	***	*** (+)	1.23
Condition factor	1.02^a	1.11^b	1.09	1.07	1.04	***	ns	***	ns	0.09
Body composition, % BW										
Cavity content, VSI	3.29^a	4.54^b	3.67	4.16	3.92	***	ns	*	** (+)	1.02
Liver, HSI	0.90^a	1.84^b	1.41	1.42	1.32	***	ns	ns	** (+)	0.38
Gonads, GSI	0.26^b	0.05^a	0.18	0.14	0.15	***	ns	*	ns	0.12
Fat, FSI	0.73	0.54	0.45^a	0.87^b	0.60^{ab}	ns	*	***	ns	0.56
Fat, FVI (% on cavity content)	17.38^b	11.28^a	13.57	16.67	12.76	*	ns	***	ns	12.50
Carcass, DY	96.71^b	95.46^a	96.33	95.84	96.08	***	ns	*	** (-)	1.02
Head	32.76^b	29.67^a	31.25	32.77	30.84	**	ns	**	*** (-)	2.04
Frame	15.70	16.29	16.48	16.76	15.85	ns	ns	ns	ns	1.49
Fillet, FY	46.57	47.89	47.21	46.40	48.09	ns	ns	ns	* (+)	2.88

RSD, residual standard deviation; BW, body weight; VSI, viscerosomatic index; HSI, hepatosomatic index; GSI, gonadosomatic index; FSI, fat somatic index; FVI, fat visceral index; DY, dressing yield; FY, fillet yield. $^{a,b}P<0.05$ within criterion; *P<0.05; **P<0.01; ***P<0.001; ns, not significant. The symbols (+) and (-) indicate the regression sign on the weight.

in cage-reared fish, while GSI was significantly higher in fish reared in tanks, consequently DY was also higher in the latter, whereas no differences in FY between rearing systems were detected.

Month of sampling evidenced high variability in morpho-biometric parameters, while the casual sampling of fish in the three days of post-mortem storage did not reveal any substantial differences, in this way indicating the homogeneity of raw material in this experimental thesis. Moreover, as BW increased, length, cavity content, liver percentage, and fillet yield increased proportionately, and only head proportion decreased.

Physical parameters

As shown in Table 2, textural analyses performed on the fillets showed that differences between rearing systems were strictly related to the site of measurement. In the epaxial zone, tank-reared fish showed significantly higher hardness values ($P<0.001$). Also the hardness measured on the caudal and ventral zones and the shear force measured only in the central zone were higher in tank fish, even though these differences were not significant. In this group of meagre, which showed higher overall hardness values, free water was released in significantly ($P<0.01$) greater amount. Similarly to texture, differences between rearing systems in colourimetric attributes were also influenced by site of measurement (Table 2). L*, a*, and b* values did not differ significantly in the epaxial zone, whereas L* and a* values in the caudal zone and a* values in the ventral zone were significantly higher in Tank fish fillets.

Although no differences in hardness among storage days were observed in the epaxial and ventral zones, hardness decreased significantly ($P<0.05$) with storage time in the caudal zone. Shear force and free water were unaffected by days of storage. With regard to colourimetric attributes, L* and a* values were significantly higher at the 2nd day than at the 1st and 3rd days, while b* differed only in the caudal zone between the 2nd and 3rd day. Body weight affected some of the physical parameters investigated; muscle free water and L* at the epaxial and caudal sites increased with rising BW. The relationship between fillet thickness and BW was obviously positive.

Proximate composition, selenium, and iron contents

The proximate composition of meagre fillets exhibited differences between rearing systems only in ether extract and total lipid content, which were lower in fish reared in tanks where the highest Fe and Se content was present (Table 3). Other factors, such as day of storage and sampling month, had little or only sporadic influence on fillet chemical composition. The influence of fish weight was more relevant; increased weight negatively affected moisture and ash content while positively affecting fillet lipid content (whether expressed as ether extract and total lipids). A positive relationship between fish weight and Se content was also observed.

Fatty acid profile

The FA profile of fillets from differently-reared fish is reported in Table 4. In both rearing systems, palmitic acid (C16:0) and oleic acid (C18:1n-9) were the predominant saturated and monounsaturated FAs (SFA and MUFA), respectively. Among polyunsaturated FAs (PUFA), C18:2n-6 (LA), C20:5n-3 (EPA), and C22:6n-3 (DHA) were the most abundant. The FA profile on the whole was strongly affected by rearing system, which did not influence only palmitic acid and SFA percentages. On the contrary, as expected, FA variation was never affected by day of storage. The influence of the sampling month, however,

Table 2. Physical characteristics of meagre. Means are estimated at average body weight of 951.5 g.

	Rearing system		Storage			Significance				RSD
	Tank	Cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Fish, n	36	36	24	24	24					
Free water, cm^2	11.90^a	10.15^b	11.58	10.70	10.80	**	ns	**	* (+)	2.22
Shear force, N	9.13	8.35	9.64	8.10	8.47	ns	ns	ns	ns	2.19
Epaxial zone										
Thickness, mm	15.04	15.56	15.46	15.17	15.29	ns	ns	***	*** (+)	1.21
Hardness, N	9.40^b	7.40^a	8.29	8.35	8.56	***	ns	*	ns	1.85
L*	31.79	31.55	27.16^a	38.64^b	29.20^a	ns	***	ns	* (+)	4.96
a*	-4.78	-5.43	-5.77^a	-3.74^b	-5.80^a	ns	***	ns	ns	1.49
b*	-0.81	-1.67	-1.37	-0.67	-1.69	ns	ns	ns	ns	2.25
Caudal zone										
Thickness, mm	7.78	8.65	8.98	7.99	7.67	ns	ns	***	*** (+)	1.91
Hardness, N	6.53	5.98	7.20^b	6.15^{ab}	5.42^a	ns	*	***	ns	2.20
L*	36.85^b	33.86^a	32.03^a	41.62^b	32.42^a	**	***	ns	ns	3.92
a*	-1.95^b	-3.80^a	-3.76^a	-1.07^b	-3.79^a	**	***	ns	ns	2.42
b*	0.94	0.64	0.73^a	1.77^b	-0.11^a	ns	*	*	ns	2.20
Ventral zone										
Thickness, mm	11.02	11.85	11.02	11.31	11.99	ns	ns	***	*** (+)	1.94
Hardness, N	10.94	9.92	10.45	8.80	12.03	ns	ns	***	ns	5.17
L*	43.41	43.57	44.19^b	46.66^b	39.62^a	ns	*	***	** (+)	7.68
a*	0.15^b	-1.92^a	-1.03^b	1.49^c	-3.11^a	*	***	ns	ns	3.51
b*	2.77	1.05	1.81	2.65	1.26	ns	ns	ns	ns	3.73

RSD, residual standard deviation; L*, lightness; a*, redness index; b*, yellowness index. $^{a,b,c}P<0.05$ within criterion; *$P<0.05$; **$P<0.01$; ***$P<0.001$; ns, not significant. The symbols (+) and (-) indicate the regression sign on the weight.

was evident. In greater detail, limiting the examination to unsaturated FAs, cage-reared fish showed a significantly higher concentration of C16:1n-7, C18:1n-9, C18:1n-7, LA, EPA, and C22:5n-3 than tank-reared fish, even though the differences in value were generally small. On the contrary, tank-reared fish showed slightly higher amounts of C20:1n-9, C22:1n-11, C20:4n-6, C18:3n-3, and a much higher amount of DHA (about 5.5 percentage points). As regards healthiness indexes, the higher percentage of PUFAn-3 observed in tank-reared fish was responsible for the superior quality of all such indexes except LA/ALA, which was lower and therefore better in cage-reared fish due to the higher percentage of C18:3n-3. Regarding the effect of fish weight, different behaviour was observed in each FA

Table 3. Chemical composition of meagre, expressed on 100 g of wet weight of fillets. Means are estimated at average body weight of 951.5 g.

	Rearing system		Storage			Significance				RSD
	Tank	Cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Fish, n	36	36	24	24	24					
Moisture, g	75.99	75.29	75.34	75.68	75.89	ns	ns	ns	*** (-)	1.14
Protein, g	21.04	20.74	21.00	21.09	20.58	ns	ns	ns	ns	0.78
Ether extract, g	1.51^{a}	2.53^{b}	2.14	1.85	2.07	***	ns	*	*** (+)	0.76
Total lipids, g	2.12^{a}	3.00^{b}	2.70	2.35	2.64	***	ns	*	*** (+)	0.69
Ash, g	1.39	1.37	1.40	1.38	1.36	ns	ns	ns	** (-)	0.06
Iron, µg	265.2^{b}	201.8^{a}	247.8	220.4	232.3	***	ns	ns	ns	43.50
Selenium, µg	18.3^{b}	15.2^{a}	16.7^{a}	14.2^{a}	19.4^{b}	*	*	***	*** (+)	4.02

RSD, residual standard deviation; ^{a,b}P<0.05 within criterion; *P<0.05; **P<0.01; ***P<0.001; ns, not significant. The symbols (+) and (-) indicate the regression sign on the weight.

Table 4. Fatty acid profile and healthiness indexes of lipids in meagre. Means are estimated at average body weight of 951.5 g.

	Rearing system		Storage			Significance				RSD
	Tank	Cage	1 d	2 d	3 d	Rearing	Storage	Sampling month	Weight	
Fish, n	36	36	24	24	24					
Fatty acids, % of total FA										
C14:0	3.20^{a}	4.06^{b}	3.63	3.57	3.68	***	ns	**	*** (+)	0.39
C16:0	17.70	17.68	17.69	17.85	17.53	ns	ns	ns	ns	0.57
C18:0	5.95^{b}	5.17^{a}	5.53	5.70	5.44	***	ns	**	*** (-)	0.54
SFA	28.29	28.38	28.31	28.57	28.13	ns	ns	ns	ns	0.86
C16:1n-7	4.29^{a}	5.85^{b}	5.03	4.99	5.19	***	ns	***	*** (+)	0.59
C18:1n-9	12.77^{a}	13.72^{b}	13.25	13.08	13.41	***	ns	*	*** (+)	0.86
C18:1n-7	2.42^{a}	2.74^{b}	2.57	2.59	2.59	***	ns	***	*** (+)	0.10
C20:1n-9	1.67^{b}	1.39^{a}	1.51	1.48	1.59	***	ns	ns	*** (+)	0.24
C22:1n-11	1.49^{b}	0.99^{a}	1.24	1.17	1.32	***	ns	ns	*** (+)	0.33
MUFA	24.56^{a}	26.47^{b}	25.49	25.14	25.92	***	ns	*	*** (+)	1.99
C18:2n-6 (LA)	10.23^{a}	11.35^{b}	10.77	10.61	10.97	***	ns	***	ns	0.58
C20:4n-6	1.57^{b}	1.10^{a}	1.33	1.37	1.30	***	ns	***	*** (-)	0.26
PUFAn-6	13.10^{a}	13.58^{b}	13.36	13.18	13.48	***	ns	***	*** (-)	0.64
C18:3n-3 (ALA)	0.94^{a}	1.18^{b}	1.06	1.01	1.11	***	ns	ns	*** (+)	0.17
C20:5n-3 (EPA)	8.58^{a}	10.12^{b}	9.28	9.35	9.42	***	ns	***	** (+)	0.62
C22:5n-3	2.22^{a}	2.32^{b}	2.26	2.31	2.25	***	ns	***	ns	0.15
C22:6n-3 (DHA)	18.54^{b}	12.95^{a}	15.86	16.17	15.21	***	ns	**	*** (-)	2.19
PUFAn-3	32.05^{b}	28.81^{a}	30.46	30.80	30.04	***	ns	ns	*** (-)	2.02
PUFA	47.14^{b}	45.08^{a}	46.17	46.26	45.91	***	ns	*	*** (-)	2.08
Healthiness indexes										
n-6/n-3	0.40^{a}	0.47^{b}	0.44	0.42	0.45	***	ns	**	ns	0.04
LA/ALA	11.98^{b}	9.58^{a}	10.85	11.09	10.40	***	ns	**	*** (-)	1.85
AI	0.44^{a}	0.49^{b}	0.46	0.46	0.46	***	ns	*	*** (+)	0.02
TI	0.09^{a}	0.14^{b}	0.12	0.11	0.12	***	ns	ns	*** (+)	0.02
HH	2.64^{b}	2.42^{a}	2.53	2.52	2.54	***	ns	*	*** (-)	0.15

RSD, residual standard devation; FA, fatty acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AI, aherogenic index; TI, thrombogenic index; HH, hypocholesterolaemic/hypercholesterolaemic fatty acids ratio. The fatty acids C12:0, C15:0, C14:1, C16:2n-4, C16:3n-4, C17:0, C17:1, C16:4n-1, C18:2n-4, C18:3n-6, C18:3n-4, C20:0, C20:1n-7, C20:2n-6, C20:3n-6, C20:3n-3, C20:4n-3, C21:5n-3, C22:1n-9, C22:4n-6, C22:5n-6, detected in percentages lower than 0.50, are considered in the composite fractions, but not reported in the table for brevity. ^{a,b}P<0.05 within criterion; *P<0.05; **P<0.01; ***P<0.001; ns, not significant. The symbols (+) and (-) indicate the regression sign on the weight.

and each FA group. All MUFA increased with the increase of BW, similarly to C14:0, ALA and EPA, whereas C18:0, C20:4n-6 and PUFAn-6, DHA and PUFAn-3 decreased with increasing BW.

Discussion

Effect of rearing system

Environmental parameters (*e.g.*, water temperature and salinity) and rearing conditions (*e.g.*, fish density) were different in the two rearing systems. Consequently, most of the different results obtained in Tank or Cage systems can be attributed to the effect of the abovementioned parameters on the metabolism and the physiological condition of the fish initially taken from the same batch.

The differences observed in morpho-biometric parameters could depend on the fact that by producing different swimming activity and feeding behaviour, rearing systems influenced fish growth and modified fish shape in different ways. In this trial, the cage-fish reared at lower density and naturally variable water temperature were less slender than tank-reared fish. Flos *et al.* (2002) found that super-intensively raised gilthead sea breams assume a very particular, more compact shape than both fish reared less intensively and wild fish, and that when compared with the latter of similar weight are shorter, wider and higher. Tulli *et al.* (2009) reported that when compared to extensively reared fish, intensively-reared sea bass showed an enlarged ventral zone resulting from reduced swimming activity and the accumulation of perivisceral fat.

Higher percentages of FVI in tank-reared meagre could be ascribed to the higher stocking density that limits swimming activity. Higher FVI was also reported in sea bass reared in inland basins when compared to those kept in off-shore marine cages (Tulli *et al.*, 2009). Conversely to FVI, the somatic indexes VSI and HSI, were higher in fish raised in cages, where more intense swimming and the higher seawater temperature in summer could have induced increased feed consumption and consequently lipid deposition in the liver and skeletal muscle rather than in the viscera in accordance with the findings of Sheridan (1988). Fish metabolism is largely based on lipids and proteins, storing lipids in the liver, viscera, and muscle, even if the detailed distribution in these body components varies between species (Love, 1970). Moreover, the liver was found to be a depository organ for energy, while muscle seemed to play a lesser role in energy storage in several Sciaenidae species (Craig *et al.*, 2000; Chatzifotis *et al.*, 2006; Shoonbee, 2006). Low HSI values have therefore been observed both after fasting periods (Chatzifotis *et al.*, 2006) and during spawning phases (Herland *et al.*, 2010). In light of these findings, it may be presumed that the physiological state of cage-reared meagre was characterized by increased feed consumption most likely promoted by higher seawater temperature in the final period of the trial. The same group of fish also showed negligible gonadal development compared to tank-reared fish, and as a result, reserves were accumulated in the liver and muscle. Differing fish physiological conditions and rearing parameters are also probably responsible for the contrasting results observed in related species in the literature available. Tulli *et al.* (2009) found higher VSI and HSI in sea bass reared in cages than in those raised in inland basins, whereas Roncarati *et al.* (2010) recorded higher VSI and HSI in land-based basins than in offshore and inshore cages.

In this trial, meagre showed particularly high DY and FY in both rearing systems that were higher than those of meagre of similar body weight analyzed in previous research by Poli *et al.* (2003). Piccolo *et al.* (2008) found lower DY and higher FY values on meagre of similar size.

Texture measurement results indicated that although rearing systems had no significant influence on hardness at the caudal and ventral sites, tank-raised fish were significantly harder in the epaxial site than cage-raised fish. It is likely that the greater thickness in the epaxial area was responsible for highlighting the difference in hardness due to the rearing system.

Current literature holds that hardness may be influenced by chemical composition, histological muscle characteristics, and animal exercise, which are greatly affected by farming density and temperature. The effect of fillet lipid content on its texture was shown in salmon by Dunajski (1979), Christiansen *et al.* (1995) and Robb *et al.* (2002) and in sea bream by Orban *et al.* (1997), which latter found flesh lipid content and hardness to be inversely related. The higher hardness of tank-reared meagre fillets might therefore be attributed to their overall lower lipid content. As concerns histological muscle characteristics, water temperature is known to influence muscle morphology by affecting the number and size of muscle fibres; more precisely, higher water temperature increases both fibre density and thinning (Ginés *et al.*, 2004; Hallier *et al.*, 2007). Higher fibre density produces an increase in hardness (Hatae *et al.*, 1990). The effect of water temperature could explain the higher hardness values detected in fish reared in tanks, where water temperatures were on average higher and more constant throughout the year than the temperatures in cages, due to the geothermal nature of water. The softer flesh of cage-reared fish may also be attributed to the more intense swimming activity enabled by lower stocking density. Physical exercise, in fact, is known to modify fish muscle structure by stimulating the fibre hypertrophy (Davison, 1997) associated with softer flesh (Hatae *et al.*, 1990; Bugeon *et al.*, 2003).

Another aspect that emerged from this study was the difference in texture in the three measurement sites. Literature reports that fillets have heterogeneous characteristics for textural properties (Botta, 1991; Reid and Durance, 1992) and lipid content (Aursand *et al.*, 1994). The heterogeneity for textural properties could be also explained by the close relationship between fillet thickness and hardness observed also in this trial. In raw salmon fillets Sigurgisladottir *et al.* (1997) found fillet thickness to be significantly and positively correlated with hardness instrumentally measured by flat cylinder method, a method similar to the one used in this study. The same Authors found a different capacity to identify fish origin through instrumental texture analysis by the different sites where the measurement is made. Although in agreement with the results of this trial, this finding runs contrary to Sigurgisladottir *et al.* (1997), who found the highest discriminating capacity at the most caudal location, whereas in our study the difference between rearing systems was most significant at the epaxial site.

The colour of tank-reared fish fillet did not substantially differ from that of cage-reared fish, apart from the L* in caudal site and the a* in caudal and ventral sites. Since the values of both chromaticity indexes a* and b* were low in all sites, the colour of the fillet was grayish on the whole. The lower a* values seen in cage-reared meagre indicate a higher green colour component tendency, which was most likely due to access to a wider variety of natural food sources and pigments in addition to artificial feed. According to observations on catfish (Hallier *et al.*, 2007) and Arctic charr (Ginés *et al.*, 2004), water temperature differences may also be responsible for colour change. In both rearing systems, epaxial sites were darker than caudal and ventral sites, whereas ventral sites had a brighter appearance with more yellowish and reddish colour.

Since a positive a* value is generally associated with the presence of hemoglobin (Chaijan *et al.*, 2005; Hallier *et al.*, 2007), the higher values of redness index at the ventral site may be attributed to a high level of vascularization in the abdominal cavity wall (Hallier *et al.*, 2007). The water-holding properties of muscle tissue are very important for commercial value and consumer acceptance. Muscle water-holding capacity is highly influenced by structural changes in muscle proteins, fibril swelling-contraction, and the distribution of fluid between intra- and extra-cellular locations (Jonsson *et al.*, 2001). In this study, tank-reared fish, which showed higher hardness values, released higher amounts of free water, thus confirming the direct relationship between these two parameters found by Jonsson *et al.* (2001) and Rawdkuen *et al.* (2010).

The rearing system significantly affected fillet proximate composition. Similar to as previously reported by Poli *et al.* (2003), Piccolo *et al.* (2008) and Grigorakis *et al.* (2011) for the same species, the fat content of the fillets that we tested was low. Moreover, cage-reared fish had higher percentages of fat than tank-reared fish, a result that contrasts with what literature commonly reports for other marine species. Sea bass (Roncarati *et al.*, 2010) and sharpsnout sea bream (Orban *et al.*, 2000) reared in cages had leaner fillets than those reared in land-based basins and tanks, respectively, even if comparing different farming systems is always difficult due to the multitude of specific and characteristic factors, however. On the other hand, Davison (1997) reported that in many cases exercise may not necessarily represent increased energy use, and that in many fish, swimming might even be a form of energy saving. An increase of total lipids in red muscle was detected after exercise training in two cyprinids by Sänger (1992), for example.

An additional assumption may be that the higher lipid content of cage-reared fish is the result of a compensatory growth induced by the consistent increase of sea temperature from the winter to summer period. In the rearing site, sea temperature drops below 20°C for half the year and is about 14°C from January to the beginning of March. Since meagre feeding activity is substantially reduced when water temperature falls below 13-15°C (El-Shebly *et al.*, 2007), caged fish may have resumed feeding in the spring. Ali *et al.* (2003), in a review on compensatory growth in teleosts, provided evidence that periods of food deprivation induce changes in fish storage reserves, particularly lipids, and that the restoration of satiation feeding is followed by significant increases in lipid content in muscles and in the liver and viscera incidences (Miglavs and Jobling, 1989). Variations in fish mineral composition are known to be closely related to seasonal and biological (species, size, dark/white muscle, age, sex, and sexual maturity) factors, area of catch, food source, environmental pollution (water chemistry, salinity, temperature and contaminant), and processing method (Erkan and Özden, 2007). In this study, the Fe and Se content of the rearing water was always very low (<0.001 and <0.01 mg/L, respectively) and without difference between the two rearing systems. Considering the low content in the water, these trace elements were derived almost entirely from the feed fed to both groups of fish. Selenium is mostly present in fish in water-extractable form and may be either unbound (*i.e.*, neutral and ionic) or bound to polar materials, such as simple amino acids, peptides, and low molecular weight proteins (Cappon and Smith, 1982). Seafood is known to be a very good source of Se, in which it is present in considerably higher quantity than in other meats (Morris and Levander, 1970). Our study showed meagre Se content to be lower than the values reported by Morris and Levander (1970) in different fish species (40-70 µg/100 g), and lower than those provided by Šatović and Beker (2004) in sea bass (21-33 µg/100 g) and by Erkan and Özden (2007) in sea bass and sea bream (28.2 and 23.6 µg/100 g, respectively). Seafood, especially marine fish and darker flesh fish, is also a reasonably good source of Fe, even if it does not represent the most important source for humans (Erkan and Özden, 2007; Peterson and Elvehjem, 1928). Tank-reared fish showed a higher Fe level than caged fish, similarly to as observed by Orban *et al.* (2000) in sharpsnout sea bream (*Diplodus puntazzo*) reared in different systems.

The fish flesh FA profile resembles that of the fish feed, and the influence of dietary composition on quality and quantity of FA is reported in the literature (Tocher, 2003). This study indicates that the rearing system also affects the FA profile however, since differences in fillet lipid content were observed in fish fed the same diet. The higher lipid content of cage-reared fish can be associated, in fact, with the higher proportion of MUFAs, which are known to abound in meagre neutral lipids (Grigorakis *et al.*, 2011). Conversely, leaner, tank-reared fish displayed higher levels of n-3 PUFAs, foremost of which DHA, which are mainly located in polar lipids, as research by Grigorakis *et al.* (2011) confirmed for this species as well. The relationship between higher n-3 PUFA content and greater leanness has also been found in other species in similar trials comparing different rearing systems, as in the case of the sea bass studied by Roncarati *et al.* (2010) and the sharpsnout sea bream studied by Orban *et al.* (2000).

These FA profiles determined better health lipid indexes in tank-reared fish. Literature offers no data on the values of these indexes in fillets from fish reared under different systems. One comparison might be made with the LA/ALA ratio calculated from the FA profiles reported by Roncarati *et al.* (2010) and by Orban *et al.* (2000) for species of fish that could share the same market niche with meagre. Roncarati *et al.* (2010) found a lower LA/ALA ratio in sea bass farmed in offshore cages than in those reared in land based basins, and this agrees with the findings on meagre in this study. A similar and very low LA/ALA ratio characterized the sharpsnout sea bream analysed by Orban *et al.* (2000) reared in tanks or cages. The only direct comparison that can be made is with the absolute values of some of these indexes obtained in studies carried out in the same species. In particular, the AI values of tank-reared meagre were abundantly lower than the value (0.69) reported for meagre reared in land-based tanks by Poli *et al.* (2003). On the contrary, the AI value of cage-reared meagre was higher than the 0.38 value detected by Grigorakis *et al.* (2011) in the same species reared in sea cages. Also on the contrary, TI values were particularly low compared to those obtained in the same species by the abovementioned studies.

Effect of storage

The deterioration of fresh fish is due to autolytic and bacterial processes (Huss, 1988). During spoilage, fish undergo changes in colour, flavour, and texture (Gram and Huss, 1996) according to an evolution affected by many factors, such as season, feeding, handling, and initial microbiological load.

As expected, the morpho-biometric (Table 1) and chemical characteristics (Table 3) of meagre analysed at different times of storage were the same. Storage had only a limited effect on fillet texture and colour. Only at the caudal site was observed a decrease in hardness, where such softening may be due to the notoriously high collagen content in the tail (Yoshinaka *et al.*, 1988; Johnston, 2001). This may explain the greater detachment of the muscle fibres from the myocommata responsible for tenderization.

The increase of L*, a*, and b* values from the 1st to the 2nd day followed by decrease at the 3rd day of storage may be due to the evolution of *rigor mortis*. The change into a more translu-

cent flesh from the 1st and the 2nd day may be attributed to muscle contraction and the altered muscle light scattering properties known to be responsible for changes in L* during *rigor* development (Erikson and Misimi, 2008). A similar variation in L*, a*, and b* values when *rigor* starts was observed by Erikson and Misimi (2008) in ice-stored salmon.

Minimal changes in the same species during the first three days of refrigerated storage were detected by Poli *et al.* (2003) after measuring non relevant variations in the dielectric properties of muscle and in the *rigor* index in the first four days after death. Also Hernandez *et al.* (2009) observed minimal changes, without detecting variations in colour properties or texture variables in the first 4 days of storage of meagre fillets. The greatest changes actually occurred during the residual period of storage. Applying the EU Sensory Scheme (Rule 2406/EEC), Poli *et al.* (2003) classified the sample of meagre analysed and stored as whole fish at 1°C under ice cover in Extra class until the 3rd day of storage, and assigned 9 days of shelf life. Equal shelf life was assigned by Hernandez *et al.* (2009) to meagre fillets stored at 4°C.

The attention in this trial was focused on the parameters that most affect the quality perceived by consumers and the storage duration corresponding to that for the mass distribution and marketing of fish from aquaculture. The overall results on maintaining product quality levels are reassuring and were undoubtedly also partially due to both the storage of meagre in whole fish form that delays changes in intrinsic properties during shelf life and the short refrigerated storage time examined. Rearing technique did not induce any different behaviour during the three days of refrigerated storage. Although the differences in hardness, lipid quantity, and fillet quality attributed to rearing technique described above could probably also induce a different evolution of quality parameters during shelf life, this was not yet evident in the short period of refrigerated storage adopted. It may also be hypothesized that variations in texture were masked by *rigor* resolution condition, and that a different susceptibility to oxidation and rancidity may be manifested only at a more advanced stage of storage.

Conclusions

In conclusion, the fish from the two rearing systems showed specific characteristics even though the differences detected were not relevant. Compared to fish reared in tanks filled with geothermal water, the fat in fish reared in mariculture cages was distributed more in the muscles than in the perivisceral areas. The higher lipid content of fillets taken from cage-reared fish probably was responsible of higher water holding capacity, lower hardness, a FA profile that was poorer in PUFAn-3, and mainly in DHA, and slightly less favorable healthiness indexes. Short time chilling did not cause significant changes in flesh quality, while the modifications in colour and texture detected can be attributed to the normal course of *rigor mortis* in the first three days after death when the whole fish is normally sold at full price. Fillets from the two rearing systems presented the same behaviour during storage.

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