

Differences in tissue fatty acid composition between reared and wild sharpsnout sea bream, *Diplodus puntazzo* (Cetti, 1777)

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The fatty acid composition and lipid content of white muscle, liver and mesenteric fat, in reared *v.* wild sharpsnout sea bream (*Diplodus puntazzo*) were compared. The mesenteric fat index ($100 \times$ mesenteric fat weight/body weight) and the lipid contents of both white muscle and liver proved consistently higher in farmed *v.* wild sharpsnout sea bream (79.0 (SE 13.1) *v.* 38.7 (SE 5.1) g/kg, 188.4 (SE 30.0) *v.* 58.2 (SE 3.9) g/kg and 27.2 (SE 3.7) *v.* 17.3 (SE 1.9) g/kg, respectively). The higher values of linoleic, eicosapentaenoic, docosahexaenoic and *n*-3 series acids in reared fish muscle make reared sharpsnout more favourable for human consumption. In reared fish mesenteric fat, polyunsaturated fatty acids reached higher levels (32.54 (SE 0.71) g/100 g total fatty acids than those found in wild fish (26.08 (SE 1.38) g/100 g total fatty acids) or even present in the diet (28.34 g/100 g total fatty acids). Compared with cultured fish, wild sharpsnout displayed a higher content of *n*-3 fatty acids in liver fat (31.67 (SE 1.13) g/100 g total fatty acids), but lower in mesenteric fat (20.35 (SE 1.41) g/100 g total fatty acids). Atherogenic index values were similar for wild and reared fish in all tissues, while the index of thrombogenicity of muscle and mesenteric fat (0.353 (SE 0.012) and 0.402 (SE 0.021) respectively) was significantly increased in wild fish probably due to the omnivorous habits of the species and/or to seasonal food variations. Depending on the time of the year or the season, reared fish could be more suitable for human consumption than wild fish.

Fatty acids: Liver: White muscle: Mesenteric fat: Aquaculture: Fish: *Diplodus puntazzo*

Sharpsnout sea bream (*Diplodus puntazzo*) is a highly valued fish species for human consumption in the Mediterranean. Since this species has proved suitable for culture, there have been serious attempts to produce it on a commercial basis (Divanach *et al.* 1993; B García-García, FM Rueda, MD Hernández-Llorente, F Aguado-Giménez, MA Egea-Nicolás and F Faraco-Munuera, unpublished results). Unlike sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), two important carnivorous species commonly grown and consumed in that region, sharpsnout is considered an omnivorous species (Sala & Ballesteros, 1997), requiring the search for new commercial diet formulations to match its nutritional demands.

Lipids are an important component of fish diets, both because of their role as energy-providing molecules and due to the essential nature of some fatty acids. Fish fatty

acids are also known to confer cardiac-health properties to human subjects and increased fish consumption has been recommended. In this sense, *n*-3 series fatty acid consumption closely correlates with a reduced incidence of several human diseases (Harris, 1989).

The lipid content of fish is highly variable between and within species (Shearer, 1994). Many factors appear to contribute to this variability, including food availability, catch location, fish size, maturity stage, biological variations, sampled tissue (Hardy & King, 1989), ration size (Kiessling *et al.* 1989) and starvation (Lie & Huse, 1992). The tissue lipid composition does not simply reflect that of the diet. Rather, the content of some fatty acids varies within defined ranges depending both on the species and the nature of the tissues under study. Upon changing the source of dietary lipids (Arzel *et al.* 1994), a relatively constant

Abbreviations: AI, atherogenic index; IT, index of thrombogenicity; MFI, mesenteric fat index; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

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fatty acid pattern is maintained in brown trout (*Salmo trutta* L.) muscle and liver total lipid content. A very similar phenomenon has been described in coho salmon, *Oncorhynchus kisutch* (Walbaum) (Yu & Sinnhuber, 1981), and rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Greene & Selivonchick, 1987). Orban *et al.* (2000) have reported fatty acid composition differences between sharpnose sea bream grown in tanks *v.* cages, particularly in terms of saturated fatty acids. Previous studies from our group have reported white muscle fatty acid composition differences between wild and reared red porgy (*Pagrus pagrus*) (Rueda *et al.* 1997), with wild fish displaying higher levels of 20:4*n*-6 and 22:6*n*-3 and reared fish muscle exhibiting much higher *n*-9 levels.

The aim of the present work was to compare the fatty acid composition and lipid content of white muscle, liver and mesenteric fat in reared *v.* wild sharpnose sea bream.

Materials and methods

Fish facilities

The reared group was comprised of wild sharpnose sea bream caught in the Mar Menor (Murcia, Spain) in April 1997 (approximately 10 g in weight) and grown under

culture conditions (distributed into several size groups) until November 1997, when one sample of five fish (mean weight 191.60 (SE 9.28) g) was killed. Animals were kept in a 5000 litre tank (raceway model) at the Centro de Recursos Marinos de Murcia and fed a commercial diet (Trouw-Mar sea bream no. 2; Trouw España S.A., Cojobar, Burgo, Spain) administered by hand five times per day to satiation. The water supply was directly pumped from the sea (final number of individuals 221, mean final weight, 200.82 (SE 9.96) g). The water temperature at that time of the year, about 16°C, was the same for the sea and the installations.

Wild animals (mean weight 193.40 (SE 15.35 g, *n* 5) were also captured in the Mar Menor in November 1997 and immediately killed, and their gut contents examined to confirm that the animals were not starving.

Chemical analysis

The composition of the diet was (g/kg): protein 455, fat 131, ash 93, water 77 and N-free extract 244. The fat content was determined by homogenisation and extraction with diethyl ether in a Soxtec system HTC extractor (Tecator, Höganöf, Sweden), whereas N was assessed by the Kjeldahl method using a 6.25 N to protein conversion factor. Moisture and ash contents were determined as sample weight differences

Table 1. Fatty acid composition of the commercial diet and white muscle, hepatic tissue and mesenteric fat of reared and wild sharpnose sea bream (g/100 g total fatty acids)†

(Mean values with their standard errors for five fish per group)

Fatty acids	Diet	White muscle				Hepatic tissue				Mesenteric fat			
		Reared		Wild		Reared		Wild		Reared		Wild	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	5.31	4.34	0.20	1.70*	0.09	2.53	0.12	1.31*	0.14	5.26	0.18	2.39*	0.20
15:0	0.36	0.29	0.01	0.54*	0.05	0.19	0.03	0.49*	0.04	0.43	0.02	0.64*	0.08
16:0	15.78	19.49	0.19	27.41*	0.28	20.48	0.58	24.98*	0.91	18.26	0.40	26.53*	0.22
18:0	2.79	3.44	0.20	5.27*	0.27	5.97	0.31	8.11*	0.57	3.01	0.16	5.73*	0.37
ΣSFA	24.23	27.66	0.23	34.92*	0.26	29.16	0.76	34.89*	0.42	26.97	0.40	35.30*	0.43
16:1 <i>n</i> -7	6.05	7.05	0.42	9.16	0.90	5.65	0.57	5.78	0.37	8.23	0.39	11.82*	0.72
18:1 <i>n</i> -9	16.29	25.95	1.33	20.18*	1.01	36.12	2.46	15.37*	0.94	27.90	0.63	22.60*	1.32
20:1 <i>n</i> -9	11.54	1.48	0.14	0.83*	0.17	2.84	0.33	0.55*	0.09	1.71	0.13	1.21*	0.17
22:1 <i>n</i> -9	12.37	1.94	0.19	2.00	0.22	2.41	0.18	1.85	0.21	2.27	0.12	2.40	0.17
24:1 <i>n</i> -9	1.18	0.53	0.02	0.63	0.09	0.36	0.10	0.66	0.16	0.38	0.03	0.61	0.22
ΣMUFA	47.43	36.95	1.34	32.80	1.58	47.38	2.34	24.22*	1.41	40.49	0.53	38.63	1.24
18:2 <i>n</i> -6	6.70	4.26	0.16	0.94*	0.12	3.26	0.25	1.01*	0.03	4.73	0.16	0.89*	0.10
18:3 <i>n</i> -6	0.12	0.11	0.01	0.30*	0.07	0.13	0.01	0.36*	0.09	0.13	0.01	0.53*	0.15
18:3 <i>n</i> -3	1.29	0.99	0.10	0.41*	0.06	0.43	0.10	0.32	0.02	1.22	0.05	0.70*	0.02
18:4 <i>n</i> -3	1.15	2.54	0.08	3.04	0.68	0.95	0.12	1.41	0.27	2.95	0.04	4.14*	0.34
20:2 <i>n</i> -6	0.87	0.51	0.12	0.63	0.09	1.18	0.10	1.13	0.12	0.40	0.07	0.55	0.09
20:3 <i>n</i> -6	0.12	0.21	0.04	0.30	0.10	0.38	0.05	0.46	0.10	0.23	0.02	0.36	0.08
20:4 <i>n</i> -6	0.50	1.02	0.09	3.24*	0.74	0.79	0.11	4.85*	0.60	0.68	0.02	1.29*	0.17
20:5 <i>n</i> -3	8.35	10.09	0.42	6.86*	0.41	4.96	0.36	6.30*	0.16	8.43	0.29	4.29*	0.23
22:4 <i>n</i> -6	0.34	0.63	0.04	2.15*	0.10	0.36	0.06	1.41*	0.08	0.71	0.02	2.11*	0.13
22:5 <i>n</i> -3	0.95	4.23	0.24	5.15*	0.23	3.66	0.68	4.51	0.34	4.21	0.13	4.73	0.32
22:6 <i>n</i> -3	7.95	10.79	0.73	9.28	1.15	7.36	1.42	19.13*	1.22	8.84	0.31	6.49*	0.75
ΣPUFA	28.34	35.39	1.50	32.29	1.70	23.45	2.96	40.89*	1.36	32.54	0.71	26.08*	1.38
Σ <i>n</i> -9	41.38	29.90	1.07	23.64*	1.18	41.74	2.60	18.44*	1.23	32.25	0.48	26.81*	1.53
Σ <i>n</i> -6	8.64	6.74	0.26	7.55	1.08	6.09	0.47	9.22*	0.82	6.89	0.13	5.73	0.64
Σ <i>n</i> -3	19.70	28.65	1.27	24.74	1.16	17.36	2.54	31.67*	1.13	25.65	0.65	20.35*	1.41
<i>n</i> -3: <i>n</i> -6	2.28	4.25	0.10	3.53	0.47	2.80	0.23	3.55	0.32	3.72	0.09	3.76	0.51
<i>n</i> -3: <i>n</i> -9	0.48	0.97	0.08	1.07	0.10	0.44	0.10	1.76*	0.17	0.80	0.03	0.78	0.10

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Mean values were significantly different from those of reared fish (*t* test): **P*<0.05.

† For details of diets and procedures, see p. 618.

before and after either desiccation at 105 (SE 1) °C (moisture) or incineration in a muffle oven at 450 (SE 2) °C (ash) until constant weight was attained. Table 1 shows the fatty acid composition of the diet employed for the investigation.

All sharpsnout specimens used in these experiments were relatively young (less than 1-year-old), yet the study was performed after the gonadal maturation season.

Similar sized muscle pieces (4–5 g) from the epiaxial muscles below the dorsal fin were taken for analysis from every sampled fish. Liver and mesenteric fat were removed and separately weighed. Mesenteric fat was expressed as mesenteric fat index (MFI) and calculated as: (mesenteric fat weight/body weight) × 100.

Fatty acid composition

In order to analyse the fatty acid composition, the total lipid content of each tissue sample from individual fish, was extracted according to Folch *et al.* (1957) using chloroform–methanol (1:1, v/v first extraction and 2:1, v/v second extraction). Fatty acid methyl esters from total lipids were prepared by the method of Stoffel *et al.* (1959) and analysed in a Hewlett-Packard 5890 GC equipped with a FID detector and a 3390 A integrator, using a SPTM 2330 glass capillary column (30 m long, 0.75 mm i.d.). N₂ served as the carrier gas at a flow rate of 5 ml/min. Peak identification was performed by comparison with known commercial standards (Sigma Chemical Co., St. Louis, MO, USA). Fatty acid relative concentrations are expressed as g/100 g total fatty acids after internal normalization of peak areas.

Lipid quality indices

Lipid quality indices were calculated according to Ulbricht & Southgate (1991). The atherogenic index (AI) was: $(12:0 + 4 \times 14:0 + 16:0) / ((n-6) + n-3)$ PUFA + 18:1 + the sum of other MUFA), where PUFA are polyunsaturated fatty acids and MUFA are monounsaturated fatty acids. 12:0 was not detected in the samples and therefore not taken into account for the calculations. The index of thrombogenicity (IT) was: $(14:0 + 16:0 + 18:0) / (0.5 \times 18:1 + 0.5 \times \text{the sum of other MUFA} + 0.5 \times n-6 \text{ PUFA} + 3 \times n-3 \text{ PUFA} + (n-3 \text{ PUFA}/n-6 \text{ PUFA}))$.

Table 2. White muscle and liver lipid content and mesenteric fat index† in reared and wild sharpsnout sea bream‡

(Mean values with their standard errors for five fish per group)

	Reared		Wild	
	Mean	SE	Mean	SE
White muscle lipid (g/kg)	75.9	13.1	38.7*	5.1
Liver lipid (g/kg)	188.4	30.0	58.2*	3.9
MFI	2.72	0.37	1.73*	0.19

MFI; mesenteric fat index.

Mean values were significantly different from those of the reared fish (*t* test):

**P* < 0.05.

† MFI = 100 × mesenteric fat weight/body weight.

‡ For details of diets and procedures, see Table 1 and p. 618.

Statistical analysis

A Student's *t* test was used in order to determine significant differences between different groups (STATGRAPHICS version 7.0, Manugistics Inc.).

Results

Tissue fatty acid composition

Table 1 shows the fatty acid composition of white muscle, liver and mesenteric fat from both reared and wild sharpsnout sea bream, as well as the fatty acid composition of the diet, assessed by GC analysis of total lipids. The fatty acids analysed were grouped as saturated (SFA), MUFA and PUFA, the latter including di-, tri-, tetra-, penta- and hexaenoic fatty acids.

In the case of muscle, only the SFA group showed significantly higher values in wild *v.* reared fish, mainly due to the 16:0 content. Although no significant differences were observed between both fish groups in the case of PUFA and MUFA acids as a whole, certain variations were detected when individual fatty acids were considered. Thus, the proportion of 18:1 *n*-9 and 20:5 *n*-3 was significantly increased in reared fish muscle when compared with wild type and also higher than that of the diet, while the proportion of 22:5 *n*-3 was higher in wild *v.* reared fish muscle and higher in all fish tissues than that of the diet. The content of MUFA acids in both fish groups was lower than that of the commercial diet, while the opposite was true for SFA and PUFA.

The liver SFA pattern was similar to that of the muscle. MUFA values were very high in reared fish, presumably reflecting the high dietary content of these acids. The difference was almost exclusively due to the 18:1 *n*-9 content. Conversely, PUFA values were very high in wild specimens, whereas PUFA values in reared fish were not high enough to match those of the diet.

In mesenteric fat, the SFA pattern was also very similar to that of muscle and liver, while the PUFA content in reared fish was significantly higher than that of either wild fish or the commercial diet. These differences were mainly due to the 18:2 *n*-6 and 20:5 *n*-3 contents.

The content of *n*-9 fatty acids for all tissues analysed was higher in reared *v.* wild fish. *n*-6 and *n*-3 fatty acid contents were both significantly (*P* < 0.05) higher in the liver of wild specimens, while the *n*-3 fatty acid mesenteric fat content was higher in the reared ones.

Fat composition

Table 2 shows the fat content of white muscle and liver, as well as the body proportion of mesenteric fat, in reared and wild sharpsnout sea bream. Significantly (*P* < 0.05) higher values were found for all three variables in the case of reared fish.

Lipid quality indices

Table 3 shows total lipid AI and IT values for white muscle, liver and mesenteric fat. Muscle and mesenteric fat IT

Table 3. Atherogenic index and index of thrombogenicity of total white muscle, liver and mesenteric fat lipids in reared and wild sharpsnout sea bream†
(Mean values with their standard errors for five fish per group)

	Atherogenic index‡				Index of thrombogenicity§			
	Reared		Wild		Reared		Wild	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
White muscle	0.509	0.012	0.526	0.008	0.242	0.009	0.353*	0.012
Liver	0.432	0.01	0.464	0.018	0.367	0.036	0.299	0.006
Mesenteric fat	0.538	0.008	0.558	0.013	0.255	0.007	0.402*	0.021

Mean values were significantly different from those of the reared fish (*t* test): **P*<0.05.

† For details of diets and procedures, see Table 1 and p. 618.

‡ Atherogenic index = (12 : 0 + 4 × 14 : 0 + 16 : 0) / ((*n*-6 + *n*-3) polyunsaturated fatty acids + 18 : 1 + the sum of the other monounsaturated fatty acids).

§ Index of thrombogenicity = (14 : 0 + 16 : 0 + 18 : 0) / (0.5 × 18 : 1 + 0.5 × the sum of the other monounsaturated fatty acids + 0.5 × *n*-6 polyunsaturated fatty acids + 3 × *n*-3 polyunsaturated fatty acids + (*n*-3 polyunsaturated fatty acids/polyunsaturated fatty acids)).

values were significantly higher in wild *v.* reared fish, mainly due to the higher 16 : 0 and 18 : 0 values and the lower 8 : 11 *n*-9 content of the wild type.

Discussion

Fish lipids are mainly distributed among mesenteric fat, liver and muscle. The results presented in the present paper show that the fat content of muscle, mesenteric and liver in reared fish are higher than those of wild fish. In general, fish that are fed commercial diets do exhibit a greater body fat content than wild specimens (Ackman, 1989; Shearer, 1994). The liver storage capacity depends on factors such as the species, the season and/or the developmental state (Henderson & Tocher, 1987; Sheridan, 1988, 1989). Mesenteric fat is the main lipid depot and is well suited for long-term lipid storage. Lipid storage in liver and muscle is secondary and probably influenced to a greater extent than mesenteric fat by life-history patterns (Sheridan, 1994).

Interestingly, the fatty acid composition that we have found in muscle total lipid content is similar to that reported by Orban *et al.* (2000) in cultured sharpsnout sea bream, in spite of the experimental design differences in terms of fish weights, time of killing and, more importantly, diet composition (particularly with respect to SFA and MUFA). This observation suggests that over and above the effects of the diet, fish may be endowed with a mechanism that limits the rate of accumulation of specific fatty acids in the tissues, and may explain why the muscle proportion of MUFA, always close to 35 g/100 g total fatty acids, proved higher than that of the feed in the study of Orban *et al.* (2000) and just the opposite in ours.

In contrast to mammals, it has been established that very little, if any, fatty acid synthesis takes place in adipose tissue (Greene & Selivonchick, 1987), and that fatty acid accretion in this tissue may reflect the composition of the diet. Thus, the fat mesenteric profile differences between wild and reared fish found in our present experiments could be mainly attributed to dietary differences. With respect to those fatty acids that are important for human nutrition (linoleic, arachidonic, eicosapentaenoic and docosahexaenoic acids), we have

observed a lower linoleic acid content in wild *v.* reared fish tissues. If mesenteric fat does in fact reflect the dietary fatty acids, it could be assumed that the linoleic acid content of the wild diet would also be lower than that of the reared fish diet, as previously described for wild gilthead sea bream (Trigari *et al.* 1997), and both wild and reared sharpsnout diets would have a low arachidonic acid content. This fatty acid, however, is markedly increased in wild fish liver and muscle in relation to the diet (as reflected in mesenteric fat), but only slightly in the case of reared fish. Thus, the arachidonic acid content is always higher in wild sharpsnout tissues and similar to that of other Mediterranean species (Zlatanov & Sagredos, 1993), although lower than previously reported for wild red porgy (Rueda *et al.* 1997). If it is assumed that mesenteric fat reflects the diet's lipid content, eicosapentaenoic acid in wild fish liver and muscle is significantly increased with respect to their diet, while in reared fish this acid is slightly increased in muscle but markedly decreased in liver. In the case of docosahexaenoic acid, wild fish exhibited a marked increase in liver and a very small increase in muscle when compared with mesenteric fat, while reared fish tissues exhibited entirely similar levels to those present in the diet. The docosapentaenoic acid content in all tissues was much higher than in the commercial diet, and the differences between reared and wild fish were only significant in muscle. This would indicate that mesenteric fat reflects the diet's composition and those fatty acids that can be obtained by active elongation from other fatty acids.

The higher values of linoleic, eicosapentaenoic, docosahexaenoic and *n*-3 series acids in reared fish muscle make reared sharpsnout more favourable for human consumption. This could be due to the omnivorous character of the species or to a seasonal availability for food that affects wild sharpsnout feeding. It should be taken into account that reared sharpsnout were fed a sea bream diet. In rainbow trout, docosahexaenoic acid accumulates in mesenteric fat to a much lesser extent than in liver or muscle (Jeziarska *et al.* 1982), and the same occurs in wild sharpsnout. In reared sharpsnout, however, docosahexaenoic acid levels in mesenteric fat and liver were very similar. Hepatic

tissue PUFA content was lower in reared specimens, however, we wonder whether this low value could be attributed to a greater MUFA deposition in liver, considering the higher fat deposition that occurs in this tissue.

The level of *n*-3 highly PUFA in farmed marine fish is usually lower than that of their wild relatives, presumably because of the lack of lipids from marine phytoplankton and other marine organism in reared fish diets (Ackman & Takeuchi, 1986). The concentration of *n*-3 highly PUFA in reared sea bass liver and muscle is significantly lower than that of samples taken from wild populations (Krajnovic-Ozretic *et al.* 1994), yet in sharpsnout this was only true for the hepatic tissue. This could also be caused by the higher fat deposition in liver.

As in red porgy (Rueda *et al.* 1997), in reared sharpsnout *n*-9 fatty acids make up the largest fatty acid fraction in all three tissues studied. The diet provided was rich in *n*-9 fatty acids, but high levels of these acids, even above those of red porgy, were also found in wild specimens. Reared sharpsnout muscle and mesenteric fat exhibited lower values than those of the diet.

Both in cultured and wild fish, *n*-3 fatty acids make up a larger fraction than *n*-6 fatty acids in all tissues analysed, as has been reported for sea fish (Greene & Selivonchick, 1987; Ackman, 1992; Tornaritis *et al.* 1993). Docosahexaenoic acid levels in sharpsnout muscle, however, are lower than those found in red porgy (Rueda *et al.* 1997). In spite of that, reared and wild fish *n*-3:*n*-6 ratios were very similar, which was not the case in red porgy.

The AI values obtained are lower than those reported for the edible parts of rabbit, lamb, veal, tuna fish, gilthead, codfish and sardine (Pérez-Llamas *et al.* 1998), for sea bass (Krajnovic-Ozretic *et al.* 1994) and for gilthead sea bream muscle (Trigari *et al.* 1997). AI values are similar to those found in red porgy muscle (Rueda *et al.* 1997). Values for the IT were lower than those of PUFA margarine and considerably lower than any of the values reported by Pérez-Llamas *et al.* (1998), but higher than those of raw mackerel (Ulbricht & Southgate, 1991). With the exception of the liver, IT and IA values were higher in wild specimens, however differences were only statistically significant ($P < 0.05$) for IT in white muscle and mesenteric fat. This fact can be explained by the relative proportions of SFA and PUFA; SFA being more abundant in wild fish muscle and mesenteric fat, while PUFA levels being lower. In turn, the omnivorous habits of the species and/or the seasonal food variation may be the principal factor modulating the lipid profile of the fish. The reported quality indices for sharpsnout reveal the higher nutritional value of both wild and reared fish. In view of the tissue fatty acid composition, depending on the time of the year or the season, reared fish could be more suitable than wild fish for human consumption.

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