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Comparison of fatty acids in the brains of wild and reared sea bass *Dicentrarchus labrax* L. and sea bream *Sparus aurata* L., and living in the same natural environment

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Abstract

This study has assessed the fatty acid contents in the brains of two perciform species, sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), and comparisons are made between them in both their natural environment and under conditions of artificial cultivation. The sea bass appears to have a more intensive brain metabolism than sea bream. This is attributed mainly to the respective genetic make-ups rather than to the feed each species may consume. Saturated and mono-unsaturated fatty acids in the brains of wild fish were at higher levels than in the brains of reared fish. The *n*-3 class were measured at about double the quantities of those found for the *n*-3 class in the wild fish of both species, but a reverse trend was apparent for the reared fish. The *n*-3/*n*-6 ratio from the brains of wild fish was almost double that from the brains in reared fish. The absence of the C20:5*n*-3 (EPA) from the brains of the wild fish, in both species, as well as from the brains of the reared sea bass probably suggests a high metabolic rate which, in these fishes, is oriented to the production of C22:6*n*-3 which is needed for the structure and function of cellular membranes in the fast developing brains of younger fish.

Keywords: Fish brain, Fatty acids, Brain composition

Introduction

The brain is a vertebrate organ with very high metabolic rates; it co-ordinates and regulates all body functions according to the external or internal stimuli that receives. In fish, the lipid composition of the brain appears to be influenced by nutrition in contrast to the mammalian brain (Pagliarani et al. 1986; Tocher et al. 1988). There is a high content of fatty acids (FA) in the fish brain with the *n*-3 class being in a higher percentage than the *n*-6 class; the cerebral fluid contains mainly mono-unsaturated FA. Glucolipids are present but in much lower proportion compared to the mammalian brains; in fish their concentration varies with the ambient temperature.

Polar species have a brain concentration of glucolipids in the range of 53-66% whereas tropical species brains contain only about 35% of glucolipids (Kappel et al. 1993). The FA profiles of fish that adapt easily to high pressures are similar to those of species adapting to low temperatures (Stoknes et al. 2004). The eicosanoids are produced at both the cellular and the somatic level as a response to stressful situations that the fish may encounter

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(Sargent et al. 1999). The change of the percentage of poly-unsaturated FA (PUFA) in the nutrition of the fish affects the percentages of the other FA and also alters the ratios between AA, EPA and DHA (Sargent et al. 1999).

The last three are deemed as necessary for the normal structure and function of the cellular membranes (Sargent et al. 1993, 1995, 1997) and, particularly for fish, EPA and DHA while AA is less needed (Castell et al. 1994; Bell et al. 1995). The long chain *n*-3 FA are quickly incorporated into the phospholipids of the cellular membranes, wherefrom they can play a major role in metabolic processes within the cells (Sinclair 2000; Dullemeijer et al. 2008), while the reverse occurs in the terrestrial mammals except for the neural tissues and the eye where DHA is required. These three FA should be assessed in correlation rather than be studied individually (Sargent et al. 1999).

Except of the known FAs that occur in the tissues of fish, there also exist their isomers which may be too many and/or short-lived and, therefore, little is known of them and even less with regard to their biochemical and bio-energetic importance (Barker 1971). This study aims at measuring the FAs composition of the brains of wild and of reared fish in two species, sea bream and sea bass, of approximately the same age and living in similar environmental conditions.

Materials and methods

Biological materials

Twenty sea bass and twenty sea bream were examined in this study. The fish were of about two years of age (1+ to 2+) and of similar natural environment, namely, the area of River Kalamas estuary (Prefecture of Thesprotia, western Greece). One half of these fish (10 sea bass and 10 sea bream) originated from a sea-cage farming facility (Bay of Valtos) and the rest were fished in a nearby lagoon (Loutsa Papadia), which is situated in the coastal area of the Ionian Sea. The fish feeds given to the farmed fish consisted (according to the manufacturer's label) of 43% proteins, 20% lipids, 16% carbohydrates, 7.4% ash, 1.6% fibres, 10% moisture, vitamins and trace elements. Raw materials were fish meal, fish oil, wheat flour, corn gluten, vitamins and minerals (with no indication as to their percentages).

The fish were all collected alive in May 2008 and in multiple numbers of the individuals finally examined (50 farmed and 100 wild specimens from both species), using seine net and steady traps. The fish were killed with thermal shock in chilled water (0 °C) and were immediately transported to the lab on ice. Subsequently, the fish were cleaned with fresh water and from the reared fish (25 sea bass and 25 sea bream, all of 22 months of age according to the farm's data) 10 were randomly selected from each species. Body weight and total length were measured to the mg and the mm, respectively, before heading and measuring the head weight to the mg, the heads were then marked and vacuum packed in plastic bags and stored at -30 ºC until examined.

Body weight and total length were also taken from all 100 wild specimens (45 sea bass and 55 sea bream), as described above, and the fish were subsequently headed, marked and the otoliths were removed from each head. Dry otoliths were placed into the plastic bags, with individual tugging, and the age was determined within 48 hours. Throughout this period, the heads were preserved at -30 ºC inside marked plastic bags until the age was determined and the final sample (10 fish from each species, of about 2 years of age) was randomly selected.

Age determination in wild specimens

Age of the wild specimens was determined in the Department of Aquaculture and Fisheries of the Technological Education Institution of Epirus, based on the annual rings of the alcohol impregnated otoliths (Panella 1971). For this, an inversed stereoscope at a magnification of 50 X was used and age was determined twice without considering the first observation. Differential determination of age led to the exclusion of the specimens in which the two observations did not coincide. Moreover, otoliths with less clear rings were also excluded. Of the total of 100 wild fish, the age was well determined for 22 sea bass and 28 sea bream (over 1 and below 3 years of age) and 10 individuals were randomly taken from each species for further investigations.

Removal of brains

After dissecting away the dorsal part of the skull and then removing the cerebral fluid by suction, the brains were removed using two small sterilized forceps. Subsequently, the brains were weighted, marked and homogenized in pairs, from each of the four experimental groups, in a small metallic homogenator, the homogenates were placed in small plastic containers and these were vacuum packed in plastic bags and preserved at -30 ºC until they were de-frozen for analysis.

Determination of total lipids and fatty acids

The homogenized brain samples (in pairs from each group of fish) were de-frozen and homogenized again in a mechanical homogenizer with metallic blades in low temperature (ice bath) for one minute. Lipid extraction was performed according to the method of Bligh and Dyer (1959), as modified by Kinsella et al. (1977), using chloroform and methanol at a 2:1 ratio. Subsequently, fatty acids were methyl-esterified with a 12% boron tri-fluoride methanol solution (BF_3-MeOH) (Folch et al. 1957).

Methyl-esters were obtained with normal hexane (Metcalfe et al. 1966). The analysis was performed using gas chromatography (Model GC-17A, Shimadzu, Kyoto, Japan) with capillary column and ionized flame detector (TRACE^{TM} TR-FAME GC Column, Thermo Fisher Scientific Inc.) and automatic sampler (HT 310A, HTA). Pure helium at a flow of 82 KPa, air of 50 KPa flow and hydrogen of 60 KPa flow were used for the analysis under the following conditions: initial temperature was 150 $^{\circ}$C for 5 minutes, followed by a 5 $^{\circ}$C/min pace until 170 $^{\circ}$C for 10 minutes and then, 5 $^{\circ}$C / min pace until 220 $^{\circ}$C for 20 minutes. The identification of fatty acids (methyl-esters) was performed by comparing the peaks printed in the special PC programme with Qalmix Fish (89-5550) and Methyl Dodecanoate (20-1200) standard fatty acids (Larodan Fine Chemicals AB).

Statistical analysis

The statistical analysis of the data obtained (average, standard deviation, ratios) was performed using Excel 2003 (Microsoft) in PC. For comparisons, the unpaired *t*-test was applied in each case, after variability comparison by F-test.

Results

The physico-chemical parameters with regard to the living conditions of the fish (Table 1) have no statistically significant differences, but the somatometric data and the weight of the total brain lipids show differences that are statistically significant (Table 1). The total lipids weight was found 0.023 grams in the wild sea bass, 0.024 grams in the wild sea bream and, in the reared fish; it was 0.112 and 0.139 grams for sea bass and sea bream respectively.

Gas chromatography separated 35-50 different fatty acids (FA) from the brains of wild sea bass and 37-42 FA from the brains of reared sea bass, while for sea bass 18-26 FA were separated from the brains of wild and 25-28 of reared sea bream. Only 18 of those FA were identified using standardized control FA.

Saturated FA from the brains of wild sea bass represented 25.20% of the total whereas they represented 23.64% from the brains of reared sea bass ($P < 0.01$); for sea bream, they were 29.57% from the brains of wild and 24.30% from the brains of reared fish ($P < 0.01$). In all cases the dominant FA was C16:0, followed by C18:0 (Tables 2 and 3).

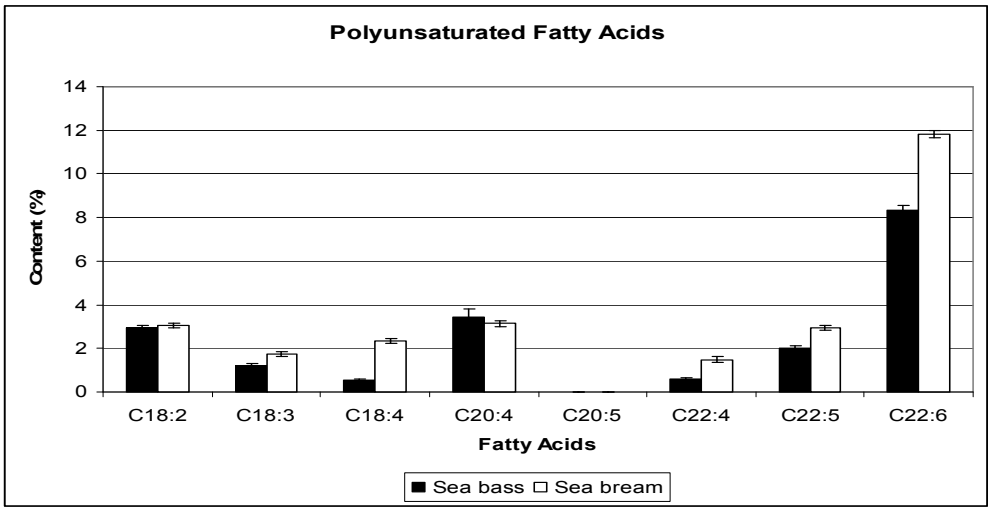

Figure 1. Polyunsaturated fatty acids composition (% of each fatty acid) of wild fish Sea bass (Black bars) and Sea bream (White bars) brains

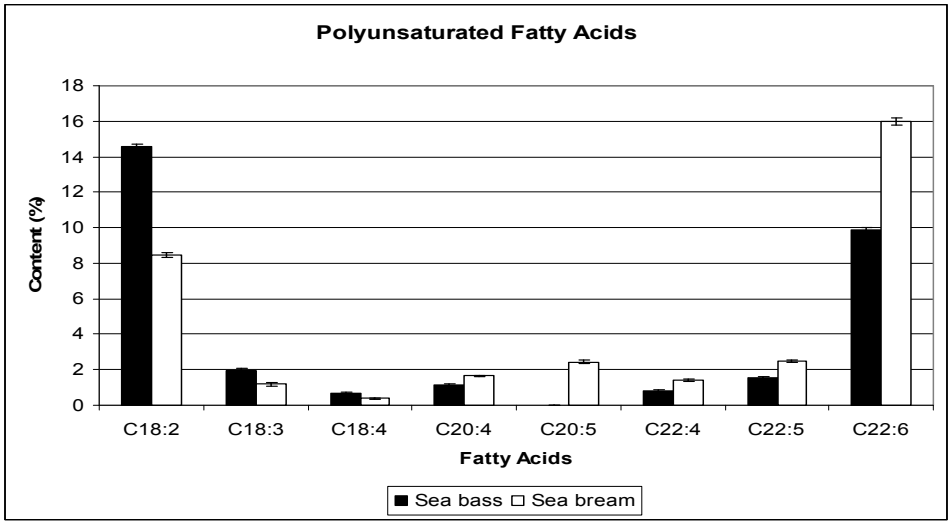

Figure 2. Polyunsaturated fatty acids composition (% of each fatty acid) of farmed fish Sea bass (black bars) and Sea bream (white bars) brains

Table 1. Mean annual values of physicochemical parameters of the marine aquatic environment, values of basic somatometric parameters, and brain fat content of wild and farmed fish. [a]

	Wild Fish		Farmed Fish		
Parameters	Sea bass[a] N=10	Sea bream[b] N=10	Sea bass[c] N=10	Sea bream[d] N=10	*t*-test
Temperature (°C)	20.81 ± 8.31	20.81 ± 8.31	19.52 ± 4.07	19.52 ± 4.07	NS
Salinity (g/l)	31.04 ± 6.99	31.04 ± 6.99	34.70 ± 0.86	34.70 ± 0.86	NS
pH	7.78 ±0 .43	7.78 ±0 .43	7.85 ± 0 .35	7.85 ± 0.35	NS
Total body length (cm)	23.71 ± 1.43	19.61 ± 0.62	33.56 ± 1.08	28.89 ± 0.39	a-b*** a-c*** b-d*** c-d***
Total body weight (g)	145.94 ± 18.24	106.12 ± 3.86	466.87 ± 32.19	418.09 ± 28.16	a-b*** a-c*** b-d*** c-d**
Total head weight (g)	24.99 ± 0.38	26.24 ± 1.28	84.14 ± 0.49	96.95 ± 0.17	a-b* a-c*** b-d*** c-d***
Total brain weight (g)	0.35 ± 0.020	0.52 ± 0.04	0.58 ± 0.01	0.87 ± 0.02	a-b^{NS} a-c*** b-d*** c-d***
Total fat (g) in brain	0.023 ± 0.003	0.024 ± 0.003	0.112 ± 0.004	0.139 ± 0.004	a-b^{NS} a-c*** b-d*** c-d ***

[a] Mean ±standard deviation, N= Number of samplings, NS = Non Significant.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Mono-unsaturated FA were found to represent 40.50% of the total from the brains of wild sea bass and 33.96% from the brains of reared sea bass ($P < 0.01$), while they were 38.61% from the brains of wild and 32.57% from the brains of reared sea bream ($P < 0.01$). In all cases C18:1 dominated, followed by C22:1 and C16:1. Poly-unsaturated FA

were found to represent 19.12% of the total from the brains of wild sea bass and 30.62% from the brains of reared sea bass ($P < 0.01$), while they were 26.50% from the brains of wild and 33.99% from the brains of reared sea bream ($P < 0.01$). In all cases the dominant FA was C22:6n-3 except for the reared sea bass where C18:2n-6 dominated, followed by C20:4n-6 and C18:2n-6, in wild sea bass and sea bream, or by C22:6n-3 and C20:4n-6, in the reared sea bass, or by C18:2n-6 and C20:5n-3, in reared sea bream. The n-3 class was recovered in double quantities with respect to the n-6 class from the brains of wild fish in both species (Fig. 1) while, conversely, in the brains of reared fish the n-6 class surpassed the n-3 class quantities (Fig. 2). The ratio n-3/n-6 was almost double in the brains from wild fish compared to that from reared fish brains.

Table 2. Fatty acid profiles of total lipids from the brain of wild and farmed fish Sea bass (*Dicentrarchus labrax*)

Fatty acid	Wild Sea bass N=10	Farmed Sea bass N=10	*t*-test
C12:0	0.28 ± 0.04	0.0 ± 0.0	***
C14:0	1.94 ± 0.15	2.80 ± 0.12	***
C15:0	0.77 ± 0.06	0.33 ± 0.05	***
C16:0	17.07 ± 0.45	16.16 ± 0.11	***
C18:0	5.15 ± 0.21	4.36 ± 0.10	***
Total saturated	25.20 ± 0.89	23.64 ± 0.37	***
C16:1 n-7 (9C)	7.56 ± 0.12	3.86 ± 0.08	***
C18:1 n-9 (9C)	15.68 ± 0.39	19.39 ± 0.08	***
C18:1 n-7 (11C)	4.96 ± 0.16	2.41 ± 0,08	***
C20:1 n-9 (11C)	2.51 ± 0.20	2.19 ± 0.10	***
C22:1 n-9 (13C)	9.79 ± 0.37	6.11 ± 0.15	***
Total mono-unsaturated	40.50 ± 1.21	33.96 ± 0.47	***
C18:2 n-6	2.94 ± 0.12	14.58 ± 0.10	***
C18:3 n-3	1.22 ± 0.07	1.97 ± 0.13	***
C18:4 n-3	0.56 ± 0.05	0.69 ± 0.06	***
C20:4 n-6	3.45 ± 0.37	1.12 ± 0.07	***
C20:5 n-3	0.0 ± 0.0	0.0 ± 0.0	-
C22:4 n-6	0.59 ± 0.06	0.81 ± 0.07	***
C22:5 n-3	2.04 ± 0.09	1.56 ± 0.06	***
C22:6 n-3	8.31 ± 0.22	9.90 ± 0,12	***
Total poly-unsaturated	19.12 ± 0.96	30.62 ± 0.59	***
Others	15.18	11.78	
Total n-3 Fatty acids	12.13 ± 0.42	14.12 ± 0.36	***
Total n-6 Fatty acids	6.99 ± 0.54	16.50 ± 0.23	***
Ratio n-3/n-6	1.74 ± 0.07	0.86 ± 0.01	***

[a]Mean ± standard deviation, N=Number of samplings, NS= Non Significant.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Discussion

The higher content of fatty acids (FA) in the brains of sea bass (95% in wild fish and 50% in reared fish) have been observed when compared to that in the brains of sea bream, and taking into the account the fact of their being living in the same environment, being of the same age and at least the reared, having the same nutrition, suggests that the sea bass has higher metabolic rates than the sea bream which is attributed to genetic reasons rather than to the food consumed by the two species.

In nearly all the quantitative and qualitative comparisons made between the four fish kinds (Tables 1, 2 and 3), statistically important differences are evident attributable to the quantities and the quality of food consumed by each kind in combination with the genetic capacity of each species.

The findings of the present study show that the saturated and the mono-unsaturated FAs levels in the brains of wild fish are higher to those in the reared fish owing, probably, to a higher consumption of feedstuffs of animal

origin and linked to phytoplankton in the natural environment in contrast to the reared fish which were fed artificial diets. The relatively high percentages of mono-unsaturated FAs in the brain are probably, to some extent, due to cerebral fluid that was retained in the brains after their removal. This finding is in agreement to that of Stochness et al. (2004) with other fish species. The poly-unsaturated FA showed higher absolute values in the brains of reared fish which can be attributed to the high content of the n-3 FA in the artificial feeds which is probably of vegetable origin given the high levels of C18:2*n*-6.

The apparent absence of C20:5*n*-3 (EPA) from the brains of the wild fish in both species and of the brains of reared sea bass is attributable to the more intense metabolism in the brains of fish of that age, with its fast conversion to DHA for reasons of increased needs of the neurons in the fast growing brain; this finding is in disagreement to those of other workers (Akman 1980; Henderson and Tocher 1988) but for other fish species and of different ages than the ones used for this study. The relatively higher content of the *n*-6 class compared to that of the *n*-3 class of FA in the brains of reared fish in both species shows that the high content of the feeds in *n*-6 FA was reflected in the FA composition of the brains. On the other hand, the *n*-3/*n*-6 ratio in the brains of wild fish is the same as the one reported by Tocher et al. (1988).

Table 3. Fatty acid profiles of total lipids from the brain of wild and farmed fish Sea bream (*Sparus aurata*)

Fatty acid	Wild Sea bream N=10	Farmed Sea bream N=10	*t*-test
C12:0	0.0 ± 0.0	0.0 ± 0.0	-
C14:0	2.41 ± 0.15	2.10 ± 0.08	***
C15:0	0.29 ± 0.05	0.57 ± 0.06	***
C16:0	18.87 ± 0.59	14.79 ± 0.32	***
C18:0	8.00 ± 0.54	6.84 ± 0.19	***
Total saturated	29.57 ± 1.28	24.30 ± 0.63	***
C16:1 n-7 (9C)	6.75 ± 0.13	3.97 ± 0.14	***
C18:1 n-9 (9C)	20.44 ± 0.79	18.43 ± 0.11	***
C18:1 n-7 (11C)	4.19 ±0.18	2.88 ± 0.09	***
C20:1 n-9 (11C)	1.07 ± 0.08	2.62 ±0.08	***
C22:1 n-9 (13C)	6.17 ± 0.26	4.68 ± 0.08	***
Total mono-unsaturated	38.61 ± 1.40	32.57 ± 0.49	***
C18:2 n-6	3.07 ± 0.11	8.46 ± 0.11	***
C18:3 n-3	1.76 ± 0.11	1.19 ± 0.10	***
C18:4 n-3	2.32 ± 0.11	0.38 ± 0.05	***
C20:4 n-6	3.14 ± 0.13	1.66 ± 0.05	***
C20:5 n-3	0.0 ± 0.0	2.45 ± 0.10	***
C22:4 n-6	1.49 ± 0.12	1.40 ± 0.09	NS
C22:5 n-3	2.93 ± 0.11	2.47 ± 0.08	***
C22:6 n-3	11.80 ± 0.16	15.98 ± 0.19	***
Total poly-unsaturated	26.50 ± 0.83	33.99 ± 0.77	***
Others	5.32	9.14	
Total n-3 Fatty acids	18.81± 0.48	22.46 ± 0.52	***
Total n-6 Fatty acids	7.69 ± 0.35	11.52 ± 0.25	***
Ratio n-3/n-6	2.45 ± 0.05	1.95 ±0.00	***

[a]mean ± standard deviation, N=Number of samplings, NS= Non Significant.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Conclusion

The higher content of fatty acids (FA) in the brains of sea bass (95% in wild fish and 50% in reared fish) have been observed when compared to that in the brains of sea bream, and taking into account the fact of their being living in the same environment, being of the same age and, at least the reared, having the same nutrition, suggests that the sea

bass have higher metabolic rates than the sea bream which is attributed to their genetic background rather than to the food consumed by the two species.

The saturated and the mono-unsaturated FAs levels in the brains of wild fish are higher to those in the reared fish owing, probably, to a higher consumption of feedstuffs of animal origin and linked to phytoplankton in the natural environment in contrast to the reared fish which were fed artificial diets.

The levels of the *n*-3 FA were double that for the *n*-6 FA in the brains of wild fish in both species whereas, conversely, the reared fish brains had a higher content in *n*-6 FA which is evidently a result of the composition of the feeds given to the reared fish, which have a high content of *n*-6 FA. The n-3/n-6 ratio from the brains of wild fish was almost double that from the brains in reared fish.

The absence of the C20:5*n*-3 (EPA) from the brains of the wild fish, in both species, as well as from the brains of the reared sea bass probably suggests a high metabolic rate which, in these fish, is oriented to the production of C22:6*n*-3 which is needed for the structure and function of cellular membranes in the fast developing brains of younger fish.

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