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Fatty acid composition of the common octopus, *Octopus vulgaris*, in relation to rearing temperature and body weight

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Abstract

Octopus vulgaris is considered a candidate for aquaculture and its growth performance is dependent upon temperature and body weight. The aim of this study was to investigate the effects of temperature on the fatty acid composition of *O. vulgaris* in relation to body weight (60–663 g). The experiments were performed in a closed seawater system with controlled temperature (15, 20 and 25 °C). The octopuses were fed on squid (*Loligo vulgaris*). *O. vulgaris* showed a high content of polyunsaturated fatty acids (51.32–57.62% total fatty acids), particularly $n-3$ highly unsaturated acids (38.01–52.73% total fatty acids). At each temperature, the proportion of some fatty acids was highly ($P<0.0001$) related to body weight. Specifically, 18:1$n-9$, 18:1$n-7$, 18:2$n-6$, 20:4$n-6$, 22:4$n-6$ and 22:5$n-6$ decreased, while 20:5$n-3$ (EPA) and 22:6$n-3$ (DHA) increased, with increasing body weight at 20 and 25 °C. The opposite was observed at 15 °C. Total polyunsaturated and $n-3$ highly unsaturated fatty acids (HUFA) decreased in larger octopuses at 15 °C, as well as in smaller octopuses at 25 °C, whereas total saturated, monounsaturated and $n-6$ HUFA increased. Thus, total unsaturated fatty acids showed a relatively small variation (60.53–63.96% total fatty acids) and the mean unsaturation index was similar at 15 and 25 °C. Arachidonic acid (2.4–9.6% total fatty acids) was inversely related to EPA and DHA, and positively to 18:2$n-6$, 22:4$n-6$ and 22:5$n-6$. It is noted that arachidonic acid is not an essential fatty acid for the common octopus. It is concluded that the fatty acid composition of *O. vulgaris* was influenced by temperature and body weight, but with an $n-3/n-6$ ratio of more than 3 and a DHA/EPA ratio of more than 1.5. The common octopus could be an excellent source of arachidonic acid, containing sufficient $n-3$ HUFA levels, in low temperatures for large individuals and in warm temperatures for small ones.

Keywords: *Octopus vulgaris*; Fatty acid composition; Temperature; Body weight

1. Introduction

Octopus vulgaris is considered an ideal candidate for rearing, as it is characterized by high fecundity (Wells, 1978), fast growth (Iglesias et al., 2000) and high feed efficiency (Mangold and von Boletzky, 1973). It also has a short life cycle of 12–18 months and a high market price (Vaz-Pires et al., 2004). Furthermore, *O. vulgaris* adapts rapidly and easily to life in captivity, and shows high acceptance of low-value natural foods (Iglesias et al., 2000). Common octopus ongrowing has recently

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begun to develop in the Mediterranean Sea (García García and Aguado Giménez, 2002; Miliou et al., 2005).

Mediterranean cephalopods contain large amounts of polyunsaturated fatty acids (PUFAs: 52.89–56.25% of total fatty acids) (Sinanoglou and Miniadis-Meimaroglou, 1998). The most characteristic PUFAs were found to be docosahexaenoic acid (DHA; 22:6$n-3$) and eicosapentaenoic acid (EPA; 20:5$n-3$), which ranged between 20% and 36%, and 8.3% and 17.6% of total fatty acids, respectively (Culkin and Morris, 1970; Gibson, 1983; Sinanoglou and Miniadis-Meimaroglou, 1998).

Polyunsaturated fatty acids consisting of 20 carbon atoms (e.g. 20:3$n-6$, 20:4$n-6$ and 20:5$n-3$) are known precursors of eicasonoids, which have a wide range of physiological actions, such as, assisting in blood clotting, the immune response, the inflammatory response, cardiovascular tone, renal function, neural function, and reproduction (Tocher, 1995). Arachidonic acid (AA; 20:4$n-6$) is the primary precursor of eicosanoids in mammals and fish, and is released from membrane phospholipids in response to several stimuli during the well documented "arachidonic cascade" (Tocher, 2003). Arachidonic acid is an essential dietary fatty acid for marine fish, although it is generally a minor component of fish cell membranes (Tocher, 2003). However, the role of AA in cephalopods has not been studied sufficiently, despite the large variation found in the proportion of this fatty acid in wild cephalopods (Culkin and Morris, 1970; Gibson, 1983; Sinanoglou and Miniadis-Meimaroglou, 1998; Passi et al., 2002).

Passi et al. (2002) studied the fatty acid composition of several species of teleosts, cephalopods and crustaceans from the Mediterranean Sea and found that all of them had an $n-3/n-6$ ratio of more than 1, confirming the great importance of these fish and shellfish as a dietary source of $n-3$ PUFA for humans. Dietary supplementation with EPA and DHA is expected to attenuate inflammatory responses by reducing AA content or inhibiting eicosanoid production (Ramesha and Pickett, 1986). Cardiovascular mortality was noted to be inversely proportionate to the intake of $n-3$ fatty acids (Kafatos et al., 1997). Wild cephalopods are characterized by relatively high DHA content and DHA/EPA ratio (Sinanoglou and Miniadis-Meimaroglou, 1998; Passi et al., 2002). However, in cultured cephalopods DHA levels are correlated with the dietary input (Koueta et al., 2002; Navarro and Villanueva, 2000, 2003; Okumura et al., 2005).

The essentiality of $n-3$ highly unsaturated fatty acids (HUFA) in fish diets is directly related to their role as components of biomembrane phospholipids, this role being well illustrated in the process of homeoviscous regulation whereby fish restructure their biomembranes in response to changes in environmental temperature (Hazel, 1984). Cold acclimation resulted in reduced proportions of saturated fatty acids and increased proportions of polyunsaturated ones (Tocher, 1995). Several ratios of fatty acids, such as unsaturated/saturated, monounsaturated/saturated and EPA/AA varied with temperature in some fish species (Sargent et al., 1989; Cordier et al., 2002). Fatty acid composition in relation to temperature in mollusks, and particularly in cephalopods, requires further investigation.

Growth in cephalopods is highly plastic and dependent on a variety of biotic and abiotic factors, particularly food and temperature (Semmens et al., 2004). Both Aguado Giménez and García García (2002) and Miliou et al. (2005) suggest temperature control for the ongrowing of *O. vulgaris*. Temperature control is also important for the achievement of a complete octopus life cycle under culture conditions (Iglesias et al., 2004).

The aim of this study is to investigate the effects of temperature on the fatty acid composition of *O. vulgaris* in relation to body weight. The nutritional quality of the cultured common octopus and the essentiality of fatty acids are evaluated.

2. Materials and methods

Eighty one octopuses (60–663 g), *O. vulgaris* Cuvier, 1797 (order Octopoda, suborder Incirrata), were collected by free diving in the Saronicos Gulf (Aegean Sea, Eastern Mediterranean). The animals were transferred (within 1 h) to the laboratory and placed to holding tanks (40 or 100 l), which were connected to a closed seawater (37.5–38.5‰) system. Octopuses were reared for 60 days after collection from the field at three temperatures (15, 20 and 25 °C). Diurnal variation in water temperature was ±0.5 °C. Photoperiod was set at 12 h light: 12 h dark. More details about the rearing conditions are given elsewhere (Miliou et al., 2005).

Specimens were reared individually and fed on frozen squid (*Loligo vulgaris*), once a day, at midday, throughout the study. The ration was 3% of body weight per day. At the end of the experiments, each octopus was wet weighed and euthanised on ice. Prior to weighing, octopuses were starved for 24 h. Each octopus was homogenized (after removal of the viscera) and freeze-dried. The same procedure was followed for frozen squid.

Table 1
Fatty acid composition (% of total fatty acids) of the common squid and the common octopus reared at three temperatures (mean±S.E.)

Fatty acid	*Loligo vulgaris*	*Octopus vulgaris*				
	$n=3$	15 (°C) $n=26$	20 (°C) $n=27$	25 (°C) $n=28$	F	P
<14:0	0.520±0.026	0.657±0.045	0.770±0.044	0.648±0.043	2.43	n.s.
14:0	1.717±0.045	1.055±0.069	1.180±0.067	1.094±0.066	0.89	n.s.
14:1	0.320±0.017	0.199±0.007	0.190±0.007	0.193±0.007	0.46	n.s.
15:0	0.523±0.023	0.523±0.034	0.501±0.033	0.495±0.033	0.20	n.s.
16:0	25.240±0.091	24.400±0.172	24.553±0.169	24.274±0.166	0.70	n.s.
16:1$n-7$	0.357±0.032	0.267±0.010	0.247±0.010	0.263±0.010	1.15	n.s.
16:2$n-4$	0.423±0.018	0.983±0.028	0.979±0.028	1.041±0.027	1.60	n.s.
17:0	0.277±0.009	1.005±0.094	1.079±0.092	1.067±0.090	0.18	n.s.
16:4$n-1$	0.080±0.010	0.088±0.004	0.090±0.004	0.091±0.004	0.17	n.s.
18:0	4.613±0.159	9.572±0.109	9.550±0.107	9.567±0.105	0.01	n.s.
18:1$n-9$	1.487±0.033	1.203±0.025	1.225±0.025	1.250±0.024	0.87	n.s.
18:1$n-7$	0.153±0.003	0.792±0.057	0.870±0.056	0.924±0.055	1.40	n.s.
18:1$n-5$	0.063±0.003	0.122±0.009	0.121±0.009	0.144±0.008	2.43	n.s.
18:2$n-6$	0.143±0.003	0.223±0.006	0.227±0.006	0.239±0.006	2.11	n.s.
18:3$n-6$	0.063±0.003	0.095±0.005	0.098±0.005	0.095±0.005	0.15	n.s.
18:3$n-3$	0.053±0.007	0.083±0.003	0.077±0.003	0.081±0.003	1.06	n.s.
20:0	0.053±0.003	0.083±0.007	0.072±0.006	0.081±0.006	0.83	n.s.
20:1$n-9$	3.907±0.028	2.439±0.099	2.517±0.097	2.545±0.095	0.32	n.s.
20:2$n-6$	0.237±0.009	0.305±0.022	0.269±0.022	0.331±0.022	2.11	n.s.
20:4$n-6$	1.857±0.003	5.338±0.362	5.427±0.355	5.981±0.349	0.97	n.s.
20:3$n-3$	0.287±0.018	0.328±0.022	0.343±0.022	0.378±0.021	1.45	n.s.
20:5$n-3$	16.683±0.125	17.095±0.390	16.429±0.383	16.205±0.376	1.44	n.s.
22:0	nd	0.262±0.020	0.306±0.019	0.273±0.019	1.41	n.s.
22:1$n-11$	0.460±0.010	0.786±0.058	0.817±0.057	0.761±0.056	0.24	n.s.
22:1$n-9$	0.580±0.040	0.475±0.038	0.572±0.038	0.586±0.037	2.53	n.s.
21:5$n-3$	0.190±0.006	0.232±0.014	0.201±0.014	0.224±0.014	1.29	n.s.
22:4$n-6$	0.093±0.012	0.548±0.040	0.582±0.040	0.638±0.039	1.32	n.s.
22:5$n-6$	0.317±0.015	0.508±0.050	0.503±0.049	0.600±0.048	1.26	n.s.
22:5$n-3$	0.083±0.009	0.987±0.038	1.070± 0.038	1.047±0.037	1.28	n.s.
22:6$n-3$	36.887±0.245	28.059±0.386	27.861±0.378	27.428±0.372	0.73	n.s.
24:1$n-9$	2.333±0.033	1.292±0.093	1.274±0.091	1.459±0.089	1.28	n.s.
SFA	32.943±0.090	37.555±0.149^{a}	38.012±0.147^{b}	37.499±0.144^{a}	3.71	*
UFA	67.057±0.090	62.445±0.149^{b}	61.988±0.147^{a}	62.501±0.144^{b}	3.71	*
MUFA	9.420±0.031	7.470±0.173	7.733±0.170	8.023±0.167	2.64	n.s.
PUFA	57.317±0.103	54.782±0.317	54.065±0.311	54.286±0.305	1.37	n.s.
UFA/SFA	2.037±0.009	1.663±0.010^{b}	1.632±0.010^{a}	1.668±0.010^{b}	3.58	*
MUFA/SFA	0.293±0.003	0.205±0.004^{a}	0.208±0.004^{a}	0.219±0.004^{b}	3.91	*
PUFA/SFA	1.740±0.010	1.461±0.014	1.424±0.014	1.449±0.014	1.83	n.s.
$n-3$ PUFA	54.183±0.110	46.783±0.761	45.981±0.747	45.361±0.733	0.91	n.s.
$n-6$ PUFA	2.710±0.017	7.017±0.450	7.105±0.442	7.884±0.434	1.18	n.s.
$n-3/n-6$ PUFA	19.997±0.111	7.853±0.562	7.065±0.552	6.471±0.542	1.57	n.s.
$n-3$ HUFA	53.843±0.133	46.372±0.761	45.561±0.747	44.903±0.734	0.97	n.s.
$n-6$ HUFA	2.267±0.003	6.395±0.446	6.511±0.438	7.219±0.430	1.05	n.s.
$n-3/n-6$ HUFA	23.753±0.033	8.802±0.685	7.692±0.672	7.214±0.660	1.46	n.s.
DHA/EPA	2.210±0.031	1.654±0.019	1.703±0.019	1.705±0.018	2.35	n.s.
EPA/AA	4.493±0.032	1.950±0.156	1.665±0.153	1.576±0.150	1.61	n.s.
18:1$^{\dagger}/n-3$ HUFA	0.032±0.001	0.047±0.003	0.049±0.003	0.053±0.003	1.07	n.s.

SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid; DHA, docosahexanoic acid (22:6$n-3$); EPA, eicosapentanoic acid (20:5$n-3$); AA, arachidonic acid (22:4$n-6$).
Means in the same row with different superscript letters are significantly different.
*$P<0.05$; n.s.: not significant ($P>0.05$).
nd: not detected.
†Includes 18:1$n-9$, 18:1$n-7$ and 18:1$n-5$.

Lipids were extracted according to Folch et al. (1957). Samples were stored at −80 °C prior to analysis. Fatty acid composition was determined for each octopus (n=81) and each batch of squid (n=3). Three aliquots from each sample were used for fatty acid analysis.

Fatty acid methyl esters were prepared by transesterification with anhydrous methanol containing 2% sulfuric acid and 0.01% (w/v) butylated hydroxytoluene (BHT) for 16 h at 50 °C (Christie, 1982). Fatty acid methyl esters were separated by gas liquid chromatography (GLC) on a Perkin Elmer Gas Chromatograph (Autosystem XL) equipped with a flame ionization detector and a split/splitless injector fitted to a fused silica capillary column (FameWax, Restek, length: 30 m, i.d.: 0.32 mm, film thickness: 0.25 μm). Helium was used as the carrier gas. Injector

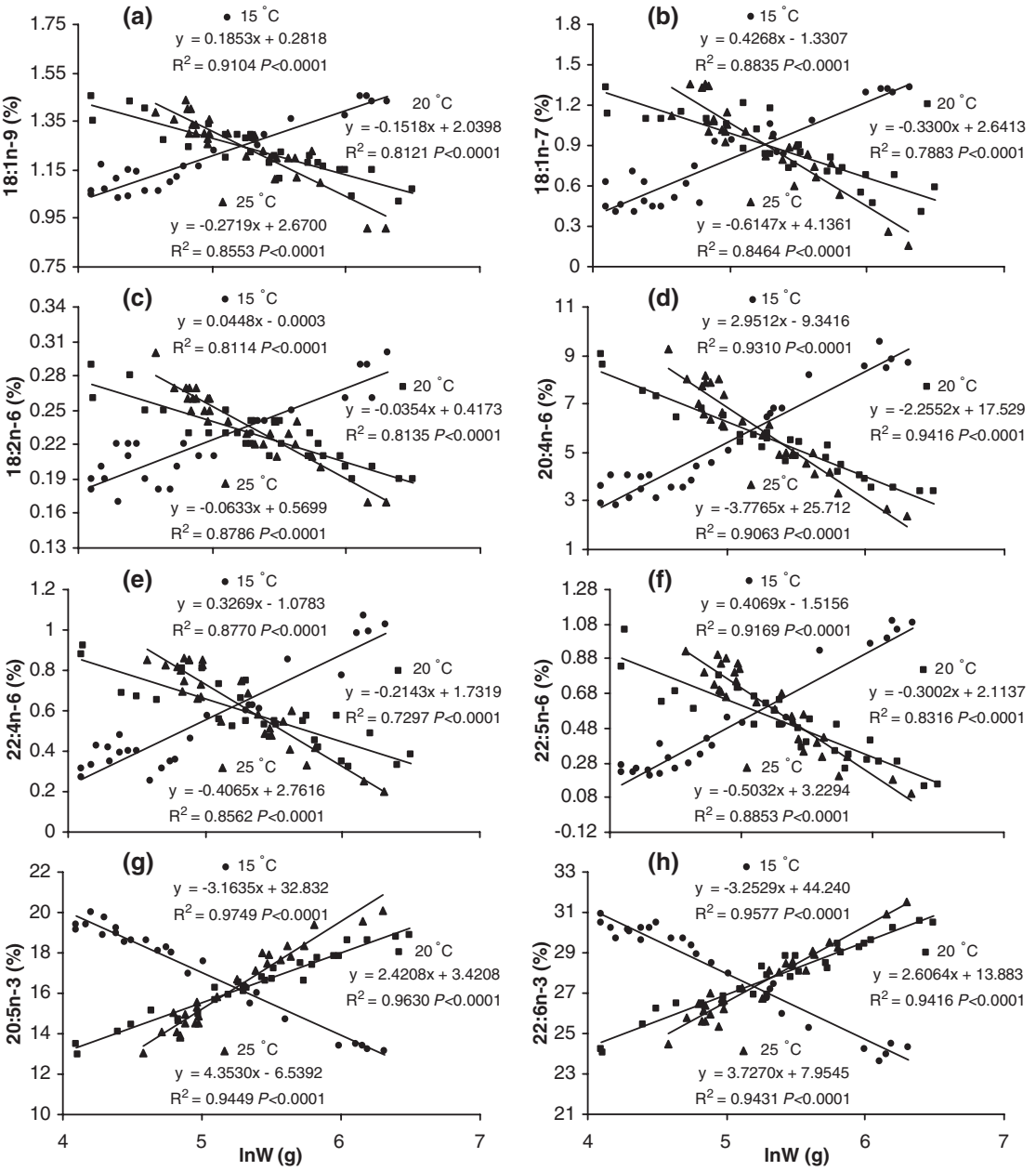

Fig. 1. Regression lines of *O. vulgaris* content (% total fatty acids) in (a) 18:1n−9, (b) 18:1n−7, (c) 18:2n−6, (d) 20:4n−6, (e) 22:4n−6, (f) 22:5n−6, (g) 20:5n−3 and (h) 22:6n−3 vs. body weight (W) at three temperatures: ● 15 °C, ■ 20 °C and ▲ 25 °C. Linear equations and regression coefficients shown.

and detector temperatures were set at 225 and 250 °C, respectively. The oven temperature was programmed to rise from 130 to 175 °C at a rate of 5 °C min^{-1} and then to 225 °C at a rate of 20 °C min^{-1}, where it was held for 10 min. Fatty acid peaks were integrated using Turbochrom Navigator Software (Version 4.1). Peak identification was performed by means of standards (Alltech, Sigma and Larodan), as well as by reference to a well characterized fish oil (menhaden oil).

Previous experiments (in the same closed system, under the same feeding conditions, at 20 °C) showed that the fatty acid composition of the common octopus (estimated from around 10 animals every 30 days) was

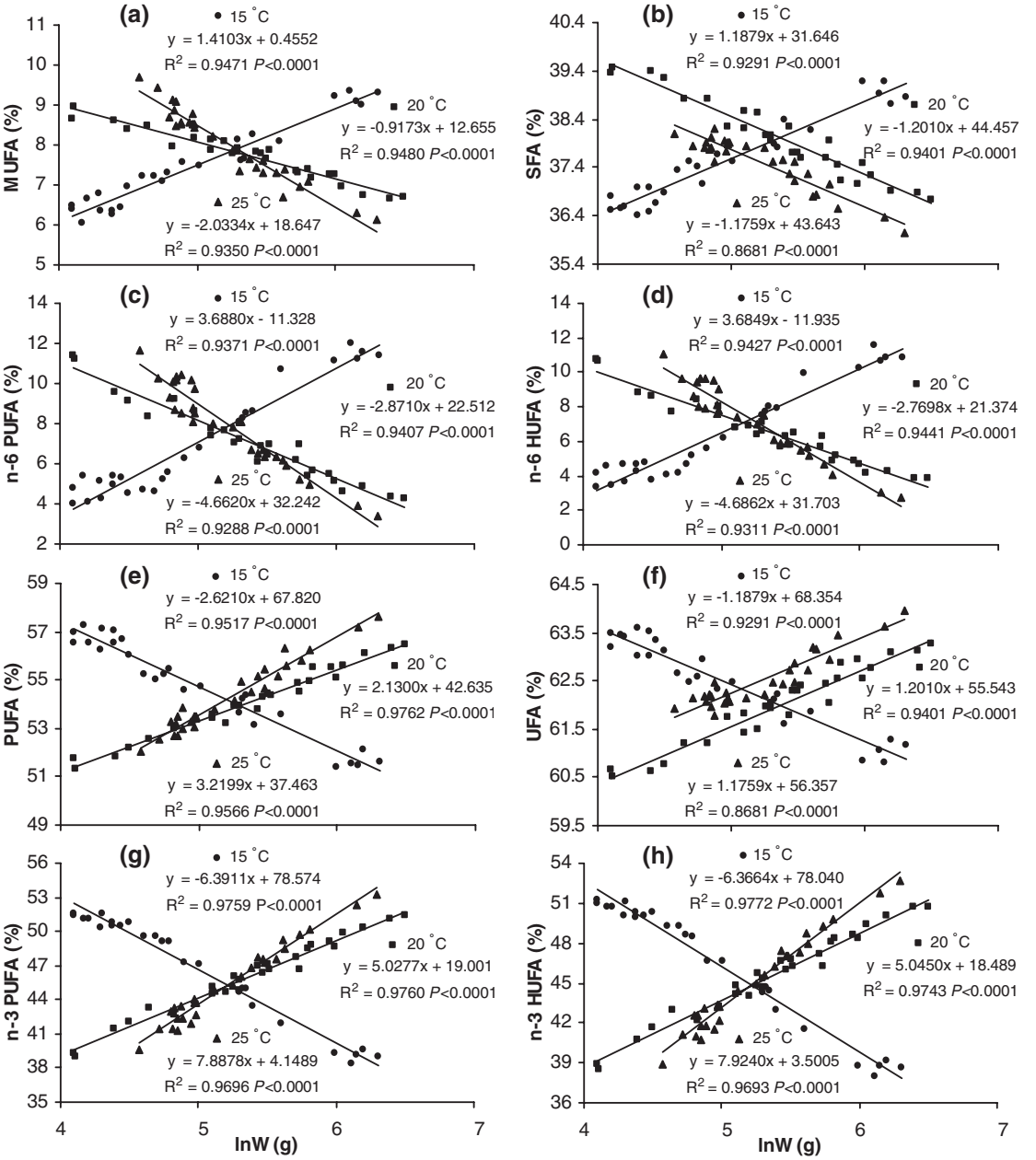

Fig. 2. Regression lines of *O. vulgaris* content (% total fatty acids) in (a) MUFA, (b) SFA, *n*−6 (c) PUFA, (d) *n*−6 HUFA, (e) PUFA, (f) UFA, (g) *n*−3 PUFA and (h) *n*−3 HUFA vs. body weight (*W*) at three temperatures: ● 15 °C, ■ 20 °C and ▲ 25 °C. Linear equations and regression coefficients shown. For abbreviations see Table 1.

not affected significantly by the rearing period (210 days after collection from the sea).

As the specimen were wild-caught, it was impossible to obtain replicates of the same body weight or even to form groups of animals having a narrow range of body weight. Regression analysis was performed between the proportion of fatty acids (Y) and final body weight (W) at each temperature ($\ln Y = a + b \ln W$). The significance ($P < 0.05$) of the differences among the regression coefficients and intercepts was tested with analysis of variance. ANOVA (LSD multiple range test) was conducted for comparing the means obtained at the

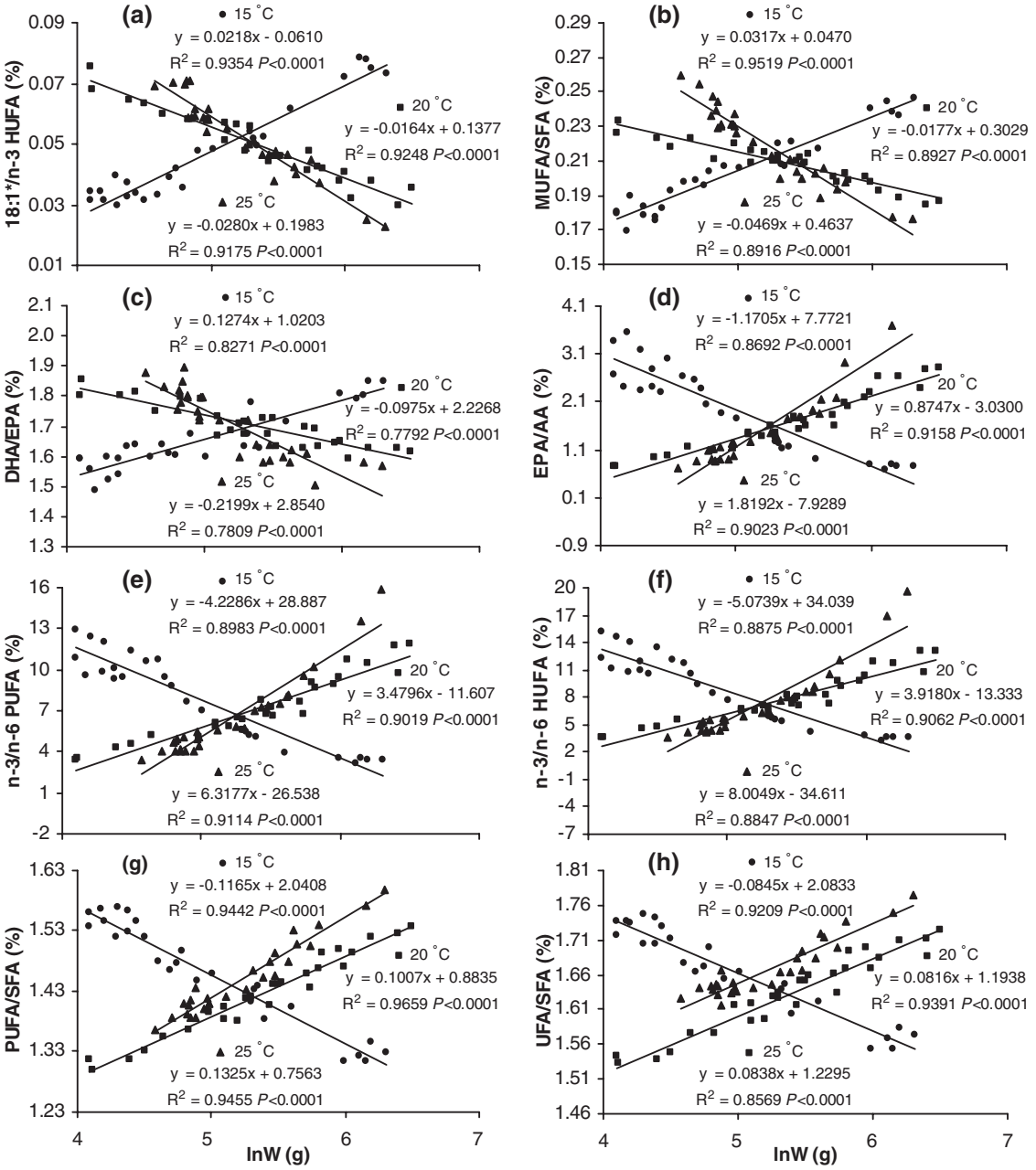

Fig. 3. Regression lines of *O. vulgaris* content (% total fatty acids) in (a) 18:1†/$n-3$ HUFA, (b) MUFA/SFA, (c) DHA/EPA, (d) EPA/AA, (e) $n-3/n-6$ PUFA, (f) $n-3/n-6$ HUFA, (g) PUFA/SFA and (h) UFA/SFA vs. body weight (W) at three temperatures: ● 15 °C, ■ 20 °C and ▲ 25 °C. Linear equations and regression coefficients shown. †: includes 18:1$n-9$, 18:1$n-7$ and 18:1$n-5$. For abbreviations see Table 1.

three temperatures. Normality of distributions (Chi-Square test, Kolmogorov–Smirnov D and Anderson–Darling A^2) and homogeneity of variances (Cochran's C and Bartlett's tests) were assessed. Where appropriate, data were transformed [$a\sin(x)$ or $a\sin(1-x/100)$]. Untransformed means ± standard error (S.E.) are presented. All statistical analyses were performed with the Statgraphics plus 4 software.

3. Results

The proportion of fatty acids (% total fatty acids) in squid did not vary among the three batches (Table 1). The mean proportion of fatty acids (% total fatty acids) in the octopuses studied did not vary significantly among the three temperatures (Table 1). In addition, no significant differences were observed in $n-3$ or $n-6$ PUFA (fatty acids having carbon chain lengths of $\geq C_{18}$), $n-3$ or $n-6$ HUFA (fatty acids having carbon chain lengths of $\geq C_{20}$ and with >3 ethylenic bonds) and the sum of polyunsaturated or monounsaturated, as well as in the ratios of $n-3/n-6$ HUFA, $n-3/n-6$ PUFA, polyunsaturated/saturated, DHA/EPA and EPA/AA, among the three temperatures. However, the sum of unsaturated and the ratio of unsaturated/saturated were significantly lower ($P<0.05$), while the sum of saturated was significantly higher ($P<0.05$), at 20 °C compared to the other two temperatures (15 and 25 °C). Moreover, the monounsaturated/saturated fatty acid ratio was significantly higher ($P<0.05$) at the higher temperature (25 °C).

At each temperature, the proportion of some fatty acids was highly ($P<0.0001$) related to the body weight. Specifically, 18:1$n-9$, 18:1$n-7$, 18:2$n-6$, 20:4$n-6$, 22:4$n-6$ and 22:5$n-6$ (Fig. 1a–f) decreased, while 20:5$n-3$ and 22:6$n-3$ (Fig. 1g–h) increased, with increasing body weight at 20 and 25 °C. In addition, some sums and ratios of fatty acids showed a significant ($P<0.0001$) relationship with body weight. Monounsaturated, saturated, $n-6$ PUFA and $n-6$ HUFA (Fig. 2a–d) decreased, whereas polyunsaturated, unsaturated, $n-3$ PUFA and $n-3$ HUFA (Fig. 2e–h) increased, with body weight at 20 and 25 °C. The ratios 18:1/$n-3$ HUFA, monounsaturated/saturated and DHA/EPA (Fig. 3a–c) decreased, but EPA/AA, $n-3/n-6$ PUFA, $n-3/n-6$ HUFA, polyunsaturated/saturated and unsaturated/saturated (Fig. 3d–h) increased, with increasing body weight at 20 and 25 °C. The opposite was observed for the aforementioned proportions, sums and ratios of fatty acids at 15 °C.

Comparison of regression lines (Table 2) estimated for 18:1$n-9$, 18:1$n-7$, 18:2$n-6$, 20:4$n-6$, 22:4$n-6$ and 22:5$n-6$, 20:5$n-3$ and 22:6$n-3$ vs. body weight proved significant differences for the slopes ($P<0.0001$) among the three temperatures. Similarly, the slopes of the regression lines estimated for all the above mentioned sums and ratios of fatty acids differed significantly ($P<0.0001$) among the three temperatures. In addition, the slopes of all the estimated regression lines differed significantly ($P<0.0001$) between 20 and 25 °C, except those for unsaturated, saturated and unsaturated/saturated. However, comparison of regression lines between 20 and 25 °C showed significant ($P<0.0001$) differences for the intercepts of the regression lines estimated for unsaturated, saturated and unsaturated/saturated fatty acids vs. body weight.

Highly significant ($P<0.0001$) linear relationships were found between the proportions of some fatty acids, being positive such as EPA vs. DHA (Fig. 4a), 18:2$n-6$ vs. AA (Fig. 4d), 22:4$n-6$ vs. AA (Fig. 4e) and 22:5$n-6$

Table 2
Comparison of regression lines estimated for selected fatty acids vs. body weight of octopuses among 15, 20 and 25 °C (A) and between 20 and 25 °C (B)

Fatty acid	A			B		
	P	*P* intercepts	*P* slopes	*P*	*P* intercepts	*P* slopes
18:1$n-9$	0.0000	0.0009	0.0000	0.0000	0.8324	0.0000
18:1$n-7$	0.0000	0.0001	0.0000	0.0000	0.8551	0.0000
18:2$n-6$	0.0000	0.0000	0.0000	0.0000	0.0654	0.0000
20:4$n-6$	0.0000	0.0000	0.0000	0.0000	0.2026	0.0000
20:5$n-3$	0.0000	0.0000	0.0000	0.0000	0.0653	0.0000
22:4$n-6$	0.0000	0.0007	0.0000	0.0000	0.4464	0.0000
22:5$n-6$	0.0000	0.0001	0.0000	0.0000	0.0557	0.0000
22:6$n-3$	0.0000	0.0000	0.0000	0.0000	0.8937	0.0000
SFA	0.0000	0.0000	0.0000	0.0000	0.0000	0.8168
UFA	0.0000	0.0000	0.0000	0.0000	0.0000	0.8168
MUFA	0.0000	0.0000	0.0000	0.0000	0.0508	0.0000
PUFA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
UFA/SFA	0.0000	0.0000	0.0000	0.0000	0.0000	0.7789
MUFA/SFA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PUFA/SFA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$n-3$ PUFA	0.0000	0.1650	0.0000	0.0000	0.0000	0.0000
$n-6$ PUFA	0.0000	0.0506	0.0000	0.0000	0.0000	0.0000
$n-3/n-6$ PUFA	0.0000	0.0000	0.0000	0.0000	0.9009	0.0000
$n-3$ HUFA	0.0000	0.0000	0.0000	0.0000	0.2533	0.0000
$n-6$ HUFA	0.0000	0.0000	0.0000	0.0000	0.0855	0.0000
$n-3/n-6$ HUFA	0.0000	0.0000	0.0000	0.0000	0.6584	0.0000
DHA/EPA	0.0000	0.0000	0.0000	0.0000	0.1618	0.0000
EPA/AA	0.0000	0.0000	0.0000	0.0000	0.2186	0.0000
18:1$^{\dagger}/n-3$ HUFA	0.0000	0.0000	0.0000	0.0000	0.7697	0.0000

For abbreviations see Table 1.

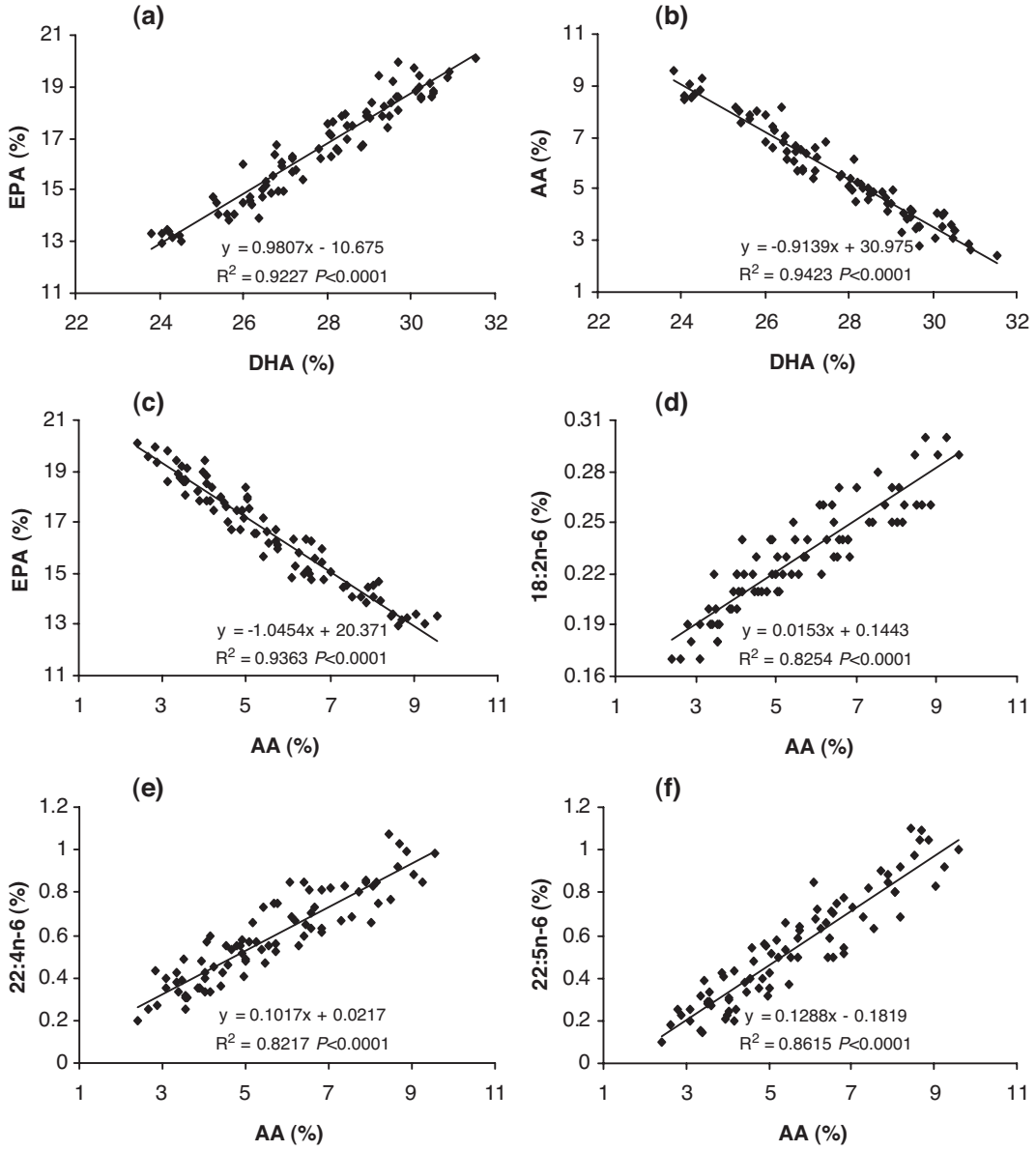

Fig. 4. Relationships between EPA and DHA (a), AA and DHA (b), EPA and AA (c), 18:2$n-6$ and AA (d), 22:4$n-6$ and AA (e) and 22:5$n-6$ and AA (f). For abbreviations see Table 1.

vs. AA (Fig. 4f), or negative such as AA vs. DHA (Fig. 4b) and EPA vs. AA (Fig. 4c).

4. Discussion

Palmitic acid (16:0), EPA (20:5$n-3$) and DHA (22:6$n-3$) were the most abundant fatty acids found in the lipids of *O. vulgaris*, as has been reported for many cephalopod species (Jangaard and Ackman, 1965; Culkin and Morris, 1970; Nash et al., 1978). The fatty acid profile observed in the common octopus, *O. vulgaris*, resembled that reported for wild individuals of the same species (Navarro and Villanueva, 2000, 2003). Specifically, the squid-fed octopuses showed a DHA/EPA ratio ranging from 1.5 to 1.9, which is above those detected (0.4–0.6) in the cultured paralarvae of the common octopus fed only *Artemia*, and is similar to that found (1.7) in the hatchlings and the mature ovary of wild *O. vulgaris* (Navarro and Villanueva, 2000, 2003). Okumura et al. (2005) found a DHA/EPA equal to 1.5 in

common octopus paralarvae fed on *Artemia* and flakes of Pacific sandeel, and suggested that this ratio is a necessary condition for the normal growth and development of these paralarvae. Thus, the fatty acid composition of the octopuses in this study matches the ideal, i.e. "natural" fatty acid profile of *O. vulgaris*.

An inverse relationship between temperature and content of unsaturated fatty acids, particularly EPA and DHA, has been reported for several poikilotherms (Hazel, 1984). In this study, the proportion of total fatty acids in EPA and DHA, and generally $n-3$ HUFA, was dependent upon octopus body weight at each temperature. Specifically, EPA and DHA decreased with increasing body weight at the low temperature (15 °C), while they increased as body weight increased at higher temperatures (20–25 °C). It has been shown that the growth rate of octopuses increased with increasing body weight at 15 °C, but the opposite occurred at 20–25 °C (Miliou et al., 2005). It seems that the decreases in EPA and DHA observed at the low temperature reflect a higher demand for EPA and DHA, for membrane synthesis of faster growing large octopuses, rather than a response for maintaining membrane permeability and plasticity. Effects of temperature on the growth rate appear to play an important role for the fatty acid composition of octopuses.

The necessity of increased fatty acid unsaturation in fish following decreases in environmental temperature is still a matter of debate (Olsen et al., 1999). Although increased unsaturation at low temperatures has been reported as a mechanism for maintaining membrane function (Tritar et al., 1997; Olsen et al., 1999; Khériji et al., 2003), several reports prove a lack of such an adaptation (Skuladottir et al., 1990; Cordier et al., 2002; Khériji et al., 2003). These differences may be influenced by the species and tissue studied, as well as by dietary (HUFA levels) and environmental factors (salinity levels). The high HUFA content of octopus, along with the high HUFA dietary input in this study, may explain in part why its fatty acid profile did not change with decreasing temperature for homeoviscous adaptation purposes. In addition, the high salinity used, preferred by *O. vulgaris*, may also help to explain this.

In this study, decreases observed in $n-3$ HUFA, at 15 °C, were accompanied by increases in 18:1$n-9$ and 18:1$n-7$. Fatty acid profiles from marine finfish exhibited an increase in 18:1$n-9$ due to essential fatty acid deficiency, resulting in an increased 18:1$n-9$/$n-3$ HUFA ratio (Ibeas et al., 1996). An increase in monounsaturated fatty acids, particularly in 18:1 isomers, has been observed in cultured *O. vulgaris* paralarvae fed on $n-3$ HUFA deficient diets (Navarro and Villanueva, 2003). However, in this study, the diet provided was rich in $n-3$ HUFA and the highest 18:1 levels were observed at the optimal experimental temperature for the growth of each octopus in relation to its weight (Miliou et al., 2005). It appears that increases in 18:1 levels were associated with the maintenance of the unsaturation index under certain temperature conditions that promote common octopus growth.

The differences observed in the fatty acid composition between the octopuses reared at 15 and 20 °C were more pronounced compared to those between 20 and 25 °C. Similarly, differences in the growth rate of cultured octopuses have been found to be more pronounced between 15 and 20 °C than between 20 and 25 °C (Miliou et al., 2005). Furthermore, larger octopuses (200–600 g) had a higher growth rate at 20 than at 25 °C, whereas smaller animals (50–150 g) at 25 than at 20 °C. This seems to explain the increased unsaturation index in the larger animals at 25 °C. The decreases in $n-3$ PUFA with increasing growth rates in smaller animals was followed by an increase in $n-6$ PUFA, but total polyunsaturated fatty acids still remain lower at 25 °C in small animals. However, the increase in 18:1 levels in smaller animals at 25 °C makes the percentages of unsaturated and the unsaturated/saturated ratio, vary with body weight in a similar way to that observed at 20 °C, being significantly higher at 25 than at 20 °C independently of body weight. This increase in 18:1 levels led to a higher monounsaturated/saturated ratio at 25 °C compared to that estimated at both other temperatures. However, these observations disagree with previous findings (Sargent et al., 1989; Tocher, 1995), according to which the unsaturated/saturated and monounsaturated/saturated ratios increased in response to lowered temperature for homeoviscous adaptation purposes.

The concentration of EPA (%) and AA (%) in the octopuses studied indicated an inverse relationship, where decreased EPA levels resulted in respective increased AA levels. Competition between EPA and AA during phospholipid esterification has been suggested to occur in fish, particularly for phosphatidylinositol (PI) where both fatty acids are major components (Bell et al., 1995). A decrease in EPA has been observed in gilthead sea bream with the increase in dietary AA (Bessonart et al., 1999; Fountoulaki et al., 2003; Van Anholt et al., 2004).

In this study, the higher levels of AA and the lower levels of EPA were observed in smaller octopuses at 20–25 °C and in larger animals at 15 °C, i.e. at temperatures that promote the growth rate in octopuses in relation to

body weight (Miliou et al., 2005). This means that increased AA levels in octopuses are associated with an improved growth of *O. vulgaris*. Arachidonic acid has been proved effective in improving egg quality (Sargent et al., 1999) and survival at the early life stages of fish (Castell et al., 1994; Bessonart et al., 1999; Koven et al., 2001). As far as the effect of temperature is concerned, a decrease in the EPA/AA ratio has been reported (Rady, 1993; Sorensen, 1993; Cordier et al., 2002) for three fish species with increasing temperature, which was followed by an increase in body weight. A similar tendency in the EPA/AA ratio was observed for smaller octopuses, which showed an increased growth rate at the higher temperatures (Miliou et al., 2005).

Octopuses and cephalopods in general are characterized by low lipid contents, with relatively large phospholipid and sterol fractions, and triacyglycerides as minor components (Nash et al., 1978; Hayashi and Yamamoto, 1987; Navarro and Villanueva, 2000). Thus, the changes in the fatty acid composition of total lipids observed in the octopuses reflected those that occurred mainly in the composition of phospholipids. In this study, AA levels showed a large variation from 2.4% to 9.6% of total fatty acids, which is consistent with that found in wild octopuses (Culkin and Morris, 1970; Gibson, 1983; Sinanoglou and Miniadis-Meimaroglou, 1998). However, in cultured marine fish, such an increase in the AA levels in the phospholipids of various tissues has been achieved by increasing the dietary AA levels (Castell et al., 1994; Bessonart et al., 1999; Fountoulaki et al., 2003). However, in this study, the diet offered to all specimens had the same fatty acid composition and moreover was poor in AA. The relatively high level of AA in octopuses appears to be an inherent characteristic of *O. vulgaris*, because it does not correlate with dietary input. In addition, *O. vulgaris* paralarvae fed on a diet poor in AA maintained this fatty acid at the levels found in hatchlings (Navarro and Villanueva, 2000).

Fish contain substantial amounts of 18:1$n-9$ and have a low 20:4$n-6$ content (Tocher, 2003) compared to that of octopuses. The role of 18:1$n-9$ and $n-3$ HUFA in modulating the unsaturation index in membrane phospholipids seems to be fundamental in fish tissues (Sargent et al., 1989). Although 18:2$n-6$ and 20:4$n-6$ are involved in the maintenance of the unsaturation index, their contribution is relatively smaller in fish (Ibeas et al., 1996). In this study, the increase in both 18:1 and $n-6$ PUFA in response to the decrease in $n-3$ PUFA caused the unsaturated/saturated ratio in the octopuses to be similar at 15 and 25 °C. However, the contribution of $n-6$ PUFA and, particularly of 20:4$n-6$, was relatively higher compared to that of 18:1 in the maintenance of this ratio, indicating a preferential desaturation and elongation of 18:2$n-6$, although 18:1$n-9$ is present in the squid in higher amounts than 18:2$n-6$. In addition, no desaturation and elongation products were obtained from 18:1$n-9$, such as 18:2$n-9$, 20:2$n-9$ and 20:3$n-9$, as has been described for another mollusc (Durazo-Beltrán et al., 2003). It seems that, in *O. vulgaris*, arachidonic acid plays an important role in the maintenance of cell membrane structure and function.

AA is the main end product of desaturation and elongation of 18:2$n-6$ and can be desaturated further and elongated to 22:5$n-6$ to some extent in the same manner as for the enzymatic conversion of 18:3$n-3$ to 22:6$n-3$ (Buzzi et al., 1997). Specifically, 20:4$n-6$ is chain elongated to 22:4$n-6$, which is then converted by $\Delta 6$ desaturation to 24:5$n-6$, which is then converted by a chain-shortening reaction in the peroxisosomes to 22:5$n-6$. The specific accumulation of 22:4$n-6$ and 22:5$n-6$, which was present in diets in small amounts and are considered intermediate metabolites of polyunsaturated fatty acid synthesis, suggests activation of $\Delta 6$ desaturase when the $n-3$ HUFA levels in octopus decreased. However, the pathway for AA biosynthesis in octopuses needs further research. Ability for enzymatic bioconversion of C18 precursors to AA has also been reported for other marine molluscs, such as the abalones *Haliotis fulgens* (Durazo-Beltrán et al., 2003), *H. laevigata* (Dunstan et al., 1996) and *H. discus hannai* (Uki et al., 1986).

Arachidonic acid levels and EPA/AA ratios similar to those of this study have been found in wild sea bream, which usually inhabit stressful environments (Alexis and Nengas, 1996). The need of AA has been mainly related to stressful reactions of fish (Sargent et al., 1999; Bessonart et al., 1999; Koven et al., 2001). Octopuses are commonly found in turbulent inshore waters and show a resistance to low water oxygen content (Cerezo Valverde and García García, 2005). They can be described as opportunistic and dim-light feeders (Vaz-Pires et al., 2004). The increase of AA in octopus tissue at preferred temperatures, i.e. warm waters for small octopuses and cold waters (deeper areas) for large ones (Katsanevakis and Verriopoulos, 2004) is evidence of the beneficial effects of arachidonic acid on this cephalopod species.

In conclusion, *O. vulgaris* reared under the experimental conditions of this study, showed a high content in polyunsaturated fatty acids (51.32–57.62% total fatty acids), particularly in DHA (23.62–31.55% total fatty acids), similar to that in wild *O. vulgaris* and *E.*

moschata from the Mediterranean Sea (Sinanoglou and Miniadis-Meimaroglou, 1998; Passi et al., 2002). Fatty acid composition of *O. vulgaris* was influenced by temperature and body weight, but with an $n-3/n-6$ ratio of more than 3 and a DHA/EPA ratio of more than 1.5. It is indicated that arachidonic acid is not an essential fatty acid for *O. vulgaris* and is inversely related to EPA and DHA. It is concluded that *O. vulgaris* could be an excellent source of arachidonic acid, containing sufficient $n-3$ HUFA levels, in warm temperatures for small individuals and in low temperatures for large individuals, i.e. at temperatures that promote growth in relation to the body weight of octopuses (Miliou et al., 2005).

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