LARVI – 2009 SPECIAL ISSUE

FATTY ACID COMPOSITION OF POLAR AND NEUTRAL LIPID

**FRACTIONS OF *Octopus vulgaris* Cuvier, 1797 PARALARVAE REARED WITH
ENRICHED ON-GROWN *Artemia***

E. Viciano¹, J. Iglesias², M.J. Lago², F.J. Sánchez², J. J. Otero² and J.C. Navarro^{1*}.

¹Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Ribera de Cabanes,
Castellón, Spain

²Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Vigo, Apartado
1552, 36200 Vigo, Spain

* Corresponding author:
Tel: +34 964319500
Fax: +34964319509
jcnavarro@iats.csic.es

Running title: polar and neutral lipid fatty acids of octopus

Keywords: *Octopus vulgaris*, paralarvae, lipids, fatty acids, *Artemia*

Abstract

Up today, rearing of common octopus, *Octopus vulgaris* paralarvae is severely limited by the lack of success during the paralarval stage, with generalised mortalities occurring before the settlement of the juveniles. Among others, a lack of balance in the lipid and fatty acid composition of the food can be at the basis of this problem. The use of on-grown *Artemia* cultured with the microalga *Isochrysis galbana* and further enriched with *Nannochloropsis* sp. has proven a certain degree of success. The present work aims at studying the effects of this rearing protocol on the fatty acid composition of paralarvae, by comparison with a diet based on on-grown *Artemia* further enriched with a high polyunsaturated fatty acid (PUFA) oil emulsion. Although apparently no clear association between the fatty acid composition of the enriched preys and that of total lipids of paralarvae could be established, further fractionation and fatty acid analysis of the total lipids of the paralarvae into polar and neutral classes, followed by PCA, revealed that irrespective of the diet, both lipid fractions showed distinct fatty acid patterns. Besides, the fatty acid composition of the polar lipids was more conservative, whereas that of the neutral lipids was more influenced by diet and showed more variation among dietary treatments.

49 Introduction

50

51 In recent years, common octopus *Octopus vulgaris* has been targeted as a promising
52 candidate for aquaculture but, up today, paralarvae rearing is severely limited by the
53 lack of success during the planktonic stage, with generalised mortalities occurring
54 before the settlement of the juveniles.

55 The life cycle of *O. vulgaris* under captivity conditions was completed for the
56 first time in 2001 (Iglesias, Otero, Moxica, Fuentes, & Sánchez 2004), using *Artemia*
57 and *Maja* zoeae as prey. Although some successful cultures of a reduced number of
58 paralarvae up to juveniles and even sub-adults have been achieved (Moxica, Linares,
59 Otero, Iglesias & Sánchez 2002; Iglesias, Otero, Moxica, Fuentes, & Sánchez 2004;
60 Carrasco, Arronte & Rodríguez 2006), these experiences have been mainly occasional,
61 anecdotic and, needless to say, economically profitless.

62 According to Navarro & Villanueva (2000 & 2003), among others, a lack of
63 balance in the lipid and fatty acid composition of the food can be at the basis of the high
64 mortalities encountered in the rearing of the paralarval stages of *O. vulgaris*. These
65 authors studied the lipid requirements of early stages of cephalopods to conclude that a
66 nutritional imbalance in the lipid and fatty acid profile of the artificial feeding protocol,
67 based on *Artemia*, may be responsible for the high mortalities encountered. *O. vulgaris*
68 should require feeding on low-lipid preys rich in polar lipids, long chain
69 polyunsaturated fatty acids and possibly cholesterol (Navarro & Villanueva 2000 &
70 2003; Okumura, Kurihara, Iwamoto & Takeuchi 2005; Seixas 2009). This closely
71 resembles the composition of a “natural” diet (totally unpractical from an aquaculture
72 approach) based on crustacean larvae (for example, *Maja* zoeae) and other marine

73 planktonic forms like copepods, but is far away from the typical composition of
74 enriched *Artemia* in any of its forms. In fact, early stages of cephalopods are particularly
75 rich in polar lipids and cholesterol, and artificial feeding under culture conditions
76 increases dramatically their content in triacylglycerides (Navarro & Villanueva 2000 &
77 2003).

78 Aside from the use of *Maja* zoeae as food, *Octopus* adult stages have been
79 reached using on-grown *Artemia* (1-1.5 mm) cultured with the microalga *Isochrysis*
80 *galbana* and further enriched with *Nannochloropsis* sp. (Hamazaki, Fukunaga, Yoshida
81 & Maruyama 1991; Moxica, Fuentes, Hernández, Iglesias, Lago, Otero & Sánchez
82 2006). The reasons for the limited success of this rearing protocol remain obscure, but a
83 striking feature of *Nannochloropsis* sp. is its high content in eicosapentaenoic acid
84 (EPA, 20:5n-3), an essential fatty acid for marine animals (Sargent, McEvoy, Estévez,
85 Bell, Bell, Henderson & Tocher 1999).

86 The present work aims at studying the effects of a rearing protocol based on
87 *Artemia* on-grown with *Isochrysis galbana* and enriched with *Nannochloropsis* sp. on
88 the fatty acid composition of paralarvae. It will be carried out by comparison with the
89 effects of a diet based on the same on-grown *Artemia* further enriched with a high
90 polyunsaturated fatty acid (PUFA) oil emulsion especially rich in docosahexaenoic acid
91 (DHA, 22:6n-3). Thus, the work will ultimately focus on the dietary effects of DHA-
92 rich and EPA-rich diets on the total, polar and neutral lipid fatty acid composition of the
93 paralarvae.

Materials and Methods

Octopus vulgaris paralarvae were obtained from broodstock kept in captivity using the technology described by Moxica *et al.* (2002). They were fed during 28 days with on-grown *Artemia* (1.5-2 mm) cultured with *Isochrysis galbana* and further enriched during 24h with either *Nannochloropsis* sp. (Nanno treatment) or a PUFA-rich oil emulsion based on a speciality oil (M70 treatment).

Cultures were carried out in 1000-l black, circular tanks (diameter 130 cm), at a larval density of 5 individuals L⁻¹ (5000 paralarvae per tank). Mean water temperature was 21-22 °C, salinity 34-35 g L⁻¹, and continuous illumination of 800-1000 lux provided with two 36 W day-light fluorescent tubes was supplied to the tanks. During the first week, the cultures were kept in a closed water circuit, with gentle central aeration and a concentration of 1 million cells mL⁻¹ of *Nannochloropsis* sp. in the rearing tank. From day 8, the water circuit was partially opened (4 hour d⁻¹) every two days.

Enriched *Artemia* was added (3 to 4 times per day) at a density of 0.5 prey mL⁻¹ until day 11, and at 0.2 prey mL⁻¹ onwards. *Artemia* was on-grown with 300.000 cells mL⁻¹ of *Isochrysis galbana* during 4 days, and further enriched during 24 h with 10 million cells mL⁻¹ of *Nannochloropsis* sp.

Dissolved oxygen, nitrites and ammonium were measured daily. Dry weights of ten paralarvae after washing with distilled water and drying at 90°C 24 h were recorded at the beginning and at the end of the experiment. Survival was recorded at day 28.

At the end of the trial, the lipids of diets and paralarvae (triplicate pools) were extracted using the method of Folch, Lees & Sloane-Stanley (1957). Total lipids were

determined gravimetrically (Mettler, 0.0001 g) and stored in chloroform/methanol (2:1, v/v) with 0.01% BHT as an antioxidant. An aliquot of total lipids was transmethylated after the addition of 19:0 as an internal standard. Fatty acid methyl esters (FAME) were purified by thin layer chromatography (Silica gel G 60, Merck) and injected on-column in a Fisons 8000 gas chromatograph equipped with a fused silica 30 m x 0.25 mm open tubular column (Tracer, TR-WAX, film thickness: 0.25 μ). Helium was used as carrier gas, and the analyses were run in a 50 to 220 °C thermal gradient. Peaks were recorded and integrated in a personal computer using Azur software (Azur, Datalys, France), and identified by comparison with well-characterised standards. A further aliquot of the total lipids was fractionated into polar and neutral lipids by thin layer chromatography, and the fatty acids transmethylated and analysed as described for the total lipids.

The fatty acid profiles thus obtained were subsequently analysed chemometrically by principal component analysis (PCA). The score plot resulting after the generation of the two principal components was used to identify patterns of similarity among the cases.

For each lipid class, the mean values of the fatty acid composition of the paralarvae from the two dietary treatments were compared with Student t-tests. Statistics were carried out using SPSS 17.0 software (SPSS Inc.)

Results and Discussion

The average weight of paralarvae from both dietary groups was not significantly different (Nanno: 1.76 ± 0.28 mg; M70: 1.88 ± 0.22 mg) and is similar to the values reported by Moxica *et al.* (2006) using the same microalgal enrichment. They are

clearly lower than those reported for paralarval of the same age (30 days post hatch) using crustacean zoeae as complementary food by Moxica, Fuentes, Hernández, Iglesias, Lago, Otero & Sánchez (2006): 2.42 mg; Iglesias, Otero, Moxica, Fuentes, & Sánchez (2004): 3.33 mg; Carrasco, Arronte & Rodríguez (2006): 2.83 mg; and higher than those obtained by other authors with different feeding regimes: Villanueva, Koueta, Riba, & Boucaud-Camou (2002) reported weights from 0.79 mg to 1.49 mg using enriched *Artemia* co-fed with millicapsules; Seixas (2009) obtained 0.83 mg paralarvae at day 25 using on-grown *Artemia* enriched with a mixture of *Isochrysis galbana* y *Rhodomonas lens*; Fuentes, Sánchez, Otero, Lago and Iglesias (2009) reported values between 0.96 mg and 1.12 mg with *Artemia* and frozen wild zooplankton as food, and Estévez, Gairín & Berger (2009) produced 0.33 mg paralarvae using live zooplankton as food.

These differences can be explained by the different culture conditions and foods. It is evident that crustacean zoeae seem to be a superior food, and the data reported here are better than those obtained with other experimental feeding protocols.

Survival was roughly estimated at 3% for M70 and 22.5% for Nanno. Culture of this last group was followed until the 35 days, reaching an average paralarval weight of 1.83 ± 0.28 mg (n=10) and a 3% survival. These data indicate that the biometrical outcome of both dietary treatments was very similar and that any hypothetical advantage of the Nanno group was lost one week later.

Lipid content of diets (% dry weight (DW)) was significantly different ($p < 0.05$): 21.01 ± 0.84 for Nanno and 18.23 ± 0.70 for M70. Analysis of the main fatty acids (Table 1) showed that main differences were due to Nanno treatment being higher in 16:1n-7 (which resulted in higher monoenes content), and 20:5n-3; whereas M70 showed higher

18:2n-6 (thus increasing total n-6 content) and 22:6n-3, which was not detected in the other diet (Table 1).

Differences in the lipid content of diets did not translate ($P>0.05$) into the lipid content of the paralarvae (Total lipid (% DW): M70= 16.31 ± 1.04 ; Nanno= 17.5 ± 0.23 ; total FAME (mg g^{-1} DW): M70= 45.71 ± 1.82 ; Nanno= 47.29 ± 1.51). Similarly, a first look into the fatty acid profiles of the total lipids of both dietary groups did not reveal striking differences between them (Table 2). Especially notable is the case of 16:1n-7, very different in the diets. Besides, the paralarvae seem to be able to cope with a 22:6n-3 deficient diet, although the poor performance of the two dietary groups could also point towards a dietary deficiency of both treatments.

A closer look into the polar and neutral lipid fatty acid composition showed the polar lipids being much richer in 20:5n-3 and 22:6n-3 (absent in the neutrals), which was reflected in a higher polyunsaturated, n-3 and HUFA n-3 content. Neutrals on their turn were richer in monounsaturated fatty acids. Within the polar lipids, M70 treatment generally increased the polyunsaturated, n-3 and HUFA n-3 content with respect to Nanno. Within the neutral lipids, Nanno treatment could be associated to higher n-6 content, whereas M70 seemed to increase HUFA n-3.

These trends were endorsed by the results of the PCA. The first component explained 76% of variance and was associated to variables 14:0, 18:1n-9, 18:2n-6 and 16:1n-7 on the positive side and to variables 20:5n-3, 20:4n-6, 22:6n-3 and 18:0 on the negative side. Second component explained only 11% of variance. The results of the score plot (Fig. 1) showed two main clouds of scores corresponding to polar (associated to variables on the negative side) and total lipids on one side, clearly distinct from neutral lipids (associated to variables on the positive side). Within the polar and neutral

lipids, the scores corresponding to the different dietary treatments were distinguishable, whereas those corresponding to the total lipids did not separate the two dietary groups. The scores of the polar lipids were more grouped than those of the neutral lipids indicating a higher similarity in the fatty acid patterns, which speaks about the structural role of this lipid class (Gurr & Hardwood 1991). Recently, Quintana (2009) has reported on the effects of the broodstock diets on the fatty acid composition of 3 days old paralarvae fed and starved, to conclude that the neutral lipids of the paralarvae reflect better than the polars any difference in the fatty acid profile of the breeders' diets. This is a further indication of the importance of the polar lipids as a structural and conservative lipid class for this species. In fact, the mantle being essentially protein and membranes, any changes in the neutral lipid fraction may be indicative of the contribution of this lipid class to the lipid composition of the digestive gland and, ultimately of the lipid metabolism and turnover linked to the dietary fatty acid composition.

To a certain point, the exposed above seems to contradict the results of Navarro & Villanueva (2003) who found that main dietary changes were already very evident in the total lipids of *O. vulgaris* paralarvae, although they were also clear in the polar lipids (unfortunately, the neutral lipid composition was not analysed). Thus, the induced dietary changes and their influence on the structural lipids may be dependent on the composition of the diets and on the coverage of the essential requirements. From this point of view both Nanno and M70 diets fulfil the essential requirements of the species, and the presence of DHA in Nanno fed paralarvae may be indicative of a certain degree of synthetic capacity.

Paralarval DHA levels not correlating with the amount in food have been reported previously (Navarro & Villanueva, 2000; Seixas 2009; Seixas, Rey-Méndez, Valente & Otero 2010). In fact, Seixas *et al.* (2010) have recently pointed out at the importance of an adequate ratio protein/lipid before the essential lipid requirements of the species may be considered. Given the generalised low performances of the paralarval cultures it is difficult to focus on a single source of variation, i.e. lipids, in a cause-effect design. Obviously, factors other than lipids may be affecting the final outcome, and even the fine-tuning of the lipid requirements may have a drastic influence. It should suffice to recall that for example, in larval sea bream (*Sparus aurata*), Rodríguez, Pérez, Díaz, Izquierdo, Fernández-Palacios & Lorenzo (1997) reported that the essential PUFA requirements were very dependent of the supply of an adequate DHA/EPA ratio. In this sense, it is interesting to note that EPA requirements of *O. vulgaris* may be particularly important, since this fatty acid has been found in very high amounts (and in 1:1 ratios with DHA) in the PC fraction of the early stages (Quintana 2009).

Conclusions

Although apparently no clear association between the fatty acid composition of the enriched preys and that of total lipids of paralarvae could be established. Further fractionation and fatty acid analysis of the total lipids of the paralarvae into polar and neutral classes, followed by PCA, revealed that irrespective of the diet, both lipid fractions showed distinct fatty acid patterns. Besides, the fatty acid composition of the polar lipids was more conservative, whereas that of the neutral lipids was more

influenced by diet and showed more variation among dietary treatments. Further research is needed to approach the lipid requirements of this species.

Acknowledgements

E. Viciano was research fellow from The “Excma. Diputación de Castellón”. Archivel Technologies helped in the elaboration of enrichments.

For Review Only

References

- Carrasco J.F., Arronte J.C. & Rodríguez C. (2006) Paralarval rearing of the common octopus, *Octopus vulgaris* (Cuvier). *Aquaculture Research* **37**, 1601-1605.
- Estévez A., Gairín J.I. & Berger E. (2009) Wild zooplankton for *Octopus vulgaris* larval rearing. In: *Larvi '09- Fish and Shellfish Larviculture Symposium* (ed by C.I. Hendry, G. Van Stappen, M. Wille & P. Sorgeloos), pp. 228-229. European Aquaculture Society Special Publication No. 38, Oostende, Belgium.
- Folch J., Lees N. & Sloane-Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497-509.
- Fuentes L., F.J. Sánchez, J.J. Otero, M.J. Lago & Iglesias, J. (2009) Utilización de zooplancton y artemia en el cultivo larvario de pulpo *Octopus vulgaris*. In: *Actas del XII Congreso Nacional de Acuicultura*, pp. 122-123.
- Gurr M.I. & Harwood J.L. (1991) Lipid Biochemistry. An introduction. Chapman and Hall.
- Hamazaki K., Fukunaga K., Yoshida Y. & Maruyama. K. (1991) Effects of marine microalgae *Nannochloropsis* sp., on survival and growth of rearing pelagic paralarvae of *Octopus vulgaris*, and results of mass culture in the tank of 20 metric tons. *Saibai – giken* **19**, 75-84.
- Iglesias J., Otero J.J., Moxica C., Fuentes, L. & Sánchez, F.J. (2004) The Completed Life Cycle of the Octopus (*Octopus vulgaris*, Cuvier) under Culture Conditions: Paralarval Rearing using *Artemia* and Zoeae, and First Data on Juvenile Growth up to 8 Months of Age. *Aquaculture International* **12**, 481-487.

- 271 Moxica C., Linares F., Otero J.J., Iglesias J. & Sánchez F.J. (2002) Cultivo intensivo de
272 paralarvas de pulpo, *Octopus vulgaris* Cuvier, 1797, en tanques de 9 m³. *Boletín*
273 *del Instituto Español de Oceanografía* **18**, 31–36.
- 274 Moxica C., Fuentes L., Hernández J., Iglesias J., Lago M.J., Otero J.J. & Sánchez, F.J.
275 (2006) Efecto de *Nannochloropsis* sp. en la supervivencia y crecimiento de
276 paralarvas de pulpo *Octopus vulgaris*. In: *Libro de Resúmenes del IX Foro dos*
277 *Recursos Mariños e da Acuicultura das Rías Galegas* (ed by M. Rey-Méndez, J.
278 Fernández & M. Izquierdo), pp. 255-260.
- 279 Navarro J.C. & Villanueva, R. (2000) Lipid and fatty acid composition of early stages
280 of cephalopods: an approach to their lipid requirements. *Aquaculture* **183**, 161–
281 177.
- 282 Navarro J.C. & Villanueva R. (2003) The fatty acid composition of *Octopus vulgaris*
283 paralarvae reared with live and inert food: deviation from their natural fatty acid
284 profile. *Aquaculture* **219**, 613-631.
- 285 Okumura, S., Kurihara, A., Iwamoto, A. & Takeuchi, T. (2005) Correlations among arm
286 sucker count, wet and dry weight of reared common octopus paralarvae.
287 *Aquaculture Science* **53**, 329–330.
- 288 Quintana D. (2009) Valoración de los requerimientos nutricionales de reproductores de
289 pulpo común (*Octopus vulgaris*). Tesis Doctoral. Universidad de La Laguna.
- 290 Rodríguez C., Pérez J.A., Díaz M, Izquierdo M.S., Fernández-Palacios H. & Lorenzo A.
291 (1997) Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus*
292 *aurata*) larval development. *Aquaculture* **150**, 77-89.

- 293 Sargent J.R., McEvoy L., Estévez A., Bell J.G., Bell M., Henderson J. & Tocher D.R.
294 (1999). Lipid nutrition of marine fish during early development: current status
295 and future directions. *Aquaculture* **179**, 217-230.
- 296 Seixas P. (2009) Composición bioquímica y crecimiento de paralarvas de pulpo
297 (*Octopus vulgaris* Cuvier, 1797), alimentadas con juveniles de *Artemia*
298 enriquecidos con microalgas y otros suplementos nutricionales. Tesis doctoral,
299 Universidad de Santiago de Compostela, 255.
- 300 Seixas P., Rey-Méndez M., Valente L.M.P. & Otero A. (2010) High DHA content in
301 *Artemia* is ineffective to improve *Octopus vulgaris* paralarvae rearing.
302 *Aquaculture* **300**, 156-162.
- 303 Rodríguez C., Pérez J.A., Díaz M., Izquierdo M.S., Fernández-Palacios H. & Lorenzo A.
304 (1997) Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus*
305 *aurata*) larval development. *Aquaculture* **150**, 77-89.
- 306 Villanueva R., Koueta N., Riba, J. & Boucaud-Camou, E. (2002) Growth and proteolytic
307 activity of *Octopus vulgaris* paralarvae with different food rations during first
308 feeding, using *Artemia* nauplii and compound diets. *Aquaculture* **205**, 269-286.
309

Figure caption

Figure 1. Score plot generated after PCA of the fatty acid patterns of *Octopus vulgaris* paralarvae fed two enriched on-grown *Artemia* diets. Nanno, M70: total lipid; Nanno N, M70 N: neutral lipid; Nanno P, M70 P: polar lipid

For Review Only

Table 1. Selected fatty acids (% of total lipid fatty acids) and total FAME of enriched on-grown *Artemia* (n=3)

Fatty acid	M70		Nanno	
	Mean	sd	Mean	sd
14:0	1.43	0.15	1.13	0.01
16:0	10.54	0.47	15.71	0.04
16:1n-7	5.19	0.13	23.69	0.62
18:0	7.26	0.38	5.40	0.12
18:1n-9	16.22	0.79	12.31	0.37
18:1n-7	9.27	0.70	7.89	0.37
18:2n-6	12.15	1.06	2.34	0.04
18:3n-3	5.05	0.13	0.38	0.01
20:4n-6	2.75	0.09	2.88	0.04
20:3n-3	0.07	0.07	nd	
20:4n-3	0.25	0.04	nd	
20:5n-3	11.99	0.43	22.55	0.52
22:5n-3	0.52	0.26	0.02	0.03
22:6n-3	8.10	1.10	nd	
Sat	20.38	0.70	23.33	0.30
Mono	31.58	0.80	44.19	0.18
Poly	44.42	1.76	28.96	0.50
n-3	27.92	1.15	23.04	0.54
n-6	16.67	1.03	5.62	0.13
HUFAn-3	20.93	0.99	22.57	0.55
HUFAn-6	3.83	0.09	3.16	0.02
FAME	60.49	4.35	116.97	12.89

Sat: saturates; Mono: monoenes; Poly: polyunsaturated; HUFA: highly unsaturated fatty acids (>20C). sd: standard deviation. FAME: total fatty acid methyl esters (mg g⁻¹ DW); nd: not detected

325 Table 2. Selected fatty acids (% of total fatty acids) of the total, polar and neutral lipid of *Octopus vulgaris*
326 paralarvae fed two enriched on-grown *Artemia* diets (n=3)
327

Fatty acid	Total Lipid				Polar Lipid				Neutral Lipid			
	M70		Nanno		M70		Nanno		M70		Nanno	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
14:0	0.74	0.06	*1.62	0.05	0.58	0.13	*1.09	0.17	2.56	0.93	*5.19	0.23
16:0	21.09	1.12	21.23	0.07	18.53	0.67	18.25	0.65	20.21	3.18	18.22	1.50
16:1n-7	5.02	0.64	4.31	0.45	2.07	0.42	2.47	0.29	16.22	4.27	13.01	1.08
18:0	12.61	0.28	12.51	0.29	14.21	0.28	13.56	0.42	10.50	3.35	8.19	1.25
18:1n-9	6.93	1.17	8.32	0.22	3.94	0.35	*5.33	0.34	12.36	1.78	*17.25	1.08
18:1n-7	5.58	0.11	*5.28	0.06	4.08	0.21	3.88	0.18	6.27	2.57	6.10	0.14
18:2n-6	1.50	0.32	*2.95	0.06	1.11	0.02	*2.08	0.21	2.52	0.63	*7.06	0.48
18:3n-3	0.94	1.20	0.77	0.04	0.22	0.11	*0.56	0.03	0.24	0.21	*1.81	0.31
20:4n-6	6.35	0.33	*5.50	0.08	7.69	0.34	*6.31	0.22	1.19	0.53	0.49	0.12
20:3n-3	1.25	0.04	*1.37	0.02	1.51	0.13	1.58	0.05	nd		nd	
20:4n-3	0.03	0.06	nd		0.01	0.02	0.05	0.05	nd		nd	
20:5n-3	21.89	1.54	20.33	0.36	25.73	0.43	*23.31	0.64	5.33	1.48	*2.31	0.14
22:5n-3	1.20	0.08	1.09	0.05	1.48	0.06	1.43	0.05	nd		nd	
22:6n-3	5.55	0.63	5.21	0.07	6.44	0.74	5.63	0.35	nd		nd	
Sat	34.57	1.28	35.54	0.15	33.73	0.59	33.35	0.59	34.62	6.19	31.94	1.96
Mono	20.20	1.26	20.45	0.66	12.81	1.02	14.55	0.82	36.41	7.43	37.19	1.76
Poly	41.57	0.54	40.48	0.89	46.06	1.66	43.42	1.11	11.96	3.83	13.99	1.20
n-3	30.98	0.62	*29.09	0.57	35.45	0.83	*32.75	0.94	5.57	1.67	4.56	0.78
n-6	9.73	0.08	*10.72	0.12	10.75	0.28	10.56	0.36	4.16	1.51	*9.25	1.29
HUFAn-3	29.92	0.87	*28.00	0.46	35.16	0.75	*32.01	0.94	5.33	1.48	*2.31	0.14
HUFAn-6	7.02	0.29	* 6.35	0.14	8.38	0.25	*7.12	0.39	1.64	1.06	1.60	1.13

328 For abbreviations see Table 1.
329 * Significant difference between means within lipid class (t-test p<0.05).
330

Figure 1