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Comparison of the lipid class and fatty acid composition between a reproductive cycle in nature and a standard hatchery conditioning of the Pacific Oyster *Crassostrea gigas*

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Abstract

The lipid composition of *Crassostrea gigas* was analyzed during the reproductive phase in natural as well as under artificial conditions. The lipid content increased and accumulated in the gonads, but to a higher extend in the naturally conditioned animals. The percentage of neutral lipid in total lipid of the gonad plus mantle was stable, high (> 70%) and equal under both conditions, underscoring that the lipid reserves were preferentially located in that organ. The composition of the polar lipid classes was stable with little variations. The fatty acid (FA) composition of the neutral lipids in all organs is influenced by diet. However, there is a different response according to organs. A high dietary impact occured in the digestive gland whereas the muscle was less affected. The polyunsaturated fatty acid (PUFA) level of the neutral and polar lipids in the gonads changed little despite the dietary conditions but the respective proportions of (n-3) and (n-6) PUFA differed drastically as a result of diet composition. There was clear evidence in all organs for a specific accumulation of 22:6(n-3) and 20:5(n-3) in the polar lipids under both conditioning diets. The proportions of 22:6(n-3) and 20:5(n-3) from neutral and polar lipids of oysters conditioned artificially were significantly lower than of those that were naturally conditioned. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Molluscs; Oyster; *Crassostrea gigas*; Gametogenesis; Nutrition; Lipids; Fatty acids; Lipid classes

1. Introduction

Basic knowledge of the key aspects of mollusc reproduction in hatchery, including broodstock nutrition and timing of the gametogenetic cycles, are lacking even for the leading commercial species *Crassostrea gigas*. Among the dietary components of the food given to broodstock, lipids affect mostly the composition of the eggs [9,30]. The lipids deposited in the eggs during broodstock conditioning play a major role as an energy source during embryonic and early larval development [7,8]. Polyunsaturated fatty acids (PUFA) with 20 and 22 carbon atoms and more than three double bonds are essential for survival and growth of molluscs [14,28,29]. The essential fatty acid composition of the broodstock's diet also influences the performance of the offspring [2,3,9,22,23,30].

The objectives of this paper are: (1) to characterize the processes of accumulation and transfer of essential fatty acids under natural and standard hatchery conditions; (2) to estimate the relative importance of those nutrients that are stored prior to conditioning and those derived directly from the broodstock's diet; and (3) to identify possible nutritional deficiencies in hatchery conditioning. The lipid class and the fatty acid composition of the polar and neutral lipids of muscle, digestive gland and mantle plus gonad will be examined.

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2. Material and methods

2.1. Broodstock conditioning

Three-year old oysters, reared on racks in the bay of Marennes Oléron, were sampled from April 20th 1997 to July 9th 1997 during the natural reproductive period every 2 weeks until the natural spawning. The temperature in the bay increased from 12 to 21°C during that period of time. The reproductive cycle under hatchery conditions (gametogenesis, spawning) was conducted from April 20th 1997 to July 2th 1997 with broodstock collected from the same origin as natural conditioning.

Three hundred oysters were divided between several flat trays and placed into two raceways in a closed sea water system.. The sea water tanks were drained and refilled daily with sea water, passed through a sand filter to remove particles greater than approximately 50 μm. The raceways and animals were also cleaned daily. Oysters were conditioned in a closed system with airlifts to control the algal quality and quantity. Temperature was maintained at 19°C after an initial acclimation period of 3 weeks. The daily dry weight algal supply was established at 6% of the mean dry oyster flesh weight according to Utting et al. [31]. The particles (< 50 μm) that accumulated due to the daily seawater change were negligible compared to the food provided. *Isochrysis affinis galbana*, *Tetraselmis suecica* and a diatom *Chaetoceros calcitrans* or *Skeletonema costatum* were fed daily in equivalent dry weight quantities. The algae used throughout the conditioning were batch cultured for at least 6 days.

Digestive gland, muscle, and the gonad plus mantle were isolated. The different organs from individual animals were collected and three pools of five organs were prepared for statistical purposes. Each pool was weighed (wet weight) and ground in a Dangoumeau grinder after freezing in liquid nitrogen at −196°C. Samples for dry weight determination were placed in pre-weighed aluminum cups, dried for 48 h at 80°C, and weighed in a Mettler micro balance with a precision of 1 μg. Total lipids were extracted after freeze-drying, according to the method of Folch et al. [6] modified by Ways and Hanahan [33], dissolved into chloroform/methanol (2:1) at a concentration of 10 mg/ml, and stored at −30°C until use.

2.2. Analysis of lipid classes

Lipid classes were separated by high-performance thin layer chromatography (HPTLC) on HPTLC glass plates (10 × 10 mm) pre-coated with silica gel 60 from Merck (Darmstadt, Germany). All solvents were of HPLC grade (C.R.B., Belgium).

A preliminary run was carried out to remove possible impurities using hexane/diethyl ether (1:1) and the plate activated for 30 min at 110°C. Lipid samples in the amount of 1.5 μl were spotted in 2 mm strokes using a 5-μl Hamilton syringe. A double development of the plate with two solvent systems was performed according to Tocher and Harvie [26]. The first system consisted of methyl acetate/iso-propanol/chloroform/-methanol/KCl 0.25% (10:10:10:4:3.6) and separated the polar lipid classes over a distance of 5.5 cm. The neutral lipids moved as one band along with the solvent front and were separated with a second solvent system containing hexane/diethyl ether/acetic acid (20:5:0.5). Lipid classes appeared as black spots after dipping plates in a cupric sulfate phosphoric acid solution and heating for 20 min at 160°C (charring). Identification was based on standards (Nu-Chek-Prep, USA, Avanti Polar Lipids, USA and Sigma–Aldrich, Belgium) and coloring techniques. Quantification was carried out with a Sharp color image scanner connected to the software system 'Image master' (Pharmacia Biotech) that was equipped with a Kodak calibration strip. Lipid classes were calculated as percentages of the total lipid as proposed by Olsen and Henderson [16].

2.3. Separation of polar and neutral lipids

The separation of the total polar and neutral lipids was carried out by micro-column liquid chromatography as described by Marty et al. [15]. Total lipids were evaporated to dryness and redissolved three times using 500 μl chloroform/methanol (98:2). Total neutral and polar lipids were separated on a silica gel micro-column (30 × 5 mm) using chloroform/methanol (98:2) and methanol successively as eluting solvents. The separated fractions were collected in tapering glass test tubes containing 100 μl of internal standard 22:2(n-6) at a concentration of 100 μg/ml.

2.4. Fatty acid composition of polar and neutral lipids

Fatty acid composition was determined using gas chromatography. Fatty acid methyl esters (FAME) from the neutral and polar lipid fractions were prepared via an acid-catalyzed esterification procedure with a mixture of 1% sulfuric acid in methanol at 50°C for 16 h. FAME were extracted with hexane/diethylether (1:1) and, after evaporation of the solvents, redissolved in iso-octane (1 mg/ml) for injection. Quantitative determination was done by a Chrompack CP9001 gas chromatograph equipped with an autosampler and a temperature-programmable on-column injector. On-column injections (1–2 μl) were performed into a polar capillary column BPX70 (50 m × 0.32 mm, 0.25 μm film thickness, SGE Australia) connected to a 2.5-m methyl deactivated pre-column. The carrier gas was H_2, at a pressure of 100 kPa with flame ionization detection

used as the detection mode. The oven was programmed to rise from the initial temperature of 85–150°C at a rate of 30°C/min, from 150 to 152°C at 0.1°C/min, then 152–172°C at 0.65°C/min, next 172–187°C at 25°C/min, and finally to stay at 187°C for 7 min. The injector was heated from 85 to 190°C at 5°C/s and stayed at 190°C for 30 min. Identification was based on standard reference mixtures (Nu-Chek-Prep, USA). Integration and calculations were done on a computer with the software program Maestro (Chrompack). The non-methylene interrupted 22:2Δ7,13 and 22:2Δ7,15 were named 22:2i and 22:2j, respectively.

2.5. Statistics

The data for dry weight, lipid content, neutral lipid percentage, and fatty acid composition of the natural and artificial conditionings were compared using the Student's t-test. Statistically significant differences were marked 'S' in the column 'sign.' of the tables.

3. Results

3.1. Dry weight (Fig. 1)

The dry weight of the gonad plus mantle increased during the first two months of conditioning and followed the same pattern in nature as in the artificial conditioning. However it differed (not statistically) at the end of gametogenesis with final values of 1.44 and 1.00 g, respectively. The dry weight in the digestive gland and in the muscle were stable over the period and were similar for both conditionings.

3.2. Total lipid percentage (expressed as a percentage of the dry weight, Fig. 2)

The total lipids in the gonad plus mantle tissue samples displayed a different pattern according to the method of conditioning The proportion of total lipid in the course of artificial conditioning increased during the first 6 weeks from 16 to 17.5%, and then stayed stable until spawning. The percentage in nature increased from 16 to 21% and was always higher than in the artificially conditioned oysters. The total lipid percentage in the digestive gland of the artificially conditioned animals increased strongly during the first 6 weeks (from 14 to 17%), but dropped again to the initial level in the next 4 weeks. The total lipid percentage under natural conditions was stable during the 13 weeks of maturation. The total lipid percentage in the muscle was similar under both conditions and stable in the course of gametogenesis. No statistically significant differences in lipid content between the two conditioning methods for all organs were observed at the end, before spawning.

3.3. Neutral lipid percentage (expressed as the percentage of fatty acids of the neutral lipids on total fatty acids, Fig. 3)

The pattern of the neutral lipid percentage in the gonad plus mantle was stable under both conditions. However, the neutral lipid percentage of the digestive gland increased under artificial conditions, especially after 6 weeks. The final value was significantly higher than that observed in nature. The neutral lipid percentage in muscle on the contrary increased over the course

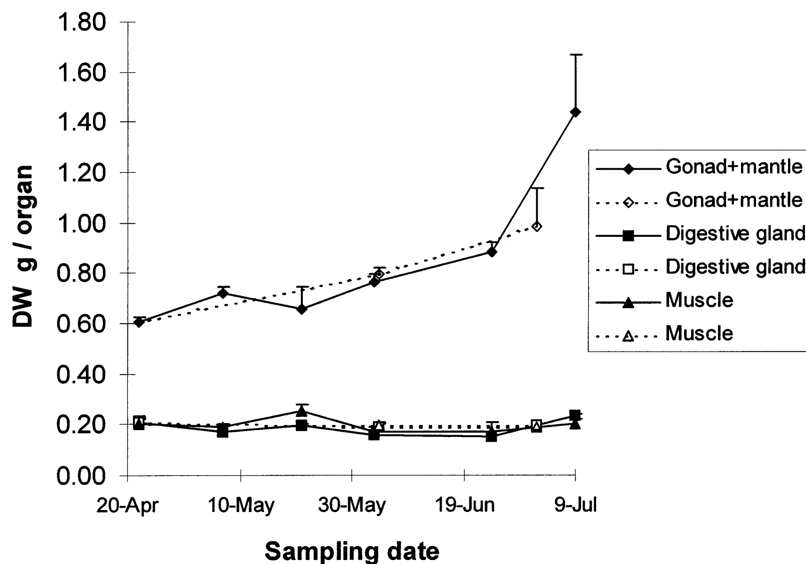

Fig. 1. Dry weight of the three organs during gametogenesis (expressed in g DW per organ; natural conditioning: filled line, artificial conditioning: dotted line; Mean, SD, $n = 3$).

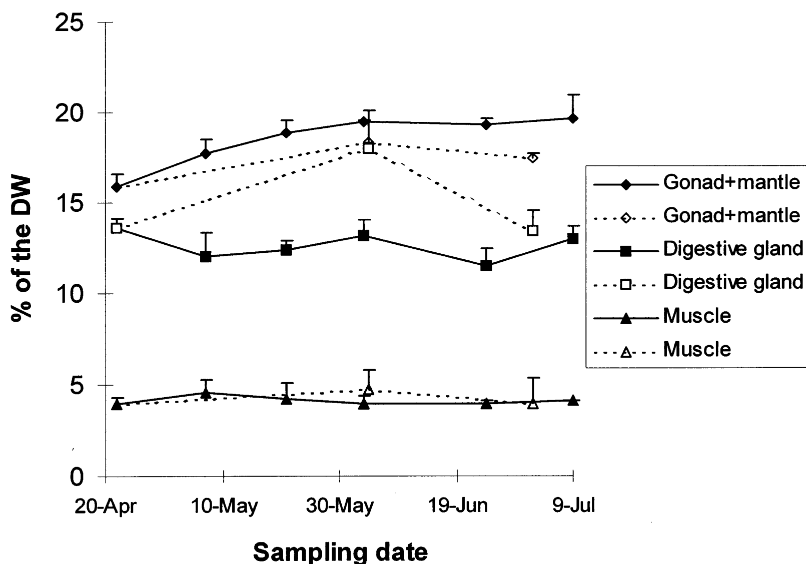

Fig. 2. Total lipid percentage in the three organs (expressed as percentage of dry weight; natural conditioning: filled line, artificial conditioning: dotted line; Mean, SD, $n = 3$).

of gametogenesis in nature. The final value was statistically higher than that observed in the artificial conditioning.

3.4. Neutral lipid class composition (Fig. 4)

The relative neutral lipid class amounts in the gonad plus mantle were similar in the artificial and natural conditions. The neutral lipid class pattern stayed stable during the experimental period, except for triacylglycerol (TAG) percentage that went from 51 in the initial sample to 67% by the end of maturation. A mean value of 12% sterols was found in the neutral lipids. The neutral lipid class patterns in the digestive gland and in the muscle were found to be stable in both natural and artificial conditioning.

3.5. Polar lipid class composition (Fig. 4)

The patterns of the polar lipids of all three organs in artificial broodstock conditioning were similar to the ones under natural conditions. The polar lipids fluctuated little, with mean phosphatidyl choline (PC) values of 34%, phosphatidyl ethanolamine (PE) 25%, phosphatidyl inositol (PI) + ceramide amino ethylphosphonate (CAEP) 11%, and phosphatidyl serine (PS) 9.5%.

3.6. Fatty acid composition of neutral lipids

The initial fatty acid composition is shown in Table 1. The percentage of monounsaturated fatty acids (MUFA) was significantly higher in all organs at the end of the gametogenesis with artificially conditioned oysters (Tables 2–4). There was a big difference within polynsaturated fatty acids (PUFA) in the pattern of (n-6) and (n-3) proportions between the two broodstock conditioning methods (Tables 2–4). The proportion of (n-3) PUFA decreased in the artificial conditioning by about 5% in all organs. There was a correlative 5% increase in (n-6) PUFA proportions (Fig. 5) as well. The proportions of (n-6) and (n-3) PUFA remained stable in the natural conditioning (Fig. 5). The final values of (n-3) and (n-6) content were significantly different between natural and artificial conditioned oyster. As a consequence opposite trends in the (n-3)/(n-6) ratios between the two groups of broodstock were observed in all organs: the ratios changed from 6.2–7.2 at the beginning moved to 8.4–9.6 and to 2.1–2.8, respectively, for the naturally and artificially conditioned broodstocks. These values were significantly different at the end of conditioning (Tables 2–4).

The percentages of essential fatty acids 22:6(n-3) and 20:5(n-3) in the neutral lipids decreased during the course of the artificial conditioning: 20:5(n-3) fell from 12.5, 13.7 and 12.3% to 8.4, 7.1 and 9.3%, 22:6(n-3) from 12.3, 9.6 and 11.9% to 7.5, 7.8 and 8.2% in, respectively, the gonad plus mantle, the digestive gland and the muscle, respectively (Fig. 6). The proportion of 20:5(n-3) in nature increased slightly during the thirteen weeks of maturation, whereas the proportion of 22:6(n-3) tended to decrease (Fig. 6). The 22:6(n-3)/20:5(n-3) ratio in the muscle and in the gonad plus mantle tended to decrease with final values of 0.7 for the natural and 0.9 for the artificially conditioned oyster. The level in the digestive gland was stable in nature (0.6), but increased to 1.1 in artificial conditioning (Table 3).

The 20:5(n-3) levels in all three organs were significantly different at the time of spawning between natu-

rally and artificially conditioned oysters. However, the level of 22:6(n-3) was significantly different in the gonad plus mantle and in the digestive gland (Table 4).

The proportion of 20:4(n-6) was similar in the three organs under both conditions. The percentage of 22:2j among non-methylene interrupted PUFA (NMI PUFA) increased during the course of gametogenesis in nature and was always higher compared to the artificial conditioning.

3.7. Fatty acid composition of the polar lipids

A significant higher proportion of MUFA was found in the gonad plus mantle and in the digestive gland of the artificially conditioned oysters. The proportion of (n-3) PUFA in the course of natural conditioning, increased from 33 to 41% in the gonad plus mantle, while it was stable in the digestive gland and in the muscle (35 and 33%, respectively) at the end of July. The proportion of (n-3) PUFA in the gonad plus mantle remained stable (33%) under artificial conditions, while it decreased in the digestive gland and in the muscle from 34 to 30% and from 33 to 30.5%, respectively. The proportion of (n-6) PUFA was stable at 4% in the gonad plus mantle and in the muscle, and 4.5% in the digestive gland during natural conditioning On the contrary, the proportion of (n-6) PUFA under artificial conditions increased from 4.5 to 9% in the gonad plus mantle, from 4.5 to 10.5% in the digestive gland, and to a lesser extend from 4 to 6% in the muscle. The (n-3)/(n-6) ratio increased from 7.1–7.9 to 8.5–9.9 in nature and decreased from 7.1–7.9 to 2.8–4.9 under artificial conditions (Tables 2–4). In both cases, less important variations were observed in the muscle. The differences in (n-3) and (n-6) content and their ratio were statistically significant in all organs.

In the gonad plus mantle, proportions of 20:5(n-3) and 22:6(n-3) increased significantly during gametogenesis (+4% compared to the initial percentage) in nature, while their respective levels remained stable under artificial conditions. The percentage of 22:6(n-3) in the digestive gland increased slightly and reached similar final values under both conditions. The percentage of 20:5(n-3) fell from 16 to 11% in the artificially conditioned broodstock, while it remained stable in nature. However, contrary to the two previous organs, the percentage of 22:6(n-3) in the muscle was higher than that of 20:5(n-3). The levels of 22:6(n-3) and 20:5(n-3) changed little in either of the two conditions. The differences observed between the two conditionings at the time of spawning were all statistically significant, except for the 22:6(n-3) content in the muscle,

The level of 20:4(n-6) in the polar lipids was higher than that of the neutral lipids. The percentage of 20:4(n-6) under natural conditions was appreciably lower but more stable than under artificial conditions. The proportion of 22:5(n-6) for artificial conditioning increased greatly in the gonad plus mantle and in the digestive gland, i.e. from 0.3 to 1.8% (six times) and in the muscle from 0.4 to 1%. However, it remained stable for oysters in the natural environment. As with the neutral lipids, the proportion of 22:2j was higher in the naturally conditioned oysters than in the artificially conditioned ones.

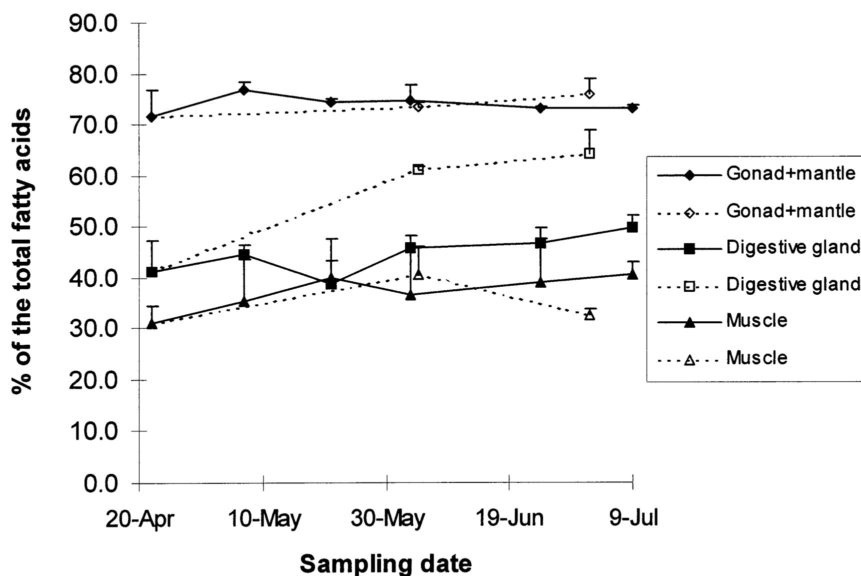

Fig. 3. Neutral lipid percentage of the organs (expressed as percentage of the total fatty acids, natural conditioning: filled line, artificial conditioning: dotted line; Mean, SD, $n = 3$).

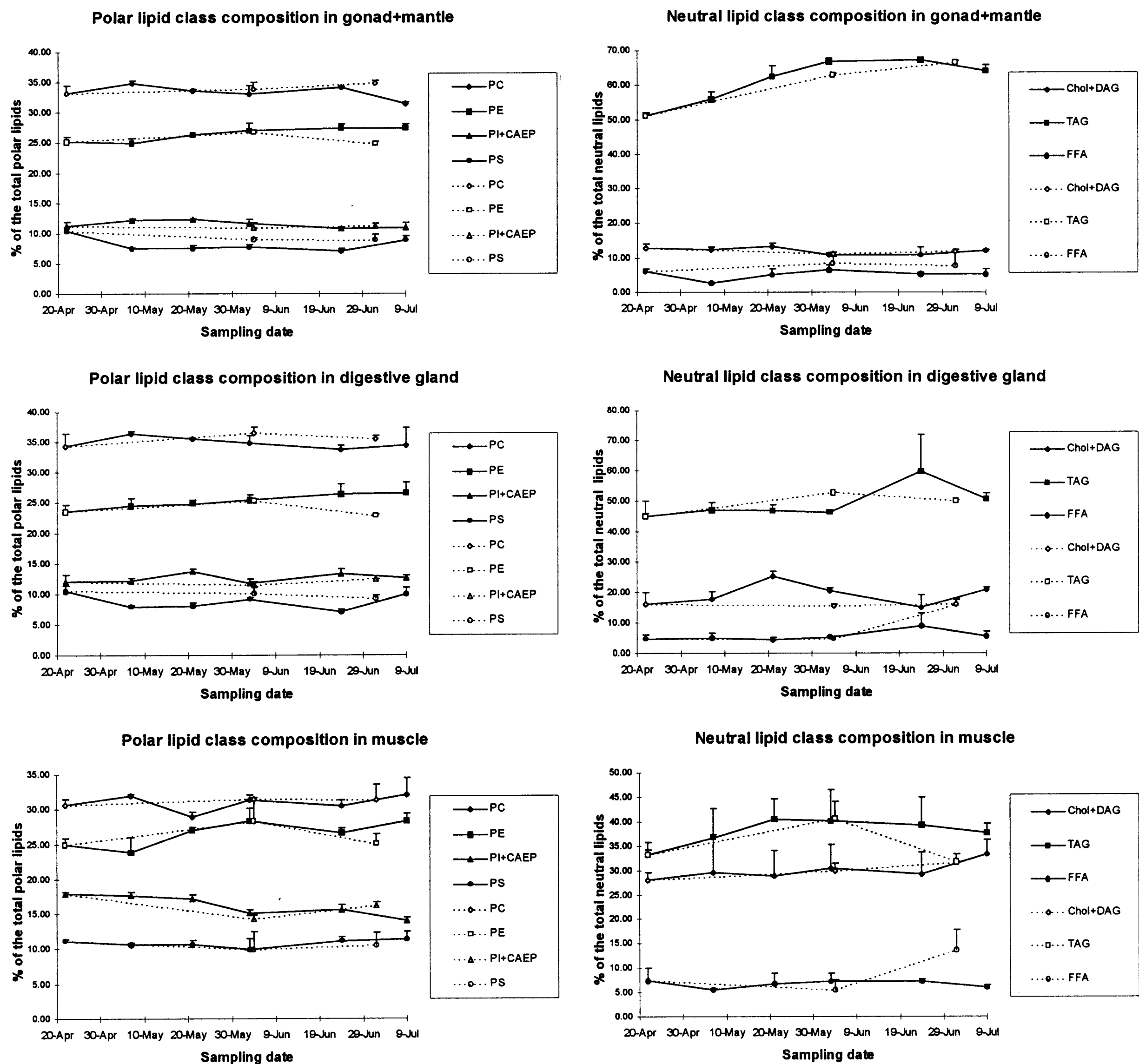

Fig. 4. Polar (left) and neutral (right) lipid class composition in the gonad plus mantle, the digestive gland and the muscle from natural (filled line) and artificial (dotted line) conditioning (expressed as percentage of the total polar lipid and of total neutral lipid, respectively, Mean, SD, $n = 3$).

4. Discussion

A constant increase in dry weight of the gonad plus mantle was noticed under both conditions. The animals that matured in nature, however, had more gonad plus mantle tissue just before spawning than those conditioned artificially. This corresponds to the terminal phase of gametogenesis (spawning in nature occurred in early August). The relatively low increase of dry weight in the digestive gland and muscle during the same period indicates that oysters spent most of their energy in gametogenesis and little in somatic growth.

The weight increase of the gonad plus mantle brought about an accumulation, or enrichment, of lipids in absolute amounts, corresponding with the female gametogenesis process. Lipid accumulation in the oocytes, which is provided by the diet, is a common process in the gametogenesis of bivalves and fish. These lipids play a major role as membrane constituents and reserve energy in embryonic development [5,7,8,27,34].

At spawning, the naturally conditioned oysters had a higher lipid content. This could be explained by two hypotheses: the developing oocytes in nature were richer in lipids, or the gonad contained a larger number of mature oocytes which suggests an advanced stage of maturation in terms of lipid storage. A significant better fecundity ($P = 0.05$; 29.6 10^6 eggs/female for the natural conditioning versus 17.6 10^6 eggs/female for artificial conditioning), and a similar egg lipid content corroborate the second hypothese.

The stable and high percentage of neutral lipids in the gonad plus mantle underscores the facts that lipid

Table 1
Fatty acid composition of the diet and of the polar and neutral lipids in the gonad+mantle (GR), the digestive gland (DG) and the muscle (MU) at the start of the broodstock conditioning[a]

% mol	Diet	Polar lipids						Neutral lipids					
		GR		G.D		MU.		GR		G.D		MU	
		Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
14:0	9.9	1.6	0.3	2.0	0.2	1.1	0.1	4.4	0.8	4.4	0.6	4.3	0.3
15:0	0.7	0.7	0.0	0.7	0.0	0.7	0.0	0.8	0.2	0.8	0.1	1.1	0.1
16:0	21.3	18.9	1.9	19.5	1.3	19.9	0.5	24.7	1.2	21.6	1.4	22.7	0.6
17:0	0.2	1.6	0.1	1.6	0.3	1.7	0.1	1.3	0.1	1.6	0.1	1.4	0.1
18:0	1.3	5.0	0.2	5.4	0.7	4.4	0.2	3.8	0.3	3.8	0.4	4.3	0.1
16:1(n-9)	–	–	–	–	–	–	–	0.5	0.1	0.6	0.1	1.1	0.2
16:1(n-7)	8.5	1.6	0.5	1.6	0.4	0.9	0.0	4.3	0.2	5.0	0.2	3.8	0.4
18:1(n-11)	–	0.3	0.1	0.2	0.0	0.1	0.0	1.0	0.0	0.6	0.1	0.9	0.2
18:1(n-9)	13.4	2.5	0.8	1.9	0.2	1.3	0.1	5.0	0.2	5.6	0.4	6.0	0.6
18:1(n-7)	2.1	3.1	0.4	4.6	0.5	3.0	0.3	3.9	0.1	4.5	0.4	3.7	0.1
20:1(n-11)	–	1.2	0.1	1.1	0.1	0.8	0.1	1.4	0.2	1.2	0.2	1.3	0.1
20:1(n-9)	–	0.3	0.2	0.4	0.1	1.1	0.5	–	–	–	–	0.3	0.1
20:1(n-7)	–	4.0	0.3	4.0	0.4	4.0	0.2	2.2	0.2	2.8	0.1	2.6	0.5
16:2(n-4)	1.5	0.2	0.1	0.3	0.0	0.1	0.0	0.3	0.1	0.7	0.1	0.3	0.1
16:3(n-4)	5.5	0.5	0.0	0.3	0.1	1.0	0.2	0.7	0.0	0.9	0.0	0.6	0.1
18:2(n-6)	3.4	1.7	0.2	1.5	0.1	0.9	0.0	2.7	0.1	3.1	0.3	2.8	0.1
18:2(n-4)	–	0.1	0.0	0.3	0.0	0.1	0.0	0.3	0.0	0.4	0.0	0.4	0.1
18:3(n-6)	0.5	0.1	0.0	0.1	0.0	0.1	0.0	0.3	0.0	0.3	0.0	0.3	0.0
18:3(n-3)	4.1	1.3	0.4	1.1	0.1	0.6	0.1	2.3	0.2	2.7	0.3	2.4	0.4
18:4(n-3)	5.4	1.8	0.1	2.9	0.2	1.2	0.1	4.5	0.3	5.0	0.3	3.6	0.6
18:5(n-3)	0.9	–	–	0.0	0.0	–	–	0.1	0.0	0.2	0.0	–	–
20:2i	–	–	–	–	–	–	–	0.1	0.0	–	–	–	–
20:2j	–	0.2	0.0	0.1	0.0	0.1	0.0	0.9	0.0	0.8	0.1	0.8	0.1
20:2(n-6)	–	0.1	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.1
20:3(n-6)	0.0	0.1	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
20:4(n-6)	0.7	2.1	0.2	2.0	0.1	2.3	0.1	0.9	0.1	0.9	0.1	1.4	0.1
20:4(n-3)	0.2	0.3	0.1	0.6	0.0	0.2	0.0	0.5	0.0	0.6	0.0	0.5	0.0
20:5(n-3)	10.4	15.6	0.9	16.2	0.9	12.3	0.5	12.5	0.5	13.8	0.9	12.3	0.1
21:5(n-3)	0.0	0.6	0.1	0.8	0.1	0.4	0.0	0.8	0.1	0.9	0.1	0.7	0.1
22:4(n-6)	0.1	0.2	0.0	0.3	0.1	0.3	0.0	0.1	0.0	0.1	0.0	0.1	0.0
22:2i	–	0.8	0.2	0.6	0.1	0.8	0.1	0.3	0.0	0.2	0.0	0.2	0.0
22:2j	–	3.5	0.4	3.4	0.0	3.3	0.4	1.7	0.2	1.6	0.2	1.5	0.1
22:5(n-6)	0.4	0.4	0.1	0.3	0.0	0.4	0.0	0.2	0.0	0.0	0.0	0.2	0.0
22:5(n-3)	0.1	1.0	0.0	1.0	0.1	1.6	0.1	0.6	0.0	0.6	0.1	0.6	0.0
22:6(n-3)	3.2	12.8	0.3	12.1	1.3	17.3	0.5	12.3	1.2	9.6	0.8	11.9	1.3
16:0DMA	–	0.8	0.2	0.7	0.0	0.8	0.1	0.2	0.1	0.3	0.0	0.6	0.3
18:0DMA	–	8.4	1.0	6.9	1.3	9.7	0.5	1.8	0.1	1.6	0.3	1.8	0.3
20:1DMA	–	1.5	0.3	1.1	0.3	1.9	0.1	0.2	0.1	0.3	0.0	0.3	0.0
T0. BR.	–	1.2	0.1	1.3	0.1	1.0	0.0	1.0	0.1	0.9	0.0	1.1	0.0
TO.SAT.	33.4	28.2	1.4	29.5	2.5	28.4	0.8	35.6	2.2	32.7	1.9	34.6	0.6
TO.DMA	–	13.9	1.3	11.2	1.4	16.4	0.8	2.5	0.2	2.8	0.5	3.1	0.5
TO.MONO	23.1	13.3	1.2	13.7	0.7	11.1	0.2	18.4	0.6	20.9	0.5	20.4	0.4
TO.(n-9)	12.4	2.8	0.7	2.2	0.3	2.4	0.4	5.4	0.3	6.2	0.3	7.4	0.8
TO.(n-7)	10.7	8.6	0.5	10.2	0.6	7.8	0.5	10.4	0.4	12.3	0.5	10.0	0.6
TO.POLY	36.7	43.3	1.1	44.2	2.4	43.2	0.6	42.5	2.3	42.8	2.2	40.8	0.9
TO.(n-4)	7.1	0.8	0.1	0.9	0.1	1.2	0.1	1.3	0.1	2.0	0.1	1.3	0.2
TO.(n-6)	5.1	4.7	0.1	4.5	0.1	4.2	0.1	4.5	0.2	4.8	0.4	5.0	0.2
TO.(n-3)	24.5	32.9	0.8	33.9	2.4	33.2	1.0	32.8	1.9	32.4	1.8	31.3	1.1
TO. NMI	–	4.4	0.6	4.1	0.1	4.2	0.5	3.1	0.3	2.6	0.1	2.5	0.0
(n-3)/(n-6)	4.8	7.1	0.2	7.5	0.6	7.9	0.3	7.3	0.3	6.8	0.2	6.2	0.5
22:6/20:5	0.3	0.8	0.0	0.7	0.1	1.4	0.1	1.0	0.1	0.7	0.0	1.0	0.1
22:5/20:4	0.5	0.2	0.0	0.2	0.0	0.2	0.0	0.3	0.0	0.0	0.0	0.2	0.0

[a] The results are expressed as percentages of total fatty acids of the fraction; Mean, SD, $n = 3$.

Table 2
Fatty acid composition of the polar and neutral lipids in the gonad+mantle at the end of the natural and artificial broodstock conditioning[a]

% mol	Polar lipids					Neutral lipids				
	Nature		Artificial		Sign.	Nature		Artificial		Sign.
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.	
14:0	1.3	0.2	1.9	0.2		8.2	0.3	9.5	0.8	
15:0	0.4	0.0	0.4	0.0		0.7	0.0	0.5	0.0	S
16:0	14.2	0.7	16.6	0.1		24.4	0.5	24.6	0.1	
17:0	1.7	0.0	1.5	0.3		1.0	0.1	0.8	0.0	S
18:0	4.2	0.1	3.9	0.1		3.7	0.1	3.3	0.1	S
16:1(n-9)	–	–	–	–		0.3	0.0	0.5	0.0	
16:1(n-7)	1.2	0.1	1.2	0.1		5.2	0.3	5.0	0.4	
18:1(n-11)	0.6	0.1	0.5	0.0		0.3	0.0	0.3	0.1	
18:1(n-9)	0.9	0.2	3.0	0.4	S	2.4	0.1	6.6	0.2	S
18:1(n-7)	2.1	0.3	2.8	0.3		4.0	0.2	4.3	0.0	
20:1(n-11)	1.9	0.1	1.6	0.1	S	0.8	0.0	0.7	0.1	S
20:1(n-9)	0.5	0.1	0.6	0.0	S	0.4	0.1	0.3	0.0	S
20:1(n-7)	4.3	0.2	3.1	0.0		2.7	0.1	1.9	0.0	S
16:2(n-4)	0.0	0.0	0.0	0.0		0.5	0.0	0.3	0.0	S
16:3(n-4)	0.0	0.0	–	–		0.1	0.0	0.1	0.0	S
18:2(n-6)	0.6	0.1	3.5	0.5	S	1.5	0.1	6.5	0.2	S
18:2(n-4)	0.3	0.0	0.2	0.0	S	0.5	0.0	0.3	0.0	S
18:3(n-6)	0.1	0.0	0.2	0.0	S	0.2	0.0	0.6	0.0	S
18:3(n-3)	0.8	0.1	1.8	0.4	S	2.2	0.1	4.2	0.1	S
18:4(n-3)	1.6	0.2	2.0	0.3		5.8	0.2	4.9	0.2	
18:5(n-3)	0.2	0.0	–	–		–	–	0.0	0.0	
20:2i	–	–	–	–		–	–	–	–	
20:2j	0.6	0.0	0.3	0.0	S	0.9	0.1	0.8	0.1	
20:2(n-6)	0.2	0.0	0.3	0.0	S	0.2	0.0	0.3	0.0	S
20:3(n-6)	0.2	0.0	0.1	0.0	S	0.2	0.0	0.3	0.0	S
20:4(n-6)	2.3	0.0	2.7	0.0	S	1.0	0.0	0.8	0.1	
20:4(n-3)	0.5	0.1	0.1	0.0	S	0.6	0.0	0.4	0.0	S
20:5(n-3)	19.9	0.5	13.7	0.1	S	13.7	0.3	8.4	0.4	S
21:5(n-3)	0.8	0.0	0.4	0.1		1.0	0.1	0.7	0.2	
22:4(n-6)	0.2	0.0	0.2	0.0		0.1	0.0	0.1	0.0	
22:2i	0.9	0.1	1.4	0.2	S	0.3	0.0	0.4	0.0	S
22:2j	5.1	0.4	3.6	0.4		2.5	0.2	1.6	0.0	S
22:5(n-6)	0.4	0.0	1.8	0.1	S	0.2	0.0	0.6	0.0	S
22:5(n-3)	1.5	0.1	0.9	0.0	S	0.8	0.0	0.3	0.0	S
22:6(n-3)	16.5	0.6	14.5	0.1	S	10.1	0.1	7.5	0.1	S
16:0DMA	0.4	0.0	0.3	0.0		0.0	0.0	0.0	0.0	
18:0DMA	10.6	0.7	11.4	1.7		1.4	0.3	0.3	0.3	
20:1DMA	1.4	0.1	1.6	0.1		0.3	0.0	0.1	0.0	
T0. BR.	0.5	0.2	0.7	0.1	S	0.8	0.1	0.9	0.0	
TO.SAT.	22.3	1.0	24.7	0.1		38.3	0.7	39.0	0.5	
TO.DMA	12.7	0.7	13.5	1.7		1.9	0.3	0.8	0.5	
TO.MONO	11.5	0.6	13.0	0.6	S	16.2	0.2	19.7	0.4	S
TO.(n-9)	1.4	0.2	3.6	0.3	S	3.1	0.1	7.4	0.1	S
TO.(n-7)	7.6	0.4	7.1	0.2		11.9	0.2	11.2	0.4	S
TO.POLY	53.1	1.0	48.1	1.0	S	42.7	0.3	39.4	0.4	
TO.(n-4)	0.4	0.0	0.2	0.0	S	1.2	0.1	0.7	0.0	S
TO.(n-6)	4.1	0.1	9.0	0.5	S	3.4	0.0	9.2	0.1	S
TO.(n-3)	40.9	0.5	33.1	0.9	S	33.2	0.1	25.6	0.3	S
TO. NMI	6.6	0.6	5.3	0.5		3.7	0.2	2.9	0.1	
(n-3)/(n-6)	9.9	0.4	3.7	0.1	S	9.6	0.1	2.8	0.1	S
22:6/20:5	0.8	0.0	1.1	0.0	S	0.7	0.0	0.9	0.0	S
22:5/20:4	0.2	0.0	0.7	0.0	S	0.2	0.0	0.8	0.1	S

[a] The results are expressed as percentages of total fatty acids of the fraction; Mean, SD, $n=3$.

Table 3
Fatty acid composition of the polar and neutral lipids in the digestive gland at the end of the natural and artificial broodstock conditioning[a]

% mol	Polar lipids					Neutral lipids				
	Nature		Artificial		Sign.	Nature		Artificial		Sign.
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.	
14:0	1.9	0.2	2.7	0.3	S	7.4	0.6	10.4	1.3	S
15:0	0.5	0.1	0.5	0.0		0.7	0.1	0.4	0.0	S
16:0	16.3	1.0	16.3	0.5		22.4	0.2	17.6	1.1	S
17:0	2.0	0.0	1.4	0.3	S	1.2	0.1	0.6	0.3	
18:0	5.6	0.1	4.5	0.1	S	3.9	0.1	2.8	0.3	S
16:1(n-9)	–	–	–	–		0.4	0.1	0.5	0.0	
16:1(n-7)	1.9	0.1	1.4	0.1		6.4	0.2	4.2	0.2	S
18:1(n-11)	0.4	0.1	0.3	0.1		0.3	0.0	0.2	0.0	
18:1(n-9)	1.1	0.1	4.2	0.3	S	2.5	0.1	10.4	0.7	S
18:1(n-7)	3.8	0.3	3.8	0.0		4.3	0.1	4.2	0.1	
20:1(n-11)	1.8	0.1	1.5	0.0	S	0.8	0.1	0.7	0.1	
20:1(n-9)	0.5	0.0	0.9	0.0	S	0.2	0.0	0.5	0.0	S
20:1(n-7)	5.0	0.1	3.6	0.2	S	3.0	0.1	2.4	0.3	S
16:2(n-4)	0.0	0.0	0.0	0.0		0.9	0.1	0.4	0.0	S
16:3(n-4)	0.2	0.0	–	–		0.0	0.0	0.1	0.0	
18:2(n-6)	0.8	0.1	4.3	0.2	S	1.5	0.0	8.8	0.4	S
18:2(n-4)	0.4	0.0	0.2	0.0	S	0.6	0.0	0.3	0.0	S
18:3(n-6)	0.1	0.0	0.3	0.0	S	0.2	0.0	0.7	0.0	S
18:3(n-3)	0.9	0.1	1.9	0.0	S	2.0	0.0	4.5	0.2	S
18:4(n-3)	2.0	0.1	2.5	0.1	S	5.3	0.1	6.1	0.5	S
18:5(n-3)	0.4	0.0	–	–		–	–	0.0	0.0	
20:2i	–	–	–	–		–	–	–	–	
20:2j	0.4	0.0	0.2	0.0	S	0.8	0.1	0.5	0.1	S
20:2(n-6)	0.3	0.0	0.6	0.1	S	0.3	0.0	0.6	0.1	S
20:3(n-6)	0.2	0.0	0.3	0.0	S	0.2	0.0	0.3	0.0	S
20:4(n-6)	2.2	0.1	3.1	0.0	S	1.1	0.1	1.0	0.1	S
20:4(n-3)	0.5	0.0	0.3	0.0	S	0.6	0.1	0.5	0.0	S
20:5(n-3)	16.7	0.1	10.8	0.2	S	15.1	0.7	7.1	0.9	S
21:5(n-3)	0.8	0.0	0.4	0.0	S	0.9	0.1	0.5	0.0	S
22:4(n-6)	0.2	0.0	0.2	0.0		0.1	0.0	0.1	0.0	S
22:2i	0.8	0.0	1.4	0.0	S	0.3	0.0	0.5	0.0	S
22:2j	5.4	0.3	3.9	0.1	S	2.3	0.1	1.5	0.2	S
22:5(n-6)	0.4	0.0	1.9	0.0	S	0.2	0.0	1.0	0.1	S
22:5(n-3)	1.2	0.1	0.7	0.1	S	0.7	0.1	0.3	0.0	S
22:6(n-3)	13.9	0.5	13.4	0.0	S	9.6	0.3	7.8	0.5	S
16:0DMA	0.2	0.3	0.2	0.0		0.0	0.0	0.0	0.0	
18:0DMA	8.6	0.6	9.0	0.2	S	1.0	0.2	0.7	0.1	S
20:1DMA	1.1	0.1	1.6	0.1		0.2	0.0	0.1	0.0	S
T0. BR.	0.8	0.1	0.8	0.1		1.4	0.1	0.7	0.0	
TO.SAT.	26.7	1.3	25.9	0.5	S	36.0	0.8	32.1	0.5	S
TO.DMA	10.3	0.7	11.3	0.4	S	1.5	0.2	1.0	0.1	S
TO.MONO	14.5	0.2	15.7	0.2	S	18.0	0.3	23.2	0.8	S
TO.(n-9)	1.5	0.1	5.1	0.3	S	3.2	0.2	11.4	0.8	S
TO.(n-7)	10.7	0.3	8.7	0.0		13.6	0.3	10.8	0.2	S
TO.POLY	47.6	0.9	46.2	0.0		42.8	1.0	42.7	0.3	
TO.(n-4)	0.6	0.0	0.2	0.0	S	1.5	0.1	0.7	0.0	S
TO.(n-6)	4.1	0.1	10.5	0.2	S	3.5	0.0	12.5	0.5	S
TO.(n-3)	35.1	0.5	29.5	0.1	S	33.4	1.0	26.3	0.7	S
TO. NMI	6.6	0.4	5.6	0.0	S	3.4	0.1	2.6	0.2	S
(n-3)/(n-6)	8.5	0.1	2.8	0.1	S	9.6	0.3	2.1	0.1	S
22:6/20:5	0.8	0.0	1.2	0.0	S	0.6	0.0	1.1	0.2	
22:5/20:4	0.2	0.0	0.6	0.0	S	0.2	0.0	1.0	0.2	S

[a] The results are expressed as percentages of total fatty acids of the fraction; Mean, SD, $n = 3$.

Table 4
Fatty acid composition of the polar and neutral lipids in the muscle at the end of the natural and artificial broodstock conditioning[a]

% mol	Polar lipids					Neutral lipids				
	Nature		Artificial		Sign.	Nature		Artificial		Sign.
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.	
14:0	1.2	0.1	2.1	0.5	S	7.6	0.9	8.0	0.3	
15:0	0.6	0.0	0.7	0.1	S	0.8	0.1	0.7	0.0	
16:0	18.1	0.8	19.8	0.4	S	22.6	1.3	22.6	1.3	
17:0	2.3	0.3	1.9	0.2		1.2	0.0	1.2	0.1	
18:0	5.0	0.0	4.8	0.2		4.1	0.2	4.2	0.2	
16:1(n-9)	–	–	–	–		0.6	0.1	0.9	0.3	
16:1(n-7)	1.5	0.0	1.3	0.1	S	4.6	0.7	4.1	0.3	
18:1(n-11)	0.6	0.2	0.5	0.1		0.3	0.1	0.4	0.0	
18:1(n-9)	0.7	0.1	1.6	0.2	S	2.6	0.1	6.6	0.2	S
18:1(n-7)	2.3	0.5	2.8	0.1	S	3.9	0.0	4.3	0.3	S
20:1(n-11)	1.3	0.0	1.2	0.1	S	0.9	0.1	0.8	0.1	
20:1(n-9)	2.0	0.1	2.3	0.2	S	0.5	0.1	0.6	0.1	S
20:1(n-7)	5.0	0.1	4.3	0.4	S	3.8	0.4	2.7	0.2	
16:2(n-4)	0.1	0.0	0.0	0.0		0.5	0.1	0.2	0.1	S
16:3(n-4)	0.1	0.0	–	–	S	0.3	0.1	0.1	0.1	
18:2(n-6)	0.5	0.0	1.7	0.0	S	1.5	0.0	6.0	0.2	S
18:2(n-4)	0.3	0.0	0.2	0.0	S	0.6	0.0	0.4	0.0	S
18:3(n-6)	0.1	0.0	0.1	0.0	S	0.2	0.0	0.5	0.0	S
18:3(n-3)	0.6	0.1	0.9	0.0	S	1.9	0.1	3.7	0.5	S
18:4(n-3)	1.2	0.1	1.1	0.1		4.9	0.3	4.4	0.5	
18:5(n-3)	0.2	0.0	–	–	S	–	–	0.0	0.0	
20:2i	–	–	–	–		–	–	–	–	
20:2j	0.2	0.0	0.2	0.0	S	0.8	0.1	0.7	0.0	S
20:2(n-6)	0.3	0.1	0.4	0.1	S	0.3	0.0	0.4	0.1	S
20:3(n-6)	0.1	0.0	0.1	0.0		0.2	0.0	0.2	0.0	
20:4(n-6)	2.3	0.1	2.6	0.1	S	1.5	0.2	1.4	0.1	
20:4(n-3)	0.2	0.0	0.1	0.0		0.6	0.0	0.3	0.1	S
20:5(n-3)	13.3	0.2	10.8	0.4	S	14.5	0.7	9.3	0.4	S
21:5(n-3)	0.5	0.0	0.3	0.0	S	0.9	0.0	0.6	0.1	S
22:4(n-6)	0.3	0.0	0.3	0.0		0.1	0.0	0.1	0.0	
22:2i	0.9	0.1	1.2	0.1	S	0.3	0.0	0.4	0.0	S
22:2j	4.5	0.2	3.9	0.1	S	2.6	0.2	1.6	0.1	S
22:5(n-6)	0.4	0.0	1.0	0.1	S	0.2	0.0	0.7	0.1	S
22:5(n-3)	1.7	0.1	1.5	0.1		0.8	0.1	0.4	0.0	S
22:6(n-3)	16.2	0.2	16.0	0.4		10.3	1.0	8.2	1.1	
16:0DMA	0.0	0.0	0.7	0.1		0.1	0.0	0.1	0.0	
18:0DMA	11.2	1.3	9.4	1.3	S	1.3	0.2	0.8	0.2	
20:1DMA	2.2	0.4	1.9	0.4		0.3	0.1	0.1	0.0	
T0. BR.	0.8	0.1	0.9	0.1		0.9	0.1	1.1	0.2	
TO.SAT.	27.7	1.1	29.8	1.2	S	36.7	1.8	37.3	1.2	
TO.DMA	14.0	1.7	12.7	1.6	S	1.9	0.2	1.4	0.2	
TO.MONO	13.6	0.5	14.1	0.8		17.2	0.2	20.4	0.4	S
TO.(n-9)	2.7	0.1	3.9	0.4	S	3.6	0.1	8.1	0.3	S
TO.(n-7)	8.8	0.4	8.4	0.5		12.3	0.4	11.1	0.3	S
TO.POLY	43.9	0.6	42.5	0.5	S	43.2	1.9	39.6	1.0	
TO.(n-4)	0.4	0.0	0.2	0.1	S	1.4	0.1	0.6	0.1	S
TO.(n-6)	3.9	0.1	6.3	0.2	S	3.9	0.3	9.3	0.2	S
TO.(n-3)	33.2	0.3	30.5	0.7	S	33.0	1.5	26.3	0.8	S
TO. NMI	5.7	0.2	5.2	0.2		3.7	0.2	2.7	0.1	S
(n-3)/(n-6)	8.5	0.2	4.9	0.2	S	8.4	0.2	2.8	0.1	S
22:6/20:5	1.2	0.0	1.5	0.1	S	0.7	0.0	0.9	0.1	
22:5/20:4	0.2	0.0	0.4	0.0	S	0.1	0.0	0.5	0.0	S

[a] The results are expressed as percentages of total fatty acids of the fraction; Mean, SD, $n = 3$.

reserves were preferentially located in that organ. The TAG percentage increased during this gametogenesis in the gonad plus mantle, which seemed to reach a plateau by the end of May (after 5–6 weeks of conditioning). In many marine species [20], the maturation of oocytes is accompanied with an accumulation of TAG. The composition of the neutral lipid classes within the neutral lipid fraction of all organs was similar under both types of conditioning. However, the polar lipid classes remained stable and seemed to be unaffected by conditioning methods. The partition of the polar lipid classes was in accordance with data found by Kostetsky and Sergeyguk [13] in different organs of several molluscs species, with PC being the most abundant lipid class followed by PE, PS and PI. Polar lipid classes, the structural components of the membranes, are considered to vary little and to be independent of external parameters and nutritional changes [18,23,28]. This stability in lipid class composition of phospholipids, despite different dietary conditions, probably reflects the necessity to stabilize structural components and corroborates results obtained with *Pecten maximus* [23,24].

There were significant differences, dependent on the method of conditioning, in the proportions of fatty acid categories in the neutral lipids of all organs. The proportion of MUFA was higher in artificially conditioned

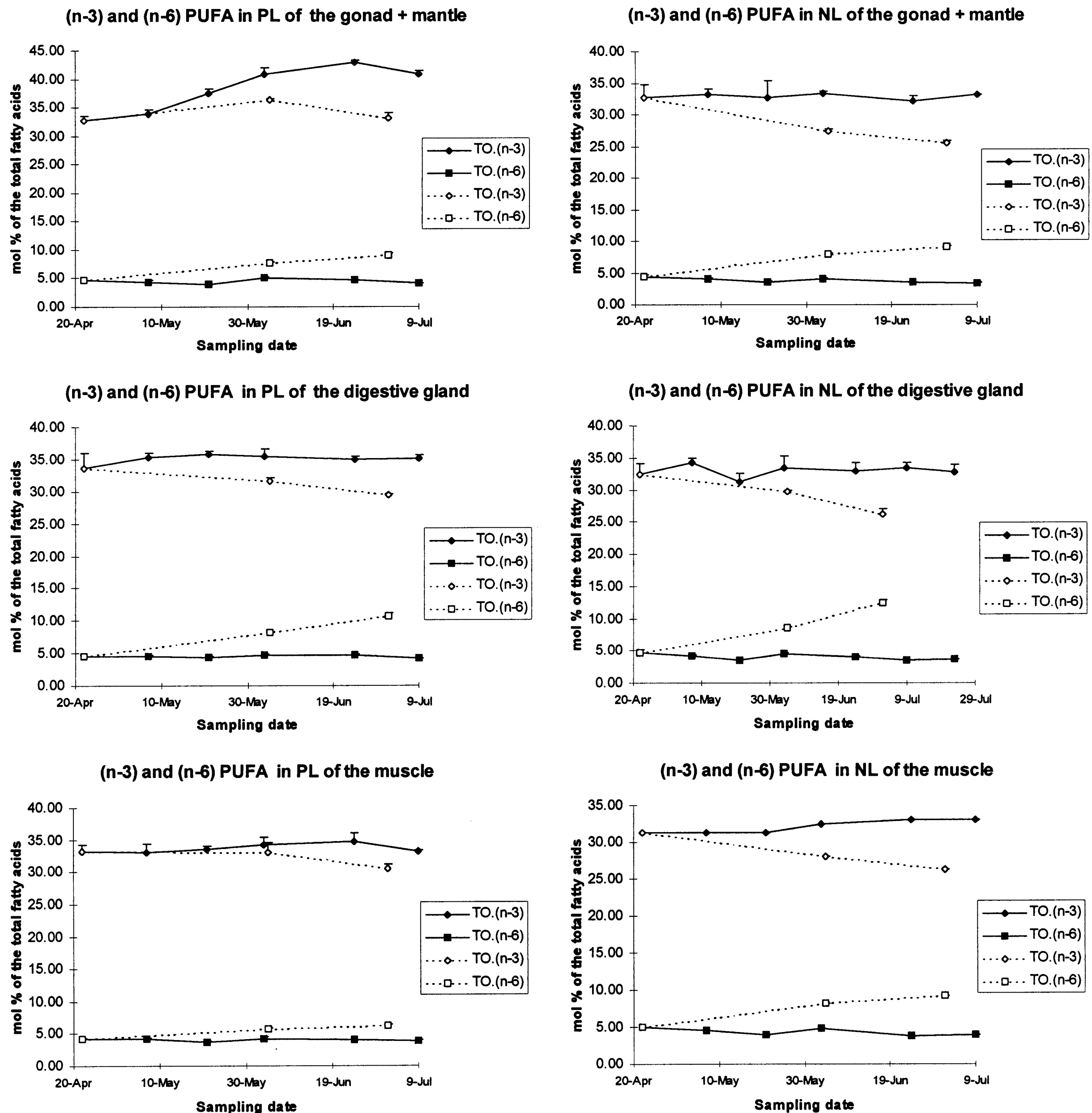

Fig. 5. Comparison of the polar (left) and neutral (right) lipid (n-3) and (n-6) PUFA contents of gonad plus mantle, digestive gland and muscle from natural (filled line) and artificial (dotted line) conditioning (expressed as percentage of the total fatty acid in polar or neutral lipids; Mean, SD, $n = 3$).

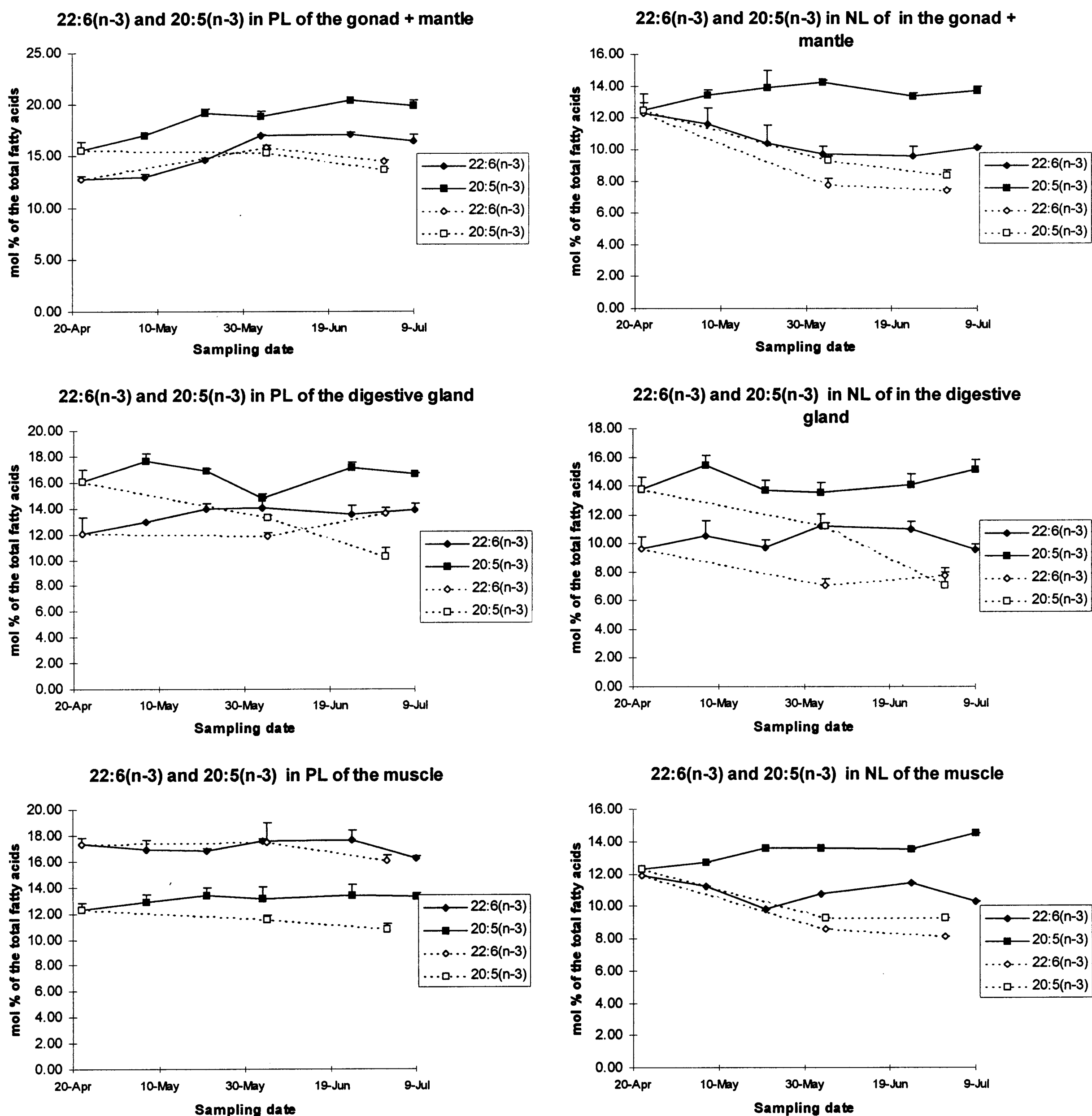

Fig. 6. Comparison of the polar (left) and neutral (right) lipid 22:6(n-3) and 20:5(n-3) contents of gonad plus mantle, digestive gland and muscle from natural (filled line) and artificial (dotted line) conditioning (expressed as percentage of the total fatty acid in polar or neutral lipids; Mean, SD, $n = 3$).

oysters probably as a result of differences between diet composition in nature and in the hatchery. The fatty acid composition of the neutral lipids in marine molluscs is considered to reflect that of the diet [4,9,22,23,30]. The impact of the diet differed from one organ to another. There was a high variation in the digestive gland, whereas the composition of the muscle was less affected. The dietary impact was also revealed in the polar lipids, but in a lesser extent than in the neutral lipids.

The artificial diet changed the respective proportions of (n-3) and (n-6) PUFA drastically in the artificially conditioned animals. Differences were found in the proportions of (n-3) and (n-6) PUFA between the two broodstock conditions in the polar lipids. The necessity to maintain a high ratio of (n-3)/(n-6) in marine fish has been found to be important for survival and growth [19]. Therefore, the low ratio which was observed in artificially conditioned oysters could have an impact on membrane functions.

There was evidence of a specific accumulation of 20–22 carbon PUFA under both broodstock conditions in the polar lipids. The amounts of 22:6(n-3) and 20:5(n-3) were higher in the polar than in the neutral lipids. This preferential incorporation of 22:6(n-3) and 20:5(n-3) occurred irrespective of their levels in the diet

and was also found in Pectinids, but to a higher degree [4,22]. The proportions of 22:6(n-3) and 20:5(n-3) in the polar lipids from the muscle were the most regulated in all studied organs of the oyster. This is probably a consequence of the low turnover of PUFA in the muscle cell membranes. The ratio 22:6(n-3)/20:5(n-3) in *C. gigas* was lower than that of *P. maximus*, suggesting that the essential fatty acid requirement could differfrom one bivalve species to another. In comparing the two methods of conditioning broodstock, the percentages of 22:6(n-3) and 20:5(n-3) with artificial conditioning were significantly lower than those in nature. The differences are even more drastic for 20:5(n-3) than for 22:6(n-3). High levels of 22:6(n-3) have been associated with a better hatching rate in *P. maximus* [21,23]. This suggests that it is possible that 22:6(n-3) plays a major role at structural and functional level of cell membranes involved in oogenesis and embryogenesis [22,23]. The specific role of 20:5(n-3) in previous studies has been related to energetic functions. An energy-yielding utilization of 20:5(n-3) has been suggested during embryogenesis in *Crassodoma gigantea* [34] and in *P. maximus* larvae [3,21].

Polar lipids contained more 20:4(n-6) than neutral lipids, and for all three organs higher levels were found in artificially conditioned oysters compared to those naturally conditioned. This fatty acid is a major precursor of prostaglandins, which influences the reproduction process in molluscs [17]. It is possible that changes in polar lipids could affected the production of eicosanoids such as prostaglandins [10].

The newly synthesized fatty acid, 22:2j (NMI PUFA) [1], was found in a higher proportion in the natural broodstock than with artificially conditioned broodstock. This suggests that its biosynthesis could be influenced by the type of conditioning. This creates a possible implication in the process of gonadal maturation which remains to be elucidated. Zhukova [35] claims that this PUFA is synthesized starting from 16:1(n-7). Thompson and Harrison [25] proved that the level of 22:2j in total lipids was correlated with the level of 16:1(n-7) in the larvae of *C. gigas*. This suggests that the synthesis of 22:2j is stimulated by the presence of 16:1(n-7) in the diet and more specifically in the neutral lipids.

The proportions of NMI PUFA in all three organs were higher in the polar than in the neutral lipids. This has previously been demonstrated before by Watanabe and Ackman [32], and by Jeong et al. [11]. It has also been suggested that NMI PUFA could play a role in the substitution of some essential FA like 20:5(n-3), 22:6(n-3) and 20:4(n-6). Klingensmith [12] observed an inverse relation between the level of 22:2j and PUFA in the polar lipids. This was not observed in this present study.

The observed change in (n-6)/(n-3) ratio for artificially conditioned broodstock could be a result of the use of *T. suecica* and T-*Isochrysis* in the diet as these algae are richer in (n-6) fatty acids than the diatom species which was used to condition broodstock in this study. The (n-3) PUFA of the two latter algae was found to contain a lot of 18:3(n-3) and 18:4(n-3) which cannot be converted by the oysters into 20:5(n-3) and 22:6(n-3) [14,28]. This could explain the lower accumulation of 20:5(n-3) and 22:6(n-3) in the gonads of hatchery conditioned broodstock than in nature, as well as the increase of percentage of total lipid in digestive gland.

These results underline the importance of the diet quality on the processes of storage and transfer to membranes of essential fatty acids. The hatchery diet can be enriched in (n-3) polyunsaturated fatty acids, especially in 20:5(n-3) to mimic the natural diet. At the same time, the supply of (n-6) via *T. suecica* and *T-Isochrysis* should be limited to provide a good (n-3)/(n-6) ratio.

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