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Fatty acids and egg quality in great scallop

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Spawners of the great scallop, *Pecten maximus*, were conditioned on three microalgal diets: T-*Isochrysis*, a mixture of four species (PTSC) and *Chaetoceros calcitrans*.

The polyunsaturated fatty acid (PUFA) composition of neutral and polar lipids of eggs was related to the fatty acid composition of the diet. However, the 20 and 22 carbon PUFAs were maintained at significant levels independent of those of the diet when these fatty acids were present in the diet at low levels. This effect was more pronounced in the polar than in the neutral lipids. A preferential incorporation of 22:6(ω–3) and 20:4(ω–6) was demonstrated in the polar lipids. This emphasizes their role in ovogenesis and embryogenesis.

The 22:6(ω–3) requirement of *P. maximus* was better satisfied by T-*Isochrysis*, which favoured the highest incorporation of this essential fatty acid in the eggs. The good success in reproduction obtained with this monospecies diet led us to modify multispecies diets by increasing the level of 22:6(ω–3).

KEYWORDS: Broodstock conditioning, Eggs, Fatty acids, Great scallop (*Pecten maximus*)

INTRODUCTION

Artificial conditioning of broodstock is a crucial step in the production of great scallop (*Pecten maximus*) spat in hatcheries but large variability is observed in the quality of the gametes obtained. During bivalve oogenesis, the oocytes acquire their lipid reserves directly from the food (Beninger *et al.* pers. comm.) and by a transfer from muscle (glycogen) and digestive gland reserves to the gonad (Vassalo, 1973; Barber and Blake, 1985).

Hatching yield has been related to lipid storage in the eggs (Helm *et al.*, 1973; Dorange *et al.*, 1989; Le Pennec *et al.*, 1990), but a high lipid level in the eggs is not always related to a good larval development (Gallager *et al.*, 1986; Delaunay, 1992). These lipids play a major role as membrane constituents and as reserve energy in the development of embryos in bivalves (Helm *et al.*, 1973; Gallager *et al.*, 1986; Whyte *et al.*, 1990) and fish (Tocher and Sargent, 1984; Falk-Petersen *et al.*, 1986). The lipid content and fatty acid spectrum of eggs and embryos, in neutral as well as in polar lipids, was strongly influenced by that of the diet on which the broodstock

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was fed for bivalves (Helm *et al.*, 1991; Delaunay, 1992; Utting and Doyou, 1992) and for fish (Watanabe *et al.*, 1984; Watanabe, 1985).

Deficiencies in essential fatty acids can alter fecundity and hatching rate and provoke anomalies in the larval fish which develop from these eggs (Shimma *et al.*, 1977; Watanabe *et al.*, 1984; Watanabe, 1985). Polyunsaturated fatty acids (PUFA) with 20 and 22 carbon and more than three double bonds are also essential for survival and growth of molluscs (Trider and Castell, 1980; Langdon and Waldock, 1981; Uki *et al.*, 1986 ; Delaunay *et al.*, 1993).

The objective of this study was to determine how the lipid quality of the broodstock diet affects the fatty acid composition of *P. maximus* eggs and to evaluate the eventual biological consequences. This was performed by monitoring the effects of the different diets on the fatty acid composition of polar lipids (membrane components) and neutral lipids (reserves) of the eggs.

MATERIAL AND METHODS

Sample preparation

Adults of *P. maximus* were collected from Brest Bay in February 1993 and transferred to IFREMER's hatchery at Argenton near Brest. After emptying their gonads by a thermal shock, the scallops were held for 11 weeks in 400 l rearing tanks with coarse sandy bottom. The circulating ambient water was filtered through a 50 μm mesh and maintained at 15°C. Three microalgal diets were tested: *Isochrysis aff. galbana* Green (clone T-iso; Tahiti *Isochrysis*), *Chaetoceros calcitrans* Takano and a hatchery standard 1:1:2:1 mixture of *Pavlova lutheri* Droop, T. *Isochrysis, Skeletonema costatum* Greville and *Chaetoceros calcitrans* (referred to as PTSC hereafter). The scallops (about 150 g whole wet weight) were fed these algal diets in a continuous flow at a rate of 8×10^9 cells per animal per day. Each experiment was duplicated, with 60 scallops per lot.

Eggs (three batches) from natural spawning in the field and 14-day-old larvae (three batches) demonstrating good developmental pattern were used as references (Delaunay, 1992).

About 200 000 eggs (from five, six and seven females for the groups T-*Isochrysis*, PTSC and *C. calcitrans* respectively) were recovered on a pre-ignited (overnight at 450 °C) GF/D filter (3 μm porosity) by filtration. The egg samples were placed separately in tubes containing a mixture of chloroform–methanol (2:1 v/v) with 1% BHT (butylated hydroxytoluene), closed under nitrogen and frozen at –20 °C.

Analysis of fatty acids

The 2:1 chloroform–methanol extracts were evaporated to dryness under vacuum and recovered with three washings of 500 μl each of 98:2 chloroform–methanol. The neutral and polar lipids were separated in a silica gel microcolumn (30×5 mm) using chloroform–methanol (98:2) and methanol respectively. The fractions were collected in tapering vials containing 20 μg of BHT and C23:0. The neutral and polar lipid fractions were transesterified (14% BF_3 in methanol – Metcalfe and Schmitz, 1961) and processed as described by Marty *et al.* (1992).

The fatty acid methyl esters (FAME) were separated in a DBWAX capillary column (25×0.32 mm; 0.2 μm film thickness). The fatty acids were identified by their retention times with reference to those of standards and designated following the formula *C*:*X* (ω–*Y*) where *C* is the number of carbon atoms, *X* is the number of double bonds and *Y* is the position of the first double bond counted from the CH_3 terminal. Dimethylacetals (DMA) are produced during the transmethylation reaction by methylation of the alk-1-enyl residues of plasmalogens in polar lipids.

Statistical analysis

Significance of differences ($p < 0.05$) in neutral and polar lipid quantities between dietary treatments were determined by ANOVA. Analyses were performed using a Statgraphics (system 2.1) computer package.

RESULTS

The three test diets gave rise to differences in embryogenesis (Fig. 1). The group T-*Isochrysis* eggs showed a better hatching rate and a smaller percentage of larvae with abnormalities.

Polar and neutral lipid quantities of the eggs were not significantly different (Fig. 2). The proportions of saturated, monounsaturated and polyunsaturated fatty acids of egg neutral and polar lipids were similar (Table 1).

The PUFA profiles of neutral lipids of the eggs (Fig. 3) reflected those of the corresponding test diets. Scallops fed diets rich in 20:5(ω–3) (PTSC and *Chaetoceros calcitrans*) and 22:6(ω–3) (T-*Isochrysis*), produced eggs with high accumulation of these fatty acids in their neutral lipids. On the other hand, a deficiency of 20:5(ω–3) and 22:6(ω–3), caused by the T-*Isochrysis* and *C. calcitrans* diets respectively, reduced (but not below 4%) the levels of both fatty acids in the neutral lipids of eggs.

The PUFA composition of the diets influenced also the PUFA profiles in eggs polar lipid (Fig. 3). The amount of 20:5(ω–3) was 3.6 times higher in group *C. calcitrans* eggs than in group T.-*Isochrysis*. Conversely, the percentage of 22:6(ω–3) was 1.9

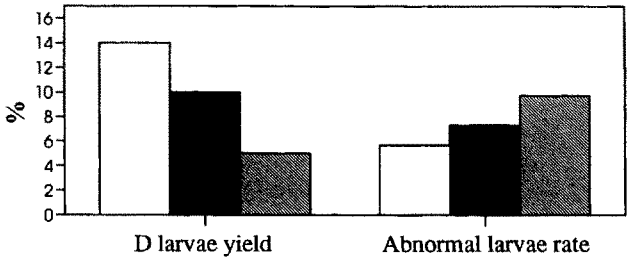

FIG. 1. Effect of broodstock conditioning diet on percentage yield of D larvae, and of abnormal larvae, from spawned eggs. No stipple, T-*Isochrysis* diet; dark shading, PTSC diet; hatching, *C. calcitrans* diet. For each conditioning, eggs from several females were pooled in one tank and no replication was made.

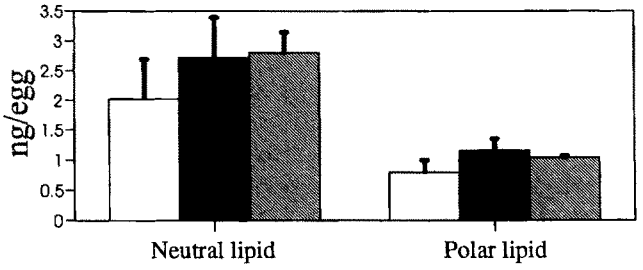

FIG. 2. Effect of broodstock conditioning diet on neutral and polar lipid quantities (ng per egg). Diets coded as in Fig. 1. SD $n = 3$.

times higher in group T-*Isochrysis* than in group *C. calcitrans*. This diet influence was, however, partial because certain fatty acids such as 22:6(ω–3) and 20:4(ω–6) showed a marked accumulation in polar lipids.

The 22:6(ω–3)/20:5(ω–3) and the 20:4(ω–6)/20:5(ω–3) ratios were consistently higher in the polar lipids than in the neutral lipids (Fig. 4) for eggs from natural and conditioned spawnings as well as for the larvae.

The percentage of 22:6(ω–3) from neutral and polar lipids (10.3% and 24.3% respectively) in group T-*Isochrysis* eggs was similar to the values in neutral and polar lipids observed in eggs from natural spawning (11.3% and 25.4% respectively) and in larvae undergoing a good development (13.3% and 26.6% respectively). On the contrary, this fatty acid in group PTSC and *C. calcitrans* eggs was present at a significantly lower percentage.

The percentage of 20:4(ω–6) from neutral and polar lipids was higher in eggs from *C. calcitrans* conditioning diet (3.0% and 6.8% respectively) than values from natural spawning (1.0% and 4.5%) and good larvae (1.0% and 2.0%).

TABLE 1 Proportions (%) of fatty acids (Sat., saturated; Mono., monounsaturated; Poly., polyunsaturated; DMA, dimethyl acetal) in neutral and polar lipids of the eggs. Data are mean (SD) $n = 3$

	Diet		
	T-*Isochrysis*	PTSC	*C. calcitrans*
Neutral lipids			
Sat.	27.9 (0.5)	30.9 (0.2)	31.7 (0.7)
Mono.	27.2 (0.1)	24.6 (0.7)	25.9 (0.2)
Poly.	44.9 (0.6)	44.5 (0.9)	42.5 (0.9)
DMA	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Polar lipids			
Sat.	23.1 (2.0)	22.6 (0.1)	27.5 (0.3)
Mono.	11.6 (1.0)	7.9 (0.4)	11.1 (0.3)
Poly.	50.2 (2.2)	54.3 (0.4)	47.1 (1.9)
DMA	15.1 (0.8)	15.2 (1.0)	14.3 (2.6)

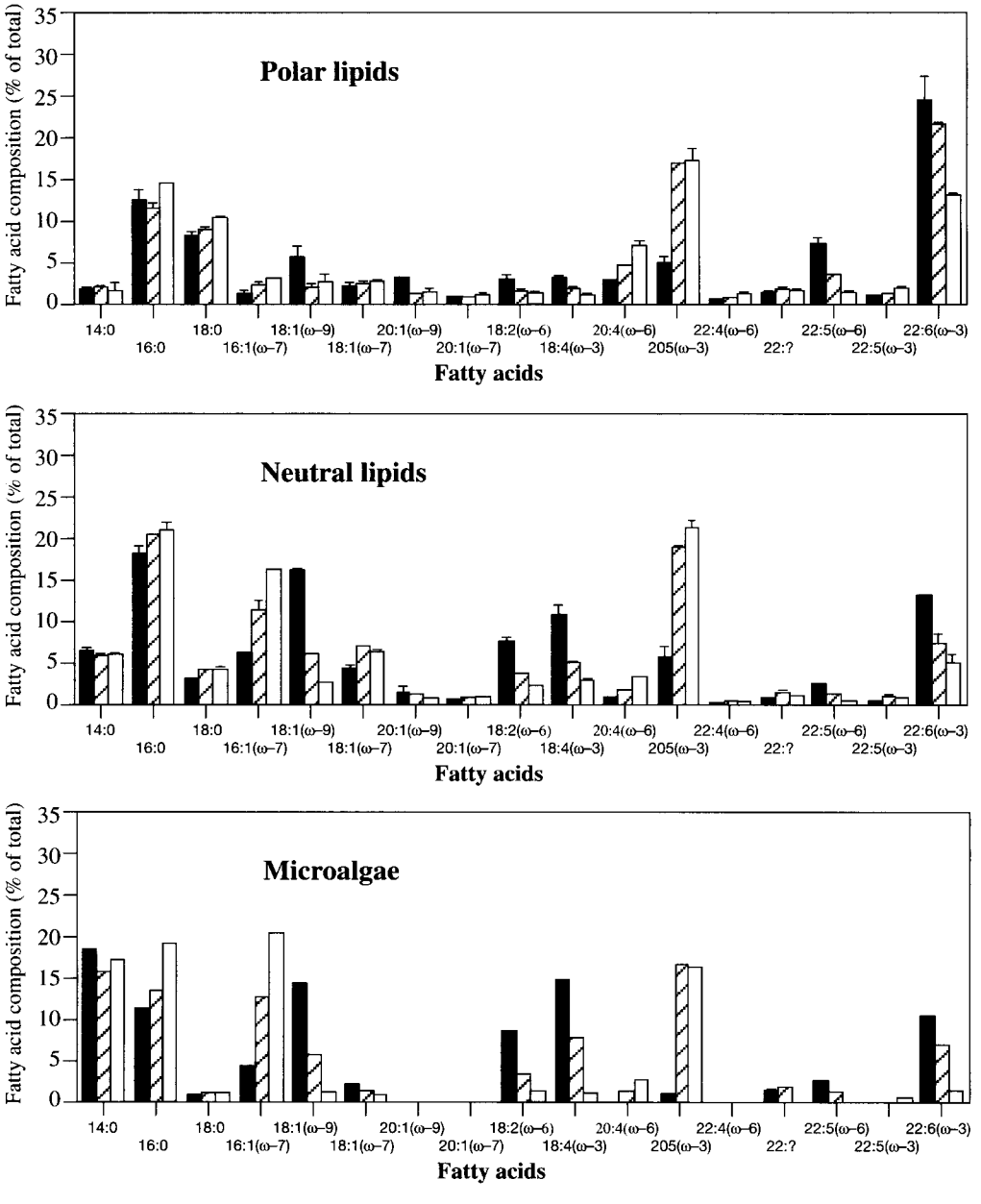

FIG. 3. Effect of broodstock conditioning diet on neutral and polar lipid fatty acid composition of eggs. Fatty acid composition of microalgal diets is also shown. Black, *T-Isochrysis* diet; no stipple, PTSC diet; striped stipple, *C. calcitrans* diet. SD $n = 3$.

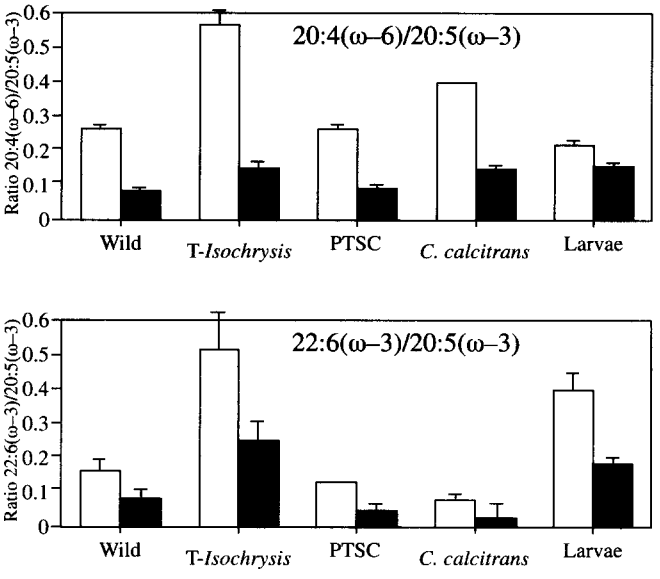

FIG. 4. Comparison of 20:4(ω–6)/20:5(ω–3) and 22:6(ω–3)/20:5(ω–3) ratios in eggs from the different broodstock conditioning diets with reference to eggs from wild spawners and to 14-day-old larvae fed the standard mixture PTSC. No shading, polar lipids; dark shading, neutral lipids. SD $n = 3$.

DISCUSSION

T-*Isochrysis* diet enhances vitellogenesis and embryogenesis more than the other two test diets. The broodstock fed this alga showed a faster rate of gametogenesis and less atresia (unpublished data). The hatching rate and the quality of the D larvae produced were also better.

The different results observed in embryogenesis were not a result of the lipid quantities in the eggs, which were similar. The same egg lipid content may possibly be explained by the isoenergetic diets provided. In the same way, we observed no difference in the relative proportions of saturated, monounsaturated and polyunsaturated fatty acids in the neutral and polar lipids, regardless of the diet. This is presumably due to a metabolic necessity to maintain an equilibrium between these different categories of fatty acids.

However, biological differences could be explained by differences in the fatty acid composition of neutral and polar lipids in the eggs. The concentration of PUFA in the neutral lipids is dependent not only on its proportion in the diet but also on the nature of the fatty acids incorporated. There is clear evidence for a specific accumulation of the 20 and 22 carbon PUFA. This specific accumulation is more accentuated in the polar lipids. In particular, the results show clearly that *Pecten maximus* develops mechanisms allowing it a specific retention of 20:4(ω–6), 22:6(ω–3) and 20:5(ω–3) in the egg lipids, even under deficient conditions. The minimum levels of 20:4(ω–6) and 22:6(ω–3) were maintained in the eggs, respectively

with T-*Isochrysis* and *C. calcitrans* diets, despite their deficiency in these algae. This result can be explained by transfers of these fatty acids from digestive gland lipid reserves to oocytes. The contribution of the digestive gland during gametogenesis has been demonstrated by Barber and Blake (1985).

Therefore, the gonad fatty acid profile represents mainly the fatty acid composition of the eggs and no change in fatty acid pattern occurs between gametogenesis and spawning (Delaunay, 1992).

The 22:6(ω–3)/20:5(ω–3) and the 20:4(ω–6)/20:5(ω–3) ratios in polar lipids compared with neutral lipids shows that the 20:4(ω–6) and the 22:6(ω–3) are incorporated more actively than 20:5(ω–3). Besnard *et al.* (1989) and Napolitano and Ackman (1993) also have shown that wild stocks of *P. maximus* and *Placopecten magellanicus* maintained higher levels of 22:6(ω–3) and 20:4(ω–6) in the polar lipids compared with neutral lipids throughout the year. Similarly, in the turbot, *Scophthalmus maximus*, fed a diet totally deprived of PUFA, the 20:4(ω–6) and the 22:6(ω–3) were selectively retained at the expense of 20:5(ω–3), which suggests that these two PUFAs have more important biochemical functions than 20:5(ω–3) in polar lipids (Bell *et al.*, 1985).

Therefore, the 22:6(ω–3) may play a major role at the structural and functional levels of cellular membranes involved in oogenesis and embryogenesis. The improved results on the hatching rate obtained with T-*Isochrysis* diet appear to be associated with a high level of 22:6(ω–3) and a high 22:6(ω–3)/20:5(ω–3) ratio.

These results are comparable to those of Xu *et al.* (1994), obtained with the Chinese prawn *Penaeus chinensis*, where the hatching rate correlated with the 22:6(ω–3) content of the polar lipids. In fish, the 22:6(ω–3) is known to be more efficient than 20:5(ω–3) in reducing the mortality and malformation of the larvae of turbot (Bell *et al.*, 1985) and gilthead seabream, *Sparus auratus* (Watanabe *et al.*, 1989). An increase of the 22:6(ω–3)/20:5(ω–3) ratio in diet was correlated significantly with an improvement of growth parameters in gilthead seabream larvae, particularly in the first week of weaning (Mourente *et al.*, 1993).

Levels of 22:6(ω–3) for the three groups demonstrated that PTSC and *C. calcitrans* diets were deficient in this fatty acid in comparison with wild eggs and larvae. In our results a 22:6(ω–3)/20:5(ω–3) ratio in the diet greater than 1 seems to be required for broodstock to avoid a deficiency. A ratio greater than 2 is recommended for marine fish (Sargent, 1995). Some specific molecules such as 20:4(ω–6) should be taken into account.

The 20:4(ω–6) is incorporated preferentially as a polar lipid component of the eggs. Its higher level in the polar lipids of eggs than of larvae suggests that it has a specific role in the polar lipids metabolism during ovogenesis. The 20:4(ω–6) is a major precursor of prostaglandins which influence reproduction in molluscs (Osada *et al.*, 1989). The 20:4(ω–6) requirements seem to be met with our standard (PTSC) and *C. calcitrans* diet but not completely with the T-Isochrysis diet.

The use of an artificial food could be a way to complement the standard microalgal diet. Some experiments demonstrated possibilities of maturation for spawners exclusively fed artificial food or dried algae (Laing and Lopez-Alvarado, 1994) or microcapsules (Robinson, 1992). Ingestion of lipid microspheres by oysters has also proved possible (Heras *et al.*, 1994), but these authors had better results when microspheres were used to complement living algae.

CONCLUSIONS AND RECOMMENDATIONS

1. The fact that the levels of 20:4(ω–6) and 22:6(ω–3) were maintained suggests that these fatty acids play an important role in reproduction: 20:4(ω–6) may have an influence on ovogenesis and 22:6(ω–3) on the differentiation of the eggs.
2. The good results obtained with the T-*Isochrysis* diet led us to modify the composition of the algal mixture currently used for conditioning the broodstock by increasing the level of 22:6(ω–3) and the 22:6(ω–3)/20:5(ω–3) ratio in the diet.
3. We recommend a change in the relative proportions of the different algae of the mixture PTSC by decreasing the abundance of diatoms in relation to T-*Isochrysis*, keeping in mind however the 20:4(ω–6) requirement supplied by diatoms.
4. Initial quality and quantity of the scallop reserves before artificial conditioning is probably necessary to meet requirements when the artificial diet is deficient. Therefore, attention should be paid to the preceding feeding status.

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