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Effect of lipid emulsions on production and fatty acid composition of eggs of the scallop *Argopecten purpuratus*

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Abstract The impact of supplementing lipid emulsions rich in eicosapentaenoic acid (EmEPA), docosahexaenoic acid (EmDHA) or saturated fatty acids (EmCOCO) to a standard algal diet [3:1 mixture of *Isochrysis galbana* (T-iso) and *Chaetoceros neogracile*, St-diet] on *Argopecten purpuratus* broodstock was evaluated. Broodstock fecundity was compared as well as the egg quality in terms of lipid content, fatty acid composition and lipid class distribution. Fecundity was defined as the number of eggs released in the spawning process, since spawning was virtually complete. Results indicated that the total lipid content of the eggs of *A. purpuratus* was diet independent. A greater energy reserve was spent on a larger number of oocytes and not on bigger sized oocytes with a higher lipid content. The lipids supplied through the emulsions were at least partially allocated to the eggs, demonstrating that the fatty acid composition of the eggs could be manipulated, especially the neutral lipid fraction. Levels of EPA changed more rapidly than DHA levels, supporting the observation that they fulfilled an energetic and structural role, respectively. The St-diet supplemented with 50%EmCOCO resulted in a significantly higher fecundity compared to the algal diet supplemented with 25%EmEPA + 25%EmDHA and the non-supplemented algal diet. It would seem that saturated fatty acids (SAFA) were more easily or preferentially incorporated in the female gonads of *A. purpuratus*. The relative content of SAFA and 18:2(*n*-6) in these eggs rose significantly. The relative content of the highly unsaturated fatty acids, EPA and DHA, on the other hand was substantially lower in the neutral lipid fraction, but hardly affected in the polar lipid fraction. It appeared that the maintenance of an adequate DHA/EPA ratio (approximately 1.2) was more important than the absolute levels of the two fatty acids, as long as a threshold value was reached.

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Introduction

The Chilean/Peruvian scallop *Argopecten purpuratus* (Lamarck 1819) has become an important aquaculture species in Chile. Because the high fishing pressure endangered the existence of the natural banks (Wolff and Alarcon 1993), extraction has been forbidden, and its culture has developed extensively during the last decade (Martínez et al. 2000). The production increased 16-fold in 10 years, from 1,182 tonne (in 1990) to 19,038 tonne (in 2000) (SERNAPESCA 2001). The scallop is a functional hermaphrodite and exhibits continuous gametogenic activity with two main spawning peaks, one in late summer and a minor one in autumn (Wolff 1988; Martínez 1991). A great variability in spawning success and larviculture results in hatcheries stimulated researchers to study the factors that influence the reproductive process of *A. purpuratus* (Martínez et al. 1992, 2000; Farías et al. 1997; Caers et al. 1999b, 2000; Navarro et al. 2000; Farías and Uriarte 2001).

Marine bivalves undergo a seasonal cycle of energy storage and utilisation that is linked to the annual reproductive cycle, which is regulated by exogenous and endogenous factors. Energy substrates stored during non-reproductive periods are used subsequently: (1) to support maintenance requirements when food is scarce, (2) to replace body mass lost at other times of the year, (3) to produce a net increase in mass over the year and (4) to produce gametes (Barber and Blake 1985b; Bayne 1985).

Bivalve broodstock conditioning in hatcheries aims at maximising fecundity of the parent animals, while maintaining egg quality and larval viability (Utting and

Millican 1997). The quality of the diets given to the broodstock explains a great portion of the variability experienced during bivalve rearing as it affects gamete composition and larval quality. Wolff (1988) suggested that, for *A. purpuratus*, high temperature, although favouring maturation and spawning, might be less critical for successful spawning than food availability. Devauchelle and Mingant (1991) found that, although temperature initiates the process of gametogenesis, fecundity of *Pecten maximus* and the quality of the eggs produced were enhanced when algal supplements were given.

During oogenesis, the oocytes acquire their lipid reserves from three main sources: (1) the adductor muscle, whereby muscle glycogen is converted to lipid (Gabbott 1975; Bayne et al. 1982; Barber and Blake 1985a, 1985b); (2) lipid transfer from reserves in the digestive gland to the female gonad (Vassalo 1973; Barber and Blake 1981,1985a); and (3) directly from food when adults are put under nutritional stress (Gallager and Mann 1986; Soudant et al. 1996b).

Stored egg reserves are catabolised initially during embryonic development from fertilised egg to straight-hinge veligers. Further catabolism occurs to maintain the larvae during the transitional stage of exogenous feeding (Whyte et al. 1990). Survival of *Mercenaria mercenaria* and *Crassostrea virginica* larvae is highly correlated with the total lipid reserves laid down in the eggs during vitellogenesis (Gallager and Mann 1986). The early development of *Mytilus edulis* larvae is correlated with the lipid level in unfertilised eggs (Bayne et al. 1975), and the viability of *Ostrea edulis* larvae is related to the lipid content at the time of liberation, particularly the neutral lipid fraction (Helm et al. 1973).

Conditioning broodstock with diets of different lipid content and quality showed the effects of specific fatty acid deficiencies in bivalve eggs and larvae (Utting and Doyou 1992; Millican and Helm 1994; Soudant et al. 1996a). Based on these findings, the aim of this study was to examine whether the supplementation of different lipid emulsions to the algal diet would enhance broodstock fecundity and to what extent it would alter the lipid content and composition of the spawned eggs. Three different emulsions were tested, either rich in 22:6(*n*-3) (EmDHA), 20:5(*n*-3) (EmEPA) or saturated fatty acids (EmCOCO).

Materials and methods

Diets

Two algal species used as standard feed, *Isochrysis galbana* (T-iso) and *Chaetoceros neogracile*, were grown in 12-l or 16-l plastic bags (batch system) with aerated, filtered (0.5 μm) and UV-treated seawater enriched with the commercial fertiliser Bayfolan. Previous to inoculation with the algae, water in the bags was additionally sterilised with chlorine (0.1 ml l^{-1}) for 24 h and then neutralised with thiosulphate (0.1 g l^{-1}) and aeration. The algae were grown under a 24-h light regime at a temperature of 26 ± 1°C and were harvested in the exponential phase.

Experimental enrichment emulsions (INVE Aquaculture NV Baasrode, Belgium) contained 62% lipid on a wet weight basis, in addition to water, vitamins, emulgators, antioxidants and preservatives. Lipids were present mainly as triglycerides. The two lipid emulsions named EmEPA and EmDHA contained approximately 30% *n*-3 highly unsaturated fatty acids (HUFAs) on total fatty acids and had a DHA/EPA ratio of 0.6 and 4, respectively. The lipid emulsion EmCOCO consisted mainly of saturated lipids, in particular 12:0, 14:0 and 16:0. It contained hardly any *n*-3 polyunsaturated fatty acids (PUFAs) (ICES 1994). The percentage of lipid supplement was calculated on basis of the dry weight (DW) of the administered algae, being 21 and 70 pg $cell^{-1}$ for *I. galbana* (T-iso) and *C. neogracile*, respectively.

Experiments

The scallops of the two experiments were sent from Coquimbo (region IV) to the field laboratory of the Universidad de Los Lagos in Calbuco (region X). The animals belonging to one treatment were divided evenly into two tanks, each with 50 l of filtered (0.5 μm) and UV-treated seawater. Aeration was provided by means of aquarium pumps. The temperature was maintained at 17°C (± 1°C). Water was changed with a flow-through system twice a week and completely renewed once a week. When changed completely, the tanks were washed with soap and diluted chlorine. The temperature of the fresh seawater was always one degree lower than the temperature in the tanks, to avoid undesired spawning. Towards the end of the conditioning period, when the animals showed developed gonads, only half the ration of algae was fed when water was changed completely. The other half was compensated for the following day by giving 1.5 times the ration. The scallops were fed 6 days a week, and food was distributed with a drip system. Antibiotic (chloramphenicol) was added with every water change at a concentration of 4 mg l^{-1}.

The animals in experiment 1 (expt 1) had been kept in the laboratory for nearly 2 months before the experiment started, receiving algae ad libitum. They were stimulated to spawn prior to the experiment, and those exhibiting completely empty gonads were selected. The experiment took place during the winter months (end July–end August). The animals had an average shell height of 78.3 mm (± 3.0 mm). Groups of ten animals were fed three different diets. Each animal received 3×10^9 alga cells daily when fed the standard diet (St) or the St-diet supplemented with 50% EmDHA (St + 50dha). The St-diet consisted of a mixture of *I. galbana* (T-iso) and *C. neogracile* (3:1, based on cell counts) that was considered to be a good maturation diet (Farías et al. 1997; Martínez et al. 2000). A third diet consisted of only half the amount of algae, maintaining the same proportion between the two algal species, supplemented with 50%EmDHA (1/2St + 50dha). After 33 days, nine animals belonging to different treatments spawned spontaneously. The remaining animals were stimulated to spawn the next day.

The animals in experiment 2 (expt 2) had an average shell height of 66.6 mm (± 2.5 mm). They arrived straight from the north and had just gone through a long and severe winter. They were devoid of any gonadal tissue. The experiment took place during springtime (early September–end November). Four diets were tested, and each treatment consisted of 14 animals. The St-diet served as the control diet. The other three diets consisted of the St-diet supplemented with lipid emulsions, either with 25%EmDHA + 25%EmEPA (St + 50hufa), 50%EmEPA (St + 50epa), or with 50%EmCOCO (St + 50coco). All animals received 2.5×10^9 alga cells daily. A higher ration, as in expt 1, was not used since it led to the production of pseudo-faeces. During the last 20 days of the conditioning period, however, the ration was increased to 3×10^9 alga cells daily without any production of pseudo-faeces.

The two tanks of the St + 50epa treatment and one tank of the St-treatment spawned spontaneously after 62 days. The released eggs were recovered by passing the water through a sieve of 37 μm, while emptying the tanks, and preserved for lipid

analysis. It was impossible however to measure all other egg characteristics. No animals were left to represent the St + 50epa-diet, and only eight animals of the St-diet were taken into further consideration. On day 82, all remaining animals were stimulated to spawn.

Analysis

Eggs of each individual animal were diluted in a known volume of seawater. Four subsamples were taken to count the eggs. Fecundity was defined as the number of eggs released in the spawning process, since spawning was virtually complete. The diameter of 50 eggs from each spawning animal was measured to determine the average size. A total of 200,000 eggs were filtered on pre-combusted and pre-tared GFC-filters to determine DW. Three filters per animal were dried at 60°C for 48 h.

Samples of eggs for lipid analysis were washed twice with 0.5 M ammonium formate to remove salts. They were freeze-dried for 48 h and subsequently stored at −30°C under nitrogen. Samples of algae for lipid analysis were concentrated by using a refrigerated centrifuge and submitting subsamples of 40 ml to a rotation speed of 8,000 rpm (*C. neogracile*) or 9,000 rpm (*I. galbana*); between 3 and 4 l of the algal culture was concentrated in this way. Tubes with the concentrated algae were frozen for 30 min at −70°C, then the algal paste was removed and washed in 0.5 M ammonium formate and kept at −70°C in a tube with a Teflon cap until analysis.

Total lipids (TL) were extracted as described by Folch et al. (1957), with some small modifications. They were extracted with the solvent mixture chloroform:methanol (2:1, v/v). Following solvent evaporation under nitrogen, lipids were determined gravimetrically. TL were dissolved again in the solvent mix at a concentration of 10 mg fat ml^{-1} solvent mix. A total of 200 μl was taken to determine the fatty acid methyl esters (FAME) and dimethyl acetals (DMA), and another 200 μl, to separate neutral from polar lipids. Esterification of fat was accomplished by the addition of freshly prepared acetylchloride-methanol (1:20) and leaving the reaction to take place for 1 h in a boiling water bath. Once cooled, FAME and DMA were extracted with hexane. For the calculation of the percentage of individual DMAs and FAMEs, "total fatty acids" refers to the sum of all DMA and FAME present in the sample. Neutral lipids (NL) and polar lipids (PL) were separated over a silicagel-microcolumn according to the method of Marty et al. (1992). FAMEs of the NL and PL fractions were prepared by transmethylation with a mixture of sulphuric acid and methanol (1:100, v/v) for 16 h at 50°C. Quantitative determination of FAME and DMA was done by a Chrompack CP9001 gas chromatograph equipped with an autosampler and a temperature-programmable, on-column injector. Injections were performed on-column into a polar, 50 m capillary column, BPX70 (SGE Australia) with a diameter of 0.32 mm and a layer thickness of 0.25 μm connected to a 2.5 m methyl-deactivated pre-column. The carrier gas was hydrogen. Identification was based on standard reference mixtures (Nu-Chek-Prep, USA). Integration and calculations were done with the software program Maestro (Chrompack). Lipid class analysis was performed with an IATROSCAN MK-5. The neutral solvent system existed of 850 parts hexane, 150 parts ether and 0.4 parts formic acid. The polar solvent system existed of 700 parts chloroform, 350 parts methanol and 35 parts water. Each sample was run three times, and identification was based on a standard reference mixture (18-5C of Nu-Chek-Prep, USA).

Data analysis

The statistical package STATISTICA 5.0 was used for data analysis. All data were log-transformed before applying any statistics, while values in percentage were arcsine–square-root transformed (Sokal and Rohlf 1995). The homogeneity of variances of means was tested by univariate tests (Cochran, Hartley, Bartlett). Significant differences were detected using one-way ANOVA, P being set at 0.05. The Newman–Keuls' test was used for post hoc comparison. In cases when data were not homogeneously distributed, the Mann–Whitney U-test was used to determine differences between each set of pairs.

Results

Diets

The major fatty acids in *Isochrysis galbana* (clone T-iso) were 14:0, 16:0, 18:1(*n*-9), 18:3(*n*-3), 18:4(*n*-3) and 22:6(*n*-3), whilst in *Chaetoceros neogracile* they were 14:0, 16:0, 16:1(*n*-7) and 20:5(*n*-3) (Table 1). The fatty acids 16:0 and 16:1(*n*-7) accounted for about 50% of the total fatty acids (TFA) of *C. neogracile*. This alga also had a relatively high amount of arachidonic acid (ARA). The emulsions EmEPA and EmDHA had, respectively, 17% and 8% EPA and 11% and 29% DHA. The saturated fatty acid (SAFA) content for both emulsions represented about 25% of TFA. EmCOCO on the other hand contained 81% SAFA, of which nearly half was made up of lauric acid (12:0). The content in 14:0 was about four times higher than in EmEPA or EmDHA. Hardly any (*n*-3)PUFAs were present in the emulsion EmCOCO. The three lipid emulsions had more or less the same FAME content, being 600–750 mg FAME g^{-1} DW.

Experiment 1

No mortality was observed during the experiment. After 33 days, all animals fed the St-diet spawned sperm and eggs, while 40% of the broodstock spawned eggs when fed the St + 50dha-diet and 30% when fed the 1/2St + 50dha-diet (Fig. 1A). Egg size and DW did not differ significantly among the treatments (Fig. 1B, C). There was however a significant difference in the number of eggs produced by the different treatments ($F_{(2,14)} = 5.05$, $P < 0.05$) (Fig. 1D). Broodstock animals receiving 1/2St + 50dha produced significantly less eggs than the broodstock fed St + 50dha [$2.2(\pm 1.8)\times10^6$ and $8.6(\pm 3.4)\times10^6$ eggs $animal^{-1}$, respectively]. There was no difference between the latter and the St-diet [$5.4(\pm 2.5)\times10^6$], although the lipid supplementation of 50%EmDHA tended to favour egg production.

Sixty percent of the fatty acid composition of eggs was made up of 16:0, 16:1(*n*-7), 18:1(*n*-9), 20:5(*n*-3) and 22:6(*n*-3) (Table 2). This corresponded well with the high levels of these fatty acids provided by the diets to the parent animals. The high content of 14:0 in the algal diet, however, was not reflected in the egg composition. The addition of 50%EmDHA to the diet clearly affected the DHA content in eggs. Eggs spawned by animals conditioned with the diets St + 50dha and 1/2St + 50dha

Table 1 *Isochrysis galbana*, *Chaetoceros neogracile*. Fatty acid composition (% of TFA, total fatty acids) of the algae and emulsions (EmEPA, EmDHA, EmCOCO) used in this study. Values are means (±SD) (*SAFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids; *DHA* docosahexaenoic acid; *EPA* eicosapentaenoic acid; *FAME* fatty acid methyl esters; *DW* dry weight; *n* number of samples; *tr* trace)

Fatty acid	*I. galbana* ($n=5$)	*C. neogracile* ($n=5$)	EmEPA ($n=6$)	EmDHA ($n=5$)	EmCOCO ($n=2$)
12:0	0.5±0.0	0.4±0.5	tr	tr	38.3±0.0
14:0	16.2±3.8	11.9±1.0	6.5±0.2	7.1±3.7	24.2±0.0
16:0	13.5±4.4	19.2±7.8	15.4±0.5	12.8±5.2	14.1±0.0
18:0	1.7±1.5	1.7±0.2	3.5±1.2	3.7±1.4	4.4±0.0
SAFA	33.7±9.0	35.5±8.1	25.4±1.1	23.7±3.9	81.1±0.0
16:1(*n*-7)	5.4±0.8	32.8±3.6	7.8±0.4	2.8±1.7	0.1±0.0
18:1(*n*-9)	14.1±4.7	0.9±0.3	11.4±0.7	11.6±2.0	10.3±0.0
18:1(*n*-7)	2.8±0.4	2.5±1.4	3.7±0.5	1.8±0.5	0.3±0.0
MUFA	23.2±5.4	38.4±2.4	22.9±0.7	16.3±3.7	10.8±0.0
18:2(*n*-6)	5.0±1.3	1.0±0.3	5.1±0.3	6.1±0.6	7.2±0.0
18:3(*n*-6)	0.4±0.2	0.8±0.3	0.2±0.0	0.1±0.0	tr
20:3(*n*-6)	tr	tr	0.1±0.0	tr	tr
20:4(*n*-6)	0.1±0.1	3.7±0.6	1.0±0.0	1.1±0.6	tr
22:5(*n*-6)	1.8±1.2	tr	0.4±0.1	1.2±0.5	tr
(*n*-6)PUFA	7.6±2.0	5.5±1.0	6.7±0.3	8.4±0.7	7.2±0.0
18:3(*n*-3)	7.6±1.4	0.2±0.1	1.3±0.2	1.0±0.1	0.6±0.0
18:4(*n*-3)	16.1±6.2	0.6±0.4	1.9±0.1	0.7±0.1	tr
20:3(*n*-3)	tr	tr	0.1±0.0	tr	tr
20:4(*n*-3)	tr	tr	0.7±0.0	0.4±0.1	tr
20:5(*n*-3)	0.9±0.4	11.2±7.3	17.0±1.6	8.0±1.9	tr
22:5(*n*-3)	1.0±1.7	tr	2.5±0.1	3.6±2.7	tr
22:6(*n*-3)	8.4±4.2	1.2±0.4	11.3±0.7	29.4±5.0	tr
(*n*-3)PUFA	34.1±13.5	13.3±8.1	34.7±1.7	43.0±8.6	0.6±0.0
DHA/EPA	8.8±4.2	0.1±0.1	0.7±0.1	3.7±0.4	tr
n-3/*n*-6	4.5±1.9	2.4±1.2	5.2±0.4	5.2±1.4	0.08±0.0
mg FAME g^{-1} DW	146.1	184.7	754.3±169.6	748.1±154.3	608.7±11.0

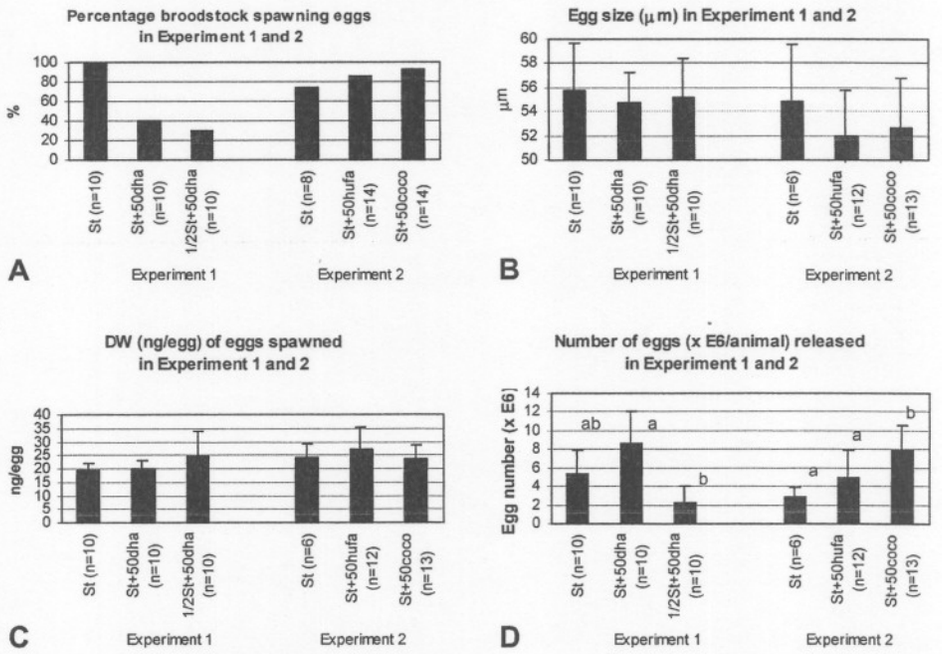

Fig. 1 *Argopecten purpuratus*. Egg-spawning results of experiments 1 and 2 (*abbreviations* for diets, see "Materials and methods"; *DW* dry weight

possessed 33% and 54% more DHA, respectively, than the eggs released by St-diet-fed animals (Table 2). This led to a significantly higher DHA/EPA ratio and a higher content of (*n*-3)PUFA. The EPA and ARA contents were not affected by the differences in the parental diet. The DMAs were dominated by 18:0. No significant difference in (18:0)DMA and total DMA was noticed among the different diets. The three broodstock diets did not lead to significant differences in SAFA and (*n*-6)PUFA levels in the eggs. The animals fed with the St-diet released eggs richest in MUFA, in particular 16:1(*n*-7) and 18:1(*n*-7).

Generally, the NL of the eggs were evenly divided between SAFA (30%), MUFA (30%) and (*n*-3)PUFA (25%) (Table 3). The remaining 15% were present as (*n*-6)PUFA (10%) and DMA (5%). The PL fraction, on the contrary, was dominated by (*n*-3)PUFA (35%), SAFA (25%) and DMA (20%). MUFA and (*n*-6)PUFA represented about 10% of the PLs. EPA was present in almost equal amounts in the NL and PL fractions, while the DHA content in the PL fraction was at least twice as high as in the NL fraction.

The EPA content in the NL and PL fractions was not affected by the diet, while the relative content of DHA in both fractions increased significantly when lipid emulsion was supplemented. As a consequence, the DHA/EPA ratio differed significantly amongst the diets, with the highest value observed for 1/2St + 50dha. In the PL fraction, the (*n*-3)PUFA content and the *n*-3/*n*-6 ratio were also significantly higher when lipids were supplemented, while DHA and ARA were preferentially incorporated in this fraction. Replacing 50% of the algae with lipid emulsion led to a significant decrease in MUFA content in the NL and PL fractions, compared to the 100% algal diet.

Table 2 *Argopecten purpuratus*. Experiment 1: fatty acid composition (% of TFA and DMA) of eggs produced by the broodstock under three different nutritional conditions (*abbreviations* for diets, see "Materials and methods"). Values are means (±SD). Values in one row followed by a *different letter* are significantly different (Newman–Keuls' test, $P<0.05$) (*DMA* dimethyl acetal; *other abbreviations*, see Table 1)

Fatty acid	St ($n=10$)	St + 50dha ($n=4$)	1/2St + 50dha ($n=3$)
14:0	2.4 ± 0.2a	2.2 ± 0.2ab	1.9 ± 0.4b
16:0	18.6 ± 1.0	19.0 ± 0.4	19.1 ± 0.9
18:0	4.2 ± 0.5	4.3 ± 0.3	4.5 ± 0.3
SAFA	28.0 ± 1.5	28.2 ± 0.6	28.9 ± 1.2
16:1(*n*-7)	13.2 ± 0.9a	11.2 ± 0.5b	8.5 ± 0.4c
18:1(*n*-9)	7.6 ± 0.5a	9.6 ± 0.3b	9.8 ± 0.3b
18:1(*n*-7)	3.6 ± 0.3a	3.2 ± 0.2b	2.9 ± 0.1b
MUFA	27.8 ± 1.5a	26.6 ± 0.8a	23.3 ± 0.3b
18:2(*n*-6)	2.3 ± 0.1a	2.6 ± 0.1b	2.9 ± 0.1c
20:4(*n*-6)	3.9 ± 0.5	3.6 ± 0.2	3.4 ± 0.2
22:5(*n*-6)	2.2 ± 0.3	2.2 ± 0.1	2.3 ± 0.2
(*n*-6)PUFA	10.3 ± 1.0	9.5 ± 0.3	9.7 ± 0.4
18:3(*n*-3)	2.0 ± 0.2a	1.5 ± 0.1b	1.4 ± 0.1b
18:4(*n*-3)	3.6 ± 0.5a	2.9 ± 0.2b	2.5 ± 0.2b
20:5(*n*-3)	8.7 ± 0.6	8.7 ± 0.2	9.0 ± 0.6
22:5(*n*-3)	0.3 ± 0.1a	0.5 ± 0.1ab	0.6 ± 0.1b
22:6(*n*-3)	12.2 ± 1.1a	16.2 ± 0.9b	18.8 ± 0.8c
(*n*-3)PUFA	27.7 ± 2.1a	30.9 ± 0.9b	32.7 ± 0.1b
(18:0)DMA	3.2 ± 0.1	3.4 ± 0.2	3.3 ± 0.2
Total DMA	5.3 ± 0.2	5.2 ± 0.2	5.4 ± 0.3
DHA/EPA	1.4 ± 0.1a	1.9 ± 0.1b	2.1 ± 0.0c
n-3/*n*-6	2.7 ± 0.5	3.2 ± 0.1	3.4 ± 0.8

Experiment 2

No mortality was observed during the experiment. There were no significant differences in egg size (52–54 μm) or egg DW (24–27 ng egg^{-1}) among the diets, while the egg quantity varied significantly (Fig. 1B, C, D). Animals that received the 50%EmCOCO supplement performed best. They released on average $7.8(\pm 2.7)\times10^6$ eggs individually, which is significantly better than the broodstock animals of the St- and the St + 50hufa-diet that produced $2.8(\pm 1.0)\times10^6$ and $4.9(\pm 3.0)\times10^6$ eggs,

Table 3 *Argopecten purpuratus*. Experiment 1: fatty acid composition (% of TFA and DMA) of neutral (*NL*) and polar (*PL*) lipids of eggs produced under three different nutritional conditions (*abbreviations* for diets, see "Materials and methods"). Values are means (±SD). Values in one row belonging to each lipid category followed by a *different letter* are significantly different (Newman–Keuls' test, $P<0.05$) (*other abbreviations*, see Table 1)

Fatty acid	NL			PL		
	St ($n=9$)	St + 50dha ($n=4$)	1/2St + 50dha ($n=3$)	St ($n=9$)	St + 50dha ($n=4$)	1/2St + 50dha ($n=3$)
14:0	3.2 ± 0.2a	2.6 ± 0.2b	2.2 ± 0.7c	1.1 ± 0.7	0.7 ± 0.3	0.4
16:0	21.0 ± 0.6	20.8 ± 0.2	20.4 ± 0.3	12.1 ± 1.9a	12.7 ± 1.4ab	8.4b
18:0	3.7 ± 0.5	4.0 ± 0.3	4.6 ± 0.1	7.5 ± 0.7	7.1 ± 0.9	7.3
SAFA	30.7 ± 0.5	30.5 ± 0.2	30.9 ± 0.9	25.6 ± 2.4	25.0 ± 1.7	21.6
16:1(*n*-7)	16.9 ± 1.4a	13.4 ± 0.8b	10.2 ± 0.0c	2.8 ± 1.4	1.9 ± 0.3	0.9
18:1(*n*-9)	9.5 ± 0.4a	11.5 ± 0.5b	12.4 ± 0.1c	2.5 ± 1.2	3.0 ± 0.7	1.8
18:1(*n*-7)	4.4 ± 0.4a	3.7 ± 0.3b	3.3 ± 0.1b	1.2 ± 0.4a	1.0 ± 0.1a	0.5b
MUFA	34.0 ± 2.0a	31.7 ± 1.2a	28.8 ± 0.1b	10.2 ± 2.4	9.0 ± 0.6	5.8
18:2(*n*-6)	2.4 ± 0.1a	2.9 ± 0.4b	3.4 ± 0.0c	0.8 ± 0.2	0.9 ± 0.1	0.7
20:4(n6)	3.1 ± 0.3	3.1 ± 0.2	3.1 ± 0.3	7.2 ± 0.8	6.1 ± 0.9	7.0
22:5(*n*-6)	1.5 ± 0.1a	1.6 ± 0.1b	1.8 ± 0.0c	4.3 ± 0.5	4.2 ± 0.3	3.8
(*n*-6)PUFA	8.5 ± 0.5a	9.1 ± 0.4ab	9.6 ± 0.3b	12.8 ± 0.9	12.0 ± 1.1	11.5
18:3(*n*-3)	2.1 ± 0.1a	1.7 ± 0.2b	1.6 ± 0.1b	0.6 ± 0.2a	0.4 ± 0.2ab	0.2b
18:4(*n*-3)	4.3 ± 0.5a	3.4 ± 0.5b	2.5 ± 0.0c	1.8 ± 0.5	1.6 ± 0.1	1.5
20:5(*n*-3)	8.7 ± 0.7	8.4 ± 0.4	9.1 ± 0.1	7.8 ± 0.7	7.3 ± 0.6	8.0
22:5(*n*-3)	0.2 ± 0.1a	0.3 ± 0.1b	0.5 ± 0.0c	0.3 ± 0.1a	0.6 ± 0.1b	0.3a
22:6(*n*-3)	8.7 ± 1.0a	11.1 ± 2.3b	14.3 ± 0.8c	21.3 ± 1.4a	26.3 ± 1.5b	27.7b
(*n*-3)PUFA	24.4 ± 2.2	25.3 ± 2.1	28.5 ± 0.7	32.0 ± 1.8a	36.4 ± 1.9b	37.7b
(18:0)DMA	1.6 ± 0.2	1.8 ± 0.2	1.4 ± 0.1	11.5 ± 2.5	11.1 ± 1.0	15.7
Total DMA	2.2 ± 0.3	2.3 ± 0.2	2.0 ± 0.7	19.0 ± 3.4	17.5 ± 1.3	23.6
DHA/EPA	1.0 ± 0.1a	1.3 ± 0.2b	1.5 ± 0.1c	2.7 ± 0.3a	3.6 ± 0.3b	3.5b
n-3/*n*-6	2.9 ± 0.4	2.7 ± 0.2	3.0 ± 0.3	2.5 ± 0.2a	3.0 ± 0.4b	3.3b

Table 4 *Argopecten purpuratus*. Experiment 2: fatty acid composition (% of TFA and DMA) of total lipids of eggs produced under four different nutritional conditions (*abbreviations* for diets, see "Materials and methods"). Values are means (±SD). Values in one row followed by a *different letter* are significantly different (Newman–Keuls' test, $P < 0.05$) (*other abbreviations*, see Table 1)

Fatty acid	St (n = 6)	St + 50epa (n = 2)	St + 50hufa (n = 9)	St + 50coco (n = 12)
12:0	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.0b	1.7 ± 0.2c
14:0	3.3 ± 0.3a	3.3 ± 0.0a	3.0 ± 0.2b	4.2 ± 0.2c
16:0	17.8 ± 0.2ab	18.3 ± 0.3a	17.6 ± 0.4b	18.4 ± 0.4a
18:0	3.7 ± 0.3a	4.1 ± 0.3b	4.1 ± 0.2b	5.0 ± 0.2c
SAFA	27.6 ± 0.3a	27.6 ± 0.6a	27.3 ± 0.5a	31.4 ± 0.7b
16:1(*n*-7)	11.7 ± 0.4a	8.2 ± 0.4b	8.7 ± 0.6b	8.2 ± 0.3b
18:1(*n*-9)	6.6 ± 0.3a	9.4 ± 0.3b	9.0 ± 0.4b	10.1 ± 0.5c
18:1(*n*-7)	4.1 ± 0.1a	3.3 ± 0.0b	3.5 ± 0.2b	2.6 ± 0.1c
MUFA	25.3 ± 0.5a	24.0 ± 0.8b	24.0 ± 0.9b	23.7 ± 0.9b
18:2(*n*-6)	2.3 ± 0.1a	2.5 ± 0.2b	2.8 ± 0.2c	5.6 ± 0.2d
20:4(n6)	3.6 ± 0.4a	2.3 ± 0.1b	3.0 ± 0.1c	2.3 ± 0.2b
22:5(*n*-6)	2.6 ± 0.1a	1.6 ± 0.0b	2.1 ± 0.1c	1.9 ± 0.1d
(*n*-6)PUFA	10.0 ± 0.5a	7.5 ± 0.0b	9.3 ± 0.3a	12.5 ± 1.8c
18:3(*n*-3)	2.1 ± 0.1a	1.3 ± 0.1b	1.5 ± 0.1c	2.5 ± 0.9d
18:4(*n*-3)	5.0 ± 0.3a	2.7 ± 0.3b	3.1 ± 0.3c	5.8 ± 0.3d
20:5(*n*-3)	10.1 ± 0.2a	15.4 ± 0.0b	13.2 ± 0.7c	7.8 ± 0.5d
22:5(*n*-3)	0.2 ± 0.1a	1.4 ± 0.0b	1.0 ± 0.1c	0.2 ± 0.0d
22:6(*n*-3)	12.8 ± 0.4a	14.7 ± 0.5a	12.1 ± 0.5a	8.8 ± 2.5b
(*n*-3)PUFA	31.3 ± 0.6a	36.4 ± 1.0b	34.5 ± 1.3c	26.6 ± 1.0d
(18:0)DMA	3.3 ± 0.5	3.0 ± 0.7	3.2 ± 0.2	3.5 ± 0.3
Total DMA	5.4 ± 0.4a	4.4 ± 0.3b	4.8 ± 0.7ab	5.3 ± 0.4a
DHA/EPA	1.3 ± 0.0a	0.9 ± 0.3b	1.1 ± 0.0c	1.2 ± 0.1a
n-3/*n*-6	3.1 ± 0.2a	4.8 ± 0.1b	3.7 ± 0.2c	2.2 ± 0.3d

respectively. No significant difference was noticed between the latter two ($P = 0.08$).

The addition of EmCOCO supplement led to eggs with the highest SAFA content in TL, i.e. 12:0, 14:0 and 18:0 (Table 4). Eggs of these parent animals also showed the highest levels of 18:2(*n*-6), reflecting the high content of this fatty acid in EmCOCO. They had the lowest levels in EPA and DHA, and consequently in (*n*-3)PUFA. Broodstock animals fed on St + 50epa and St + 50hufa released eggs with a clear increase in 20:5(*n*-3) and 22:5(*n*-3) content. EPA content increased from 10.1% (St) to 15.4% and 13.2%, respectively, and 22:5(*n*-3) content increased from 0.2% to 1.4% and 1.0%, respectively. The DHA content on the other hand was not affected.

The distribution of the fatty acids over the different lipid groups was the same as found in expt 1 (Table 5). In the NL fraction, SAFA, MUFA and (*n*-3)PUFA represented each 30%, while (*n*-6)PUFA represented only 10%. The importance of MUFA in PL on the other hand was much less (10%), while DMA represented 21%.

The same differences in the fatty acid (FA) composition of the TL were found again in the FA composition of the NL fraction. Eggs of the St + 50coco-diet have significantly more SAFA and (*n*-6)PUFA and less (*n*-3)PUFA than the eggs of the other diets. The diets St + 50epa and St + 50hufa led to a higher content in EPA and in EPA and DHA, respectively, than the St-diet. The eggs resulting from the St-diet had the highest MUFA content. With regard to the PL fraction, there was no significant difference in SAFA and MUFA among the diets. The (*n*-6)PUFA fraction was also similar among the diets, except for the St + 50epa-eggs with a significant lower content. The diets St + 50epa and St + 50hufa resulted in eggs with a higher (*n*-3)PUFA content (more specifically EPA) in PL than the diets St and St + 50coco. The PL fraction had at least twice as much 22:6*n*-3 and at least seven times as much DMA as the NL fraction.

Lipid class analysis

Despite the addition of lipid emulsions during the conditioning of the adults, no significant increase in total lipid content was observed in the eggs compared to the eggs released by the animals fed with the St-diet (Table 6). The NL fraction represented roughly 85% of the total egg lipids and was dominated by triglycerides (TAG, roughly 90%). Free sterols and sterol esters made up the remaining 10%. The most important PL class was phosphatidylcholine (PC), representing about 71% (range 66–79%) of the PLs. The other 29% corresponded to phosphatidylethanolamine (PE, roughly 21%) and phosphatidylserine + phosphatidylinositol (PS + PI, roughly 6%).

Reducing the algal ratio by half (1/2St + 50dha, expt 1) led to a significantly lower PS + PI content in the eggs. The lower TAG content in eggs from animals fed with the St-diet in expt 2 led to a significantly lower content in total NL and a relatively higher content in PL; this was not the case for expt 1.

Discussion

The fatty acid profiles of the microalgae (*Isochrysis galbana* (T-iso), *Chaetoceros neogracile*) used in this work were similar to those previously reported (Napolitano et al. 1990; Delaunay et al. 1993; Thompson et al. 1993; Tzovenis et al. 1997). *C. neogracile* had a relatively

Table 5 *Argopecten purpuratus*. Experiment 2: fatty acid composition (% of TFA and DMA) of neutral (*NL*) and polar (*PL*) lipids of eggs produced under four different nutritional conditions (*abbreviations* for diets, see "Materials and methods"). Values are means (±SD). Values in one row belonging to each lipid category followed by a *different letter* are significantly different (Newman–Keuls' test, $P<0.05$) (*other abbreviations*, see Table 1)

Fatty acid	NL				PL			
	St (n=6)	St+50epa (n=2)	St+50hufa (n=9)	St+50coco (n=12)	St (n=6)	St+50epa (n=2)	St+50hufa (n=9)	St+50coco (n=12)
12:0	0.1±0.1a	0.1±0.0a	0.1±0.2a	1.9±0.3b	0.2±0.1	0.2±0.1	0.1±0.2	0.1±0.1
14:0	3.9±0.4a	3.7±0.1a	3.5±0.3a	5.2±0.2b	1.0±0.4	1.0±0.1	0.9±0.3	1.1±0.4
16:0	18.7±0.5a	20.0±0.0b	18.3±1.0a	20.2±0.5b	11.0±2.8	12.4±1.3	12.1±1.6	12.3±1.8
18:0	3.6±0.3a	4.2±0.1b	3.9±0.2a	4.8±0.2c	4.9±1.3a	4.8±0.8ab	6.0±0.4b	7.2±0.9c
SAFA	29.1±0.6a	30.3±0.1a	28.6±1.6a	34.2±0.6b	21.0±3.0	21.5±2.7	22.0±2.2	23.6±1.9
16:1(*n*-7)	13.7±0.7a	9.5±0.4b	9.9±0.5b	9.9±0.6b	2.6±1.8	2.0±0.3	2.0±0.7	1.9±0.5
18:1(*n*-9)	8.0±0.3a	10.9±0.3cd	10.5±0.6c	11.7±0.6d	2.5±1.6a	3.0±0.3ab	3.6±1.1ab	4.4±0.8b
18:1(*n*-7)	4.7±0.1a	3.8±0.1b	4.13±0.2b	3.2±0.2c	1.7±0.7	1.3±0.1	1.6±0.5	1.2±0.2
MUFA	29.6±0.9a	27.2±0.8b	28.4±1.2b	28.1±0.9b	10.7±4.0	9.7±0.7	10.6±2.1	10.7±1.5
18:2(*n*-6)	2.7±0.1a	2.7±0.0a	3.35±0.3b	6.3±0.3c	0.8±0.4a	0.9±0.1a	1.2±0.6a	2.7±1.0b
20:4(n6)	3.1±0.1a	2.1±0.1b	2.54±0.1c	1.8±0.1d	5.1±1.1a	3.1±0.1b	4.3±0.5a	4.4±0.8a
22:5(*n*-6)	2.0±0.2a	1.2±0.0b	1.6±0.2c	1.2±0.1b	5.0±0.5a	3.0±0.1b	3.7±0.3c	4.4±0.3d
(*n*-6)PUFA	9.6±0.5a	7.2±0.1b	8.9±0.4c	11.5±0.3d	12.0±0.6a	8.6±1.63b	10.8±2.3a	12.7±0.8a
18:3(*n*-3)	2.5±0.1a	1.4±0.1b	1.6±0.1c	2.7±0.1d	0.9±0.6	0.4±0.1	0.6±0.2	1.7±1.3
18:4(*n*-3)	5.4±0.4a	2.7±0.3b	3.3±0.4c	6.1±0.2d	2.3±0.5a	1.8±0.3a	1.7±0.3a	3.1±0.5b
20:5(*n*-3)	10.1±0.3a	15.3±0.1b	13.1±1.0c	7.4±0.4d	8.6±0.8a	12.0±0.3b	10.6±0.9c	8.0±0.8a
22:5(*n*-3)	0.2±0.1a	1.1±0.1b	0.8±0.1c	0.1±0.0d	0.3±0.1a	1.8±0.3b	1.3±0.4c	0.4±0.1a
22:6(*n*-3)	9.9±0.6a	11.8±0.3b	12.0±1.0b	6.3±0.6c	22.2±1.6a	23.2±0.5a	23.5±1.6a	19.8±1.9b
(*n*-3)PUFA	28.8±0.5a	33.2±0.8b	31.2±1.8c	23.5±1.0d	34.8±2.1a	40.0±1.5b	38.6±2.3b	33.6±2.1a
(18:0)DMA	1.8±0.5	1.15±0.1	1.6±0.2	1.6±0.4	12.0±4.5	13.7±1.2	11.8±2.0	13.3±1.3
Total DMA	2.4±0.7	1.85±0.1	2.5±0.3	2.5±0.6	21.1±8.1	20.9±2.1	18.8±3.3	19.0±1.7
DHA/EPA	1.0±0.1a	0.8±0.3b	0.9±0.0ac	0.8±0.1bc	2.6±0.4a	1.9±0.0b	2.2±0.3ab	2.5±0.2a
n-3/*n*-6	3.0±0.2a	5.5±0.1b	3.5±0.3c	2.0±0.1d	2.9±0.2a	4.8±1.1b	3.7±0.6c	2.7±0.2a

Table 6 *Argopecten purpuratus*. Lipid classes of eggs spawned in experiments 1 and 2 (*abbreviations* for diets, see "Materials and methods"). Values are means (±SD). Values in one row belonging to the column experiment 1 or experiment 2 followed by a *different letter* are significantly different (Newman–Keuls' test, $P<0.05$). Significant differences for total NL and total PL were determined by Mann–Whitney *U*-test because data were not homogeneous (*Chol.ester* cholesterol esters; *TAG* triglycerides; *FFA* free fatty acids; *Chol* cholesterol; *DAG* diglycerides; *NL* neutral lipids; *PE* phosphatidylethanolamine; *PA* phosphatic acid; *PS* phosphatidylserine; *PI* phosphatidylinositol; *PC* phosphatidylcholine; *PL* polar lipids; *TL* total lipids)

	Experiment 1			Experiment 2			
	St (*n*=6)	St+50dha (*n*=4)	1/2St+dha (*n*=1,**n*=2)	St (*n*=6)	St+epa (*n*=2)	St+hufa (*n*=9)	St+coco (*n*=12)
Chol.ester	3.3±0.6	3.6±0.6	2.9	4.3±1.0	2.9±0.7	3.4±1.0	2.9±0.8
TAG	76.8±2.9	78.4±0.6	76.9	70.±5.9a	78.0±2.6b	77.8±4.3b	78.6±3.0b
FFA	0.1±0.2	0.0±0.6	0.0	1.2±1.6a	3.2±1.5b	0.5±0.8a	0.7±0.8a
Chol+DAG	3.4±0.7	3.9±0.6	4.2	5.8±2.6	5.0±0.5	3.2±0.6	3.6±1.2
Total NL	83.7±1.8	86.0±1.2	84.1	81.4±2.4a	89.1±0.2b	85.0±4.4bc	85.9±1.6c
PE+PA	3.7±0.7	2.7±0.6	2.8	4.5±0.6a	2.5±0.9b	3.7±2.4b	3.2±0.6ab
PS+PI	1.3±0.3a	0.7±0.6ab	0.5b	1.1±0.4	0.8±0.4	0.9±0.4	0.7±0.3
PC	11.3±1.1	10.6±0.6	12.6	12.7±1.6a	7.2±1.1b	10.4±2.0c	10.1±1.2ac
Total PL	16.3±1.8	14.0±1.2	15.9	18.5±2.4a	10.9±0.2b	15.0±4.4bc	14.1±1.6c
TL	24.6±1.7	24.6±0.6	24.0±1.1*	21.1±2.6	24.4±0.1	23.4±2.4	22.4±3.4

high amount of arachidonic acid, which also corresponded with the findings of Napolitano et al. (1990).

Egg size

Both experiments demonstrated that the broodstock diet did not affect the size of the eggs or the DW of *Argopecten purpuratus*. Even the diet with only half the amount of algae (1/2St+50dha) resulted in equally sized eggs. This is in agreement with Caers et al. (2002), who found no difference in egg size of *Crassostrea gigas* among the mixed algal control diet, a single diet of *Dunaliella tertiolecta* and a single diet of *D. tertiolecta* supplemented with an emulsion rich in EPA and DHA. Bayne and Newell (1983) and Devauchelle and Mingant (1991) concluded that egg size is a species-specific characteristic. Farías and Uriarte (2001) found that the egg size of *A. purpuratus* fed high and normal protein diets was similar and concluded that the additional

protein in the high protein diet is mainly used for the production of a larger number of eggs and not for larger eggs. The same observation was made for *Placopecten magellanicus* when grown in poor environmental conditions (Napolitano et al. 1992). Nevertheless, other authors have observed that environmental conditions can affect the size and subsequently the viability of eggs (Bayne 1985; Gallager and Mann 1986).

Although the broodstock animals of expt 1 were considerably larger in size than those of expt 2 (78.3 and 66.6 mm, respectively), the egg size was very similar and varied between 52 and 56 μm. This would indicate that the egg size of *A. purpuratus* was independent of the size of fully mature parent animals. The average egg size observed in this study was rather low in comparison to other studies with the same species: 66.8 (Farías et al. 1998), 61.3 and 62.4 μm (Uriarte et al. 1996).

Fecundity

Millican and Helm (1994) showed that broodstock diet had an impact on the fecundity of *Ostrea edulis*. Laing and Lopez-Alvarado (1994) found that the higher lipid reserves in clams fed live diets released more eggs per female. Higher numbers of eggs per female were collected from oysters fed on algal and lipid supplements compared to those from non-fed *C. gigas kumamoto* (Robinson 1992). Our study demonstrated that supplementation of emulsions rich in HUFA to the St-diet improved fecundity of *A. purpuratus*, though not significantly (161% for St+50dha and 173% for St+50hufa when St=100%). This is in agreement with the findings of Caers et al. (1999b). Supplementation with 50%EmCOCO in expt 2, however, led to significantly higher egg production compared to the St-diet (278%) and St+50hufa-diet (161%). Saturated lipids seem more effective in increasing fecundity than polyunsaturated fatty acids. If energy supply is the key factor during gametogenesis, then broodstock animals were in need of an accessible energy source. Since energy of fats is more efficiently released via β-oxidation of saturated than unsaturated fats (Langdon and Waldock 1981; Lehninger 1982, cited in Thompson et al. 1993), adults may have benefited more from the extra supply in short-chained fatty acids and may have built up the greater energy reserves necessary for gamete production. The emulsion EmCOCO was also rich in 18:2(*n*-6), the levels of which increased significantly in the egg lipids of the NL and PL fractions when broodstock was fed St+50coco. This may be important since Santiago and Reyes (1993) demonstrated the impact of linoleic acid on fecundity for the Nile tilapia (*Oreochromis nilotica*).

When the algal diet was reduced to half (1/2St+50dha), fecundity dropped sharply. The EmDHA could not replace the efficiency of live algae at such a high replacement percentage. Low fecundity was possibly caused by resorption of oocytes, since the animals were put under nutritional stress. Such phenomena have been observed in *A. purpuratus* by Martínez et al. (1992) and in *O. edulis* by Lane (1989). Another possibility, however, is that the scallops reduced the clearance rate because chlorophyll concentrations were depleted below a certain threshold (threshold feeding response), as observed for *Argopecten irradians irradians* (Rheault and Rice 1996) and *Mytilus edulis* (Thompson and Bayne 1972). The findings of Martínez et al. (2000) support this idea, since they found more spawning animals when the scallops were fed the lipid-supplemented algal diet (70% microalgae+30%EmDHA) compared to the pure algal diet, only when the feeding ratio was set at 6% of dry biomass per day and not at 3%. The latter was considered to be an insufficient food supply.

A significant difference in fecundity was recorded between the St-diet groups of the two experiments, i.e. broodstock of expt 1 spawned an average of 5.36×10^6 eggs individually, while the animals of expt 2 spawned only 2.81×10^6 eggs. The size of the parent animals is very likely to have played an important role. Avendaño (1993, cited in Le Pennec et al. 1998) showed a positive correlation between the shell height of *A. purpuratus* and the number of eggs spawned. A substantial difference in condition and energy reserves between the two groups is also a possible reason, since the animals of expt 2 had just gone through a severe winter period under "natural" conditions (lantern net in Tongoy Bay), while the animals of expt 1 had received algae at libitum in the laboratory for 2 months previous to the experiment. The impact of the season on fertility in our study was negligible in comparison to the impact of the nutritional condition, since lower fecundity was expected for the "winter experiment (expt 1)", while the opposite occurred.

Egg total lipids and fatty acids

Lipids play an important role during embryogenesis prior to the development of a functional mechanism for particulate feeding. A study of the rock scallop *Crassadoma gigantea* demonstrated that 56.9% of the total energy of the egg was expended during embryogenesis and that lipid, protein and carbohydrate were catabolised linearly to contribute 46.7%, 43.5% and 9.8% of the total energy used, respectively (Whyte et al. 1990). Several authors believe that lipids are also an essential factor in the exotrophic phase and determine larval growth. This hypothesis seems to be validated in *M. edulis* (Bayne et al. 1975), *O. edulis* (Helm et al. 1973) and *Ostrea chilensis* (Wilson et al. 1996). However, it does not hold true for *Crassostrea gigas* (Gallager et al. 1986), *Crassostrea virginica* (Gallager and Mann 1986), *Mercenaria mercenaria* (Gallager and Mann 1986) and *Pecten maximus* (Delaunay et al. 1992).

The total lipid content of *A. purpuratus* eggs was approximately 23.5% on a DW basis or 5.5 ng egg^{-1}. This value is comparable to the 24.8% found by Caers

et al. (1999b) for eggs from *A. purpuratus* broodstock fed pure algae and in agreement with the 5.1 ng egg^{-1} found by Farías et al. (1998). The different maturation diets in our study did not change the total lipid content of the eggs, which is in disagreement with Caers et al. (1999b). They found that lipid supplementation resulted in a significant increase of the TL content in *A. purpuratus* eggs. Similarly, Gallager and Mann (1986) found that variations in the broodstock-conditioning protocol of *M. mercenaria* and *C. virginica* induced large fluctuations in egg lipid levels. Caers et al. (2002), however, demonstrated that the lipid content of *C. gigas* is diet independent, and Utting and Millican (1997) suggested that bivalves adjust their fecundity in order to maintain a consistent lipid level in the eggs. Millican and Helm (1994) and Laing and Lopez-Alvarado (1994) found that *O. edulis* and *Tapes philippinarum* maintain their egg production of a particular quality rather than produce a large number of eggs.

The essential fatty acid composition of the gonad and egg lipids in bivalves is related to the fatty acids in the microalgae fed to broodstock during hatchery conditioning (Whyte et al. 1990, 1991; Marty et al. 1992; Napolitano et al. 1992; Utting and Millican 1997; Caers et al. 1999b, 2000, 2002). Soudant et al. (1996a) demonstrated that the lipid composition of the eggs of *P. maximus* reflects the fatty acid profile of the gonad, and no change in fatty acid pattern occurs between gametogenesis and spawning. In the present work, the abundance of 16:0, 16:1(*n*-7) and 18:1(*n*-9) in the TL of the eggs reflected the high percentage of these fatty acids in the algae, but substantial amounts of algal 14:0 were not fully expressed in the fatty acid profile of the eggs. The significant increase of 20:5(*n*-3), 22:6(*n*-3) and 12:0 in the eggs of broodstock fed the lipid emulsions EmEPA, EmDHA and EmCOCO, respectively, illustrated that recently ingested fatty acids did significantly contribute to the lipid accumulation process in the maturing female gonad. This confirms the findings of Caers et al. (1999b). Although the lipid emulsions altered the fatty acid composition of the eggs, the relative abundance of the different lipid groups, i.e. SAFA, MUFA, (*n*-3)PUFA and (*n*-6)PUFA, was maintained in NL and PL fractions.

The high percentage in the PL fraction of 20:4(*n*-6) and especially 22:6(*n*-3) (twice as much as in the NL fraction) supports the idea of an important functional role for these fatty acids. It also indicates that the scallops could at least partially control which fatty acids were incorporated in the PL fraction. DHA would be important during egg differentiation, while ARA is important for the process of oogenesis (Soudant et al. 1996b). ARA is also the precursor to prostaglandins, which play an active role in water transport and osmoregulation in marine invertebrates (Freas and Grollman 1980, cited in Whyte et al. 1991).

The addition of 50%EmDHA (expt 1) led to a significant increase in DHA content in the TL (33%), NL (27%) and PL (23%) fractions of the released eggs, as compared to the St-diet eggs. The EPA content was not affected, in contrast to the findings of Caers et al. (1999b), who found a significantly lower EPA content when 50%EmDHA was supplemented to the algal diet of *A. purpuratus* broodstock. The 1/2St + 50dha-diet resulted in eggs with significantly less 16:1(*n*-7), due to the fact that less *C. neogracile* was offered to the parent animals. The loss in 16:1(*n*-7) content was compensated for by an increase in relative DHA content, originating from the emulsion.

Incorporation of additional EPA in eggs (expt 2) increased steadily in line with higher amounts offered to the parent animals. EPA levels increased by 30% and 23% in the NL and PL fraction, respectively, when 25%EmEPA (St + 50hufa) was supplemented and by 51% and 39%, respectively, when 50%EmEPA (St + 50epa) was added. This is in disagreement with the findings of Caers et al. (1999b), who found that the relative content of 20:5(*n*-3) in the PL fraction of *A. purpuratus* eggs was not changed when 50%EmEPA was supplemented to the algal diet.

When the conditioning diet contained high EPA levels, DHA was also being selectively incorporated into the NL fraction. This was illustrated by an increase of 19% and 21% in DHA content when the broodstock was offered the St + 50epa- and St + 50hufa-diet, respectively. It would seem that the DHA level in the NL fraction varied in order to maintain an appropriate DHA/EPA ratio of approximately 1.2 for TL, while the PL fraction was only affected when very high levels of additional DHA were offered (St + 50dha). In general, the EPA concentration in eggs was more readily affected by dietary (*n*-3)HUFA than DHA. This could be related to the fact that EPA is used as an energy source during embryogenesis (Whyte et al. 1990, 1991).

Compared to the St-diet, the supplementation of EmCOCO (expt 2), rich in 12:0 and 18:2(*n*-6), led to a significant increase of these fatty acids in the eggs and hence to higher SAFA (117%) and (*n*-6)PUFA (120%) contents in the NL fraction. The increase in these lipid fractions was compensated for by a decrease in EPA (73% of St-diet eggs) and DHA (63% of St-diet eggs). Such a trade-off was not seen in the PL fraction of the eggs, which maintained basically the same relative composition as the PL of the St-diet eggs. The DHA/EPA ratio in the TL fraction was maintained at 1.2. It would seem that the DHA/EPA ratio was more important than the absolute levels of DHA and EPA as long as a minimum threshold level was maintained.

The high percentage of 20:5(*n*-3) and 22:6(*n*-3) in the eggs of scallops fed solely algae (St-diet) cannot fully be explained by the algal content. It has been well described that fatty acids of the NL fraction of the digestive gland are transferred to the female gonad during gametogenesis (Vassallo 1973; Taylor and Venn 1979; Barber and Blake 1981, 1985b; Napolitano and Ackman 1993). Caers et al. (2000) suggested that because of the similarities between the fatty acid composition of the female gonad and the digestive gland of *A. purpuratus*, lipids

were transferred from the lipid-rich digestive gland to the female gonad.

Molluscs in general are rich in plasmalogens, which are primarily found in the PL fraction (PE and PC) and to a lesser extent in the NL fraction (Sargent 1989). Our data confirm that plasmalogens represented roughly 20% of the FAMEs in the polar fraction of *A. purpuratus* eggs. Comparable high levels of DMA in the polar lipids have been found in eggs of *C. gigas* (Caers et al. 2002). The fatty acid 18:0 is the most important alkenyl ether chain, regardless of the broodstock diet. Caers et al. (1998, 1999b) and Sargent (1989) also found that 18:0 was dominant in *A. purpuratus*, *C. gigas* and *T. philippinarum*, independent from organ (gonads, digestive gland, adductor muscle, gills, or mantle) or development stage (egg, spat, adult).

The exact function of plasmalogen and the importance of the fatty acid chain composition are not very clear yet, but they probably have a critical role in membrane integrity (Chapelle 1987; Caers et al. 1999a) and/or are involved in some type of transmembrane transport process (Rapport 1961). A role as endogenous antioxidant to protect polyunsaturated fatty acids against peroxyl-radical-promoted oxidation has been proposed by Hahnel et al. (1999) in mammalian cells.

Egg lipid classes

In general, the diets did not change the relative content of the lipid classes, except for expt 2, where the St-diet resulted in eggs with less TAG compared to the lipid-supplemented diets. This was not true for expt 1. Further confirmation is needed to prove that this difference was related to the broodstock diet.

Pecten maximus has a NL/PL ratio of 2–2.5 (Soudant et al. 1996a), while eggs of *Crassadoma gigantea* and *Patinopecten yessoensis* have a value of 3–4 (Whyte et al. 1990, 1991). Napolitano et al. 1992 found a value of 1.7–1.9 in *Placopecten magellanicus*. The eggs of *A. purpuratus* in our study had a ratio of 5–6, which is quite high in comparison to the above-mentioned values for other scallops. The high level of the NL fraction (about 85% of TL) in the *A. purpuratus* eggs was dominated by TAG (86–92%) and may imply a very high energy demand during embryogenesis. Several researchers have confirmed the findings of Gallager and Mann (1986) that, although phospholipids are partially catabolised, TAG is the predominant fuel for the process of embryogenesis (Bayne et al. 1975; Whyte et al. 1990). PC (71%) and to a minor degree PE (22%) were the quantitatively most important polar lipid classes. Caers et al. (2002) found that PC and PE were the dominant lipid classes in *Crassostrea gigas* eggs, each representing about 30% of the PL.

Although there was no follow-up of the fertilisation rate and D-larval survival in this study, Martínez et al. (2000) did not find a difference for these two parameters when comparing eggs from *A. purpuratus* broodstock fed the pure microalgal diet with those from broodstock fed the diet consisting of 70%algae + 30%EmDHA. This would be in line with our findings, since no difference in TL content was observed in the eggs resulting from non-supplemented and lipid-supplemented diets.

Conclusions

Results indicated that the total lipid content of the eggs of *A. purpuratus* was diet independent. A greater energy reserve was spent in a larger number of oocytes and not in bigger sized oocytes with a higher lipid content. The lipids supplied through the emulsions were at least partially allocated to the eggs, demonstrating that the fatty acid composition of the eggs could be manipulated, especially the NL fraction. Levels of EPA changed more rapidly than DHA levels, supporting the observation that they fulfilled an energetic and structural role, respectively. The St-diet supplemented with 50% EmCOCO resulted in significantly higher fecundity compared to the algal diet supplemented with 25%EmEPA + 25%EmDHA and the non-supplemented algal diet. It would seem that SAFAs were more easily or preferentially incorporated in the female gonads of *A. purpuratus*. The relative content of SAFA and 18:2(*n*-6) in these eggs rose significantly. The relative content of the highly unsaturated fatty acids EPA and DHA, on the other hand, was substantially lower in the NL fraction, but hardly affected in the PL fraction.

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