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Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus, 1758). Part II. New formulated feeds offer new perspectives to commercial hatcheries

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Abstract Five diets were compared for their efficiency at maturing the gonads of the blue mussel, *Mytilus edulis*. The diets consisted of a 1:1:1 mixture of *Isochrysis galbana* (T-Iso), *Pavlova lutheri*, and *Chaetoceros calcitrans* given at concentrations of 2.4×10^{11} cells day^{-1} for the positive control treatment (PF) and 3.0×10^{10} cells day^{-1} (=1/8) for the negative control treatment (NF). The other three treatments, MB10+, MyStock+, and Frippak+, consisted of the NF diet supplemented with one of the micro-encapsulated diets MB10 (mixture of dried algae), MyStock (formulated diet), and FRiPPAK® Fresh #1 CAR (larval shrimp diet) at a level of 0.2% of the live weight (LW). Treatments PF, MB10+, and MyStock+ led to high percentages of spawning animals (80, 78, and 85%, respectively) and large numbers of eggs (on average 3.0×10^6 eggs female^{-1}). Females given the NF and Frippak+ treatments produced only half the number of eggs per female, and only 17 and 6%, respectively, of the animals spawned. A high hatching rate was observed for all treatments, 71% for the pure algae diets PF and NF and more than 80% for the micro-encapsulated diets. The larvae resulting from the NF treatment were smaller, with 41% of D-larvae measuring less than 90 μm, whereas with the other treatments only 5–11% belonged to that size category. The four most important fatty acids found in mussel eggs were 16:0, 16:1($n-7$), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA); these represented at least 50% of the fatty acids in all treatments. The high DHA content of MB10 and MyStock was not reflected in the fatty acid composition of the eggs whereas the high concentration of linoleic acid in MyStock was. To our knowledge, this is the first time that mussels are successfully conditioned with formulated feeds.

Keywords Algae replacement · Blue mussel · Broodstock · Conditioning · Formulated diet · *Mytilus edulis*

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Abbreviations

LW	Live weight
PF	Positive control treatment
NF	Negative control treatment
MB10+	NF + 0.2% formulated diet MB10
MyStock+	NF + 0.2% formulated diet MyStock
Frippak+	NF + 0.2% FRiPPAK® Fresh #1 CAR
FAME	Fatty acid methyl esters
DW	Dry weight
EPA	Eicosapentaeneoic acid
DHA	Docosahexaenoic acid

Introduction

The commercial cultivation of *Mytilus edulis* is often hindered by the irregular supplies of seed for restocking ropes or for re-laying on the seabed. Huge amounts of mussel seed are now required to supply Europe's industry, but the growers are struggling to obtain sufficient supplies because of irregular spatfalls, predation, tidal forces, and increasing interference from the environmental lobby (Edwards 2005). As a consequence there is an increasing interest in hatchery-produced mussel seed, not only as a safety net against nature's capriciousness but also because it offers the opportunity to select animals for specific characteristics.

Many techniques are used to bring adult bivalves into spawning condition in a hatchery environment (Utting and Millican 1997). Conditions of utmost importance are temperature, and feed quantity and quality. Much work has been done with *Pecten maximus* (Soudant et al.1996a, b; Utting and Millican 1998), *Argopecten purpuratus* (Farías and Uriarte 2001; Martínez and Pérez 2003; Nevejan et al. 2003), *Ostrea edulis* (Helm et al. 1973; Berntsson et al. 1997), *Crassostrea gigas* (Chávez-Villalba et al. 2002; Fabioux et al. 2005) and *Placopecten magellanicus* (MacDonald and Thompson 1985; Pernet et al. 2003).

Because there are major differences in biochemical composition among different algal species, mixing several cultured species of unicellular algae as food for broodstock undoubtedly provides greater variation in amino acid and fatty acid composition. However, it is not cost effective for the industry to produce the large quantities of algae necessary for conditioning broodstock. Conditioning bivalves with complete or partial replacement of live algae diets with formulated diets may lead to substantially reduced production costs (Robinson 1992; Laing and Lopez-Alvarado 1994; Knauer and Southgate 1999). Robinson (1992) found that supplementary feeding with lipids or live algae during conditioning of *Crassostrea gigas Kumamoto* in both cases led to increased numbers of eggs released per female and increased spat yields, compared with unfed animals. *Tapes philippinarum* broodstock was successfully conditioned on a diet of 100% dried *Tetraselmis suecica* or 70% *T. suecica* + 30% *Cyclotella cryptica* although fecundity was lower than with live algae (Laing and Lopez-Alvarado 1994).

Live algae have been successfully replaced with dried algae (Langdon and Önal 1999) and formulated feeds (Nevejan et al. 2007) in trials with *Mytilus edulis* spat but, as far as we are aware, no literature is available about conditioning of *Mytilus edulis* broodstock with partial replacement of live algae. Conditioning of mussel broodstock with different mixtures of algae at different concentrations was described in Part I (Pronker et al. 2007); the usefulness of formulated feeds is described in this second part.

Materials and methods

Experimental set-up

The experimental set-up is described in detail by Pronker et al. (2007). Graded mussels were randomly divided into six conditioning groups of approximately 140 individuals each, weighing exactly 3 kg. The 40-l tanks received 1 μm UV-filtered seawater from a header tank at a constant rate of 1.2 l min^{-1}. During the first week the temperature was kept at 8.2°C (±0.9°C). During the second week temperature was raised gradually to reach the final temperature of 18°C. Detailed comparison is made between three different algae diets in Part I (Pronker et al. 2007) whereas in Part II emphasis is put on the performance of three formulated diets in relation to the two flagellate-dominated diets consisting of a mixture of *Isochrysis galbana* (T-Iso), *Pavlova lutheri* and *Chaetoceros calcitrans* (1:1:1, based on cell count).

The daily algae ratio did not change during the experiment and was set at a total of 2.4×10^{11} cells for the positive control treatment (PF; DW algae = 1.6% DW meat) and at 3.0×10^{10} cells for the negative control treatment (NF). The other three treatments MB10+, MyStock+, and Frippak+ consisted of the NF diet supplemented with one of three micro-encapsulated diets: the experimental diet MB10, consisting mainly of a mixture of dried algae, the experimental diet MyStock, a formulated diet without algae and with proteins of vegetable origin, and the commercially available diet FRiPPAK® Fresh #1 CAR (Frippak), designed for shrimp in their early larval Zoea stages. The proximate analysis of the formulated diets is given in Table 1. Frippak is richer in fat than the experimental diets and MyStock contained 10% more crude protein than the other two diets.

The daily amount of formulated feed (6 g) was equal to 0.2% of the live weight (LW). The daily amounts of algae and INVE diet were mixed and diluted with filtered seawater to attain a volume of 50 l. The feeding tanks were aerated to minimize sedimentation. The feed suspension was continuously pumped into the experimental tanks at a rate of 37 ml min^{-1}.

Details of the spawning procedure and larval rearing are fully described in Part I (Pronker et al. 2007). A total of 100 animals from each treatment were induced to spawn by temperature shock. Spawning females belonging to one treatment were divided into three sub-groups. The eggs were collected per individual female for counting and put together per sub-group afterwards. Egg samples were taken for fatty acid methyl ester (FAME) analysis and kept at −18°C. Each sub-group was fertilized with sperm from males belonging to the same treatment. Mussels that did not spawn were sacrificed and

Table 1 Biochemical analysis of the formulated diets (% DW)

Diet	Crude protein	Crude carbohydrate	Crude fat
MB10	53.6	7.3	15.4
MyStock	62.9	8.6	11.2
Frippak	53.3	4.6	19.5

Source: INVE Technologies, Hoogveld 93, 9200 Dendermonde, Belgium

DW: Dry weight

Crude carbohydrate was measured as starch

histologically checked for sex and state of gonadal ripeness. The embryos of each subgroup were stocked in 100-l tanks at a concentration of 15 embryos ml^{-1}. After 48 h, the number of hatched larvae was counted per tank and samples for size measurements were taken.

Analysis

The hatching rate was defined as described in Part I (Pronker et al. 2007).

FAME analysis of the formulated feeds, algae mixtures, and mussel eggs (frozen samples) was carried out as described in Nevejan et al. (2007). No eggs were collected from the Frippak+ treatment though, because insufficient eggs were available. Algae samples for FAME analysis were prepared by centrifuging a total of 0.5 l algae mixture at 14,000 rpm for 7 min. The algae paste was kept frozen (−18°C) until analysis.

The homogeneity of variances of means was tested by the Univariate test (Levene's test). Significant differences were detected using the one-way ANOVA, P being set at 0.05. The Tukey HSD test was used for post-hoc comparison. Percentages were asin$\sqrt{}$ transformed before ANOVA-analysis.

Results

Spawning success

Treatments PF, MB10+, and MyStock+ led to a high percentage of spawning animals (80, 78, and 85%, respectively) and large numbers of eggs (on average 3.0×10^6 eggs $female^{-1}$). Females of the NF and Frippak+ treatment produced only half the number of eggs per female (Table 2) and only 17 and 6%, respectively, of the animals spawned. As a result, total egg production per treatment varied from 9×10^7 (PF, MB10+, MyStock+) to $4–13 \times 10^6$ (Frippak+, NF) as illustrated in Fig. 1.

There was much variability in eggs produced by female mussels belonging to the same treatment. Production varied from less than 1.0×10^6 to more than 11.2×10^6 eggs per female. Therefore no significant difference could be detected between the treatments ($F(4,98) = 1.09$, $P = 0.37$). However, by categorizing the broodstock animals according to the number of eggs produced, differences between treatments became clear. Figure 2 illustrates that mussels that received the NF and the Frippak+ diet were less fertile.

Table 2 Spawning success of *Mytilus edulis* per treatment

	PF	NF	MB10+	MyStock+	Frippak+
Total animals	98	94	99	100	99
Total ♀ animals	36	44	40	39	52
Total ♂ animals	62	43	56	57	36
Unidentified animals	0	7	3	4	11
% spawning ♀ animals	75	18	80	85	6
% spawning ♂ animals	82	19	80	91	17
% spawning total animals	80	17	78	85	6
Average eggs/female ($\times 10^6$)	3.6	1.6	3.0	2.8	1.6

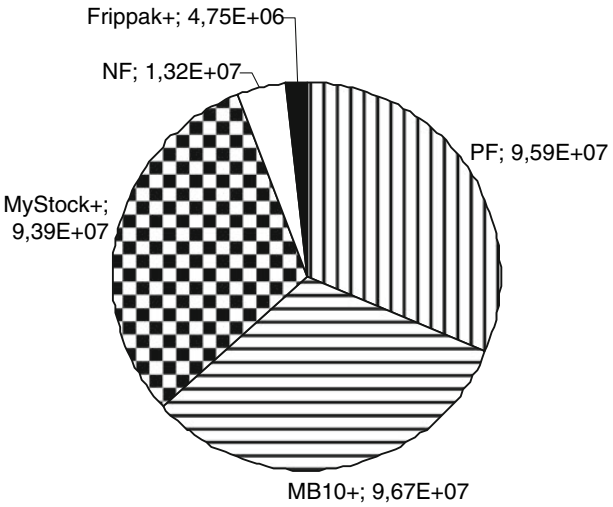

Fig 1 Total egg production of *Mytilus edulis* females conditioned with five different diets. *PF*, positive flagellate diet (2.4×10^{11} algal cells; T Iso: *P. lutheri*:*C. calcitrans* 1:1:1); *NF*, negative flagellate diet (3.0×10^{10} algal cells; T-Iso: *P. lutheri*:*C. calcitrans* 1:1:1); *MB10+*, NF + 0.2% MB10; MyStock+, NF + 0.2% MyStock; Frippak+, NF + 0.2% Frippak

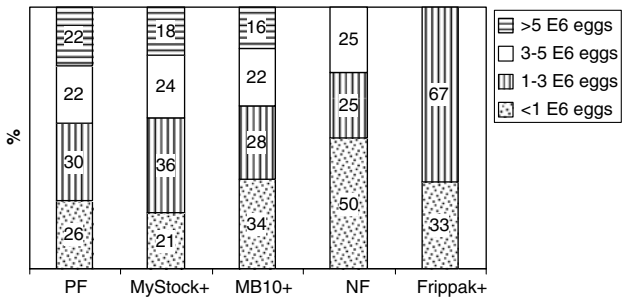

Fig 2 Percentage of spawning female mussels per category of recovered eggs. *PF*, positive flagellate diet (2.4×10^{11} algal cells; T Iso:*P. lutheri*:*C. calcitrans* 1:1:1); *NF*, negative flagellate diet (3.0×10^{10} algal cells; T-Iso:*P. lutheri*:*C. calcitrans* 1:1:1); *MB10+*, NF + 0.2% MB10; MyStock+, NF + 0.2% MyStock; Frippak+, NF + 0.2% Frippak

Animals of the NF treatment never produced more than five million eggs and half of the animals produced less than one million eggs. The three spawning females that were fed on Frippak+ never produced more than three million eggs. On the other hand, the pattern of egg production was similar for treatments PF, MyStock+, and MB10+, where 22, 18, and 16% of the females produced more than five million eggs.

Hatching rate and larval development

The hatching rate was very high for all treatments (Table 3). Of the 1.5 million embryos that were stocked in the tanks, more than 70% was recovered two days later as trochophore larvae or D-larvae. A significantly higher hatching rate (88.9%) was observed for broodstock fed MyStock+ than for those fed the pure algae diets PF and NF ($F(4,9) = 5.2$, $P = 0.019$).

The larvae resulting from the NF treatment were smaller than the others (Fig. 3). A total of 41% of D-larvae of the NF treatment measured less than 90 μm whereas only 5–11% of the other treatments belonged to that size category. Although fertility was very low for the

Table 3 Hatching rate of fertilized eggs spawned by mussel broodstock (*Mytilus edulis*) conditioned with five different diets

	% hatching rate (average ± SD)
PF ($n = 3$)	71.9 (±4.4)[a*]
NF ($n = 3$)	71.0 (±4.9)[a]
MB10+ ($n = 3$)	82.9 (±3.6)[a,b]
MyStock+ ($n = 3$)	88.9 (±7.8)[b]
Frippak+ ($n = 2$)	80.1 (±0.5)[a,b]

* Values followed by different superscript letters are significantly different (Tukey HSD test, $P < 0.05$)

PF: Positive flagellate diet (2.4×10^{11} algal cells; T-Iso:*P. lutheri*:*C. calcitrans* 1:1:1)

NF: Negative flagellate diet (3.0×10^{10} algal cells; T-Iso: *P. lutheri*:*C. calcitrans* 1:1:1)

MB10+: NF + 0.2% MB10

MyStock+: NF + 0.2% MyStock

Frippak+: NF + 0.2% Frippak

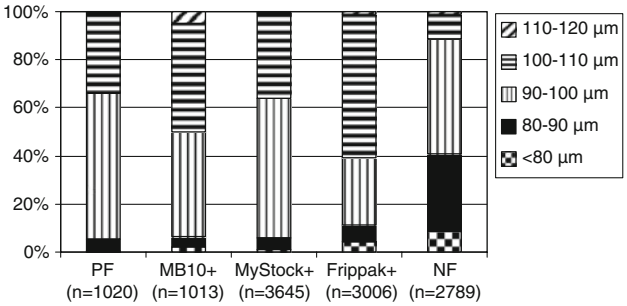

Fig 3 Distribution of size of two-day-old D-larvae per treatment. *PF*, positive flagellate diet (2.4×10^{11} algal cells; T Iso:*P. lutheri*:*C. calcitrans* 1:1:1); *NF*, negative flagellate diet (3.0×10^{10} algal cells; T-Iso: *P. lutheri*:*C. calcitrans* 1:1:1); *MB10+*, NF + 0.2% MB10; MyStock+, NF + 0.2% MyStock; Frippak+, NF + 0.2% Frippak

Frippak+ treatment, D-larvae size was not affected. In contrast, 61% of the larvae were bigger than 100 µm, while treatments PF, MB10+ and MyStock+ resulted in only 34, 50, and 36% of larvae, respectively, bigger than 100 µm.

Fatty acid analysis

The total amount of identified fatty acids was very similar for the algae mixture, MyStock, and MB10, and amounted to 96–115 mg gDW^{-1} (Table 4). The formulated diets MyStock and MB10 differed from the algae diet in the sense that they were richer in polyunsaturated fatty acids (PUFAs) and poorer in saturated fatty acids (SFAs)—the formulated diets contained approximately 60% PUFAs and between 15 and 22% SFAs whereas the algae mixture contained only 37% PUFAs and 36% SFAs. The algae mixture was particularly rich in 14:0. Frippak had a higher total FAME content (164 mg gDW^{-1}) than all the other diets, and consisted of 44% PUFAs and 29% SFAs.

Table 4 Fatty acid profile of formulated feeds and algae mixture PF/NF

$n = 1$	PF/NF		MyStock		MB10		Frippak	
	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}
14:0	15.83	16.90	0.95	0.90	0.80	0.90	3.64	5.90
16:0	15.86	17.00	9.58	8.70	15.78	18.70	19.62	31.50
16:1 ($n-7$)	18.89	22.40	1.56	1.60	2.06	2.70	4.55	8.10
17:0	1.98	2.30	0.07	0.10	0.19	0.30	0.11	0.20
18:0	0.43	0.50	4.05	3.70	4.10	4.90	4.69	7.50
18:1 ($n-9$)	1.19	1.40	12.75	12.90	7.66	10.10	14.17	25.30
18:1 ($n-7$)	1.84	2.20	1.87	1.90	2.02	2.70	2.37	4.20
18:2 ($n-6$)	1.15	1.40	19.54	19.70	4.74	6.30	8.76	15.70
18:3 ($n-3$)	1.19	1.40	1.47	1.50	2.53	3.30	1.18	2.10
18:4 ($n-3$)	3.73	4.40	1.09	1.10	0.97	1.30	0.93	1.70
20:1 ($n-9$)	0.12	0.10	1.47	1.50	1.27	1.70	1.46	2.60
20:4 ($n-6$)	0.78	0.90	1.45	1.50	1.61	2.10	1.56	2.80
20:4 ($n-3$)	0.00	0.00	0.73	0.70	0.84	1.10	0.75	1.30
20:5 ($n-3$)	16.35	19.40	13.78	13.90	15.18	20.00	7.01	12.50
22:1 ($n-9$)	0.00	0.00	1.44	1.40	1.33	1.80	0.48	0.90
21:5 ($n-3$)	0.00	0.00	0.71	0.70	0.84	1.10	0.34	0.60
22:5 ($n-6$)	0.80	1.00	0.81	0.80	0.95	1.30	0.76	1.40
22:5 ($n-3$)	0.10	0.10	2.27	2.30	2.60	3.40	2.44	4.40
22:6 ($n-3$)	7.01	8.70	17.30	18.30	17.47	24.20	14.82	27.80
SUM	89.00	102.30	95.40	95.60	88.20	114.70	93.90	164.10
$n-3 \geq 20:3$		28.40		36.20		50.10		46.90
$n-6 \geq 18:2$		4.20		22.40		13.20		22.50
$n-3/n-6$		6.78		1.62		3.79		2.08
DHA/EPA		0.50		1.30		1.20		2.20
Total FAME		102.00		96.00		115.00		164.00
SFA		37.00		14.00		26.00		48.00
MUFA		26.00		20.00		20.00		43.00
PUFA		38.00		61.00		68.00		73.00

PF/NF: Flagellate diet (T-Iso:*P. lutheri*:*C. calcitrans* 1:1:1)

Concerning the PUFAs, the eicosapentaenoic acid (EPA) content of the diets was very similar, varying between 12.5 (Frippak) and 20.0 mg gDW^{-1} (MB10). In contrast, the docosahexaenoic acid (DHA) content of Frippak, MB10, and MyStock was at least twice that in the algae mixture, reaching 27.8, 24.2, and 18.3 mg gDW^{-1}, respectively. MyStock was characterized by a high linoleic acid content of 19.7 mg gDW^{-1}.

Although the total mono-unsaturated fatty acid content was not very different, the fatty acid composition was. The algae mixture was 9 to 12 times richer in 16:1(n-7) than MB10 and MyStock. On the other hand, the formulated diets were 6 to 10 times richer in oleic acid and more than 10 times richer in 20:1(n-9).

Looking at the profile of the eggs, a first observation to make was the relative high content of total FAMEs in the eggs compared with the diets (Table 5). Fat was clearly

Table 5 Fatty acid profile of mussel eggs (*Mytilus edulis*) spawned by broodstock conditioned on four different diets

$n = 2$	PF		NF		MyStock+		MB10+	
	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}
14:0	4.45 (0.38)	7.9 0 (0.20)	2.70 (0.11)	3.70 (0.60)	2.20 (0.34)	3.40 (0.30)	2.20 (0.14)	3.20 (0.10)
16:0	21.01 (1.02)	37.20 (0.50)	20.06 (0.17)	27.20 (3.20)	17.70 (0.59)	28.10 (1.00)	20.20 (0.19)	30.00 (3.30)
16:1 ($n-7$)	13.96 (0.07)	27.50 (1.60)	12.85 (3.03)	19.20 (2.40)	9.30 (1.08)	16.50 (0.80)	10.60 (0.56)	17.60 (0.90)
17:0	0.27 (0.22)	0.50 (0.50)	0.15 (0.07)	0.20 (0.10)	0.50 (0.2)	0.90 (0.00)	0.30 (0.21)	0.50 (0.30)
18:0	1.92 (0.02)	3.40 (0.20)	1.81 (0.19)	2.50 (0.50)	1.90 (0.01)	3.00 (0.20)	2.00 (0.02)	2.90 (0.30)
18:1 ($n-9$)	2.62 (0.47)	5.10 (0.60)	3.74 (0.96)	5.60 (0.80)	5.10 (0.02)	9.10 (0.60)	4.10 (0.27)	6.80 (0.20)
18:1 ($n-7$)	3.34 (0.07)	6.60 (0.30)	2.98 (0.14)	4.50 (0.30)	2.80 (0.3)	5.00 (0.20)	2.80 (0.00)	4.70 (0.40)
18:2 ($n-6$)	0.91 (0.02)	1.80 (0.10)	1.01 (0.04)	1.60 (0.30)	6.00 (0.03)	10.60 (0.70)	1.70 (0.11)	2.90 (0.40)
18:3 ($n-3$)	0.59 (0.01)	1.20 (0.10)	0.60 (0.01)	0.90 (0.10)	0.60 (0.06)	1.10 (0.00)	0.90 (0.08)	1.50 (0.00)
18:4 ($n-3$)	1.07 (0.01)	2.10 (0.20)	0.68 (0.23)	1.00 (0.50)	0.60 (0.21)	1.10 (0.30)	0.70 (0.05)	1.20 (0.00)
20:1 ($n-9$)	1.49 (0.05)	2.90 (0.10)	2.47 (0.16)	3.70 (0.20)	2.80 (0.12)	4.90 (0.20)	2.50 (0.17)	4.20 (0.10)
20:4 ($n-6$)	2.24 (0.23)	4.40 (0.20)	2.19 (0.15)	3.30 (0.60)	2.20 (0.14)	3.90 (0.50)	2.30 (0.31)	3.80 (0.90)
20:4 ($n-3$)	0.11 (0.01)	0.20 (0.00)	0.14 (0.04)	0.20 (0.00)	0.10 (0.00)	0.20 (0.00)	0.20 (0.02)	0.30 (0.00)
20:5 ($n-3$)	16.16 (0.66)	31.80 (0.70)	14.65 (0.75)	22.10 (3.60)	12.70 (0.66)	22.40 (0.40)	15.10 (1.03)	25.00 (4.20)
22:1 ($n-9$)	0.34 (0.64)	0.70 (1.30)	0.04 (0.02)	0.10 (0.10)	0.21 (0.00)	0.40 (0.00)	0.10 (0.01)	0.20 (0.00)
21:5 ($n-3$)	0.46 (0.03)	0.90 (0.00)	0.61 (0.03)	0.90 (0.20)	0.70 (0.07)	1.20 (0.10)	0.60 (0.02)	1.10 (0.10)
22:5 ($n-6$)	0.60 (0.13)	1.20 (0.20)	0.56 (0.05)	0.80 (0.00)	0.60 (0.05)	1.10 (0.00)	0.70 (0.04)	1.10 (0.20)
22:5 ($n-3$)	1.01 (0.12)	2.00 (0.10)	1.33 (0.04)	2.00 (0.20)	1.70 (0.12)	3.00 (0.00)	1.90 (0.08)	3.10 (0.40)
22:6 ($n-3$)	8.25 (0.00)	17.00 (1.10)	9.25 (0.65)	14.60 (0.60)	10.70 (1.20)	19.80 (0.90)	11.20 (0.11)	19.50 (2.10)
SUM	85.00 (2.20)	162.60 (6.10)	82.40 (3.20)	121.00 (8.70)	82.50 (5.10)	142.90 (0.80)	84.50 (0.60)	136.80 (14.60)
$n-3 \geq 20:3$		52.20 (1.40)		39.90 (4.60)		46.60 (0.60)		49.10 (6.90)
$n-6 \geq 18:2$		9.00 (0.0)		6.70 (1.00)		17.70 (1.20)		9.30 (1.70)
$n-3/n-6$		5.79 (0.11)		5.97 (0.28)		2.60 (0.20)		5.30 (0.24)
DHA/EPA		0.50 (0.00)		0.70 (0.10)		0.90 (0.00)		0.80 (0.00)

Table 5 continued

$n = 2$	PF		NF		MyStock+		MB10+	
	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}	Area (%)	mg gDW^{-1}
Total FAME		163.00 (6.00)		121.00 (8.00)		142.90 (1.00)		136.80 (14.00)
SFA		51.00 (2.00)		36.00 (5.00)		37.20 (1.00)		38.70 (4.00)
MUFA		47.00 (3.00)		37.00 (2.00)		39.20 (0.00)		37.00 (2.00)
PUFA		64.00 (1.00)		49.00 (7.00)		66.60 (1.000)		61.10 (8.00)

PF: Positive flagellate diet (2.4×10^{11} algal cells; T-Iso:*P. lutheri*:*C. calcitrans* 1:1:1)

NF: Negative flagellate diet (3.0×10^{10} algal cells; T-Iso:*P. lutheri*:*C. calcitrans* 1:1:1)

MB10+: NF + 0.2% MB10

MyStock+: NF + 0.2% MyStock

accumulated in the eggs in the PF treatment, and the FAME content was increased by 60%. Although the broodstock of the NF treatment received only 1/8 of the PF diet, the FAME content still amounted to 75% of the content of PF eggs. Addition of MB10 and MyStock to the NF diet raised the FAME content of eggs further, with 10 and 14%, respectively.

The four most important fatty acids found in mussel eggs were palmitic acid 16:0, 16:1 ($n-7$), EPA, and DHA. They represented at least 50% of the fatty acids in all treatments.

The EPA content of the eggs of all treatments reflected the concentration of EPA present in the diet, which was pretty constant, varying between 13 and 16%. In absolute values, the EPA content of eggs increases considerably because of the higher lipid content of eggs compared to the feed. In contrast, the DHA content of the formulated diets MB10 and MyStock was more than twice that of the other diets but did not affect the DHA content of the spawned eggs. The relative and absolute amounts of DHA in the eggs were very similar among all treatments, being 8–11% and 14.6–19.8 mg gDW^{-1}, respectively.

Oleic acid was also accumulated in the eggs, even more so when the broodstock received the diets MyStock or MB10 in addition to the algae. The oleic acid content of the algae was 1.4 mg gDW^{-1} whereas 5.6 mg gDW^{-1} was recovered from the eggs of diet NF and 9.1 mg gDW^{-1} from the eggs of diet MyStock. The high linoleic acid content of the MyStock diet is reflected by that of the eggs—10.6 mg gDW^{-1} linoleic acid compared with 1.6 mg gDW^{-1} for eggs of the NF treatment.

Discussion

Taking into account an average dry weight of the algal mixture of 22.6 (±1.8) pg per cell (Pronker et al. 2007), animals of the PF treatment received a total of 1.81 g DW algae day^{-1} kg LW^{-1}, while the NF diet only provided 0.22 g DW algae day^{-1} kg LW^{-1}. The artificial diets contributed another 2 g DW day^{-1} kg LW^{-1} or 90% of the total feed DW for the supplemented treatments MB10+, MyStock+, and Frippak+.

Despite this very high replacement factor, a total of 78% (MB10+) and 85% (MyStock+) of the animals spawned, which was very similar to the 80% obtained with the 100% algae diet PF. This very high percentage indicates that the conditioning method was appropriate for the blue mussel. Animals of the PF, MB10+, and MyStock+ treatments produced, respectively, 3.6, 3.0, and 2.8 million eggs per female on average.

The negative control treatment NF demonstrated clearly that underfed mussels were less fertile, because only 17% of the broodstock animals spawned after six weeks conditioning and the number of eggs per female amounted to 1.6 million only. Bayne et al. (1978) observed that mussels under stress from high temperature and lack of food still developed new gametes during conditioning but that there was simultaneous regression and resorption of previously formed gametes. Oocyte lysis was also linked to energy deficiency caused by low food availability for *Pecten maximus* (Lubet et al. 1987).

There was hardly any difference in spawning success between male and female animals, except for the Frippak treatment where three times more males spawned than females (17 and 6% respectively).

Although broodstock animals fed with Frippak+ had a very similar biochemical composition and tissue DW (results not shown) to the animals receiving diet PF, MB10+, and MyStock+, the former spawned very poorly. Visual inspection of the Frippak+ animals that did not spawn revealed thick fatty mantel tissues without gametes. This was in big contrast with the animals of the NF treatment which lost tissue weight and had a very transparent

and thin mantel. The weight loss was probably because the inadequate food ration resulted in utilization of body reserves (Lane 1989). Despite Frippak being suitable for mollusks in terms of particle size and gross biochemical composition, maturation of gonads in mussel broodstock was not achieved. The animals performed even worse than the negative control treatment NF. It seemed that the mussels were capable of ingesting and digesting the Frippak diet (hence elevated DW and thick mantel tissue) but that some specific nutrients were missing to trigger them to use the energy for gamete development. The size of the larvae resulting from the Frippak+ treatment was surprisingly large, but one needs to consider that larvae of only three females were measured, hardly representing a population average.

Lane (1989), however, found that oysters (*O. edulis*) fed *Pavlova lutheri*, as a full (6% DW/W) or half ration, supplemented with FRiPPAK CAR capsules produced the largest number of broods, and that significantly more larvae were produced from oysters fed the mixture of algae and capsules. One must consider however that only 25,000–38,000 larvae per oyster were produced in that experiment which was very low and, in fact, comparable with the production of their unfed oyster broodstock (20,000 larvae oyster^{-1}). Furthermore, a third-generation FRiPPAK® Fresh #1 CAR product was used in our trial, which may differ in ingredients.

The different fatty acid composition of the diets was not directly reflected in the fatty acid composition of the eggs. A higher DHA content was expected in the eggs from treatments MB10+ and MyStock+, because the diets contained twice as much DHA as the algal diet, but these eggs had a DHA content of 11.2 and 10.7% respectively, quite similar to the 8.25 and 9.25% of the PF and NF eggs. Mussels seem to possess a controlling mechanism to even-out excesses of a particular fatty acid. Similar observations were made by Nevejan et al. (2007), in mussel spat for arachidonic acid, and by Whyte et al. (2002), who suggested blue mussels were capable of selectively achieving steady-state concentrations of individual fatty acids, with excess dietary acids being egested.

The high concentration of linoleic acid in the MyStock+ diet (19.7 mg gDW^{-1}) was, however, also found in the eggs, which contained six times more linoleic acid (10.6 mg gDW^{-1}) than eggs resulting from the 100% algal diets PF and NF (1.8 mg gDW^{-1}). This observation indicates that the lipids incorporated in the eggs do not only originate from the adult's energy stored before the start of the gametogenesis but that recently ingested food also contributes to the energy build-up of the eggs. Nevejan et al. (2003) also concluded that the fatty acid composition of *A. purpuratus* eggs, especially the neutral lipid fraction, could be manipulated by addition of emulsions during conditioning. Helm et al. (1991) suggested that PUFAs in flat oyster larval phospholipids originated from the parent's fatty acid pool, stored prior to or during the early stages of oogenesis. The PUFA content of the neutral lipids, however, was a reflection of what was deposited in the oocytes, during the later stages of development, from the diet available to adults in that period. Soudant et al. (1996a) also observed that the PUFA composition of neutral and polar lipids of *Pecten maximus* eggs was related to the fatty acid composition of the diet.

The total amount of FAMEs recovered in the eggs of the NF treatment amounted to 75% of the amount found in PF eggs, although the amount of algae fed to the broodstock was reduced to 1/8. On the other hand, larvae of the NF treatment were smaller than those of the other treatments (41% <90 μm), suggesting a negative effect of nutritional stress on larval condition. Bayne et al. (1978) also concluded that when mussel eggs are produced under stressful conditions their biochemical composition expressed as μg of biochemical component per mg DW, is maintained within fairly narrow limits but the eggs are fewer and contain less organic matter. Especially the lipid content per egg seemed to be greatly

affected, leading to less viable larvae. Unfortunately, DW and size of eggs were not determined in this experiment, so the larval performance was the only indicator that could be used to confirm this statement by Bayne et al. (1978). This is in contrast with the findings of Millican and Helm (1994), Laing and Lopez-Alvarado (1994), and Nevejan et al. (2003) who suggested that broodstock of the flat oyster (*O. edulis*), Manila clam (*Tapes philippinarum*), and Chilean scallop (*Argopecten purpuratus*), respectively, which possess lower lipid reserves produce fewer eggs/larvae (in the case of *O. edulis*) to maintain a particular quality of eggs/larvae.

Not only did the formulated feeds result in a similar broodstock fecundity as the positive flagellate diet, the hatching rate was very high (80%) and the resulting larvae followed the same growth pattern.

To our knowledge, this is the first time that mussels are successfully conditioned with formulated feeds. It allows hatcheries to condition mussels with only a fraction of the amount of algae normally necessary to get the animals in spawning condition. Although results are comparable with the PF diet, Part I revealed that a 2/3 diatom+1/3 flagellate diet (PD-diet) resulted in the best spawning yields in terms of number of eggs produced per female. It is, therefore, suggested that use of a diatom-dominated algae diet in addition to MB10 or MyStock may enhance the performance of the formulated diets even further. Following on that, a low-food control (NF) was used instead of an unfed control because the presence of algae was considered necessary. Ward and Targett (1989) mentioned that chemical stimulation by epiparticulate compounds on the surface of algae cells or digestive feed-back from ingested algae could play an important role in the stimulation of filtration. Further testing is necessary, however, to confirm both hypotheses.

In general, the amounts of the individual fatty acids of the formulated diets MB10 and MyStock corresponded well with the fatty acid composition of the algae diet, which may at least partly explain their success in replacing algae. The small overall changes in the fatty acid profile of the mussel eggs would seem to suggest that the lipid requirements of mussels are satisfied by the formulated diets.

The use of experimental diets MB10 and MyStock as a conditioning diet for the blue mussel led to highly comparable results, although they contain different ingredients. The algae-free diet MyStock is of particular interest since a constant quality can be easily guaranteed and the formulation cost is much lower (INVE Technologies, personal communication).

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