Use of centrifuged and preserved microalgae for feeding juvenile Manila clam (*Tapes philippinarum*): effects on growth and fatty acid composition

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ABSTRACT

The objective of the present study was to assess the nutritional value of three diets containing commercial preserved microalgae pastes and the relationship of their biochemical composition to the growth rate and fatty acids profile of juvenile *Tapes philippinarum*.

The feeding period was 6 weeks. At a daily ratio of 1% dry weight live weight of the clams^{-1}, the trispecific diet with 40% of *I. galbana*, 20% of *T. suecica* and 40% of *C. muelleri* supported greater growth of animals than either a mixture of 80% of *I. galbana* and 20% *T. suecica* or *I. galbana* alone (P< 0.05). Even the fatty acid profile was significantly modified depending on the composition of the diets. In particular a decrease of EPA was observed in clams with the lowest growth rate.

Key Words: Manila clam, *Tapes philippinarum*, Algal pastes, Bivalve nutrition, Fatty acids.

RIASSUNTO
UTILIZZO DI ALGHE MONOCELLULARI CONCENTRATE E CONSERVATE NELL'ALIMENTAZIONE DELLA VONGOLA VERACE (*TAPES PHILIPPINARUM*): EFFETTI SULL'ACCRESCIMENTO E SULLA COMPOSIZIONE IN ACIDI GRASSI

Lo scopo di questo studio è stato quello di determinare il valore nutrizionale di tre diete contenti prodotti commerciali costituiti da alghe monocellulari concentrate e l'influenza della loro composizione sugli accrescimenti e il profilo in acidi grassi di giovanili di vongole veraci Tapes philippinarum. *Il periodo di alimentazione è durato 6 settimane. Utilizzando una razione giornaliera pari a 1% di peso secco di alimento peso vivo degli animali^{-1}, la dieta costituita da una miscela di tre alghe con il 40% di* I. galbana, *20% di* T. suecica *e 40% di* C. muelleri *ha dato una crescita statisticamente superiore rispetto alla dieta costituita dall'80% di* I. galbana *e il 20% di* T. suecica *o alla dieta costituita esclusivamente da* I. galbana *(P< 0.05). Anche il profilo in acidi grassi è stato modificato in modo significativo in relazione alla dieta utilizzata. In particolare è stato osservato un calo di EPA nelle vongole con il tasso di crescita più basso.*

Parole chiave: *Vongola verace,* Tapes philippinarum, *Alghe concentrate, Nutrizione dei bivalvi, Acidi grassi.*

Introduction

The mass production of microalgae is identified as a major cost in mollusc rearing in hatcheries and nurseries. In fact, this practice requires special expertise, manpower and devoted facilities, covering 20-50% of the overall seed production costs (Coutteau and Sorgeloos, 1992; Borowitzka, 1999). In order to reduce the expenses for producing algae in hatcheries, many authors have tried to find alternative feeds. There would be many benefits in using substitute products in mollusc feeding: a guaranteed food supply, even during peak demand or in case of a loss of fresh algae cultures, standardized feed quality, better control of food bacteria load, a reduction in overhead costs, a reduction in space and labour demands. Over the past few years, inert food such as microcapsules (Jones *et al.*, 1974), yeast and bacteria (Nell *et al.*, 1996), cornstarch (Fernandez-Reiriz *et al.*, 1999) or preserved microalgae (Knauer and Southgate, 1996; Robert *et al.*, 2001; Brown and Robert, 2002) have been studied. To evaluate the efficacy of a new product, many nutritional factors could be taken into account, but among all the other parameters, the fatty acids profile (Fernandez-Reiriz *et al.*, 1999) and gross biochemical composition (Albentosa *et al.*, 1996b) play an important role in determining different growth rates of molluscs. At present, among all the alternative feeds, preserved microalgae seem to be the most promising "off the shelf" products for molluscs (Robert *et al.*, 2001; Brown and Robert, 2002). Preserved microalgae are similar to fresh cells in terms of size, biochemical composition and fatty acid profile even though the concentration and preservation procedures can modify their nutritive and biochemical characteristics. Although the production of dried microalgae and microalgae pastes is relatively simple and many products are already on the market, only few studies have been carried out to determine their nutritive value and most of the data available, which originate from commercial hatcheries, remain elusive or confidential (Robert and Trintignac, 1997).

The objective of this study was to assess the nutritional value of three diets containing commercial preserved microalgae pastes and the relationship of their biochemical composition to the growth rate and fatty acids profile of juvenile *Tapes philippinarum*.

Material and methods

Spat

The experiment was carried out with juvenile Manila clam spat (*Tapes philippinarum*) obtained from broodstock of the Almar (Marano Lagunare - Udine, Italy) hatchery. From the stock, spat with an initial live weight of about 34.45 ± 13.40 mg ind^{-1}, and a size of 5.20 ± 0.71 mm ind^{-1} were selected and randomly distributed between the experimental groups.

Diets

Three commercial concentrate microalgae were used in this study: *Isochrysis galbana* (clone T-ISO), *Tetraselmis suecica* (Ply429) and *Chaetoceros muelleri* (CHGRA) marked as "Isochrysis 1200 Premium®" (Iso), "Tetraselmis 3600 Premium®" (Tetra), and "Chaetoceros 1000 Premium®" (Chaeto), respectively (Reed Mariculture Inc., USA). The algae produced in the Reed Mariculture Inc. (San Jose, CA) facility, were autotrophically cultured in f/2 medium using closed recirculating photobioreactors, harvested, concentrated by centrifugation and stored at 4°C according to manufacture instructions. The concentrated microalgae were produced and immediately sent to the hatchery where the trial was carried out and stored in hermetically sealed plastic boxes until the beginning of the experiment. There was a one-week interval between microalgae production and the start of the experiment. *Isochrysis* cells have a particle size range of 5-6 μm, *Tetraselmis* cells have a particle size range of 8-16 μm while *Chaetoceros* cells have a particle size range of 6-9 μm (manufacturers information). Three different diets were supplied at a daily ratio expressed as DW (dry weight) of 1% of the spat live weight: (1) monospecific (100% *I. galbana*/DW, [Diet M]), which has been used as a control diet in other studies (Pèrez-Camacho *et al.*, 1998; Fernàndez-Reiriz *et al.*, 1999; Albentosa *et al.*, 2002); (2) bispecific (80% *I. galbana* + 20% *T. suecica*/DW, [Diet B]); (3) trispecific (40% *I. galbana* + 20% *T. suecica* + 40% *C. muelleri*/DW, [Diet T]). The weighed amounts of each concentrated

microalgae were re-suspended in seawater, gently mixed and added to the tanks. The biomass increase resulting from spat growth was taken into account to maintain a constant feed/clams ratio. The animals were fed once a day over a period of 6 weeks.

Culture conditions and experimental design

The experimental system consisted of 500-l tanks filled with seawater and provided with an air stone to avoid food settlement. Each tank contained two down welling silos with an air-water lift to carry the water from the tank into the silos. The initial concentration of the spat was 500 g LW (live weight) per silo. Twice a week the animals were harvested, rinsed with tap water and restocked with new culture water. Water temperature was maintained at 18°C by heating the room. The cultures were maintained under continuous light conditions. Spat biomass was monitored every second week by weighing each group after draining and drying for 10 minutes at room temperature. The daily growth rate (DGR) was calculated according to Caers *et al.* (1998). All treatments were carried out in four replicates. To make a comparison between the results obtained with the experimental diets and a hatchery practical diet, another group of clams allocated in 4 silos presenting the same culture conditions of the others, was fed fresh algae (40% *I. galbana* + 20% *T. suecica* + 40% *C. muelleri*) throughout the experiment.

Table 1. Proximate analysis (as g 100g^{-1}) of microalgae pastes fed to juvenile *T. philippinarum*.

| | Microalgae pastes | | |
	Iso	Tetra	Chaeto
Protein	48.2	55.3	32.3
Lipid	5.9	11.4	6.1
Carbohydrate	9.8	7.7	5.8

Iso = Isochrysis 1200 Premium®,
Tetra = Tetraselmis 3600 Premium®,
Chaeto = Chaetoceros 1000 Premium® (Reed Mariculture Inc., USA)

Analytical methods

At the end of the experiment, spat were starved for 24 hours to empty the content from the gut . Dry weight (DW as % of LW, 24 h at 60°C), ash (as % of DW, 24 h at 450°C), ash free dry weight (AFDW, considered as the difference between DW and ash) were determined on a randomly selected group of 100 juveniles per silo. Analysis of microalgae was made at the beginning of the experiment. The dry weight of the microalgae concentrate was determined after drying known volumes at 60° for 24 h. For proximate analysis of the microalgae, total protein and total carbohydrates were determined using the Kjeldahl method (nitrogen X 6.25) and according Dubois *et al.* (1956), respectively. Lipids of algae and clams were extracted using the method of Folch *et al.* (1957). Fatty acid methyl esters (FAME) of total lipid were prepared by acidified methylation with 1% sulphuric acid in methanol (v/v). Fatty acid composition of both algae and clams was determined in a GC Varian 3380 01 gas chromatograph fitted with a 30m_0.25μm_0.325mm capillary column (DB-23 J&W Scientific). Nitrogen was used as a carrier gas. Individual FAME was identified by comparison with known standards (Sigma-Aldrich) and a well-characterised oil (Supelco).

Statistical analysis

Data were expressed as means ± S.D. (standard deviation). Results were subjected to a one-way ANOVA with a level of significance of $P < 0.05$. Differences between each one of the treatments were compared by Newmann-Keuls multiple comparison test using the software–programme GraphPad Prism 3.00 (Graph Pad Software, San Diego, CA).

Results and discussion

Proximate composition of the microalgae pastes

The proximate composition (g 100g^{-1}) of ingredients used in the trial, is shown in Table 1. Protein was a major constituent in all the products. Values ranged from 55.3 g 100g^{-1} in Tetra and 48.2 g 100g^{-1} in Iso to 32.3 g 100g^{-1} in Chaeto. The lipid content of Tetra (11.4 g 100g^{-1}) was higher than that of Chaeto (6.1 g 100g^{-1}) and Iso (5.8 g

100g^{-1}) while the highest proportion of carbohydrates was found in Iso (9.8 g 100 g^{-1}) followed by Tetra (7.7 g 100g^{-1}) and Chaeto (5.8 g 100g^{-1}).

Fatty acid composition of the microalgae pastes

The fatty acid composition of each ingredient (as percentage of the total fatty acid) is shown in Table 2. The highest proportion of saturated fatty acids was found in Chaeto (33.0%) followed by Iso (30.8%) and Tetra (20.5%). Monounsaturated fatty acids were the major family in Chaeto (29.8%) while the content of these acids was lower in Iso (16.4%) and in Tetra (11.5%). Fatty acids of the polyunsaturated family were abundant in Tetra (57.5%) and also in Iso (49.6%). Chaeto contained a lower content of these fatty acids (31.8%). The percentage of n-6 fatty acids in Chaeto was 4.3% whereas in Tetra and Iso, it was 3.2 and 11.6%, respectively. The n-3/n-6 ratio was 3.2, 17.0 and 6.3 in Iso, Tetra and Chaeto, respectively. The fatty acids predominant in Iso were 18:4 n-3 and 14:0. The fatty acids 18:3 n-3, 16:0 and 16:4 n-3 were the major fatty acids in Tetra. Chaeto showed a high content of 16:1 and 20:5 n-3. Differences were also recorded in the contents of some of the essential fatty acids, that is, EPA, DHA and AA. Iso had a high content of DHA (9.0%) but very low amount of EPA (0.8%). Tetra had a very low content of DHA (0.1%) but EPA was detected in relatively high amounts (4.7%). Chaeto showed a high content of EPA (23.6%) and a low proportion of DHA (1.6%). The highest percentage of AA was found in Chaeto (1.2%) while Iso and Tetra contain 0.2 and 0.3%, respectively.

Growth

The average DGR of the spat fed the various diets during 6 weeks is given in Table 3. No mortality was observed during the experimental period. The diet T supported the best growth throughout the experiment when compared to the other groups. In fact, the average DGR of clams fed diet T (0.61) was significantly (P<0.05) faster than the other two groups. Significant differences were also noted among the three diets during the weeks 1-2 while during the weeks 3-4 significant differences were observed between clams fed diet T and clams fed diet M and B. The DGR decreased during the 6 weeks of feeding for all the treatments. The final

weight of the spat (Table 4) differs depending on the composition of the diets, even though differences were not statically significant. In fact, the dry weight of clams at the end of the experiment varies between 50.2 ± 5.4 mg DW ind^{-1} fed diet T, 46.8 ± 3.2 mg DW ind^{-1} fed diet B and 43.28 ± 4.20 mg DW ind^{-1} fed diet M. The percentage of DW (on LW) and of AFDW (on DW) were similar for any group, with values around 60% and 14%, respectively. Also the lipid content expressed both as mg g DW^{-1} or as percentage of AFDW was not significantly different between groups.

Fatty acid composition of the clams

The fatty acid composition of the clams fed the experimental diets is given in Table 5. As previously studied in the diets, polyunsaturated fatty acids were the most abundant fatty acid group, followed by the saturated fatty acids and monounsaturated fatty acids. The content of monounsaturated fatty acids of clams fed diet T (21.3%) was significantly different (P<0.05) from those fed diets B and M (19.0% and 18.6%, respectively). The main fatty acids present in the clams were 16:0 and 22:6 n-3 in all the groups. Among the most abundant fatty acids, the diet had a significant influence on 14:0, 18:1 n-9, 18:1 n-7, 18:3 n-3, 18:4 n-3 and 20:5 n-3. Because of the statistically different amount of EPA, even the DHA/EPA and EPA/AA ratios showed significant differences among groups. The highest DHA/EPA ratio was found in clams fed diet M (3.7) while clams fed diet B and T presented a value of 3.0 and 1.7, respectively, while the EPA/AA ratio of clams fed diet M (1.0) was significantly lower than that of clams fed diets T (1.7) and B (1.8).

Our study showed that the three diets used to feed juvenile *Tapes philippinarum* are characterized by a different nutritional value. In fact, the average daily growth rate of the animals fed Diet T was significantly higher than those fed Diet B or Diet M. Considering the feeding ratio (1% DW LW of the clams^{-1}) was the same for all the treatments, this result may be related to a different food quality of the three microalgae pastes used in the experiment. The three algae species evaluated are the most used in the hatchery and nursery of bivalves, as reported in an international questionnaire made from Coutteau and Sorgeloos (1992).

The fact that the trispecific diet (diet T) achieved the highest growth rate is not surprising since the diets of mixed species generally produce greater growth than monospecific diets. This has been attributed to the fact that these diets provide a more balanced mix of essential nutrients (Webb

Table 2. Fatty acid composition (% of the total fatty acid) of the microalgae pastes used in the feeding experiment with juvenile *T. Philippinarum*.

| Fatty acid* | Microalgae pastes | | |
| | Iso | Tetra | Chaeto |
	%	%	%
14:0	18.8	0.4	12.0
14:1	0.2	-	1.3
15:0	0.3	0.7	0.8
15:1	0.5	-	1.2
16:0	10.0	19.1	19.8
16:1	5.9	2.3	25.3
16:4 n-3	-	18.3	-
17:0	1.4	1.4	-
18:1 n-9	9.0	6.8	1.5
18:1 n-7	0.5	1.4	0.5
18:2 n-6	11.1	2.3	1.4
18:3 n-6	0.5	0.6	1.7
18:3 n-3	6.8	24.9	-
18:4 n-3	20.5	5.7	2.0
20:1 n-9	-	1.0	-
20:4 n-6	0.2	0.3	1.2
20:5 n-3	0.8	4.7	23.6
22:4 n-3	0.7	-	-
22:6 n-3	9.0	0.1	1.6
Σ Saturated	30.8	20.5	33.0
Σ Monounsaturated	16.4	11.5	29.8
Σ Polyunsaturated	49.6	57.5	31.8
Σ n-6	11.6	3.2	4.3
Σ n-3	37.8	54.3	27.2
n-3/n-6	3.2	17.0	6.3
DHA/EPA	11.2	0.0	0.1
EPA/AA	4.0	15.7	19.7

Iso = Isochrysis 1200 Premium®, Tetra = Tetraselmis 3600 Premium®, Chaeto = Chaetoceros 1000 Premium® (Reed Mariculture Inc., USA)
*Fatty acids detected at levels < 0.5%: 17:1 n-7, 18:0, 18:1 n-11, 20:4 n-3, 22:5 n-3
∑ Saturated = total saturated fatty acids; ∑ Monounsaturated = Total monoenoic fatty acids;
∑ Polyunsaturated = Total polyunsaturated fatty acids; ∑ n-6 = total n-6 fatty acids; ∑ n-3 = total n-3 fatty acids.
n-3/n-6 = ratio between total n-3 fatty acids and total n-6 fatty acids content; DHA/EPA = ratio between docosahex-aenoic acid and eicosenoic acid content; EPA/AA = ratio between eicosenoic acid and arachidonic acid content.

Table 3. Daily growth rate (DGR, %/day) of juvenile *T. philippinarum* fed experimental diets over 6 consecutive weeks.

Clams	Week			
	1-2	3-4	5-6	Average
M	0.71±0.06[c]	0.41±0.28[b]	0.19±0.00[a]	0.29±0.00[c]
B	0.82±0.01[b]	0.38±0.07[b]	0.19±0.04[a]	0.38±0.01[b]
T	0.98±0.04[a]	0.91±0.11[a]	0.35±0.13[a]	0.61±0.06[a]

Means ± SD; n=4 silos. M = monospecific diet; B = bispecific diet; T= trispecific diet.
Means on the same column followed by the same superscript do not differ at P < 0.05 (Newmann-Keuls Test)

Table 4. Dry weight content (DW), expressed as mg ind^{-1} and as percentage of live weight (LW), ash-free dry weight (AFDW) and lipid content (LC), expressed as mg g DW^{-1} and % of AFDW of juvenile *T. philippinarum* fed the experimental diets.

Clams	DW		AFDW	LC	
	mg ind^{-1}	% LW	% DW	mg g DW^{-1}	% AFDW
Initial	34.4±13.4	64.1±0.7	14.2±2.1	7.2±0.6	5.1±0.4
M	43.3±4.2	60.4±0.6	14.2±0.8	6.3±0.0	4.4±0.6
B	46.8±3.2	60.9±1.5	13.6±0.3	6.4±0.2	4.7±0.4
T	50.2±5.4	60.9±0.6	14.5±1.5	6.7±0.4	4.6±0.2

Means ± S.D. n=4 silos. M = monospecific diet; B = bispecific diet; T= trispecific diet.
No statistical differences were detected

and Chu, 1983). In fact, among all factors, the nutritive value of a diet depends upon its chemical composition. Dietary constituents have to provide the energy required for growth and sustenance, but also those compounds unable to be synthesized by the animal (Albentosa *et al.*, 1996a). In particular, many studies showed that the nutritional value of the diet for molluscs could depend on the presence of certain fatty acids e.g. EPA and DHA (Fernandez-Reiriz *et al.*, 1999). In comparison with the content of the initial clams (6.8%), Diet T maintained the 20:5n-3 content (4.9%) more than diet B (3.2%) and M (2.7%). The very low (0.8%) content of EPA in Iso might hence explain the lower nutritive value of diet M in comparison with diet B and T. Fernandez-Reiriz *et al.* (1998) confirmed the essential nature of EPA and/or DHA in the spat of R. decussatus and observed that a decrease in the amount of DHA present in the diet has a negative effect on the growth rate of the spat. According to Langdon and Waldock (1981) both DHA and EPA can alternatively meet the requirements of bivalves for n-3 PUFA. Freites *et al.* (2002) identify EPA as one of the fatty acids of well-known energetic importance in marine bivalves. The requirements for essential fatty acids in molluscs seem to be species-dependent (Albentosa *et al.*, 1996a). For example, *Tapes semidecussata* and *Mercenaria mercenaria* require high levels of DHA while *Crassostrea gigas* shows a preferential requirement for EPA (Helm, 1990). Despite this, minimum EPA and DHA concentrations are needed to ensure normal development in every species (Caers *et al.*, 1999). At the end of the trial, all the experimental groups were also characterised by the presence of 20:1 n-9 with values

Table 5.   Effect of a 6-week feeding period with the experimental diets on the fatty acid composition (% of total fatty acids) of juvenile *T. philippinarum*.

Fatty acid	Initial	Microalgae pastes M	B	T
	%	%	%	%
14:0	6.1±1.5	3.9±0.5a	2.9±0.23b	2.7±0.7b
14:1	0.2±0.0	0.4±0.0a	0.4±0.05a	0.5±0.1a
15:0	0.6±0.2	0.6±0.0a	0.6±0.1a	0.6±0.2a
15:1	0.5±0.1	0.2±0.0a	0.2±0.0a	0.1±0.1a
16:0	22.4±1.6	15.7±1.8a	16.0±0.1a	17.2±3.6a
16:1	4.1±1.1	3.6±0.3b	3.0±0.3b	5.±1.0a
16:4 n-3	4.9±0.6	6.0±0.1a	6.6±0.1a	6.0±0.2a
17:0	-	0.1±0.0c	0.2±0.0b	0.3±0.1a
17:1 n-7	0.5±0.2	0.2±0.0a	0.2±0.0a	0.6±0.1a
18:0	6.8±0.2	6.4±0.3b	6.4±0.2b	7.0±0.1a
18:1 n-11	4.2±0.4	-	-	-
18:1 n-9	5.3±1.3	3.9±0.3b	4.5±0.0a	3.8±0.1b
18:1 n-7	2.6±0.3	3.7±0.1b	3.8±0.1b	4.5±0.2a
18:2 n-6	1.5±0.5	0.9±0.1a	0.9±0.1a	0.7±0.0a
18:3 n-6	-	0.1±0.0b	0.1±0.1b	0.2±0.0a
18:3 n-3	1.6±0.2	2.8±0.1c	4.3±0.0a	3.1±0.4b
18:4 n-3	3.1±0.6	6.9±0.3a	5.3±0.1b	3.1±0.5c
20:1 n-11	0.6±0.2	2.2±0.1a	2.1±0.0a	2.3±0.3a
20:1 n-9	1.8±0.3	2.5±0.2b	3.0±0.1a	2.6±0.0b
20:3 n-3	-	0.3±0.1a	0.4±0.0a	0.3±0.0a
20:4 n-6	3.3±0.6	2.7±0.2a	2.7±0.1a	2.8±0.2a
20:4 n-3	-	1.0±0.0a	0.8±0.0b	0.6±0.1c
20:5 n-3	6.8±1.1	2.7±0.3b	3.2±0.2b	4.9±0.4a
20:2 n-6	1.2±0.2	2.0±0.2a	2.1±0.1a	1.8±0.2a
20:3 n-6	0.3±0.1	0.2±0.0b	0.2±0.0b	0.3±0.0a
20:1 n-7	0.5±0.1	1.6±0.1a	1.4±0.1a	1.4±0.1a
22:1	0.4±0.1	0.3±0.0a	0.4±0.0a	0.4±0.1a
22:4 n-6	0.9±0.2	1.3±0.2a	1.2±0.0a	1.2±0.4a
22:4 n-3	1.5±0.1	2.0±0.3a	1.9±0.1a	1.7±0.4a
22:5 n-3	0.5±0.2	0.6±0.1a	0.7±0.0a	0.7±0.2a
22:6 n-3	10.1±1.3	10.0±1.6a	9.5±0.6a	8.4±2.1a
∑ Saturated	35.9±1.8	26.7±2.6a	26.1±0.7a	27.8±4.2a
∑ Monounsaturated	20.7±1.2	18.6±0.5b	19.0±0.4b	21.3±1.0a
∑ Polyunsaturated	35.7±3.9	39.5±3.7a	39.9±1.4a	35.9±5.1a
∑ n-6	7.2±0.6	7.2±0.7a	7.2±0.5a	7.0±1.2a
∑ n-3	23.6±1.3	26.3±2.1a	26.1±0.6a	22.8±2.8a
n-3/n-6	3.3±0.1	3.7±0.1a	3.6±0.2a	3.2±0.2b
DHA/EPA	1.5±0.2	3.7±0.2a	3.0±0.0b	1.7±0.3c
EPA/AA	2.1±0.0	1.0±0.0b	1.8±0.0a	1.7±0.0a

Iso = Isochrysis 1200 Premium®, Tetra = Tetraselmis 3600 Premium®, Chaeto = Chaetoceros 1000 Premium® (Reed Mariculture Inc., USA)
*Fatty acids detected at levels < 0.5%: 17:1 n-7, 18:0, 18:1 n-11, 20:4 n-3, 22:5 n-3
∑ Saturated = total saturated fatty acids; ∑ Monounsaturated = Total monoenoic fatty acids;
∑ Polyunsaturated = Total polyunsaturated fatty acids; ∑ n-6 = total n-6 fatty acids; ∑ n-3 = total n-3 fatty acids.
n-3/n-6 = ratio between total n-3 fatty acids and total n-6 fatty acids content; DHA/EPA = ratio between docosahexaenoic acid and eicosenoic acid content; EPA/AA = ratio between eicosenoic acid and arachidonic acid content.

ranging from 3.0% in clams fed diet B to 2.5% in clams fed diet M. The values of the clams at the end of the experiment were higher than those of initial clams (1.8%). This fact could seem surprising because, except for Tetra, which contains a low amount of this fatty acid (1.0%), the other ingredients ack it. According to Soudant *et al.* (1998) this could be due to an elongation mechanism of the fatty acid 18:1 n-9, which is quite abundant in Iso (9.0%) and Tetra (6.8%), indeed.

Besides the fatty acid composition, the nutritional value of the diets is to correlate to other parameters. The three microalgae pastes are characterized by a different proximate composition with a protein content ranging from 55.3% in Tetra and 48.2% in Iso, to 32.3% in Chaeto. The lipid content ranged from 11.4% in Tetra, to 6.1% in Chaeto and 5.9% in Iso. Despite this, even though the fatty acid composition showed statistical differences, the gross lipid content of the clams didn't seem to be excessively influenced by the diets. In fact the lipid content was very similar with values around 6 mg g DW-1 and 4% AFDW-1 for any group. Albentosa *et al.* (2002) observed that the amount of protein and lipid available for the spat had a significant influence on its growth. High dietary protein level provides better growth for juvenile mussel Mytilus trossulus (Kreeger and Langdon, 1993). This was not true in our trial where the best DGR was achieved with the Diet T, where Chaeto, which contains the lowest protein content of the three ingredients (32.3%), represents 40% of the diet. In evaluating the nutritional value of a new diet for molluscs besides the gross/fatty acid composition, many other interspecific differences between different algal diets (Caers *et al.*, 1998) could be taken into account. According to Albentosa *et al.* (1997) the differences observed in the ingestion, absorption and digestion of the various species of different microalgae may provide an explanation for differing growth rates in mollusc feeding. This may be truer when we consider preserved algae as substitute food for fresh algae. In fact, the process of preserving the microalgae may in some way alter the cell wall or compact the cells, thus making it more difficult to digest (Albentosa *et al.*, 1997). In recent years, a number of studies on preserved microalgae in mollusc feeding have been reported.

Brown and Robert (2002) demonstrated that flocculated concentrates of *Chaetoceros calcitrans* and *Chatecoceros* sp. ("*tenuissimus*-like") can be used effectively as major components of the diet for larval and juvenile oysters. According to Albentosa *et al.* (1997), the nutritional quality of the microalga *Isochrysis* for the seed of *Ruditapes decussatus* was not dramatically altered when it was centrifuged to produce a concentrate. Also McCausland *et al.* (1999) found that only limited nutritional loss occurred during the pasting, transportation and storage of the *S. costatum* and *C. calcitrans* algal pastes compared to live microalgal diets. Haesmann *et al.* (2000) demonstrated that microalgae concentrate diets, when appropriately produced, have the potential of replacing fresh microalgae culture diets in the feeding of larval and juvenile bivalves. They also found that particular types of microalgae concentrates that provide the best result with one life stage of a reared species would not necessarily apply to another life stage. At the life stage used in our trial, clams are usually transferred to nurseries waiting to be moved to the natural environment. In these facilities the need for food is much greater than in the hatchery, reaching $10 - 40$ m^3 of extensive microalgal cultures (Baud and Bacher, 1990) and up to 85% of the total production costs (Bolton, 1982). For that reason, a cost effective substitute of live algae at this stage should be very useful in reducing space and labour demands. Our experiment showed that the microalgae pastes were able to obtain good DGR of clams with a value of 0.98 for diet T during the weeks 1-2. However, after the second week of feeding, DGR started to decrease until weeks 5-6 where a generalized arrest of growth was observed in all the groups.

It's interesting to note that the polyunsaturated fatty acid profile of this kind of concentrated microalgae remains unchanged at 4°C for a month (Molina Grima *et al.*, 1994; Montaini *et al.*, 1995). Consequently, the lower larval growth rate recorded in our trial in weeks 3-4 and 5-6 is not linked to a deficiency in essential fatty acids. Furthermore, according to other authors, concentrates produced by centrifugation and refrigerated at 2-4°C have been successfully used for larval or juvenile bivalves for 1-8 weeks (McCausland *et al.*, 1999;

Haesmann *et al.*, 2000). Though we only partially characterised the chemical composition of the ingredients used in the experiment, we did not find a relationship between the chemical composition and diet performance of the microalgae pastes along the experiment. To make a comparison with an "in the field" feeding protocol, another group of four silos was fed a fresh algae diet (*I. galbana*, 40%, *T. suecica*, 20%, *C. calcitrans*, 40%) all through the experiment (data not published). The group fed fresh algae showed a never-decreasing growth rate with an average DGR of 1.2 ± 0.1 all through the experiment, twice that of the equivalent paste. The higher value is probably partially due to a higher feeding ratio of this diet whose DW was not easily detectable and comparable to that of microalgae pastes. In any case this fact confirmed the good valence of the experimental design demonstrating that the problem was principally related to the diets. Among the three microalgae pastes, Iso should be the most perishable among the three ingredients because of the fragility of its cell wall (Albentosa *et al.*, 1997). The highest decrease in the DGR was noticed in diet M, hence the main reason for the decrease in DGR could have resulted from an unexpected deterioration of this alga.

Conclusions

The unpredictable drops in molluscs growth and the current high costs of most preserved products, limit the use of preserved algae in the field to a partial substitution of fresh algae in anticipation of better standardized preserved products. The success in using non-living diets for spat rearing will depend on standard reliability and costs that are comparable to those associated with algal production.

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