Fatty Acid Composition of *Ruditapes decussatus* Spat Fed on Different Microalgae Diets

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ABSTRACT. The fatty acid composition of the *Ruditapes decussatus* spat fed on three different microalgal diets during 4 weeks was determined. The fatty acid pattern of each diet was also analysed. The diets used were *Isochrysis galbana*, clone T-ISO, *Tetraselmis suecica*, and *Phaeodactylum tricornutum*. The fatty acid composition of the spat was usually well correlated with that of the diet supplied. Major differences among spat cultures were found in 14:0, 16:0, 16:1n-9, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:5n-3, 22:5n-6 and 22:6n-3 fatty acids. These differences were correlated with the particular fatty acid content of each diet supplied. It has been shown that *R. decussatus* spat have a very low capacity to elongate and desaturate linolenic acid to n-3 PUFA, so when 20:5n-3 or 22:6n-3 were not present in the diet, they were also absent, at least in measurable amounts, in the clams. The absence of any of the “essential” fatty acids, 20:5n-3 in T-ISO or 22:6n-3 in *Tetraselmis*, did not limit spat growth, so their role as “essential” fatty acids might be a matter for discussion. Finally, the nutritive value of each diet was discussed in terms of its fatty acid composition.

KEY WORDS. Fatty acids, microalgae diets, clam spat

INTRODUCTION

Lipids of marine animals have a high degree of unsaturation in their fatty acids, which is related to membrane fluidity at low temperatures (26). Polyunsaturated fatty acids (PUFA) are essential as they can not be synthesized “de novo.” Some n-3 fatty acids,

such as linolenic acid (18:3n-3), eicosapentanoic acid (20:5n-3) and docosahexanoic acid (22:6n-3), are very abundant in marine lipids (1,27,9). The fatty acid composition of different bivalves species has been previously reported (see 34,35 for *Crassostrea virginica* and *Ostrea edulis*; 6 for *Chlamys islandica*; 7 for *Tapes decussatus* and *T. Philippinarum*; 19 for *Myth edulis*; and 4 for *Venerupis pullastra*). These studies have been focussed mostly on adults, and only a few of them have included larval or spat stages (32,10,23,15 on larvae of various oyster spp.; and 20 or 4 on small size spat).

The nutritive value of microalgal diets for molluscs seems to depend upon the presence of essential fatty acids. Trider and Castell (29) showed that n-3 fatty acids were preferentially required over n-6 fatty acids by *C. virginica*. The growth rate of *C. gigas* spat fed on diets deficient in C20 and C22 highly unsaturated fatty acids can be considerably increased if these fatty acids are additionally supplied within microcapsules (20). Elongation and desaturation activities of fatty acids were not high enough to sustain optimal growth rates in juvenile oysters (31). Thus, these authors pointed out that highly unsaturated fatty acids, such as 20:5n-3 or 22:6n-3, must be supplied in the diet. Elongation and desaturation of linolenic acid to n-3 PUFA were also highly limited in the *V. pullastra* clam spat, so when n-3 PUFAs were not present in the diet, they were not found in the spat (4).

This present work aimed to study the fatty acid composition of the clam spat, *Ruditapes decussatus*, and how the fatty acid profile of the diet had influence on that of the clams. Three microalgal diets with a different fatty acid composition were used: *Isochrysis galbana* (clone T-ISO), *Tetraselmis suecica* and *Phaeodactylum tricornutum*.

MATERIALS AND METHODS

Spat were obtained from a broodstock conditioned at the Instituto Español de Oceanografía (La Coruña, Spain). Spawning induction and larval cultures were realized according to Perez-Camacho et al. (24).

Determinations of size, live weight (LW), dry weight (DW) and organic matter content (considered as ash-free dry weight, AFDW) were done on a selected spat group. The clams used in this experiment had the following initial characteristics: 769 ± 93 pm of

size, 0.151 ± 0.003 mg live weight ind^{-1} , 0.084 ± 0.001 mg dry weight ind^{-1} , and $14.50 \pm 0.01\%$ of the dry weight was organic matter content.

The spat cultures were carried out in 61 plastic beakers. Clams were laid on the bottom of the beakers and a slight stream of air was applied to minimize the sedimentation rate of microalgae. Cultures were grown under isothermal conditions at $20 \pm 1^\circ\text{C}$ during 4 weeks. Sea water passed through 1 μm filters and sterilised with U.V. was used as culture water and changed daily. Spat growth, measured as live weight, was monitored weekly by weighing the total biomass after drying onto absorbent paper (10 min) at room temperature. Live weight, dry weight and organic matter content were also determined after 4 weeks. Dry weight and organic matter content were determined after 24 hr at either 80°C or 450°C , respectively.

The diets used were *Isochrysis galbana* (T-ISO), *Tetraselmis suecica* and *Phaeodactylum tricornutum*. The sedimentation rate of each diet was determined by adding it to a culture without clams. The experiments were carried out in triplicate for each diet. The microalgal diets were cultured in 61 glass bottles under isothermal conditions at 18°C , and a continuous illumination of 9900 lux. Salinity was kept constant at 33 ppm. The culture medium used was that described by Walne (33). Microalgae were harvested from the early stationary phase of growth and supplied as food. The daily food ration, expressed as microalgal organic weight, was 2% of the spat live weight. The daily biomass increase resulting from spat growth was taken into account to maintain a constant food ration.

Samples from each diet were collected, centrifuged and then washed in 0.5 M ammonium formate. Subsequently, they were freeze-dried and stored at -30°C until required for analyses of fatty acid composition.

Lipids were firstly extracted with chloroform and methanol (1:2), and after centrifuging at $3246 \times g$, re-extracted from the precipitate with chloroform and methanol (2:1). Both supernatants were washed into a chloroform, methanol and water solution (8:4:3) according to Folch et al. (14). Solvents contained 0.05% butylated hydroxytoluene (BHT). The total lipid content was determined gravimetrically on a Sartorius MSP balance ($\pm 1 \mu\text{g}$) by evaporating the solvent from 200 μl of purified extract spread on

aluminum plates heated at 60-80°C. The purified extract was stored under nitrogen at -70°C until required for fatty acid analyses.

Fatty acids from total lipid were transesterified to methyl esters by using methanolic hydrogen chloride (8) and subsequently analysed by gas chromatography (GC). A gas chromatograph (Perkin Elmer 8500) fitted with a fused silica capillary column (Supelco SP-2330, 30 m length, 0.25 mm i.d.) was used. Nitrogen was used as carrier gas at 10 psi. The injector temperature was set at 275°C and the temperature of the column was increased from 140°C to 210°C at a rate of 1°C min⁻¹. Nonadecanoic acid was used as an internal standard, and a response factor was determined for each fatty acid for quantitative analysis. Fatty acids were identified by co-injection of the sample with fatty acid methyl esters standards and GC-MS (Hewlett Packard 5971 Mass Detector). Chemicals, solvents and fatty acid standards were supplied by E. Merck, Darmstadt, and Sigma Chemical Co.

The results of the fatty acid composition of the spat fed on each diet was analysed by ANOVA to establish statistical significances. The relative percentage of each fatty acid was the mean value of hundreds of individuals, so that a normal distribution was guaranteed. Variance homogeneity was tested by the Barlett's test. If this was not the case, the angular transformation on the relative percentages was applied. The Student-Newman-Keuls' test (37) was performed to do multiple comparisons among the different treatments. Correlation analyses between the fatty acid composition of the diet and that of the spat fed on it were also performed. The Statgraphics statistical package was used for the statistical analyses.

RESULTS

Fatty Acid Composition of the Diets

The fatty acid composition of each microalgal diet is shown in Table 1. The content of each fatty acid was expressed as both the percentage relative to the total fatty acid content, and as pg per mg dry weight. The total lipid contents of T-IS0 (28.78% of the organic matter) and *Phaeodactylum* (25.25%) were considerably higher than that of *Tetraselmis* (11.92%). *Phaeodactylum* and *Tetraselmis* lipids had a higher fatty acid

proportion, that is, 85.49% and 66.63%, respectively, than T-IS0 lipids, with 52.43%. Nonetheless, in absolute values, the fatty acid content of T-IS0 (148.84 µg) was higher than that of Tetraselmis (74.36 µg) as a result of the lower lipid content of the latter microalga.

Saturated fatty acids were the major fatty acid group in all the diets, with values around 40% of the total fatty acid content. The highest proportion of polyunsaturated fatty acids was found in T-IS0 (33.30%), followed by Tetraselmis (27.22%). *Phaeodactylum* showed a lower PUFA content (17.07%), although presenting the highest monosaturated fatty acid content (39.33%). Fatty acids of the n-3 family were abundant in T-IS0 (25.69%), as well as those of the n-9 family (19.01%). Tetraselmis contained a higher proportion of n-9 (26.72%) than of n-3 fatty acids (20.55%), although the latter were also abundant. The n-3 and n-9 fatty acid contents of *Phaeodactylum* were lower (12.88 and 5.38%, respectively) than in the other microalgae and n-7 fatty acids were the major fatty acid family (33.96%). Most n-3 fatty acids of *Phaeodactylum* were n-3 HUFA (highly unsaturated fatty acids), (nearly 80%), especially 20:5n-3. Tetraselmis and TIS0 contained a much lower n-3 PUFA proportion, (around 35% of the n-3 fatty acid content). The n-3/n-6 ratio was very similar for all the diets, with a value around 3.

The fatty acids predominant in T-IS0 and Tetraselmis were 16:0 and 18:1n-9. 16:1n-7 and 16:0 were the major fatty acids in *Phaeodactylum*, but 18:1n-9 was found in a low proportion. T-IS0 showed a high content of myristic acid 14:0, and so did *Phaeodactylum*, but not Tetraselmis, this microalgae was rich in linolenic acid (18:3n-3), whereas T-IS0 had a lower content and *Phaeodactylum* hardly any of this fatty acid. Differences were also found in the linoleic acid content (18:2n-6). T-IS0 had a 18:4n-3 content much higher than the other diets. Differences were also recorded in the contents of some of the essential fatty acids, that is, 20:5n-3 and 22:6n-3. T-IS0 had a high 22:6n-3 content (7.95%), but very low amounts of 20:5n-3 (0.54%). Tetraselmis did not have 22:6n-3, but 20:5n-3 was detected in relatively high amounts (5.63%). *Phaeodactylum* showed a high content of 20:5n-3 (9.61%) and a low proportion of 22:6n-3 (0.74%).

Fatty Acid Composition of the Spat

The fatty acid composition of the spat fed on each diet during 4 weeks is given in Table 2. The lipid and the fatty acid contents of *R. decussatus* spat were similar for any diet, with values around 20% of the organic matter and 30-40% of the total lipid content, respectively.

Saturated fatty acids were the most abundant fatty acid group in the clams, followed by the polyunsaturated (PUFA) and monounsaturated fatty acids. However, when *Phaeodactylum* was used as a diet, monounsaturated fatty acids were accumulated in a higher proportion than PUFAs. Significant differences ($p < 0.01$) were found in the contents of monounsaturated and polyunsaturated fatty acids as a result of the diet supplied. Monounsaturated fatty acid contents of *Phaeodactylum* and *Tetraselmis* were similar (ANOVA, $p > 0.05$), but significantly different from that of T-ISO. PUFA contents of T-ISO and *Tetraselmis* were significantly different from that of *Phaeodactylum*. The more abundant fatty acid families in spat fed on T-ISO or *Tetraselmis* were the n-3 and n-9 families, whereas the n-7 family was the most abundant in clams fed on *Phaeodactylum*. Most of the n-3 fatty acids of spat fed on T-ISO or *Phaeodactylum* were PUFAs, but a high proportion of n-3 acids was not accumulated when fed on *Tetraselmis* (nearly 40%). The n-6, n-7 and n-9 fatty acid contents of clams fed on the different diets were significantly different. The n-6 and n-7 fatty acid contents of spat fed on *Tetraselmis* or T-ISO were different from that of spat fed on *Phaeodactylum*, which had a lower n-6 content and a higher n-7 content. Differences among the n-9 fatty acid contents of each culture were also found.

The diets had an important and significant influence on the content of the following fatty acids: 14:0, 16:1n-9, 16:1n-7, 18:1n-9, 18:3n-3, 18:4n-3, 20:3n-6, 20:5n-3, 22:5n-6 and 22:6n-3. The content of 16:1n-7 was very low in clams fed on T-ISO or *Tetraselmis* (1.78 and 1.56%, respectively), but high when the diet was *Phaeodactylum* (14.48%). T-ISO and *Tetraselmis* diets also gave rise to large amounts of 18:1n-9 in clams (11.81 and 17.88, respectively) but not *Phaeodactylum* (4.59%). Linolenic acid was abundant in spat fed on *Tetraselmis*, and 18:4n-3 in those fed on T-ISO. *Phaeodactylum* diets gave rise to low contents of both acids. Clams fed on T-ISO contained a high 22:6n-3 content, but hardly had 20:5n-3, whereas if fed on *Tetraselmis* or *Phaeodactylum* had a high content of 20:5n-3, but low of 22:6n-3.

Effects of the Diet on the Fatty Acid Composition of the Spat

The fatty acid composition of the spat reflects basically that of the diet. Thus, similar data were obtained for the different fatty acid groups of each diet and the seeds that were supplied to, as shown in Tables 1 and 2. Similar patterns also resulted among the different unsaturated fatty acid families. A large content of n-7 fatty acids in the *Phaeodactylum* diet was reflected in the clams fed on it. Total n-9 fatty acids were the major family in *Tetraselmis* and, so, in the clams fed on this diet. Therefore, the proportions of the different fatty acid groups and families of each diet determine similar proportions in the clams fed on them.

The main significant differences ($p < 0.01$) found in the fatty acid content of the spat were well correlated with the proportions of those same fatty acids in the diets, i.e. 14:0, 16:1n-9, 18:1n-9, 16:0, 16:1n-7, 18:2n-6, 18:3n-3, 18: 4n-3, 20: 5n-3, 22: 5n-6 and 22:6n-3.

DISCUSSION

The fatty acid pattern of each microalgal diet was comparable to results previously reported (see 25,30, or 22 for *Isochrysis*; 30 or 13 for *T. suecica*; and (5) or 28 for *P. tricornutum*). The nutritive value of a microalga species depends, among other factors, upon its chemical composition (36). Dietary constituents have to provide not only the energy required for growth and sustenance, but also those compounds unable to be synthesized by the animal, such as essential fatty acids. Data obtained in the present study showed that nutritive value of each of the three microalgae used for *R. decussatus* spat is different.

In this sense, *R. decussatus* spat fed on *Tetraselmis* showed the highest growth and, then, if fed on T-ISO. On the contrary, the nutritive value of *Phaeodactylum* was lower for *R. decussatus* (Table 3, modified from 3). None of these diets has both fatty acids, although as was pointed out by Langdon and Waldock (20), any of them could satisfy the requirements for n-3 HUFAs. The requirement for essential fatty acids seems to be species-dependent. *Tapes semidecussata* and *Mercenaria mercenaria* required 22:6n-3, whereas *Crassostrea* spp. Showed a preferential requirement for 20:5n-3 (16). The

absence of 22:6n-3 in *Tetraselmis* lipids might explain the lower nutritive value of this diet, in comparison with T-ISO, for the clam *Venerupis pullastra* (4). These authors also pointed out that a mixed diet, containing both microalgae, gave rise to a higher growth than a simple diet, partially due to the presence of both “essential” fatty acids in the mixed diet. According to these criteria, T-ISO had a lower nutritive value than *Tetraselmis* for *R. decussatus* spat as a consequence of its higher requirement for 20:5n-3. Nonetheless, *Phaeodactylum*, having a high 20:5n-3 content, was not suitable for *R. decussatus*. This points out that some other criteria, for example, ingestion rate, absorption efficiency, etc., should be used to explain the nutritive value of a diet (3).

The effect of the fatty acid composition of the diet on the fatty acid profile of bivalves has been studied previously. Some initial studies showed that the fatty acid composition of two oysters species did not depend on the diet (34,35). However, the diet had a clear influence on the fatty acid pattern of *Mesodesma mactroides* according to De Moreno et al. (11). Similar results were found for larvae and spat of *C. gigas* (specially for the triglyceride fraction) (32,20) and for *V. pullastra* spat fed on a number of diets (4). Thus, these latter authors indicated that some fatty acids, such as n-3 HUFAs, were present in the spat only if they were also present in the diet. The present study has also shown that spat lipids reflect the fatty acid composition of the diet.

Clams fed on *Phaeodactylum* had a relatively high 18:1n-7 content (7%). However, this fatty acid was contained in very small amounts in *Phaeodactylum* cells, so it must result from elongation of 16:1n-7 within the spat, since the latter is highly abundant in this diet (32.75%). Clams fed on any diet had 20:1n-9, but this fatty acid was not present in T-ISO or *Phaeodactylum* diets, although it could have been formed by elongation of 18:1n-9. This is supported by the higher 20:1n-9 content in spat fed on T-ISO than in those on *Phaeodactylum*, what seems to be related to the higher 18:1n-9 content in T-ISO than in *Phaeodactylum*. Although it was not detected in any of the diets, spat fed on *Tetraselmis* had a 20:4n-6 content higher than the other clam cultures. This fatty acid could have resulted from desaturation of 20:3n-6, which is more abundant in *Tetraselmis*.

With regard to NMID, and though their function is not clearly discerned, it seems that their biosynthesis is regulated both functionally and physiologically. Actually, high amounts of these acids were observed in the membranes of sponges (21) and molluscs (2), which could indicate a functional and a structural role in the biological system. Klingensmith (18) working with clams clearly indicates a competitive incorporation between NMID and PUFAs, especially 20:5n-3 and 22:6n-3. Fang et al. (12) found that mussels from the Gulf of Mexico had a high NMID content, but not essential PUFA. In our study, however, we have found low levels of NMID.

The requirements of fish and crustacea for fatty acids is related to the bioconversion capacity to transform fatty acids from the diet in highly unsaturated fatty acids. The capacity to elongate and desaturate dietary 18:3n-3 to 20:5n-3 and 22:6n-3 is higher in freshwater fish than in marine fish or in crustacea (17). Radioactivity studies carried out on *C. gigas* juveniles by marking dietary fatty acids with ^{14}C showed that they could elongate and desaturate n-3 fatty acids to produce n-3 HUFAs. However, this capacity was too low to maintain optimal growth rates (31). Similar results were found in *C. virginica*, with no detection of 20:5n-3 or 22:6n-3 resulting from ^{14}C -marked 18:3n-3 (9). Albentosa et al. (4) indicated that *V. pullastra* spat could not elongate and desaturate 18:3n-3 to 20:5n-3 and this latter to 22:6n-3, as when these fatty acids were not present in the diet, they were not found in the spat either. *Ruditapes decussatus* spat seem not to be able to convert 18:3n-3 to n-3 PUFA as a consequence of very low $\Delta 5$ and $\Delta 4$ desaturase activities. In this way, T-IS0 contained 18:3n-3 (3.23%), but nearly lacks 20:5n-3 (0.54%). Spat fed on T-IS0 did also have a low content of 20:5n-3 (1.48%), reflecting the low content of this fatty acid in the diet rather than a low bioconversion capacity for 18:3n-3. *Tetraselmis* had a high content of 18:3n-3 (10.43%) and of 20:5n-3 (5.63%), but lacked 22:6n-3, so clams fed on this diet showed a notable 20:5n-3 content (6.86%), but a small proportion of 22:6n-3 (1.04%). *Phaeodactylum* had a high 20:5n-3 content (9.61%) and a slight 22:6n-3 content (0.74%), but almost no 18:3n-3 (0.52%). Spat fed on *Phaeodactylum* were rich in 20:5n-3 (12.54%) and had a low content of 22:6n-3 (2.65%). *R. decussatus* spat have a very limited ability to elongate and desaturate 20:5n-3 to 22:6n-3, so when this latter fatty acid was not present in the diet, it was only found in a very small proportion, if any, in spat lipids. Furthermore, when fatty acids such as 20:5n-3 in TIS0 or 22:6n-3 in *Tetraselmis* were not present, the growth of *R. decussatus* spat was not limited, so that their essentiality might be a matter

for discussion. Consequently, criteria other than “essential” fatty acid content must be looked through in order to give an explanation to the different nutritive value of the microalgal diets tested (3).

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TABLE 1. Fatty acid composition of the three microalgal diets tested as food for *Ruditapes decussates* spat: *Isochrysis galbana* (clone T-ISO), *Tetraselmis suecica* and *Phaeodactylum tricornutum*

Fatty acid	I. galbana, T-ISO		T. suecica		P. tricornutum	
	%	µg/mgDW	%	µg/mgDW	%	µg/mgDW
14:0	14.31	21.30	1.50	1.11	6.12	12.57
15:0	0.91	1.35	0.92	0.68	0.71	1.46
16:0	17.04	25.36	27.54	20.48	28.20	57.96
16:1n-9	1.42	2.11	2.40	1.78	0.66	1.35
16:1n-7	2.72	4.05	1.01	0.75	32.75	67.29
16:4n-3	0.16	0.24	0.07	0.05	0.21	0.42
17:0	0.92	1.37	1.15	0.85	0.67	1.37
17:1n-7	0.34	0.51	0.53	0.40	0.66	1.35
18:0	9.91	14.75	7.54	5.60	7.79	16.01
18:1n-9	17.59	26.18	22.65	16.85	4.27	8.78
18:1n-7	1.12	1.67	5.67	4.22	0.55	1.14
18:2n-6	2.76	4.11	2.69	2.00	1.14	2.34
18:3n-6	0.24	0.36	0.28	0.21	0.25	0.52
18:3n-3	3.23	4.81	10.43	7.76	0.52	1.07
18:4n-3	11.68	17.39	3.51	2.61	1.40	2.87
18:5n-3	1.10	1.64	0.41	0.31	n.d.	n.d.
20:0	0.42	0.62	0.21	0.15	0.12	0.24
20:1n-9	n.d.	n.d.	1.67	1.24	n.d.	n.d.
20:3n-6	2.34	3.49	3.11	2.31	2.08	4.27
20:4n-6	0.80	1.18	0.43	0.32	0.48	0.99
20:4n-3	0.77	1.14	0.50	0.37	0.40	0.82
20:5n-3	0.54	0.80	5.63	4.18	9.61	19.75
22:5n-6	1.47	2.19	0.16	0.12	0.24	0.50
22:5n-3	0.26	0.39	n.d.	n.d.	n.d.	n.d.
22:6n-3	7.95	11.83	n.d.	n.d.	0.74	1.51
24:1n-9	n.d.	n.d.	n.d.	n.d.	0.44	0.91
Σ n-3	25.69	38.24	20.55	15.28	12.88	26.44
Σ n-6	7.61	11.33	6.67	4.96	4.19	8.62

Σ n-7	4.18	6.23	7.21	5.37	33.96	69.78
Σ n-9	19.01	28.29	26.72	19.87	5.37	11.04
Σ n-3HUFA	8.75	13.02	5.63	4.18	10.35	21.26
n-3/n-6	3.38		3.08		3.07	
Σ Sat	43.51	64.75	38.86	28.87	43.61	89.61
Σ Mono	23.19	34.52	33.93	25.24	39.33	80.82
Σ PUFA	33.30	49.57	27.22	20.24	17.07	35.06
Total FA	52.52*	148.84	66.62	74.35	85.49	205.49
Lipids	28.78†	283.39	11.92	111.60	25.25	240.38

*Total fatty acids (FA) are noted as the percentage of total lipids. †Lipids are expressed as the percentage of the ash-free dry weight (AFDW). Undetected fatty acids are noted as n.d.

Data are expressed as % of the total fatty acid content and as μg per mg dry weight.

TABLE 2. Fatty acid composition of *R. decussatus* spat fed on *Isochrysis galbana* (clone T-SO), *Tetraselmis suecica* and *Phaeodactylum tricornutum*

Fatty acid	Initial		I. galbana T-IS0		T. suecica		P. tricornutum	
	%	µg/mgDW ⁻¹	%	µg/mgDW ⁻¹	%	µg/mgDW ⁻¹	%	µg/mgDW ⁻¹
14:0	3.44	0.48	5.44 ± 0.63	0.57 ± 0.08	1.10 ± 0.22	0.10 ± 0.03	2.63 ± 0.29	0.19 ± 0.02
15:0	0.87	0.12	0.72 ± 0.23	0.08 ± 0.03	0.51 ± 0.14	0.05 ± 0.02	0.50 ± 0.05	0.04 ± 0.01
16:0	20.91	2.91	19.28 ± 0.17	2.01 ± 0.05	23.82 ± 0.31	2.11 ± 0.29	21.55 ± 0.76	1.58 ± 0.18
16:1n-9	1.07	0.15	1.32 ± 0.25	0.14 ± 0.03	2.62 ± 0.12	0.23 ± 0.04	0.89 ± 0.12	0.07 ± 0.01
16:1n-7	1.15	0.16	1.78 ± 0.06	0.19 ± 0.01	1.56 ± 1.09	0.15 ± 0.12	14.48 ± 0.72	1.06 ± 0.06
16:4n-3	0.21	0.03	0.22 ± 0.06	0.03 ± 0.01	0.20 ± 0.01	0.02 ± 0.00	0.28 ± 0.06	0.02 ± 0.01
17:0	0.89	0.13	0.97 ± 0.14	0.10 ± 0.02	0.51 ± 0.09	0.05 ± 0.02	0.63 ± 0.13	0.05 ± 0.01
17:1n-9	1.42	0.20	0.82 ± 0.12	0.09 ± 0.01	0.72 ± 0.03	0.07 ± 0.01	1.92 ± 0.07	0.14 ± 0.01
18:0	32.43	4.51	13.17 ± 3.29	1.37 ± 0.34	8.81 ± 0.38	0.78 ± 0.10	14.14 ± 3.44	1.05 ± 0.33
18:1n-9	8.97	1.25	11.81 ± 0.83	1.23 ± 0.09	17.88 ± 0.85	1.58 ± 0.18	4.59 ± 0.82	0.34 ± 0.06
18:1n-7	1.96	0.28	3.12 ± 0.16	0.33 ± 0.02	3.11 ± 0.11	0.28 ± 0.05	7.00 ± 0.37	0.51 ± 0.03
18:2n-6	1.53	0.22	1.96 ± 0.06	0.21 ± 0.01	1.56 ± 0.09	0.14 ± 0.02	1.07 ± 0.10	0.08 ± 0.01
18:3n-4	0.06	0.01	0.17 ± 0.15	0.02 ± 0.02	0.23 ± 0.02	0.02 ± 0.01	0.39 ± 0.02	0.03 ± 0.01
18:3n-3	2.53	0.35	2.93 ± 0.46	0.31 ± 0.05	7.90 ± 0.43	0.70 ± 0.06	0.62 ± 0.11	0.05 ± 0.01

18:4n-3	3.67	0.51	7.67 ± 0.32	0.80 ± 0.05	3.58 ± 0.08	0.32 ± 0.04	0.73 ± 0.24	0.06 ± 0.02
20:0	0.75	0.11	0.33 ± 0.06	0.04 ± 0.01	0.28 ± 0.03	0.03 ± 0.00	0.49 ± 0.07	0.04 ± 0.01
20:1n-11	0.17	0.03	0.15 ± 0.13	0.02 ± 0.02	0.22 ± 0.06	0.02 ± 0.01	0.33 ± 0.06	0.03 ± 0.01
20:1n-9	1.51	0.21	2.67 ± 0.10	0.28 ± 0.01	3.57 ± 0.14	0.32 ± 0.03	0.77 ± 0.10	0.06 ± 0.01
20:1n-7	0.27	0.04	0.50 ± 0.04	0.05 ± 0.01	0.31 ± 0.02	0.03 ± 0.01	0.96 ± 0.06	0.07 ± 0.01
20:2NMID1	0.40	0.06	0.57 ± 0.08	0.06 ± 0.01	1.42 ± 0.10	0.13 ± 0.01	0.53 ± 0.40	0.04 ± 0.03
20:2NMID2	0.12	0.02	0.29 ± 0.04	0.03 ± 0.01	0.43 ± 0.04	0.04 ± 0.01	0.77 ± 0.18	0.06 ± 0.02
20:2n-6	0.69	0.10	1.25 ± 0.04	0.13 ± 0.01	1.04 ± 0.03	0.09 ± 0.01	1.01 ± 0.13	0.08 ± 0.01
20:3n-6	1.34	0.19	1.64 ± 0.04	0.17 ± 0.01	1.18 ± 0.07	0.11 ± 0.02	1.61 ± 0.27	0.12 ± 0.02
20:4n-6	0.88	0.13	1.33 ± 0.29	0.14 ± 0.03	3.11 ± 0.20	0.28 ± 0.02	0.89 ± 0.30	0.07 ± 0.02
20:4n-3	0.41	0.06	0.96 ± 0.12	0.10 ± 0.02	1.10 ± 0.07	0.10 ± 0.02	0.40 ± 0.15	0.03 ± 0.01
20:5n-3	1.66	0.23	1.48 ± 0.71	0.15 ± 0.07	6.86 ± 0.05	0.61 ± 0.08	12.54 ± 0.27	0.92 ± 0.08
22:1n-11	0.20	0.03	0.22 ± 0.04	0.03 ± 0.01	0.22 ± 0.03	0.02 ± 0.00	0.19 ± 0.10	0.02 ± 0.01
22:2NMID1	1.59	0.22	1.59 ± 0.16	0.17 ± 0.02	2.28 ± 0.05	0.20 ± 0.03	1.18 ± 0.07	0.09 ± 0.01
22:2NMID2	0.34	0.05	0.46 ± 0.08	0.05 ± 0.01	0.40 ± 0.04	0.04 ± 0.01	1.88 ± 0.11	0.14 ± 0.02
22:4n-6	1.57	0.22	0.55 ± 0.18	0.06 ± 0.02	0.48 ± 0.56	0.05 ± 0.06	0.25 ± 0.03	0.02 ± 0.01
22:5n-6	1.08	0.15	2.22 ± 0.14	0.23 ± 0.02	0.51 ± 0.09	0.05 ± 0.01	0.38 ± 0.09	0.03 ± 0.01
22:5n-3	0.31	0.05	0.27 ± 0.24	0.03 ± 0.03	0.58 ± 0.44	0.05 ± 0.03	0.75 ± 0.21	0.06 ± 0.02
22:6n-3	5.11	0.71	10.74 ± 0.45	1.12 ± 0.08	1.04 ± 0.04	0.09 ± 0.01	2.65 ± 0.43	0.20 ± 0.04
24:1n-9	0.51	0.07	1.38 ± 0.67	0.15 ± 0.07	0.86 ± 0.16	0.08 ± 0.02	1.03 ± 0.48	0.08 ± 0.04

Σ n-3	13.90	1.94	24.27 ± 1.42	2.54 ± 0.15	21.26 ± 0.97	1.89 ± 0.16	17.97 ± 1.07	1.34 ± 0.14
Σ n-6	7.09	1.01	8.95 ± 0.14	0.94 ± 0.02	7.88 ± 0.18	0.72 ± 0.11	5.21 ± 0.76	0.40 ± 0.06
Σ n-7	3.38	0.48	5.40 ± 0.21	0.57 ± 0.03	4.98 ± 1.19	0.46 ± 0.17	22.44 ± 1.11	1.64 ± 0.09
Σ n-9	13.68	1.91	18.22 ± 1.36	1.92 ± 0.13	25.87 ± 0.89	2.30 ± 0.26	9.39 ± 1.02	0.71 ± 0.11
Σ n-11	0.17	0.03	0.15 ± 0.13	0.02 ± 0.02	0.22 ± 0.06	0.02 ± 0.01	0.33 ± 0.06	0.03 ± 0.02
Σ n-3PUFA	7.08	0.99	12.49 ± 1.06	1.30 ± 0.13	8.48 ± 0.58	0.75 ± 0.11	15.94 ± 0.96	1.18 ± 0.13
n-3/n-6	1.96		2.71 ± 0.12		2.70 ± 0.16		3.45 ± 0.29	
Σ NMID	2.45	0.35	2.91 ± 0.24	0.31 ± 0.02	4.53 ± 0.07	0.41 ± 0.04	4.36 ± 0.17	0.33 ± 0.03
Σ Sat	59.29	8.26	39.91 ± 3.56	4.17 ± 0.25	35.03 ± 0.45	3.12 ± 0.45	39.94 ± 3.74	2.95 ± 0.50
Σ Mono	17.23	2.42	23.77 ± 1.63	2.51 ± 0.16	31.07 ± 0.61	2.78 ± 0.41	32.16 ± 1.63	2.38 ± 0.19
Σ PUFA	23.50	3.31	36.30 ± 1.94	3.81 ± 0.20	33.90 ± 0.98	3.04 ± 0.31	27.93 ± 2.21	2.10 ± 0.24
Total FA	51.47*	13.99	40.56 ± 2.82	10.49 ± 0.25	36.74 ± 4.05	8.94 ± 1.16	33.67 ± 1.92	7.43 ± 0.76
Lipids	18.74†	27.18	20.03	25.86	16.84	24.33	17.01	22.07

*Total fatty acids % (FA) are noted as a percentage of total lipids and † lipids % are expressed as percentage of the organic matter.

Data are expressed as % of the total fatty acid content and as μg per mg dry weight. Average values of the three replicates and ($\pm$) standard deviations are shown.

TABLE 3. Live and dry weights, organic contents (considered as ash-free dry weight, AFDW) and size of *R. decussatus* spat fed on three microalgal diets: *I. galbana*, clone T-ISO, *T. suecica* and *P. tricornutum* during 4 weeks

Diet	Live weight	Dry weight	% AFDW	Size
	mg/ind			mm
<i>I. galbana</i> , T-ISO	0.883 ± 0.070	0.534 ± 0.014	13.8 ± 1.55	1.42 ± 0.02
<i>T. suecica</i>	1.102 ± 0.048	0.609 ± 0.048	14.5 ± 0.29	1.51 ± 0.05
<i>P. tricornutum</i>	0.664 ± 0.028	0.370 ± 0.003	12.9 ± 1.57	1.20 ± 0.05

Data obtained from 3.

Average values of the three replicates and (±) standard deviations are shown.