

Nutritional effect of substituting microalgal concentrates with microencapsulated diets in *Mytilus galloprovincialis* spat

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ABSTRACT

The expansion of mussel aquaculture is limited by the availability of spat and production on land is dependent on costly cultures of microalgae as feed. Substitution of algae with microencapsulated feeds can reduce feed contaminations, ease the storage and extend the shelf life, ensuring stable production of consistent quality at sustainable costs. This study investigated the effects of the substitution of commercial preserved microalgal concentrates (A) with different inclusion levels of *Schizochytrium*-microencapsulated feed (BioBullets, B) for *Mytilus galloprovincialis* spat: no substitution, A (100%A; 0%B); low substitution, ABL (40%A; 60%B); medium substitution, ABM (20%A; 80%B); complete substitution, B (0%A; 100 %B). For 8 weeks, spat of <1 cm were cultured under dietary treatments and survival, growth, condition index, digestive gland atrophy and adipogranular cell index were compared, using spat unsupplemented with any feeds as negative control (NC). Diet had a significant effect on spat growth and tissue biochemical and fatty acid composition, demonstrating the assimilation of microencapsulated diets. Spat growth was positively correlated with dietary and spat tissue protein, monounsaturated fatty acids and eicosapentaenoic acid content and was highest in spat fed A and ABL. <10% dietary proteins equally sustained *M. galloprovincialis* spat growth through dietary replacement with higher proportions of lipids (x3) and carbohydrates (x2). With manufacturing improvements, 60% substitution of preserved microalgal concentrates by microencapsulated *Schizochytrium* can be considered in attempting to enhance economic feasibility in spat production.

1. Introduction

Marine bivalves, with characteristically high levels of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Tan et al., 2020), provide opportunities as health-promoting food for humans (Caldar, 2018; Khan and Liu, 2019; Willer and Aldridge, 2019b, 2020a). Global aquaculture production of bivalves reached 17.7 MT in 2018 (FAO, 2022) and they are increasingly being consumed worldwide (Khan and Liu, 2019; FAO, 2020). In particular, mytilidae mussels contributed to 7% of total production, with Spain being the major producer in Europe (FAO, 2022; EUFOMA, 2019). The extensive production of the Mediterranean mussel

Mytilus galloprovincialis is highly environmentally sustainable (Gentry et al., 2020; van der Schatte Olivier et al., 2020) and economically valuable (~470 million euros year⁻¹; Robert et al., 2013), with satisfactory consumer acceptability (Azpeitia et al., 2017a). Despite the numerous benefits of *Mytilus* as a food source, mussel aquaculture in Europe, which has mostly relied on wild spat (Kamermans et al., 2013), witnessed a production decline over the last two decades, largely due to the strict dependence on more variable environmental conditions, an increased frequency of algal blooms and spread of diseases (Wijsman et al., 2018; Avdelas et al., 2021), which hamper a stable supply of spat (Kamermans and Capelle, 2018). Thus, the availability and reliability of natural spat, which are collected from the shore or from collectors

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(Azpeitia et al., 2017b; Fernández-Reiriz et al., 2016), remains a constraining issue for mussel aquaculture (Wijsman et al., 2018; Avdelas et al., 2021).

Auxiliary inland hatcheries and nursery facilities can, not only support the production, by ensuring known quantity and quality of spat each year, regardless of uncertain coastal environmental conditions, but can also free up space on offshore longlines and raft systems for the collection of additional recruits (Helm and Bourne, 2004; Kamermans and Capelle, 2018). Especially in offshore cultures in suspended ropes, already subjected to adverse environmental conditions such strong storms (Rasilla et al., 2018), this can reduce density-dependent mortality and secondary settlement, which can cause high losses at sea (from 50% up to 95%; South et al., 2017, 2022), particularly of small sized individuals (South et al., 2017, 2022; Skelton et al., 2021). Inland systems are, however, still costly, as spat are principally fed with live microalgae (Brown et al., 2002; Chauton et al., 2015; Roy and Pal, 2015; Skelton et al., 2021), whose cultivation requires the use of expensive growth media and labour-intensive cultivation procedures, which can drive the costs of bivalve hatchery production from 30 to 40% up to 91% (Coutteau and Sorgeloos, 1992; Knauer and Southgate, 1997a; Carrasco et al., 2014; Chauton et al., 2015; Cheng et al., 2020). High costs and high risks of culture contaminations (Prado et al., 2010) have spurred major interests towards the substitution of live algae with more convenient, mass-produced inert feeds with prolonged shelf-life and consistent nutritional quality, such as dried algae (Knauer and Southgate, 1997a; Arney et al., 2015), concentrated algal pastes (Brown and Robert, 2002; Southgate et al., 2016; Supono et al., 2023), formulated and microencapsulated feeds (Knauer and Southgate, 1997b; Numaguchi, 2002; Nevejan et al., 2007; Willer and Aldridge, 2019a, 2019b; Yang et al., 2019; Skelton et al., 2021; Zhu et al., 2022), bacteria and yeasts (Coutteau and Sorgeloos, 1992; Nell et al., 1996; Nevejan et al., 2016; Tanyaros et al., 2016; Yarnold et al., 2019; Loo et al., 2021). While live microalgae appear essential for bivalve larval nutrition (Brown et al., 2002; Willer and Aldridge, 2019a), positive spat growth can be supported by dry and/or microencapsulated feeds (e.g. Boeing, 1997; Knauer and Southgate, 1997a, 1997b; Langdon and Önal, 1999; Önal et al., 2005; Nevejan et al., 2007; Gui et al., 2016; Willer and Aldridge, 2019a; Skelton et al., 2021; Campanati et al., 2022). For example, a 75% substitution of live algae with a formulated feed (MySpat™) in nursery settings, achieved the same productivity of green-lipped mussel spat *Perna canaliculus* with a ~ 40% reduction of total feed costs (Gui et al., 2016).

The value of feeds is largely determined by its biochemical composition (i.e. Proteins, lipids, carbohydrates) and the ability of mussel spat to grow is then determined by their ability to digest and utilise these compounds as energy substrates (Aranda-Burgos et al., 2014). Positive correlations between dietary protein content, usually in the range of 12–50% (g/100 g dw) in microalgae, and spat growth have been observed in several species (Kreeger and Langdon, 1993; Knauer and Southgate, 1999; Chen et al., 2021). Protein requirements, however, although being species-specific, seemingly reduce with growth throughout ageing (i.e. higher in larvae, 30–60% g/g dw, lower in spat and adults, 13–20% g/g dw; Knauer and Southgate, 1999). Low dietary protein content combined with elevated dietary PUFA levels were found to be beneficial for the growth of *Crassostrea gigas* larvae (Utting, 1986; Thompson and Harrison, 1992), *Ostrea edulis* (Willer and Aldridge, 2019a) and *Mytilus galloprovincialis* (Campanati et al., 2022) spat. For example, 9% of protein (g/100 g dw) were sufficient to sustain high survival rates and growth of *C. gigas* spat, possibly supported by increased dietary carbohydrates and n-3 PUFAs (Carboni et al., 2016). Feeds with lower proteins compared to carbohydrates (25 and 45% respectively) were also found to be optimal for the growth of juvenile pearl oysters, *Pinctada fucata martensii*, compared to higher protein (40%)-lower carbohydrates (35%) diets (Yang et al., 2019).

Successful partial and complete replacement of live and concentrated algal diet has been reported in previous studies, which used dried or

spray-dried microalgae (Boeing, 1997; Langdon and Önal, 1999; Önal et al., 2005) and microencapsulated diets (Nevejan et al., 2007; Gui et al., 2016; Wang et al., 2016; Willer and Aldridge, 2019a; Campanati et al., 2022) containing Thraustochytrids (e.g. *Schizochytrium* sp.). These feeds, with limited culture requirements (Raghukumar, 2008; Aasen et al., 2016), can be valuable sources of n-3 LC-PUFAs (especially DHA), providing promising opportunities for lower-cost carbon sources as growth of medium (Gupta et al., 2012; Kralovec et al., 2012). *M. galloprovincialis* cultured on a spray-dried algal diet containing 50% of *Schizochytrium* and 50% *Spirulina* showed similar growth rate to mussels fed on live microalgae (Langdon and Önal, 1999). The inclusion of *Schizochytrium*-sp. into microcapsules showed further potential for optimising bivalve feeding formulation, delivering high nutritive and energetic values for lower biomass, while reducing capital and maintenance expenditures, and extending product shelf life (Southgate et al., 1992; Robert and Trintignac, 1997; Langdon and Önal, 1999; Willer and Aldridge, 2017, 2019b). Microencapsulated feeds, containing *Schizochytrium* sp. and *Undaria pinnatifida*, which provided ~7% dietary proteins (g/ 100 g dw), were able to sustain survival and growth at similar rates as with conventional microalgal feeds (52% protein; g/100 g dw) in *M. galloprovincialis* spat cultured at laboratory scale (Campanati et al., 2022).

In this study we conducted a feeding trial on *Mytilus galloprovincialis* spat, designed to identify the potential substitution level of commercial microalgal inert diet (partial or complete: 0%; 60%; 80%; 100%) with *Schizochytrium* sp.-microencapsulated feeds (BioBullets Ltd., Cambridge, UK). The treatments were designed to compare inert diets, readily off-the shelves. We tested the dietary efficiency in terms of survival, growth and condition enhancement of juvenile mussels. By substituting commercially available preserved inert microalgal concentrates by *Schizochytrium* -microencapsulated feeds (BioBullets), the dietary protein availability is decreased while dietary carbohydrate and lipid content are increased. The aim of this study was to assess the substitution level of commercial preserved inert microalgal concentrates by microencapsulated 'BioBullets' in mussel spat feeds, which could uplift mussel production by reducing feed costs and thus increase the economic viability of nursery systems.

2. Material and methods

2.1. Spat collection, staining and initial conditions

Mytilus galloprovincialis spat (shell length 0.5–0.8 cm) were obtained from natural settlement on ropes from ACUIMAR SERVICIOS MARÍTIMOS (Benalmádena, Malaga, Spain), in August 2020. The mussels were transported, machine seeded to culture ropes and deployed in a longline facility in Mendexa (Basque Country, Spain; Azpeitia et al., 2016, 2017a) for a week, before being brought to the laboratory in Plentzia Marine Station (PiE-UPV/EHU facilities; Basque Country, Spain). Natural seawater was sand filtered in uptake wells and pumped into the laboratory facility, providing a flow-through system of seawater entering the experimental units with ~1000 particles/min, with particles size <3 µm (Beckman Coulter Counter Z2). In the laboratory, animals were acclimated for 10 days in a 1000 L- tank receiving natural filtered seawater, through the aforementioned flow-through systems, and not provided with any additional food. After the acclimation period, mussels were manually detached from the ropes, shells were cleaned of foulers with the help of scalpels and tweezers, and size range selected. Calcein staining, carried out through immersion of spat in 25 L- tanks with staining solution (150 mg L⁻¹) for 24 h (Campanati et al., 2022), was conducted before the start of the experiment to mark time 0 (t0) on the shell and retrieve shell thickness and height growth after the eight weeks trial (e.g. Stemmer et al., 2013). To determine the initial (t0) shell length (anterior-posterior axis) and width (dorsal-ventral axis; Muñoz-Moya et al., 2022), a group of 30 individuals were also photographed alongside a reference scale and measurements were obtained through

Image J (Schneider et al., 2012). The same individuals were then dissected for initial (t0) measurements of tissue and shell weight. Shells and tissues were first dried at 60 °C (dry weight, dw) in an oven and then at 450 °C in a muffle furnace to reduce the samples to ashes. Tissue ash-free dry weight (afdwt) and shell dw were used to calculate the initial condition Index (CI: Tissue afdwt/Shell dw *100). Additionally, other ~260 mussels (> 1 g wet tissue weight) were stored at -20 °C for further analyses on the initial tissue biochemical composition. Triplicate samples of the feeding treatments (commercial microalgae and inert microdiet) were also collected and stored at -20 °C for further biochemical analysis.

2.2. Dietary treatments

Following calcein staining, 3375 individuals were distributed among five experimental groups that received different feeding treatments for 8 weeks (56 days):

- 1) NC (Negative Control): supplied only with natural filtered seawater (FSW) and no additional feed;
- 2) A (Algae; Conventional singular diet): supplied with FSW and 100% commercial preserved microalgal concentrates (Shellfish Diet 1800, Reed Mariculture, California, USA), which was composed of a mixture of *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii* and *Thalassiosira pseudonana*;
- 3) B (BioBullets as alternative singular diet): supplied with FSW and 100% *Schizochytrium* BioBullets (BioBullets Ltd., Cambridge, UK; see below);
- 4) ABL (Mixed diet: Algae + low BioBullets inclusion): supplied with FSW and 60% BioBullets and 40% commercial preserved microalgal concentrates
- 5) ABM (Mixed diet: Algae + medium BioBullets inclusion): supplied with FSW and 80% BioBullets and 20% preserved microalgal concentrates.

Based on previous results (Willer and Aldridge, 2017, 2019a), BioBullets particles were produced at large commercial scale (>20 kg) using *Schizochytrium* sp. (50% by weight) by BioBullets Ltd. (Cambridge, UK). A premix slurry was prepared containing the encapsulant and active ingredient under conditions of controlled shear, as described in Aldridge et al. (2006) and Tang and Aldridge (2019). The spherical encapsulant particles (size distribution in Fig. S1), displayed near-neutral buoyancy in seawater for 24 h. Throughout this time, particles remained stable in seawater as only light dissolution of the particles surface was observed.

Each treatment group was represented by three replicate tanks (20L) with 225 spat/each and received sand- FSW flowing at rates of 40 mL min⁻¹. All feeding treatments were provided once a day (9:00 am) at a rate of 5% dw of feed per live weight of bivalve. The mixed diets (ABL and ABM) were prepared by homogenising the two dietary ingredients at the appropriate proportion before being distributed to the tanks. After feed administration, the water flow was stopped for 5 h to allow mussels to feed. At the same time, the water flow was equally stopped in NC tanks with non-fed mussels. Every day, the remaining feed was siphoned out of each tank to preserve good water quality. Total water change was conducted every 3 days for all the 15 tanks. Water parameters were monitored to ensure optimal conditions: Salinity = 32.97 ± 0.04; Temperature = 18.92 ± 0.08 °C; pH = 8.09 ± 0.03; Dissolved oxygen = 7.26 ± 0.17 mg L⁻¹. Photoperiod throughout the experiment was set at 12:12 h light:dark.

2.3. Survival, growth and final condition (CI)

Spat rearing tanks were inspected every day for mortalities. Dead individuals were promptly removed from the tank and cumulative mortality (%) was determined throughout the 8 weeks experiment. At

weekly intervals all individuals from each tank ($n = 3$ tanks/treatment) were blotted-weighted (i.e. live weight, WW) to determine singular tank feed rations for the following week. As for the initial spat samples, after 8 weeks under different dietary treatments, 10 mussels per tank (30 mussels/treatment = Σ 150 mussels) were photographed alongside a reference material to measure the final shell length (anterior-posterior axis) and width (dorsal-ventral axis; Muñoz-Moya et al., 2022). The same individuals were then dissected to obtain the final tissue and shell weight (afdwt and dw respectively) and calculate the final condition index. Growth rates (GR) in shell length (SL), shell width (SW), individual live weight (WW), tissue AFDW and shell DW were calculated as final-initial measurements/days of cultures (i.e. 56). Changes in condition index (CI) are expressed as percentages change from the initial value ((final-initial)/initial *100). Shells of other 10 mussels per tank (30 mussels/treatment = Σ 150 mussels) were rinsed with freshwater and dried at room temperature, before being examined for shell growth. Shells were embedded in resin (Epofix, Struers), sectioned through the longest shell dimension with a granite blade (Diamant Boart) and calcein marks were photographed under a fluorescent microscope (Leica MZ16F) using blue light ($\lambda_{exc} = 460-490$ nm) to evaluate shell thickness (ST) and height (SH) accretion throughout the experiment, considered as the increment of growth perpendicular and parallel to the longest direction of growth from the calcein mark to the outer shell, respectively (Fig. S2; e.g. Stemmer et al., 2013; Campanati et al., 2022). Shell thickness and height measurements obtained from the calcein marks were divided by the days of culture to calculate mussel ST and SH growth rates (GR).

2.4. Histology: spat digestive gland atrophy and adipogranular (ADG) cell index

At the start of the experiment (t0), 10 mussel spats were dissected, the soft tissue fixed with 4% neutral buffered formaldehyde for 24 h, and then stored in 70% EtOH at 4 °C for histological processing. After 8 weeks under different dietary treatments, four mussels from each replicate tank (i.e. 12 mussels/treatment; Σ 60 mussels), were dissected and their soft tissues stored in the same way as for t0 samples. Spat tissues were then dehydrated through ethanol series, cleared in xylene at room temperature and embedded in paraffin. Samples were cut with a microtome (Leica RM2125 RTS) to thin sections (5 μ m), then mounted on glass slides and stained with haematoxylin-eosin with an automated stainer (Leica CV 5030). Histological samples were assessed for the semi-quantification of the level of digestive gland atrophy and adipogranular (ADG) cell index. The atrophy index of digestive diverticula was assigned to samples according to Kim et al. (2006), rating the digestive diverticula's epithelium thickness from 0 (i.e. normal tubule wall thickness with lumen nearly occluded) to 4 (i.e. tubule wall extremely thin). The ADG cell index was determined by scoring the histological samples from 0 (i.e. absent adipogranular cells within the connective tissue) to 4 (i.e. adipogranular cells represent the majority of cells within the connective tissue), using the grading system by Bignell et al. (2008).

2.5. Biochemical analyses

Proximal biochemical composition was assessed in the diets and in spat tissues at the beginning (t0) and at the end of the feeding period (8 weeks). From each experimental tank of each treatment group, the tissue of different individuals (100–250) was pooled to reach 1 g of tissue, snap-frozen in liquid nitrogen and stored in -80 °C for further analyses. All samples were sent in dry ice to IU_ECOAQUA (Las Palmas de Gran Canaria, Spain) for biochemical and fatty acid composition analyses. Determination of moisture, protein and crude lipids were carried out following standard analytical methods (AOAC, 1995; Folch et al., 1957). Carbohydrates (%) were calculated by subtracting moisture (%), ash (%), proteins (%) and lipids (%) from 100%. Fatty acid methyl esters were obtained by trans methylation of crude lipids as described by

Christie (1982). Fatty acid methyl esters were separated by GLC (GC-14A, Shimadzu, Tokyo, Japan) following the procedure previously described by Izquierdo et al. (1990). An internal standard (21:0, Sigma Aldrich, MO, USA) was added for diet samples before methylation of crude lipids. Fatty acids were identified by comparison to previously characterised standards and GLC-MS (POLARIS QTRACE™ Ultra: Thermo Fisher Scientific). The results were expressed as relative abundance of the total pool of fatty acids. For dietary treatment B, individuals from different replicates were pooled to reach 1 g of tissue needed for the biochemical analyses. Thus, the number of samples analysed for this group is one.

2.6. Statistical analyses

All statistical analyses and graphics were performed in the R environment for statistical computation (R Core Team, 2022) and the level applied for significance in each statistical analysis was 0.05. Prior to any parametric test, normality of data was tested with Shapiro-Wilk test and homogeneity of variances through Levene's test. Spat survival and growth, proximal and fatty acids (FA) composition were compared among dietary treatment groups with one-way ANOVA followed by post hoc Tukey's test for pairwise comparisons. The level of digestive gland atrophy and the ADG cell index were compared between the initial (t0) and final (i.e. after 8 weeks) values with Kruskal-Wallis test. Relationships between dietary and tissue FA composition were determined through polynomial regressions. Initial and final (i.e. after 8 weeks) FA composition of spat mussels were also combined into principal component analysis (PCA) through factoMineR (Lê et al., 2008), with sum and ratios of FA included as supplementary quantitative variables and treatment as a categorical variable. Pearson's correlations were conducted between singular FA and principal components, to assess fatty acids contribution to group separation. Spearman's correlations analyses were conducted between spat growth parameters and changes in biochemical and FA tissue and visualised using corrplot package. Further, regressions estimating the effects that major FA have on spat growth variables are presented.

3. Results

3.1. Dietary treatments proximate and fatty acid composition

Proteins were provided at the highest proportion in the commercial microalgal paste treatment (A; ~20%), with dietary percentages decreasing with the inclusion of BioBullet (Table 1). Likewise, ash content decreased significantly, from ~56% (A) to ~5% (B) with Bio-Bullet substitution (Table 1). However, dietary lipids and carbohydrates increased significantly, from ~12% (A) to ~59% (B) and from ~11% (A) to ~34% (B) respectively, as microalgae were substituted by Bio-Bullet in dietary treatments (Table 1).

Total PUFAs, followed by total saturated fatty acids (SFAs) and total monounsaturated fatty acids (MUFAs) were relatively the most abundant fatty acids in dietary treatment A (Table 2). Among PUFAs, diet A presented the highest content of n-3 PUFAs, including also the highest % of n-3 LC-PUFAs: EPA (20:5n-3; 13.4%), DHA (22:6n-3; 8%) and also of α -linolenic (18:3n-3; 5.4%) and stearidonic (18:4n-3; 12%) acids. Among n-6 PUFAs, diet A contained the highest linoleic acid (18:2n-6; 4%) proportions among dietary treatments. BioBullets inclusion in the diet (ABL, ABM, B) provided relatively higher dietary SFAs due, particularly, to palmitic (16:0) and stearic (18:0) acids proportions. B dietary treatment, with relatively lower EPA contents compared to the other treatments (Table 2), provided the lowest dietary EPA/DHA ratio (Table 2). ABM and ABL treatments provided a mixture of A and B fatty acid relative profiles: improving n-3 PUFA, n-3 LC-PUFA, especially increasing proportions of EPA and arachidonic acid (AA) and EPA/AA and EPA/DHA ratios, with decreasing B (BioBullet) substitution (Table 2).

Table 1

Proximate nutritional biochemical compositions of dietary treatments (%/ g/ 100 g dry weight; mean $\pm$ SD, $n = 3$). A: Algae; B: BioBullet; ABL (40% A + 60% B); ABM (20% A + 80% B). ABL and ABM proximal composition are theoretical values calculated from the proportion of A (algae) and B (BioBullet) in each diet. Different letters represent significant differences found through pairwise comparisons following one-way ANOVA ($p < 0.05$). *Estimated dietary gross energy was calculated from dry weight of lipids, protein and carbohydrates using standard equations (Reg. EU N. 1169/2011).

	Diets			
	A	ABL	ABM	B
Ingredients (%)				
Microalgae (Shellfish Diet)	100	40	20	0
BioBullet (<i>Schizochytrium</i> sp.)	0	60	80	100
Proximal composition (% dw)				
Proteins	20.23 $\pm$ 0.22 ^a	8.64 $\pm$ 0.11 ^b	5.10 $\pm$ 0.09 ^c	1.71 $\pm$ 0.09 ^d
Lipids	12.22 $\pm$ 0.18 ^a	41.64 $\pm$ 0.24 ^b	50.62 $\pm$ 0.35 ^c	59.22 $\pm$ 0.46 ^d
Carbohydrates	11.06 $\pm$ 0.72 ^a	23.53 $\pm$ 0.30 ^b	29.94 $\pm$ 0.36 ^c	34.17 $\pm$ 0.49 ^d
Ash	56.49 $\pm$ 0.36 ^a	24.19 $\pm$ 0.12 ^b	14.34 $\pm$ 0.07 ^c	4.89 $\pm$ 0.02 ^d
Gross energy (kcal/100 g feed)*	235.12 $\pm$ 0.95 ^a	511.43 $\pm$ 1.37 ^b	595.74 $\pm$ 1.83 ^c	676.56 $\pm$ 2.26 ^d
(kJ/100 g feed)*	983.97 $\pm$ 4.09 ^a	2121.57 $\pm$ 5.52 ^b	2468.62 $\pm$ 7.36 ^c	2801.27 $\pm$ 9.05 ^d

3.2. Spat survival and growth

Mussels from all treatments sustained high survival throughout the experiment (i.e. mortality was lower than 10%) with no differences found among treatments at the end of the 8 weeks of feeding ($F_{4,10} = 1.737$; $p = 0.218$). Non-fed mussels (NC) showed similar survival to the fed groups. Changes in NC mussels shell and tissue growth from initial values were, though, almost nil compared to fed mussels.

Mussels fed with algae (A) and algae and BioBullets at low inclusion (ABL) showed the highest daily growth rates in shell length, width, height and thickness (Fig. 1A-D). Throughout 8 weeks of experiment, shell thickness increment was lower than shell height increment and was similar among mussels fed A, ABL and ABM (Fig. 1C-D). Non-fed mussels (NC) showed the lowest increment in both shell height and thickness (Fig. 1C-D). Shell weight of mussels fed A and ABL were also higher compared to mussels fed 100% BioBullets (B) (Fig. 1G; Table 3). Mussels fed with A and ABL also presented a higher live weight increase (daily increase of 0.34 and 0.24 mg day⁻¹, respectively) compared to mussels of the rest of the treatment groups (Fig. 1E; Table 3). Throughout the experimental period, mussels fed A and ABL increased 0.65 and 0.71 mg in tissue AFDW, respectively, compared to non-fed individuals (NC) which lost mass since the start of the experiment (Fig. 1F). NC mussels significantly reduced the condition index from the start of the experimental period compared to the fed groups (Fig. 1H; Table 3).

3.3. Spat digestive gland atrophy and adipogranular (ADG) cell index

Different levels of digestive gland atrophy were found among groups (Fig. S3 A; KW: $\chi^2 = 27.7$, $df = 5$, $p < 0.001$). After 8 weeks, NC mussel spat presented the highest level of digestive gland atrophy, whereas mussels fed microalgae (A) showed the lowest level, significantly different from initial values, NC and B groups (Fig. S3 A; $p < 0.05$). No differences in ADG cell index were found among groups and neither between the initial and final samplings (Fig. S3 B; KW: $\chi^2 = 5.21$, $df = 5$, $p = 0.391$).

Table 2

Fatty acid composition (% FA of total fatty acids) of different diets provided to *M. galloprovincialis* spat for 8 weeks (A = Algae; ABL = Algae + BioBullets at low inclusion; ABM = Algae + BioBullets at medium inclusion; B = BioBullets; Table 1). SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; LC-PUFAs = long chain polyunsaturated fatty acids.

FA \ Diets	A	ABL	ABM	B
14:0	7.843	3.312	1.802	0.291
15:0	0.709	0.314	0.182	0.050
16:0	13.902	15.298	15.763	16.229
16:1n-7	9.189	3.713	1.888	0.063
16:1n-5	1.094	0.438	0.219	0.000
16:2n-4	3.034	1.214	0.607	0.000
17:0	3.314	1.326	0.663	0.000
16:3n-1	1.202	0.489	0.251	0.014
18:0	0.441	40.849	54.318	67.787
18:1n-9	7.506	5.176	4.399	3.622
18:1n-7	1.118	0.544	0.353	0.162
18:2n-6	3.959	3.159	2.892	2.625
18:3n-6	0.966	0.403	0.216	0.028
18:3n-3	5.440	2.210	1.134	0.057
18:4n-3	11.629	4.670	2.351	0.031
20:0	0.040	0.803	1.057	1.311
20:1n-9	1.064	0.434	0.224	0.014
20:1n-7	0.237	0.119	0.080	0.041
20:4n-6	0.527	0.361	0.306	0.251
20:5n-3	13.450	5.416	2.738	0.061
22:5n-6	2.478	1.921	1.735	1.549
22:5n-3	0.140	0.081	0.062	0.042
22:6n-3	8.001	6.371	5.828	5.284
ΣSFA	26.250	61.901	73.785	85.668
ΣMUFA	21.142	10.948	7.551	4.153
ΣPUFA	47.589	25.106	17.612	10.118
Σ n-3 PUFA	38.931	18.913	12.240	5.567
Σ n-3 LC PUFA	21.862	12.032	8.755	5.479
Σ n-3	39.420	19.128	12.364	5.600
Σ n-6	8.196	6.001	5.269	4.537
n-3/n-6	4.809	3.188	2.347	1.234
EPA + DHA	21.451	11.787	8.566	5.345
EPA/DHA	1.681	0.850	0.470	0.011
EPA/ARA	25.514	14.985	8.943	0.242

3.4. Spat proximate and fatty acid composition

Dietary composition affected mussel's tissue composition, as feeding on higher dietary proteins, especially in A and ABL treatments, promoted higher tissue protein content in mussels (Table 1; Table 4). However, although not provided with external food, NC mussels showed similar final protein content to ABL, ABM and B mussels, being ~ + 3% higher than the initial values (Table 4). Mussels of all groups increased their tissue lipid content in 8 weeks (i.e. +3% in NC; +5% in A, ABL and ABM; +9% in B), although final lipid tissue contents of fed mussels were not significantly different among replicated treatments (Table 4). ABL, ABM and B-fed mussels, provided with 24–33% carbohydrates (Table 1), increased their tissue carbohydrate proportion from ~0.5 to ~3% from initial values (Table 4). A-fed mussels, however, provided with the lowest amount of dietary carbohydrates among the feeding groups (Table 1), showed the lowest content of tissue carbohydrates (~7% lower than the initial value; Table 4). <1% reduction of tissue carbohydrate content was found in NC mussels with respect to the initial contents (Table 4). All mussels lost ash and gained tissue moisture content in 8 weeks cultivation at experimental conditions (Table 4). NC and A mussels showed similar ash and moisture tissue contents after 8 weeks (Table 4). At the end of the experiment, moisture was significantly higher in the tissue of spat fed on diets with BioBullets inclusion (ABL, ABM) compared to non-fed spat (NC) and spat fed A (Table 4).

The major fatty acids (FA) constituting spat tissue FA profile before and after 8 weeks-culture under different dietary treatments are presented (as % of total FA) in Table 5. Mussels fed A and ABL increased their tissue proportion of MUFAs after 8 weeks of culturing (~ + 12.5%

and ~ 9.5% from t0; Fig.2A; Table 5). Among SFAs, stearic acid (18:0) was found at highest percentages in BioBullets-fed groups (ABL, ABM, B) compared to A-fed mussels. Dietary treatment A contained, in fact, a low proportion of 18:0 compared to the other treatments (Table 2; Table 5). On the other hand, palmitic acid (16:0), which was provided in similar proportions in all diets (14–16%), was mostly reduced (~ – 4%) with respect to initial tissue content in B-fed mussels after 8 weeks. PUFAs, particularly n-6 PUFAs, and the sum of EPA and DHA, were retained in highest proportions (+12.4%; +10.2%; +3% respectively from t0; Fig. 2A) in B-fed mussels. B-fed mussels, after 8 weeks of culture, presented the lowest proportions, among the feeding groups, of tissue EPA/DHA and EPA/ARA ratios, (Fig. 2A; Table 5). Regarding n-6 PUFAs, B-fed mussels presented the highest content of linoleic acid (18:2n-6), and docosapentanoic acid (22:5n-6), with a 7% and 5% increase in proportion of total FA, respectively, from initial values (Fig. 2B; Table 5). All feeding groups showed decreased arachidonic acid (ARA; 20:4n-6) proportions from initial values (~ – 1 to – 2.4%), whereas this FA was found to be highly retained in non-fed mussels (NC; ~ + 2% from t0) (Fig. 2B; Table 5). Among n-3 PUFAs, A-fed mussels presented the highest final tissue proportions of α-linolenic (18:3n-3), stearidonic (18:4n-3) and EPA fatty acids (Table 5), all of which increased in spat tissues from t0 (Fig. 2C). EPA was, however, reduced in non-fed (NC) and B-fed mussels (Fig. 2C; Table 5), provided with <0.1% in B diet (Table 2). Mussels of all treatment groups showed a decrease in tissue DHA, in the range of ~ – 7 to – 11.5% from t0, except from B-fed mussels (+5% from t0; Fig. 2D), which were provided with the lowest dietary proportion (Table 2; Table 5).

PCA plot and spat tissue FA profiles correlation with principal components also showed clear differences among groups, at the start and after 8 weeks under dietary treatments (Fig. S4, Table S3). A cumulative ~74% of total variance of tissue FA composition among groups is explained by the first two principal components of the PCA plot (Fig. S4), achieving 82% of cumulative variance explained once PC3 is also considered (Table S3). Non-fed and fed mussels are represented by different clusters separated by both PC1 and PC2 (Fig. S4), which strongly correlated with various FAs (Fig. S4B; Table S3). A-fed mussels and B-fed mussels groups well separated through PC2, which was positively correlated with MUFAs, EPA, EPA/DHA and EPA/ARA proportions, more strongly contributing to A-fed mussels (Fig. S4; Table 5; Table S3), and negatively correlated with DHA, n-3 LC-PUFAs, n-6 s, EPA + DHA proportions, more strongly contributing to B-fed mussels (Fig. S4; Table 5; Table S3). These FAs were, in fact, highly retained after 8 weeks in soft tissues of B-fed mussels (Fig. S4; Table 5; Table S3). ABL and ABM-fed mussels, grouped in one cluster (Fig. S4), were mainly characterised by higher percentages of stearic acid (18:0), 18:1n-9, SFAs. Non-fed mussels (NC) were mostly defined by PC1, with the highest percentages of ARA (20:4n-6) among groups (Fig. S4; Table 5; S3).

After 8 weeks culture, the FA composition of fed *M. galloprovincialis* spat (i.e. NC excluded) was dependent on the respective dietary FA profile (Table S1; $R^2 = 0.6$; $F_{3,224} = 109.2$; $p < 0.001$). Particularly, significant positive relationships were found between dietary proportions of EPA (log-log: $R^2 = 0.85$; $F_{1,8} = 44.1$; $p < 0.001$), MUFAs (log-log: $R^2 = 0.76$; $F_{1,8} = 24.8$; $p < 0.001$) and the n-3/n-6 ($R^2 = 0.67$; $F_{1,8} = 16.98$; $p = 0.003$), EPA/ARA (log-log: $R^2 = 0.87$; $F_{1,8} = 53.41$; $p < 0.001$) and EPA/DHA ($R^2 = 0.89$; $F_{1,8} = 67.43$; $p < 0.001$) ratios, and their respective proportion in spat tissues after 8 weeks culture. On the other hand, dietary proportions of DHA were negatively related (log-log linearised regressions) to the spat tissue proportions ($R^2 = 0.57$; $F_{1,8} = 10.7$; $p = 0.011$).

3.5. Role of fatty acids and biochemical composition on spat growth

In mussels fed A and ABL, growth parameters were positively correlated to significant changes in tissue composition of protein content, and relative proportions of MUFAs, EPA and EPA/ARA ratios ($p <$

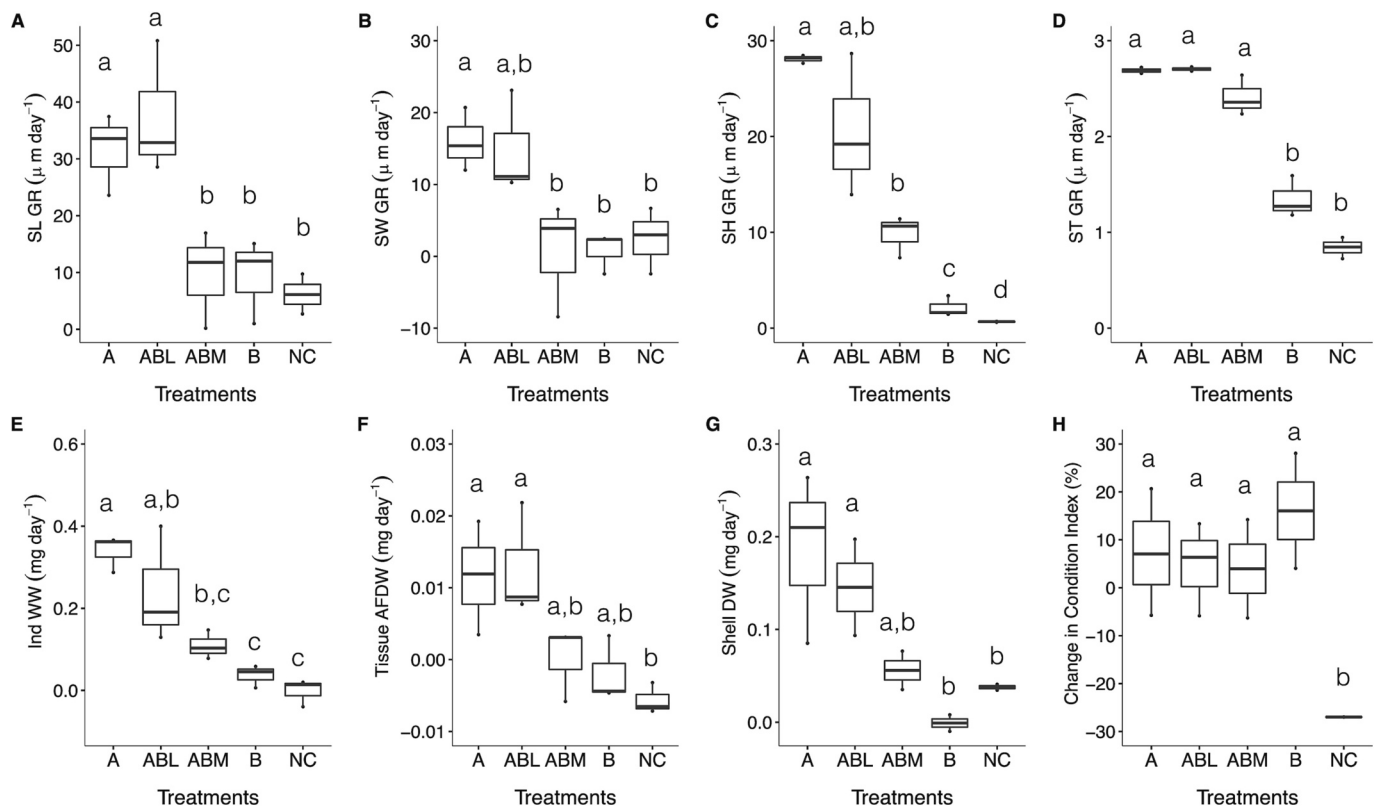


Fig. 1. *M. galloprovincialis* spat growth over 8 weeks under different dietary treatments (i.e. A, ABL, ABM, B, NC). Comparisons of growth rates of (A) shell length (SL), (B) shell width (SW), (C) shell height (SH) and (D) shell thickness (ST) are presented with growth of (E) individual live weight, (F) tissue ash-free dry weight (afdwt), (G) shell dry weight (dw) and (H) Change in condition Index (% change from Initial CI). Median ($\pm$ 95% confidence intervals) with $n = 3$ replicate tanks are reported for all treatment groups. Different letters represent significant differences ($p < 0.05$) found through Tukey's and least-square mean (for CI) post hoc pairwise comparisons following ANOVA results (Table 3).

0.05; Fig. 3, Table S4), during the 8-weeks period (Tables 4, 5). Spat growth was, however, negatively correlated to significant changes in tissue ARA and DHA (Fig. 3, Table S4), which decreased in all treatment groups except in NC and B groups after 8 weeks of culture (Table 5).

4. Discussion

4.1. Effect of starvation on *M. galloprovincialis* spat

Although survival was not affected by relative starvation, non-fed mussels (NC) showed almost nil tissue and shell growth compared to mussels fed different dietary treatments. Likewise, the condition index of NC mussels was significantly reduced throughout the experimental period. Several FA proportions changed from the initial values in this mussel group. For example, a reduction of $\sim 9\%$ n-3 PUFAs and n-3 LC-PUFAs tissue content was observed from the start of the experiment. Similarly, a decrease of $\sim 11\%$ n-3 PUFAs was observed in adult mussels starved for 72 h following depuration (Ruano et al., 2012), whereas the FA profile of mussels starved for 30 days was found unchanged in another study (Pirini et al., 2007). Compared to the fed groups, NC-mussels preferentially retained ARA in their tissues. Similar findings were shown in other studies for starved individuals (e.g. Pirini et al., 2007; Supono et al., 2022) or during stress response (i.e. increased temperature; Pernet et al., 2007). The authors suggested that the FA was made available in the tissue for its involvement in the synthesis of highly biologically active hormones, prostaglandins (Coutteau et al., 1996; Knauer and Southgate, 1997a, 1997b; Ventrella et al., 2013). Pernet and Tremblay (2004) also showed that larvae of the scallop *Placopecten magellanicus* fed with a diet deficient in ARA, accumulated this FA at three times higher concentration compared to the dietary content.

Some other FAs, such as SFAs, among which, stearic acid (18:0) particularly, increased from initial values in NC-mussels. The FSW flowing in the experimental system retained particles larger than $3 \mu\text{m}$ in size. From a monitoring study of phytoplankton assemblages, developed in the Nervion River estuary, which is relatively close to Plentzia, picoplankton ($< 3 \mu\text{m}$ size) was found to account for $< 5\%$ of the total phytoplankton pigments sampled (Seoane et al., 2006). Among the picoplankton sampled, however, pigment ratios suggested a high presence of *Nannochloropsis*-like eustigmatophytes (Seoane et al., 2006). The presence of *Synechococcus* cyanobacteria were also found in noticeable quantities in August (Urrutxurtu et al., 2003; Orive et al., 2004) in a marine area close to Plentzia and coinciding with the experimental period (August to October 2020). In this context, picoplankton species and bacteria could have contributed to the mussel sustainment and change in tissue FA composition in the NC group, as for example, levels of 16:1n-7 and 18:1n-7 paired with a decrease in n-3 PUFAs have been suggested to be indicators of bacterial input in the environment (Zhukova, 2019). Nevertheless, most studies agree that the retention efficiency increases with particle diameter and most bivalves cannot effectively retain particles smaller than $4 \mu\text{m}$ (Ward and Shumway, 2004). Recent experiments demonstrated that the retention efficiency in *Mytilus edulis* varies with changes in the seston size, as observing that when small particles dominated, mussels preferably retained particles between 7 and $15 \mu\text{m}$ diameter, albeit they had some capacity to retain particles between 1 and $4 \mu\text{m}$ diameter (Strohmeier et al., 2012).

The capacity to overcome long-term starvation (i.e. 2 and 8 months) was also found in *Mytilus edulis* adults (Harbach and Palm, 2018) and juveniles (Diehl et al., 1986) respectively, with associated reduced rates of metabolism. The fact that no significant increase in mortality has been observed in non-fed (NC) mussels compared to fed mussels could imply

Table 3

Results of one-way ANOVA testing the effect of dietary treatment (i.e. NC, A, ABL, ABM, B) on mussel growth rates of shell dimensions: Shell length (SL), width (SW), height (SH), thickness (ST); weight: Individual wet weight (WW), tissue ash-free dry weight (afdw), shell dry-weight (dw) and condition index (CI; % change from Initial CI). Results in bold represent significant differences found among groups and were further investigated with post hoc analyses (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Variable	Source of variance	df	Sum Sq	Mean Sq	F value	p-value	
SL_GR (µm/day)	Treatment	4	2524.00	630.90	9.53	0.002	**
	Residuals	10	662.00	66.20			
SW_GR (µm/day)	Treatment	4	727.60	181.90	5.58	0.013	*
	Residuals	10	326.10	32.61			
SH_GR (µm/day)	Treatment	4	30.15	7.54	94.46	<0.001	***
	Residuals	10	0.80	0.08			
ST_GR (µm/day)	Treatment	4	8.73	2.18	105.70	<0.001	***
	Residuals	10	0.21	0.02			
Ind WW (mg)							
WK 0	Treatment	4	285.90	71.48	2.15	0.149	
	Residuals	10	333.00	33.30			
WK 1	Treatment	4	225.30	56.33	1.81	0.204	
	Residuals	10	311.80	31.17			
WK 2	Treatment	4	311.10	77.77	2.11	0.154	
	Residuals	10	368.20	36.82			
WK 3	Treatment	4	424.20	106.05	2.81	0.084	
	Residuals	10	377.40	37.74			
WK 4	Treatment	4	453.10	113.27	2.43	0.116	
	Residuals	10	466.50	46.65			
WK 5	Treatment	4	563.20	140.80	3.09	0.067	
	Residuals	10	456.00	45.60			
WK 6	Treatment	4	738.10	184.52	3.79	0.040	*
	Residuals	10	487.20	48.72			
WK 7	Treatment	4	1126.60	281.66	6.12	0.009	**
	Residuals	10	460.10	46.01			
WK8	Treatment	4	1405.60	351.40	6.50	0.008	**
	Residuals	10	540.70	54.10			
Ind WW_GR (mg/day)	Treatment	4	0.24	0.06	12.09	<0.001	***
	Residuals	10	0.05	0.01			
Tissue AFDW_GR (mg/day)	Treatment	4	8.23×10^{-4}	2.06×10^{-4}	5.85	0.011	*
	Residuals	10	3.51×10^{-4}	3.51×10^{-5}			
Shell DW_GR (mg/day)	Treatment	4	0.15	0.04	5.30	0.015	*
	Residuals	10	0.07	0.01			
Change in CI (%)	Treatment	4	0.70	0.18	3.86	0.038	*
	Residuals	10	0.46	0.05			

Table 4

M. galloprovincialis spat tissue proximal composition (%/ wet weight; ww) at t0 and after 8 weeks of culture under different dietary treatments (i.e. NC, A, ABL, ABM, B). Initial values (t0) and final values of NC, A, ABL and ABM represent averages ($\pm$ SD) of $n = 3$ replicates but BioBullets $n = 1$. Different letters represent significant differences among replicated treatments after 8 weeks found through pairwise comparisons following one-way ANOVA.

(%/ww)	Initial (t0)	Final tissue composition				
		NC	A	ABL	ABM	B
Proteins	61.24 ± 0.08	63.80 ± 0.32 a,c	68.17 ± 0.45 b	63.97 ± 0.51 a,b	57.94 ± 2.37 c	56.44
Lipids	6.26 $\pm$ 0.19	9.41 $\pm$ 0.37	11.51 ± 0.23	11.64 ± 0.21	10.65 ± 1.76	15.94
Carbohydrates	12.77 ± 0.11	11.92 ± 0.65 a	6.04 $\pm$ 0.39 b	13.26 ± 0.70 a	15.99 ± 0.63 c	15.68
Moisture	1.37 $\pm$ 0.14	2.21 $\pm$ 0.27 a	1.77 $\pm$ 0.22 a	3.87 $\pm$ 0.44 b	4.94 $\pm$ 1.01 b	1.46
Ash	18.36 ± 0.21	12.65 ± 0.16 a	12.52 ± 0.43 a	7.26 $\pm$ 0.22 b	10.49 ± 0.26 c	10.54

that the condition and energy reserves of mussels at t0 were adequate to sustain maintenance costs during a relatively long-term period without feed supplementation, a phenomenon also observed by other authors (Hawkins et al., 1989). It has been shown that under-nourished mussels had lower RNA content, reflecting suppression of protein turnover, increased catabolism and autophagy (González-Fernández et al., 2017). Survival maintenance in NC mussels could, in fact, also be explained by an enhanced autophagy process. The severe digestive gland atrophy

revealed in NC mussels reflects the stress condition and digestion disturbance (Marigómez et al., 2017).

4.2. Effect of the dietary treatment composition on *M. galloprovincialis* spat

Mussels fed only with commercial microalgal concentrates (A) and with microalgae combined with BioBullets at low inclusion (ABL) treatments showed similar growth performances, indicating that substitution of preserved commercial microalgae (A) up to 60% with *Schizochytrium* sp.(B) can be considered. Dietary energy was not limiting for spat growth, as A fed spat showed the best growth performance throughout the experimental period, although containing 2-fold less energy than ABL, or 2.5-fold and 3-fold less than ABM and B diets, respectively. Likewise, dietary lipids were neither limiting for spat growth, as energy and lipid content increased with the substitution of A by B in dietary treatments. Although providing a more balanced dietary lipids and protein content, diet A, with ~20% (% dw) protein promoted similar growth to ABL-diet, which provided ~8% protein. In this context, as correlated with growth, dietary protein content <8% (% dw) in ABM and B diets could be below the nutritional requirements for *M. galloprovincialis* spat, limiting tissue and shell growth. Micro-encapsulated diet provided at 100% (B group), proved suboptimal for *M. galloprovincialis* spat (i.e. low tissue and shell growth), possibly due to the low protein content and excess of lipids in the treatment.

Our study supports many others that reported a strict dependence of dietary FAs composition on bivalves' tissue composition (Langdon and Waldock, 1981; Albentosa et al., 1994, 1996; Caers et al., 1999, 2002; Knauer and Southgate, 1997b; Delaporte et al., 2005; Fernández-Reiriz

Table 5

Fatty acid composition (% FA of total fatty acids) of *M. galloprovincialis* spat tissue at t0 (Initial) and after 8 weeks culture under different dietary treatments (i.e. NC, A, ABL, ABM, B). Values are reported as mean ($\pm$ SD) of $n = 3$ replicates but BioBullet and Initial samples have $n = 1$. Different superscript letters represent significant differences found for each fatty acid through one-way ANOVA and pairwise post-hoc tests among the replicated treatments (Table S2). Not significant differences are left blank. SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; LC-PUFAs = long chain polyunsaturated fatty acids.

FA \ Treatm	Initial	NC $\pm$ SD	A $\pm$ SD	ABL $\pm$ SD	ABM $\pm$ SD	B
14:0	0.714	0.363 $\pm$ 0.054 ^a	1.866 $\pm$ 0.361 ^b	1.342 $\pm$ 0.350 ^{b,c}	1.093 $\pm$ 0.248 ^c	0.298
15:0	0.670	0.904 $\pm$ 0.102 ^a	0.834 $\pm$ 0.213 ^a	0.532 $\pm$ 0.066 ^b	0.478 $\pm$ 0.047 ^b	0.210
16:0	13.317	12.601 $\pm$ 0.689	15.701 $\pm$ 1.376	15.183 $\pm$ 1.602	14.519 $\pm$ 1.618	9.733
16:1n-7	0.764	1.675 $\pm$ 0.198 ^a	7.360 $\pm$ 1.669 ^b	4.973 $\pm$ 1.132 ^{b,c}	4.002 $\pm$ 0.447 ^c	1.762
16:1n-5	0.762	1.264 $\pm$ 0.032 ^a	1.370 $\pm$ 0.110 ^a	0.0861 $\pm$ 0.133 ^b	0.751 $\pm$ 0.120 ^b	0.251
16:2n-4	0.056	0.676 $\pm$ 0.063 ^{a,b}	0.859 $\pm$ 0.139 ^a	0.486 $\pm$ 0.130 ^{b,c}	0.346 $\pm$ 0.056 ^c	0.017
17:0	1.321	1.255 $\pm$ 0.141 ^a	0.521 $\pm$ 0.052 ^b	0.165 $\pm$ 0.075 ^c	0.163 $\pm$ 0.049 ^c	0.176
16:3n-1	21.053	15.847 $\pm$ 1.984 ^a	7.976 $\pm$ 0.891 ^b	7.229 $\pm$ 0.553 ^b	7.217 $\pm$ 0.486 ^b	6.071
18:0	5.380	10.259 $\pm$ 0.250 ^a	4.710 $\pm$ 0.524 ^b	11.482 $\pm$ 1.302 ^{a,b}	13.464 $\pm$ 1.627 ^c	11.046
18:1n-9	1.672	3.101 $\pm$ 0.333 ^a	7.286 $\pm$ 2.041 ^b	8.070 $\pm$ 0.798 ^b	8.088 $\pm$ 0.766 ^b	9.141
18:1n-7	0.919	2.370 $\pm$ 0.308 ^a	4.536 $\pm$ 0.240 ^b	3.915 $\pm$ 0.159 ^{a,b}	4.219 $\pm$ 0.870 ^{a,b}	2.852
18:2n-6	1.761	2.122 $\pm$ 0.173 ^a	4.774 $\pm$ 0.838 ^b	5.612 $\pm$ 0.494 ^b	5.775 $\pm$ 0.355 ^b	8.508
18:3n-6	0.099	0.120 $\pm$ 0.021 ^a	0.394 $\pm$ 0.047 ^b	0.255 $\pm$ 0.025 ^c	0.207 $\pm$ 0.033 ^c	0.091
18:3n-3	0.516	0.389 $\pm$ 0.073 ^a	3.128 $\pm$ 0.470 ^b	2.008 $\pm$ 0.337 ^c	1.537 $\pm$ 0.281 ^c	0.137
18:4n-3	0.508	0.266 $\pm$ 0.017 ^a	1.425 $\pm$ 0.281 ^b	0.770 $\pm$ 0.145 ^c	0.561 $\pm$ 0.081 ^c	0.057
20:0	0.086	0.175 $\pm$ 0.014 ^a	0.086 $\pm$ 0.021 ^b	0.177 $\pm$ 0.031 ^a	0.201 $\pm$ 0.014 ^a	0.128
20:1n-9	3.974	4.018 $\pm$ 0.323 ^a	4.041 $\pm$ 0.719 ^a	0.880 $\pm$ 0.090 ^b	0.755 $\pm$ 0.138 ^b	0.683
20:1n-7	5.087	5.671 $\pm$ 0.279 ^a	6.04 $\pm$ 0.118 ^b	3.450 $\pm$ 0.632 ^c	2.776 $\pm$ 0.929 ^c	1.815
20:4n-6	7.258	9.236 $\pm$ 0.438 ^a	4.922 $\pm$ 0.778 ^b	4.840 $\pm$ 0.944 ^b	4.953 $\pm$ 0.569 ^b	6.256
20:5n-3	5.722	3.804 $\pm$ 0.286 ^a	8.401 $\pm$ 0.825 ^b	6.618 $\pm$ 0.726 ^c	6.032 $\pm$ 0.598 ^c	3.759
22:5n-6	2.071	1.955 $\pm$ 0.141 ^a	3.496 $\pm$ 0.858 ^{a,b}	4.379 $\pm$ 0.690 ^b	4.719 $\pm$ 0.485 ^b	7.055
22:5n-3	1.107	1.040 $\pm$ 0.114 ^a	0.694 $\pm$ 0.152 ^b	0.510 $\pm$ 0.145 ^b	0.483 $\pm$ 0.073 ^b	0.735
22:6n-3	21.407	13.971 $\pm$ 1.241	9.938 $\pm$ 1.924	12.304 $\pm$ 2.516	14.144 $\pm$ 2.189	26.996
Σ SFA	21.489	25.557 $\pm$ 0.803	23.739 $\pm$ 1.778	28.881 $\pm$ 3.100	29.918 $\pm$ 3.314	21.592
Σ MUFA	14.153	20.726 $\pm$ 0.308	26.639 $\pm$ 2.470	23.599 $\pm$ 0.979	21.916 $\pm$ 2.016	17.150
Σ PUFA	42.494	35.822 $\pm$ 2.434	39.730 $\pm$ 4.075	39.189 $\pm$ 4.277	40.037 $\pm$ 2.830	54.936
Σ n-3	29.380	19.695 $\pm$ 1.678	24.008 $\pm$ 2.432	22.485 $\pm$ 2.983	23.010 $\pm$ 1.908	31.929
Σ n-3 LC PUFA	28.355	19.040 $\pm$ 1.636	19.455 $\pm$ 2.773	19.707 $\pm$ 3.405	20.912 $\pm$ 2.104	31.734
Σ n-3	29.911	20.639 $\pm$ 1.683	24.450 $\pm$ 2.322	22.693 $\pm$ 3.027	23.195 $\pm$ 1.896	32.013
Σ n-6	12.575	15.114 $\pm$ 0.705	14.961 $\pm$ 1.560	16.179 $\pm$ 1.323	16.604 $\pm$ 0.956	22.825
n-3/n-6	2.379	1.364 $\pm$ 0.058 ^a	1.635 $\pm$ 0.039 ^b	1.399 $\pm$ 0.074 ^a	1.396 $\pm$ 0.033 ^a	1.403

Table 5 (continued)

FA \ Treatm	Initial	NC $\pm$ SD	A $\pm$ SD	ABL $\pm$ SD	ABM $\pm$ SD	B
EPA + DHA	27.128	17.775 $\pm$ 1.506	18.338 $\pm$ 2.592	18.922 $\pm$ 3.242	20.176 $\pm$ 2.081	30.755
EPA/DHA	0.267	0.273 $\pm$ 0.010 ^a	0.857 $\pm$ 0.109 ^b	0.545 $\pm$ 0.055 ^{c,d}	0.434 $\pm$ 0.090 ^{a,d}	0.139
EPA/ARA	0.788	0.412 $\pm$ 0.027 ^a	1.733 $\pm$ 0.310 ^b	1.385 $\pm$ 0.135 ^b	1.224 $\pm$ 0.126 ^b	0.601

et al., 1998, 1999, 2006; Nevejan et al., 2007; Pirini et al., 2007; Rivero-Rodríguez et al., 2007; Ventrella et al., 2013; Geng et al., 2016). *Mytilus edulis* spat growth positively correlated with higher dietary triacylglycerol content, MUFAs and PUFAs, among which DHA was suggested as preferred essential FA by mussels rather than ARA/EPA (Laudicella et al., 2020). In our study, *M. galloprovincialis* spat growth was positively correlated with dietary and tissue change in protein content, as well as levels of EPA, MUFAs, and EPA/ARA and EPA/DHA ratios. Positive correlations between growth and tissue proteins and MUFAs were also found in a study feeding different microalgal diets to the freshwater mussel *Hyriopsis cumingii* (Chen et al., 2021). After 8 weeks of culture, mussels fed with B presented the highest tissue content of n-3 PUFAs and n-3 LC PUFAs. For example, DHA was retained at the highest percentages in B-fed mussels (i.e. $\sim +6\%$ from t0) although provided with the lowest amount in the B dietary treatment. Thus, DHA could have been preferentially retained in B group compared to EPA, and/or have been derived from elongation and desaturation of EPA, reduced from the initial value (-2%). This capacity, also found in *C. gigas* spat (Waddock and Holland, 1984), was possibly too low to meet DHA essential requirements and sustain mussel spat growth. Thus, results indicate that the dietary proportion of DHA could beneficially be increased in the encapsulated feeds (e.g. 12% of total FA; Laudicella et al., 2020), and especially its proportion to EPA, ideally reaching EPA/DHA ratios of around 0.85.

Higher dietary provisioning of stearic acid (18:0) with diets containing B (ABL, ABM, B), led to small increased percentages of this FA in the mussels' tissues. Possible involvements of delta 9 fatty acyl ($\Delta 9$) desaturase activities could justify the gain in oleic acid (18:1n-9) in the tissues of these mussel groups, which surmounted the dietary inputs. The activity of $\Delta 9$ desaturase has been described as ubiquitous in all living organisms (Zhukova, 2019), whereas the successive desaturation stage of $\Delta 12$ desaturase in producing linoleic (18:2n-6) acid is uncertain. B-fed mussels, however, showed a 7% increase in linoleic acid proportion of total FA from initial values (t0). All groups showed a reduction of 16:3n-1 from the initial value and feeding groups showed a reduction to a lesser extent in 20:1n-7 and 20:1n-9 from t0, which could have been used as precursors for non-methylene interrupted (NMI) dienolic FAs (da Costa et al., 2015; Zhukova, 2019), with a double bond insertion commonly mediated by $\Delta 5$ desaturase in invertebrates (Zhukova, 1991; da Costa et al., 2015). This consideration, however, remains speculative as the bulk lipid analyses of spat tissues did not detect these FAs, usually found at higher concentrations in phospholipids (Zhukova, 1991). Further work would be necessary to discern FA usage in neutral (storage) or polar (structural) lipid fractions in bivalves (e.g. Fernández-Reiriz et al., 2015; Willer et al., 2020).

Additionally, species-specific essential FA requirements and utilisation of energy sources (e.g. Albentosa et al., 1996; Delaporte et al., 2005; Milke et al., 2006; Ruano et al., 2012), as well as different particle capture capacity (Rosa et al., 2015; Pales Espinosa et al., 2016) have been reported for different filter feeders. Nevertheless, the resemblance of tissue biochemical and FA composition with the dietary profile, confirm the digestion of encapsulated *Schizochytrium* by *M. galloprovincialis* spat. The larger size of the particles compared to microalgae could, however, have reduced the feeding and retention capacity of the microcapsules, requiring a higher energy for their assimilation (Rosa et al., 2015; Pales Espinosa et al., 2016).

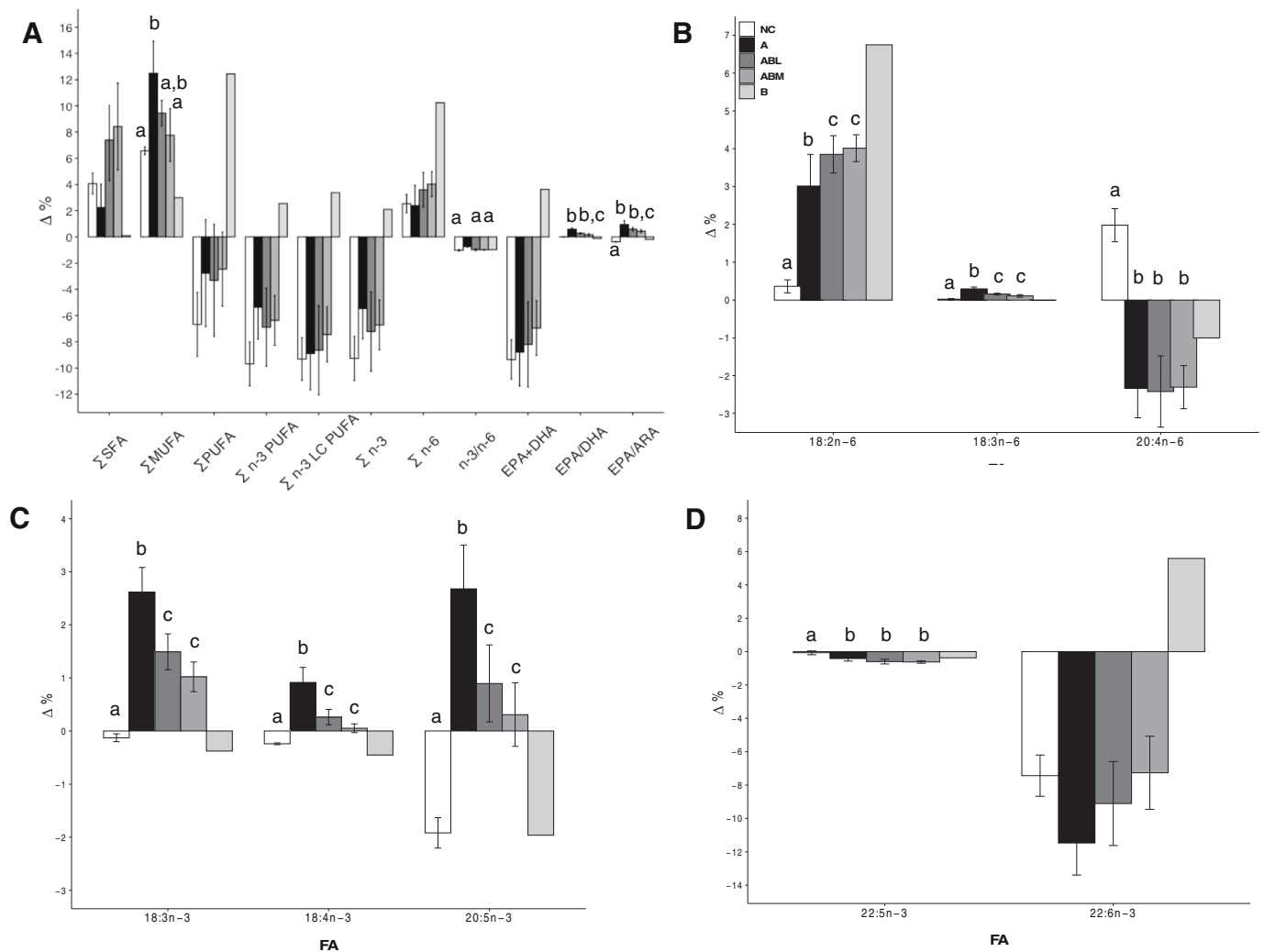


Fig. 2. (A) Variation of fatty acids (FA) of *M. galloprovincialis* spat cultured for 8 weeks under different dietary treatments (i.e. NC, A, ABL, ABM, B). (B) Variation in spat tissue arachidonic acid (ARA), (C) EPA, (D) DHA and their precursors. Values ($\pm$ SD) are expressed in percentage of fatty acid gains or losses from initial values. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LC-PUFA = long-chain PUFAs ($C \geq 20$). Different letters represent significant differences ($p < 0.05$) found through Tukey's post hoc pairwise comparisons following ANOVA results.

In grow-out conditions seeded spat of 15–25 mm showed a growth increment between 0.2 and 0.36 mm day⁻¹ (Camacho et al., 1995; Robert et al., 2013; Kamermans et al., 2013). In our study, we observed limited spat growth compared with studies that tested in the laboratory the feeding of larger size spat of this mussel species (16–25 mm spat; e.g. Fernández-Reiriz et al., 2016). However, our results of spat growth, measured through calcein staining and fluorescent microscopy, are comparable to growth rates observed in studies with smaller-sized spats (e.g., 2.6–3 mm; Skelton et al., 2021; 5–8 mm; Campanati et al., 2022). The relatively small growth rates observed over our 8 weeks experimental period could result from elevated energy expended in the digestion of microencapsulated diets at this small size stage (e.g. Skelton et al., 2021). Although not tested here, differences in feeding rates and efficiency of pre-ingestive particle selection and retention processes could have caused differences in metabolic efficiencies and thus in energy expenditure for growth in the different feeding treatment groups (e.g. Tamayo et al., 2016; Prieto et al., 2020). Likewise, different digestibility of the tested diets (e.g. Milke et al., 2006) could have also contributed to differences in the nutritional utilisation of the dietary treatments.

It is possible that a quality difference between live microalgae and microalgal paste could have allowed a higher substitution with

microcapsules without causing significant effects on the spat growth. For example, feeding *Ostrea edulis* spat with live microalgae alone led to the same improvements in spat growth as combining live microalgae and microcapsules, whereas a better growth was obtained when feeding *O. edulis* spat with a combination of microalgal paste and microcapsules compared to microalgal paste alone (Willer and Aldridge, 2019a). Nevertheless, high-quality concentrated algal pastes (e.g., *Isochrysis* sp., *Thalassiosira pseudonana*, *Tetraselmis* sp.) have long been considered promising replacements for live algal feeds (Schienner et al., 2015; Yarnold et al., 2019) and have previously been used in laboratory (e.g. Schienner et al., 2015; Campanati et al., 2022) and hatchery settings (e.g. Southgate et al., 2016), supporting normal growth and development of bivalve early stages.

Although mussel spat are usually transferred to open water at a size of 0.5–1 cm shell length, a trade-off exists between greater mussel losses linked to early transfer to the field and costs related to longer nursery period (Kamermans et al., 2013; Gui et al., 2016). Slower growth rates of spat reared in inland nurseries can require longer time to reach adequate size for grow-out phases on rafts, although this cost, could balance against the greater seed loss at sea because of adverse natural environmental conditions (predation, toxins, storms, etc) disproportionately impacting small sized spat culture (Kamermans et al., 2013; McFarland

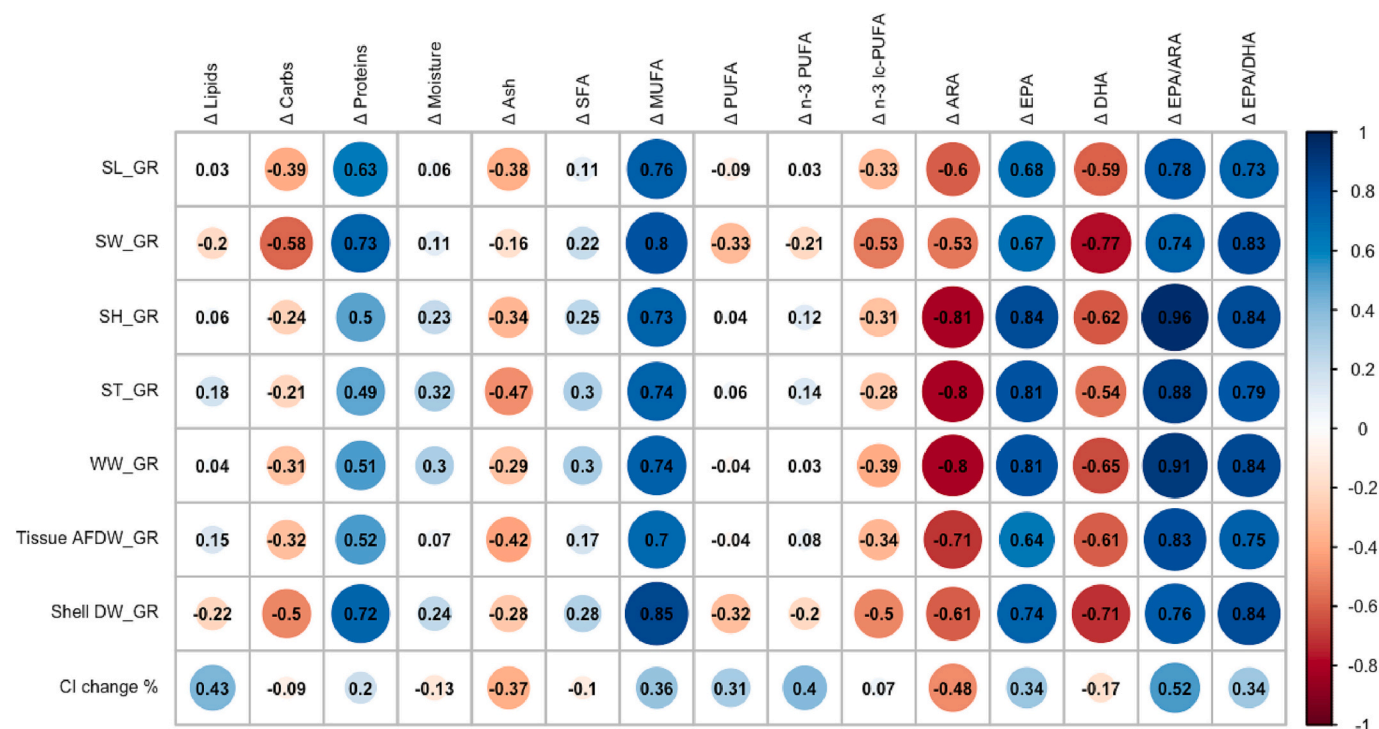


Fig. 3. Spearman's correlation plots between mussel growth rates (GR) (shell length, SL; shell width, SW; shell height, SH; shell thickness, ST; Individual wet weight, WW; tissue dry weight, afdw; shell dry weight, dw), change in condition index (% change from initial CI) with changes (final-initial) in tissue biochemical composition. Positive correlations are displayed in blue whereas negative correlations in red. Correlation coefficients are also displayed with corresponding colour intensity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2016; South et al., 2017, 2022). Losses in the field can be reduced by seeding larger juveniles (Supono et al., 2020; Skelton et al., 2021). Tailor-made feeds could then provide longer nursery periods due to more economically feasible production, and, at the same time, could free space for the natural settlement on ropes, in offshore longlines or raft systems, accommodating additional recruits (Kamermans and Capelle, 2018; Campanati et al., 2022).

4.3. Path towards more sustainable feeds

The BioBullets formulation can reduce feed costs by 30% compared to commercially available microalgal paste, which would additionally require a refrigerated storage (0–5 °C), with a limited shelf life of 4 months (Shellfish Diet® data) versus a shelf life of 12 months at room temperature for BioBullet microcapsules stored in metallic packaging (data from BioBullets, Ltd). Furthermore, at comparable production scale, heterotrophic cultivation can result in higher biomass, growth rates, cell densities and lipids content compared to autotrophic cultivation (van der Voort et al., 2017; Ruiz et al., 2022). *Schizochytrium* sp. can proliferate at industrial scale in heterotrophic conditions, in which the main expenses, usually glucose, used as energy source, can be replaced by alternative lower-cost substrates (van der Voort et al., 2017; Ruiz et al., 2022) such as industrial side streams (Campanati et al., 2021). Examples include wastewaters derived from food and agro-industrial wastes (Pleissner et al., 2013; Santos et al., 2017; Mo et al., 2018; Pleissner and Smetana, 2020), or aquaculture waste concentrates (Tanyaros et al., 2016; Campanati et al., 2021). *Schizochytrium limacinum* growing on saline waste medium instead of standard artificial seawater medium, reduced the cost by 84–85% per kg of dry mass and kg of DHA produced (Roque Fortes, 2016). Likewise, *Schizochytrium mangrovei* can be cultured on food waste and reach higher protein and lipid levels than strains cultured in conventional mediums (Pleissner et al., 2013). *Thaustochytrium stiatum* ONC-T18 also showed potential for commercialization given the low requirements for nitrogen and salt

concentrations (Burja et al., 2006) and the ability of producing higher percentages (i.e. 80 vs 50%) of total FAs compared to *Schizochytrium* sp.

Macroalgae and derived detritus, retrievable from aquaculture wastewaters and as by-product from the biorefinery industry, also showed promising results as economic alternatives for bivalve nursery feeds (e.g. Tanyaros and Chuseingjaw, 2014; Schiener et al., 2015; Carboni et al., 2016; Peña-Rodríguez et al., 2020; Omont et al., 2021; Campanati et al., 2022) and can provide energy sources in the form of carbohydrates (up to 32–60% of dw; Biris-Dorhoi et al., 2020) for lipogenesis from low-cost raw and recycled material. In order to reduce operational costs and improve the efficiency for bivalve spat production, the best alternatives to substitute live microalgae by inert feeds require continued searching for more sustainable inert feeds while optimising species-specific nutritional composition.

5. Conclusions

The results demonstrate the capacity of *M. galloprovincialis* spat to incorporate and digest macromolecules and fatty acids from micro-encapsulated diets. This study supports the possibility of replacing commercial microalgal concentrates with up to 60% of micro-encapsulated *Schizochytrium* sp. inclusion in *M. galloprovincialis* mussel spat dietary treatments resulting in equivalent growth. Dietary protein contents <8% (g/100 g dw) can compromise tissue and shell growth of mussel spat. Likewise results support a high dependency of mussel spat tissue FA composition on diet provisioning, with special concern on balancing dietary EPA and DHA as essential FAs for spat growth. Similar to other studies, a higher dietary provisioning of proteins, MUFAs (18:1n-9), essential fatty acids as EPA, DHA and ARA, and their balanced dietary proportions (EPA/DHA and EPA/ARA) resulted in higher mussel growth.

Given the limited spat growth in our dietary treatments, further research is required before the results of this study could be extended commercially at large scale by industry. However, microencapsulated

diets appear to offer considerable economic promise; extensive work has demonstrated the potentials of microcapsules in providing shellfish with micronutrients, vitamins (Willer and Aldridge, 2020b) and therapeutics, such as probiotics (Martínez et al., 2012; Willer and Aldridge, 2019b).

In order to achieve optimal mussel nursery performances in a cost-efficient manner, future work would have to further consider balancing the microencapsulated diets with a balanced composition of essential fatty acids to meet species specific requirements, as higher proportions of DHA. This could be achieved through encapsulation of a wider range of algal species or their products that collectively provide a more balanced nutritional profile. Finally, given that diet optimization is a key factor for economic feasibility of shellfish production, improvements in the manufacturing of microcapsules to reduce particle size, as well as the undertaking of particle digestibility trials, are recommended in order to enhance spat growth performances with higher substitution of live algae or commercial microalgal pastes by encapsulated diets.

CRedit authorship contribution statement

Camilla Campanati: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Funding acquisition, Investigation, Supervision, Writing – review & editing. **Leire Arantzamendi:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Izaskun Zorita:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Tifanie Briau-deau:** Investigation, Writing – review & editing. **Xabier Lekube:** Investigation, Writing – review & editing. **Urtzi Izagirre:** Conceptualization, Methodology, Supervision, Writing – review & editing. **David C. Aldridge:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

D.C.A. is Managing Director of Biobullets Ltd.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2023.739879>.

References

Aasen, I.M., Ertesvåg, H., Heggset, T.M.B., Liu, B., Brautaset, T., Vadstein, O., Ellingsen, T.E., 2016. Thraustochytrids as production organisms for docosahexaenoic acid (DHA), squalene, and carotenoids. *Appl. Microbiol. Biotechnol.* 100, 4309–4321.

Albentosa, M., Labarta, U., Pke-Camacho, A., Fernández-Reiriz, M.J., Beiras, R., 1994. Fatty acid composition of *Venerupis pullastra* spat fed on different microalgal diets. *Comp. Biochem. Physiol. IOEA* 4, 639–648.

Albentosa, M., Labarta, U., Fernández-Reiriz, M.J., Perez-Camacho, A., 1996. Fatty acid composition of *Ruditapes decussatus* spat fed on different microalgal diets. *Comp. Biochem. Physiol.* 113, 113–119.

Aldridge, D., Elliot, P., Moggridge, G., 2006. Microencapsulated BioBullets for the control of biofouling zebra mussels. *Environ. Sci. Technol.* 40, 975–979. <https://doi.org/10.1021/es050614>.

AOAC, 1995. *Official Methods of Analysis*, 16th ed. Association of Official Analytical Chemists, Arlington VA, USA.

Aranda-Burgos, J.A., da Costa, F., Nóvoa, S., Ojea, J., Martínez-Patiño, D., 2014. Effects of microalgal diet on growth, survival, biochemical and fatty acid composition of *Ruditapes decussatus* larvae. *Aquaculture* 420–421, 38–48. <https://doi.org/10.1016/j.aquaculture.2013.10.032>.

Arney, B., Liu, W., Foster, I.P., Scott, McKinley R., Pearce, C.M., 2015. Feasibility of dietary substitution of live microalgae with spray-dried *Schizochytrium* sp. or *Spirulina* in the hatchery culture of juveniles of the Pacific geoduck clam (*Panopea generosa*). *Aquaculture* 444, 117–133.

Avdelas, L., Avic-Mravlje, E., Borge Marques, A.C., Cano, S., Capelle, J.J., Carvalho, N., Cozzolino, M., Dennis, J., Ellis, T., Fernández Polanco, J.M., Guillen, J., Lasner, T., Le Bihan, V., Llorente, I., Mol, A., Nicheva, S., Nielsen, R., van Oostenbrugge, H., Villasante, S., Visnic, S., Zhelev, K., Asche, F., 2021. The decline of mussel aquaculture in the European Union: causes, economic impacts and opportunities. *Rev. Aquac.* 13, 91–118. <https://doi.org/10.1111/raq.12465>.

Azpeitia, K., Ferrer, L., Revilla, M., Pagaldai, J., Mendiola, D., 2016. Growth, biochemical profile, and fatty acid composition of mussel (*Mytilus galloprovincialis* Lmk.) cultured in the open ocean of the Bay of Biscay (northern Spain). *Aquaculture* 454, 95–108. <https://doi.org/10.1016/j.aquaculture.2015.12.022>.

Azpeitia, K., Ríos, Y., García, I., Pagaldai, J., Mendiola, D., 2017a. A sensory and nutritional validation of open ocean mussels (*Mytilus galloprovincialis* Lmk.) cultured in SE Bay of Biscay (Basque Country) compared to their commercial counterparts from Galician Rías (Spain). *Int. Aquat. Res.* 9 (2), 89–106. <https://doi.org/10.1007/s40071-017-0159-0>.

Azpeitia, K., Ortiz-Zarragoitia, M., Revilla, M., Mendiola, D., 2017b. Variability of the reproductive cycle in estuarine and coastal populations of the mussel *Mytilus galloprovincialis* Lmk. from the SE Bay of Biscay (Basque Country). *Int. Aquat. Res.* 9, 329–350. <https://doi.org/10.1007/s40071-017-0180-3>.

Bignell, J.O., Dodge, M.J., Feist, S.W., Lyons, B., Martin, P.D., Taylor, N.G.H., Stone, D., Travalent, L., Stentiford, G.D., 2008. Mussel histopathology: effects of season, disease and species. *Aquat. Biol.* 2, 1–15.

Biris-Dorhoi, E.-S., Michiu, D., Pop, C.R., Rotar, A.M., Tofana, M., Pop, O.L., Socaci, S.A., Farcas, A.C., 2020. Macroalga-A sustainable source of chemical compounds with biological activities. *Nutrients* 12 (10), 3085. <https://doi.org/10.3390/nu12103085>.

Boeing, P., 1997. Use of spray-dried *Schizochytrium* sp. as a partial algal replacement for juvenile bivalves. *J. Shellfish Res.* 16, 284.

Brown, M., Robert, R., 2002. Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oyster (*Crassostrea gigas*). *Aquaculture* 207 (3–4), 289–309.

Brown, M.R., Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G., 2002. Nutritional value of microalgae for aquaculture. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M.G., Simoes, N. (Eds.), *Avances en Nutrición Acuicola VI. Memorias del VI Simposium Internacional de Nutrición Acuicola*. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

Burja, A.M., Radianingtyas, H., Windust, A., Barrow, C.J., 2006. Isolation and characterization of polyunsaturated fatty acid producing *Thraustochytrium* species: screening of strains and optimization of omega-3 production. *Appl. Microbiol. Biotechnol.* 72, 1161–1169.

Caers, M., Coutteau, P., Sorgeloos, P., 1999. Dietary impact of algal and artificial diets, fed at different feeding rations, on the growth and fatty acid composition of *Tapes philippinarum* (L.) spat. *Aquaculture* 170, 307–322.

Caers, M., Utting, S.D., Coutteau, P., Millican, P.F., Sorgeloos, P., 2002. Impact of the supplementation of a docosahexaenoic acid-rich emulsion on the reproductive output of oyster broodstock, *Crassostrea gigas*. *Mar. Biol.* 140 (6), 1157–1166. <https://doi.org/10.1007/s00227-002-0798-5>.

Calder, P.C., 2018. Very long-chain n-3 fatty acids and human health: fact, fiction and the future. *Proc. Nutr. Soc.* 77 (1), 52–72. <https://doi.org/10.1017/S0029665117003950>.

Camacho, A.P., Labarta, U., Beiras, R., 1995. Growth of mussels (*Mytilus edulis galloprovincialis*) on cultivation rafts: influence of seed source, cultivation site and phytoplankton availability. *Aquaculture* 138 (1–4), 349–362. [https://doi.org/10.1016/0044-8486\(95\)01139-0](https://doi.org/10.1016/0044-8486(95)01139-0).

Campanati, C., Willer, D., Schubert, J., Aldridge, D.C., 2021. Sustainable intensification of aquaculture through nutrient recycling and circular economies: more fish, less waste, blue growth. *Rev. Fish. Sci. Aquac.* 1–50. <https://doi.org/10.1080/23308249.2021.1897520>.

Campanati, C., Arantzamendi, L., Zorita, I., Juez, A., Aldridge, D.C., 2022. Microencapsulated diets using thraustochytrids and macroalgae side streams for nursery rearing of *Mytilus galloprovincialis* spat. *J. World Aquacult. Soc.* 1–19. <https://doi.org/10.1111/jwas.12912>.

Carboni, S., Clegg, S.H., Hughes, A.D., 2016. The use of biorefinery by-products and natural detritus as feed sources for oysters (*Crassostrea gigas*) juveniles. *Aquaculture* 464, 392–398. <https://doi.org/10.1016/j.aquaculture.2016.07.021>.

Carrasco, A., Astorga, M., Cisterna, A., Farias, A., Espinoza, V., Uriarte, I., 2014. Pre-feasibility study for the installation of a Chilean mussel *Mytilus chilensis* (Hupe, 1854) seed hatchery in the Lakes Region, Chile. *Fish. Aquac.* J. 05 (03).

Chauton, M.S., Reitan, K.I., Norsker, N.H., Tveterås, R., Kleivdal, H.T., 2015. A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities. *Aquaculture* 436, 95–103. <https://doi.org/10.1016/j.aquaculture.2014.10.038>.

Chen, Q., Jiang, X., Han, Q., Sheng, P., Chai, Y., Peng, R., Jiang, M., Mao, Z., Wu, S., 2021. Growth, calcium content, proximate composition, and fatty acid composition

- of triangle sail mussel (*Hyriopsis cumingii*) fed different microalgal diets. *Aquaculture* 530, 73719. <https://doi.org/10.1016/j.aquaculture.2020.735719>.
- Cheng, P., Zhou, C., Chu, R., Chang, T., Xu, J., Ruan, R., Chen, P., Yan, X., 2020. Effect of microalgal diet and culture system on the rearing of bivalve mollusks: nutritional properties and potential cost improvements. *Algal Res.* 51, 102076 <https://doi.org/10.1016/j.algal.2020.102076>.
- Christie, W.W., 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* 23, 1072–1075.
- Coutteau, P., Sorgeloos, P., 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. *J. Shellfish Res.* 11 (2), 467–476.
- Coutteau, P., Castell, J., Ackman, R., Sorgeloos, P., 1996. The use of lipid emulsions as carriers for essential FA in bivalves juvenile *Placopecten magellanicus*. *J. Shellfish Res.* 15 (2), 259–264.
- da Costa, F., Robert, R., Quéré, C., Wikfors, G.H., Soudant, P., 2015. Essential fatty acid assimilation and synthesis in larvae of the bivalve *Crassostrea gigas*. *Lipids* 50 (5), 503–511. <https://doi.org/10.1007/s11745-015-4006-z>.
- Delaporte, M., Soudant, P., Moal, J., Kraffe, E., Marty, Y., Samain, J.F., 2005. Incorporation and modification of dietary fatty acids in gill polar lipids by two bivalve species *Crassostrea gigas* and *Ruditapes philippinarum*. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* 140 (4), 460–470. <https://doi.org/10.1016/j.cbpb.2005.02.009>.
- Diehl, W.J., Gaffney, P.M., Koehn, R.K., 1986. Physiological and genetic aspects of growth in the mussel *Mytilus edulis*. I. Oxygen consumption, growth, and weight loss. *Physiol. Zool.* 59 (2), 201–211.
- EUFOMA, 2019. Case Study. Mussel in the EU. Price Structure in the Supply Chain. Focus on Spain, France, Italy and Ireland. www.eumofa.eu.
- FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome. <https://doi.org/10.4060/ca9229en>.
- FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome. <https://doi.org/10.4060/cc0461en>.
- Fernández-Reiriz, M.J., Labarta, U., Albentosa, M., Pérez-Camacho, A., 1998. Effect of microalgal diets and commercial wheatgerm flours on the lipid profile of *Ruditapes decussatus* spat. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* 119 (1), 369–377.
- Fernández-Reiriz, M.J., Labarta, U., Albentosa, M., Pérez-Camacho, A., 1999. Lipid profile and growth of the clam spat, *Ruditapes decussatus* (L), fed with microalgal diets and cornstarch. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 124, 309–318.
- Fernández-Reiriz, M.J., Labarta, U., Albentosa, M., Pérez-Camacho, A., 2006. Lipid composition of *Ruditapes philippinarum* spat: effect of ration and diet quality. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 144 (2), 229–237. <https://doi.org/10.1016/j.cbpb.2006.02.015>.
- Fernández-Reiriz, M.J., Irisarri, J., Labarta, U., 2015. Feeding behaviour and differential absorption of nutrients in mussel *Mytilus galloprovincialis*: responses to three microalgal diets. *Aquaculture* 446, 42–47. <https://doi.org/10.1016/j.aquaculture.2015.04.025>.
- Fernández-Reiriz, M.J., Irisarri, J., Labarta, U., 2016. Flexibility of physiological traits underlying inter-individual growth differences in intertidal and subtidal mussels *Mytilus galloprovincialis*. *PLoS One* 11 (2). <https://doi.org/10.1371/journal.pone.014824>.
- Folch, J., Lees, M., Stanley, S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509. [https://doi.org/10.1016/s0021-9258\(18\)64849-5](https://doi.org/10.1016/s0021-9258(18)64849-5).
- Geng, S., Zhou, C., Chen, W., Yu, S., Huang, W., Huan, T., Xu, J., Yan, X., 2016. Fatty acid and sterol composition reveal food selectivity of juvenile ark shell *Tegillarca granosa* Linnaeus after feeding with mixed microalgae. *Aquaculture* 455, 109–117. <https://doi.org/10.1016/j.aquaculture.2016.01.012>.
- Gentry, R.R., Alleway, H.K., Bishop, M.J., Gillies, C.L., Waters, T., Jones, R., 2020. Exploring the potential for marine aquaculture to contribute to ecosystem services. *Rev. Aquac.* 12, 499–512. <https://doi.org/10.1111/raq.12328>.
- González-Fernández, C., Albentosa, M., Sokolova, I., 2017. Interactive effects of nutrition, reproductive state and pollution on molecular stress responses of mussels, *Mytilus galloprovincialis* Lamarck, 1819. *Mar. Environ. Res.* 131, 103–115. <https://doi.org/10.1016/j.marenvres.2017.08.011>.
- Gui, Y., Zamora, L., Dunphy, B.J., Jeffs, A.G., 2016. Evaluation of the formulated diet MySpat for feeding hatchery reared spat of the green-lipped mussel, *Perna canaliculus* (Gmelin, 1791). *Aquac. Res.* 47 (12), 3907–3912. <https://doi.org/10.1111/are.12841>.
- Gupta, A., Barrow, C.J., Puri, M., 2012. Omega-3 biotechnology: Thraustochytrids as a novel source of omega-3 oils. *Biotechnol. Adv.* 30, 1733–1745.
- Harbach, H., Palm, H.W., 2018. Development of general condition and flesh water content of long-time starved *Mytilus edulis*-like under experimental conditions. *AACL Bioflux* 11 (2). <http://www.bioflux.com.ro/aacl>.
- Hawkins, A.J.S., Widdows, J., Bayne, B.L., 1989. The relevance of whole-body protein metabolism to measured costs of maintenance and growth in *Mytilus edulis*. *Physiol. Zool.* 62 (3), 745–763. <https://www.jstor.org/stable/30157925>.
- Helm, M.M., Bourne, N., 2004. Hatchery Culture of Bivalves: A Practical Manual. Food and Agriculture Organization, FAO Fisheries and Aquaculture Technical Paper, Rome, Italy.
- Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C., 1990. Optimum EPA levels in *Artemia* to meet the EPA requirements of red sea bream (*Pagrus major*). In: Takeda, M., Watanabe, T. (Eds.), Status of Fish Nutrition in Aquaculture. Proc. Third International Symposium on Feeding and Nutrition. In Fish. Japan Translation Center Ltd, Tokyo, pp. 221–232.
- Kamermans, P., Capelle, J.J., 2018. Provisioning of mussel seed and its efficient use in culture. In: Goods and Services of Marine Bivalves. Springer International Publishing, pp. 27–49. https://doi.org/10.1007/978-3-319-96776-9_3.
- Kamermans, P., Galley, T., Boudry, P., Fuentes, J., McCombie, H., Batista, F.M., Blanco, A., Domínguez, L., Cornette, F., Pincot, L., Beaumont, A., 2013. Blue mussel hatchery technology in Europe. In: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Elsevier, New York, NY, pp. 339–373.
- Khan, B.M., Liu, Y., 2019. Marine mollusks: food with benefits. *Compr. Rev. Food Sci. Food Saf.* 18 (2), 548–564. <https://doi.org/10.1111/1541-4337.12429>.
- Kim, Y., Ashton-Alcox, A., Powell, E.N., 2006. Histological techniques for marine bivalve molluscs: update NOAA technical memorandum. Natl. Centers Coast. Ocean Sci. 27, 76.
- Knauer, J., Southgate, P.C., 1997a. Growth and fatty acid composition of Pacific oyster (*Crassostrea gigas*) spat fed a spray-dried freshwater microalga (*Spongiococcus excentricum*) and microencapsulated lipids. *Aquaculture* 154, 293–303.
- Knauer, J., Southgate, P.C., 1997b. Growth and fatty acid composition of pacific oyster (*Crassostrea gigas*) spat fed a microalga and microcapsules containing varying amounts of eicosapentaenoic and docosahexaenoic acid. *J. Shellfish Res.* 16 (2), 447–453.
- Knauer, J., Southgate, P.C., 1999. A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. *Rev. Fish. Sci.* 7, 241–280. <https://doi.org/10.1080/10641269908951362>.
- Kralovec, J.A., Zhang, S., Zhang, W., Barrow, C.J., 2012. A review of the progress in enzymatic concentration and microencapsulation of omega-3 rich oil from fish and microbial sources. *Food Chem.* 131, 639–644.
- Kreeger, D.A., Langdon, C.J., 1993. Effects of dietary protein content on growth of juvenile mussels, *Mytilus trossulus* (Gould, 1850). *Biol. Bull.* 185, 123–139.
- Langdon, C., Onal, E., 1999. Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilus galloprovincialis*. *Aquaculture* 180, 283–294.
- Langdon, C.J., Waldoock, M., 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. *J. Mar. Biol. Assoc. U.K.* 431–448.
- Laudicella, V.A., Beveridge, C., Carboni, S., Franco, S.C., Doherty, M.K., Long, N., Mitchell, E., Stanley, M.S., Whitfield, P.D., Hughes, A.D., 2020. Lipidomics analysis of juveniles' blue mussels (*Mytilus edulis* L. 1758), a key economic and ecological species. *PLoS One* 15 (2). <https://doi.org/10.1371/journal.pone.0223031>.
- Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. *J. Stat. Softw.* 25 (1), 1–18. <https://doi.org/10.18637/jss.v025.i01>.
- Loor, A., Bossier, P., Nevejan, N., 2021. Dietary substitution of microalgae with the *Saccharomyces cerevisiae* mutant, Δmn9, for feeding Pacific oyster (*Crassostrea gigas*) juveniles. *Aquaculture* 534, 736253.
- Marigómez, I., Múgica, M., Izagirre, U., Sokolova, I.M., 2017. Chronic environmental stress enhances tolerance to seasonal gradual warming in marine mussels. *PLoS One* 12 (3). <https://doi.org/10.1371/journal.pone.0174359>.
- Martínez, Cruz P., Ibáñez, A.L., Monroy Hermosillo, O.A., Ramírez Saad, H.C., 2012. Use of probiotics in aquaculture. *ISRN Microbiol.* 916845 <https://doi.org/10.5402/2012/916845>.
- McFarland, K., Jean, F., Thébault, J., Volety, A.K., 2016. Potential impacts of blooms of the toxic dinoflagellate *Karenia brevis* on the growth, survival and juvenile recruitment of the non-native green mussel *Perna viridis* in southeastern United States. *Toxicol.* 109, 94–102. <https://doi.org/10.1016/j.toxicol.2015.11.017>.
- Milke, L.M., Bricelj, V.M., Parrish, C.C., 2006. Comparison of early life history stages of the bay scallop, *Argopecten irradians*: effects of microalgal diets on growth and biochemical composition. *Aquaculture* 260 (1–4), 272–289. <https://doi.org/10.1016/j.aquaculture.2006.06.004>.
- Mo, W.Y., Man, Y.B., Wong, M.H., 2018. Use of food waste, fish waste and food processing waste for China's aquaculture industry: needs and challenge. *Sci. Total Environ.* 613–614, 635–643.
- Muñoz-Moya, E., García-Herrera, C.M., Lagos, N.A., Abarca-Ortega, A.F., Checa, A.G., Harper, E.M., 2022. Evaluation of remodeling and geometry on the biomechanical properties of nacreous bivalve shells. *Sci. Rep.* 12, 710. <https://doi.org/10.1038/s41598-021-04414-1>.
- Nell, J., Diemar, J., Heasman, M., 1996. Food value of live yeasts and dry yeast-based diets fed to Sydney rock oyster *Saccostrea commercialis* spat. *Aquaculture* 145, 235–243.
- Nevejan, N., Davis, J., Little, K., Kilona, A., 2007. Use of a formulated diet for mussel spat *Mytilus galloprovincialis* (Lamarck 1819) in a commercial hatchery. *J. Shellfish Res.* 26 (2), 357–363. [https://doi.org/10.2983/0730-8000\(2007\)26\[357:UOAFDF\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2007)26[357:UOAFDF]2.0.CO;2).
- Nevejan, N., De Schryver, P., Wille, M., Kristof, D., Kartik, B., Gilbert Van, S., 2016. Bacteria as food in aquaculture: do they make a difference? *Rev. Aquac.* 10, 180–212. <https://doi.org/10.1111/raq.12155>.
- Numaguchi, K., 2002. Effect of an artificial diet on early spat growth of the Japanese pearl oyster *Pinctada fucata martensii*. *Fish. Sci.* 68, 694–696.
- Omout, A., Py, C., Gamboa-Delgado, J., Nolasco-Soria, H., Spanopulos-Zarco, M., Peña-Rodríguez, A., 2021. Nutritional contribution of seaweed *Ulva lactuca* single-cell detritus and microalgae *Chaetoceros calcitrans* to the growth of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 541, 736835.
- Önal, E., Langdon, C., Önal, U., 2005. The evaluation of spray-dried microalgae in diets for juvenile Manila clam, *Tapes philippinarum*. *J. Shellfish Res.* 24 (4), 1061–1065.
- Orive, E., Franco, J., Madariaga, I., Revilla, M., 2004. Bacterioplankton and phytoplankton communities. In: Borja, A., Collins, M. (Eds.), Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, Amsterdam, pp. 367–393.
- Pales Espinosa, E., Cerrato, R.M., Wikfors, G.H., Allam, B., 2016. Modeling food choice in the two suspension-feeding bivalves, *Crassostrea virginica* and *Mytilus edulis*. *Mar. Biol.* 163 (2), 1–13. <https://doi.org/10.1007/s00227-016-2815-0>.
- Peña-Rodríguez, A., Morales-Alvarado, G., Elizondo-González, R., Mendoza-Carrión, G., Tovar-Ramírez, D., Escobedo-Fregoso, C., 2020. Seaweed single cell detritus effects

- on the digestive enzymes activity and microbiota of the oyster *Crassostrea gigas*. J. Appl. Phycol. 32, 3481–3493. <https://doi.org/10.1007/s10811-020-02167-4>.
- Pernet, F., Tremblay, R., 2004. Effect of varying levels of dietary essential fatty acid during early ontogeny of the sea scallop *Placopecten magellanicus*. J. Exp. Mar. Biol. Ecol. 310 (1), 73–86. <https://doi.org/10.1016/j.jembe.2004.04.001>.
- Pernet, F., Tremblay, R., Comeau, L., Guderley, H., 2007. Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids. J. Exp. Biol. 210 (17), 2999–3014. <https://doi.org/10.1242/jeb.006007>.
- Pirini, M., Manuzzi, M.P., Pagliarini, A., Trombetti, F., Borgatti, A.R., Ventrella, V., 2007. Changes in fatty acid composition of *Mytilus galloprovincialis* (Lmk) fed on microalgal and wheat germ diets. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 147 (4), 616–626. <https://doi.org/10.1016/j.cbpb.2007.04.003>.
- Pleissner, D., Smetana, S., 2020. Estimation of the economy of heterotrophic microalgae- and insect-based food waste utilization processes. Waste Manag. 102, 198–203.
- Pleissner, D., Lam, W.C., Sun, Z., Lin, C.S.K., 2013. Food waste as nutrient source in heterotrophic microalgae cultivation. Bioresour. Technol. 137, 139–146.
- Prado, S., Romalde, J.L., Barja, J.L., 2010. Review of probiotics for use in bivalve hatcheries. Vet. Microbiol. 145, 187–197. <https://doi.org/10.1016/j.vetmic.2010.08.021>.
- Prieto, D., Tamayo, D., Urrutxurtu, I., Navarro, E., Ibarrola, I., Urrutia, M.B., 2020. Nature more than nurture affects the growth rate of mussels. Sci. Rep. 10 (1), 3539. <https://doi.org/10.1038/s41598-020-60312-y>.
- R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Raghukumar, S., 2008. Thraustochytrid marine protists: production of PUFAs and other emerging technologies. Mar. Biotechnol. 10, 631–640.
- Rasilla, D., García-Codron, J., Biarnandia, C., Herrera, S., Rivas, V., 2018. Extreme wave storms and atmospheric variability at the Spanish coast of the Bay of Biscay. Atmosphere 9 (8), 316. <https://doi.org/10.3390/atmos9080316>.
- Rivero-Rodríguez, S., Beaumont, A.R., Lora-Vilchis, M.C., 2007. The effect of microalgal diets on growth, biochemical composition, and fatty acid profile of *Crassostrea corteziensis* (Hertlein) juveniles. Aquaculture 263, 199–210. <https://doi.org/10.1016/j.aquaculture.2006.09.038>.
- Robert, R., Trintignac, P., 1997. Substitutes for live microalgae in mariculture: a review. Aquat. Living Resour. 10, 315–327.
- Robert, R., Sánchez, J.L., Pérez-Parallé, L., Ponis, E., Kamermans, P., O'Mahoney, M., 2013. A glimpse on the mollusc industry in Europe. Aquac. Eur. 38 (1), 5–11.
- Roque Fortes, L.A., 2016. Heterotrophic Cultivation of *Thraustochytrids* Using Glycerol and Saline Medium from a Dairy Effluent. Master thesis., Universidade do Algarve.
- Rosa, M., Ward, J.E., Ouvrard, M., Holohan, B.A., Pales Espinosa, E., Shumway, S.E., Allam, B., 2015. Examining the physiological plasticity of particle capture by the blue mussel, *Mytilus edulis* (L.): confounding factors and potential artifacts with studies utilizing natural seston. J. Exp. Mar. Biol. Ecol. 473, 207–217. <https://doi.org/10.1016/j.jembe.2015.09.005>.
- Roy, S.S., Pal, R., 2015. Microalgae in aquaculture: a review with special references to nutritional value and fish dietetics. Proc. Zool. Soc. London 68 (1). <https://doi.org/10.1007/s12595-013-0089-9>.
- Ruano, F., Ramos, P., Quaresma, M., Maria Bandarra, N., Pereira da Fonseca, I., 2012. Evolution of fatty acid profile and condition index in mollusc bivalves submitted to different depuration periods. RPCV 111, 581–582.
- Ruiz, J., Wijffels, R.H., Domínguez, M., Barbosa, M.J., 2022. Heterotrophic vs autotrophic production of microalgae: bringing some light into the everlasting cost controversy. Algal Res. 64, 102698. <https://doi.org/10.1016/j.algal.2022.102698>.
- Santos, A., Roso, G., Menezes, C., Queiroz, M., Zepka, L., Jacob-Lopes, E., 2017. The bioeconomy of microalgal heterotrophic bioreactors applied to agroindustrial wastewater treatment. Desalination Water Treat. 64, 12–20.
- Schiener, P., Attack, T., Wareing, R., Kelly, M.S., Hughes, A.D., 2015. The by-products from marine biofuels as feed source for the aquaculture industry: a novel example of the biorefinery approach. Biomass Conv. Bioref. 6, 281–287. <https://doi.org/10.1007/s13399-015-0190-6>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Seoane, S., Laza, A., Orive, E., 2006. Monitoring phytoplankton assemblages in estuarine waters: the application of pigment analysis and microscopy to size-fractionated samples. Estuar. Coast. Shelf Sci. 67, 343–345. <https://doi.org/10.1016/j.ecss.2005.10.020>.
- Skelton, B.M., McKay, W.J.G., Yang, K., Wu, Z., Jeffs, A.G., 2021. Investigation of the potential of liposome and microparticle feeds to partially replace microalgae in the nursery rearing of green-lipped mussels (*Perna canaliculus*). Aquac. Nutr. 27 (5), 1730–1737. <https://doi.org/10.1111/anu.13310>.
- South, P.M., Floerl, O., Jeffs, A.G., 2017. Differential effects of adult mussels on the retention and fine-scale distribution of juvenile seed mussels and biofouling organisms in long-line aquaculture. Aquac. Environ. Interact. 9, 239–256. <https://doi.org/10.3354/aei00230>.
- South, P.M., Delorme, N.J., Skelton, B.M., Floerl, O., Jeffs, A.G., 2022. The loss of seed mussels in longline aquaculture. Rev. Aquac. 14 (1), 440–455. <https://doi.org/10.1111/raq.12608>.
- Southgate, P.C., Lee, P.S., Lucas, J.S., 1992. Development of artificial diets for bivalve larvae. In: Allan, G.L., Dall, W. (Eds.), Proceedings of the Aquaculture Nutrition Workshop, pp. 156–162. Salamander Bay, Australia.
- Southgate, P.C., Beer, A.C., Ngaluaf, P., 2016. Hatchery culture of the winged pearl oyster, *Pteria penguin*, without living micro-algae. Aquaculture 451, 121–124.
- Stemmer, K., Nehrkke, G., Brey, T., 2013. Elevated CO₂ levels do not affect the shell structure of the bivalve *Arctica islandica* from the Western Baltic. PLoS One 8 (7), e70106. <https://doi.org/10.1371/journal.pone.0070106>.
- Strohmeier, T., Strand, Ø., Alunno-Bruscia, M., Duinker, A., Cranford, P.J., 2012. Variability in particle retention efficiency by the mussel *Mytilus edulis*. J. Exp. Mar. Biol. Ecol. 412, 96–102.
- Supono, S., Dunphy, B., Jeffs, A., 2020. Retention of green-lipped mussel spat: the role of body size and nutritional condition. Aquaculture 520, 735017. <https://doi.org/10.1016/j.aquaculture.2020.735017>.
- Supono, S., Yu, X., Skelton, B.M., McKay, W.J.G., Jeffs, A., 2022. Effect of starvation on the nutritional condition of juvenile green-lipped mussels of different sizes. Aquaculture 560, 738580. <https://doi.org/10.1016/j.aquaculture.2022.738580>.
- Supono, S., Mugica, M., Spreitzenbarth, S., Jeffs, A., 2023. Potential for concentrated microalgae as replacement diets for juvenile green-lipped mussels, *Perna canaliculus*. Aquac. Res. 2023, 9841172.
- Tamayo, D., Azpeitia, K., Markaide, P., Navarro, E., Ibarrola, I., 2016. Food regime modulates physiological processes underlying size differentiation in juvenile intertidal mussels *Mytilus galloprovincialis*. Mar. Biol. 163 (6). <https://doi.org/10.1007/s00227-016-2905-z>.
- Tan, K., Ma, H., Li, S., Zheng, H., 2020. Bivalves as future source of sustainable natural omega-3 polyunsaturated fatty acids. Food Chem. 311. <https://doi.org/10.1016/j.foodchem.2019.125907>.
- Tang, F., Aldridge, D.C., 2019. Microcapsulated biocides for the targeted control of invasive bivalves. Sci. Rep. 9 (1). <https://doi.org/10.1038/s41598-019-55392-4>.
- Tanyaros, S., Chuseingjau, S., 2014. A partial substitution of microalgae with single cell detritus produced from seaweed (*Porphyra haitanensis*) for the nursery culture of tropical oyster (*Crassostrea belcheri*). Aquac. Res. 47 (7), 2080–2088. <https://doi.org/10.1111/are.12662>.
- Tanyaros, S., Sujarit, C., Jansri, N., Tarangkoon, W., 2016. Baker's yeast as a substitute for microalgae in the hatchery rearing of larval and juvenile tropical oyster (*Crassostrea belcheri*, Sowerby 1871). J. Appl. Aquac. 28, 35–46. <https://doi.org/10.1080/10454438.2016.1163312>.
- Thompson, P.A., Harrison, P.J., 1992. Effects of monospecific algal diets of varying biochemical composition on the growth and survival of Pacific oyster (*Crassostrea gigas*) larvae. Mar. Biol. 113, 645–665.
- Urrutxurtu, I., Orive, E., de la Sota, A., 2003. Seasonal dynamics of ciliated protozoa and their potential food in an eutrophic estuary (Bay of Biscay). Estuar. Coast. Shelf Sci. 57, 1169e1182.
- Utting, S., 1986. A preliminary study on growth of *Crassostrea gigas* larvae and spat in relation to dietary protein. Aquaculture 56, 123–138.
- van der Schatte Olivier, A., Jones, L., le Vay, L., Christie, M., Wilson, J., Malham, S.K., 2020. A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac. 12 (1), 3–25. <https://doi.org/10.1111/raq.12301>.
- van der Voort, M.P.J., Spruijt, J., Potters, J., de Wolf, P.L., Elissen, H.J.H., 2017. Socio-economic assessment of algae-based PUFA production. In: Public Output Report of the PUFACHain Project, Göttingen, 2017. IFUEU, p. 79. www.pufachain.eu.
- Ventrella, V., Pagliarini, A., Nesci, S., Trombetti, F., Pirini, M., 2013. Dietary enhancement of selected fatty acid biosynthesis in the digestive gland of *Mytilus galloprovincialis* Lmk. J. Agric. Food Chem. 61 (4), 973–981. <https://doi.org/10.1021/jf304505d>.
- Waldock, M.J., Holland, D.L., 1984. Fatty acid metabolism in young oysters, *Crassostrea gigas*: polyunsaturated fatty acids. Lipids 19 (5), 332–336.
- Wang, Q.H., Yang, C.Y., Du, X.D., Liu, X.W., Sun, R.J., Deng, Y.W., 2016. Growth performance and biochemical composition of juvenile pearl oyster *Pinctada martensii* fed on artificial diets. Aquac. Int. 24, 995–1005.
- Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130. <https://doi.org/10.1016/j.jembe.2004.03.002>.
- Wijnsman, J.W.M., Troost, K., Fang, J., Roncarati, A., 2018. Global production of marine bivalves. Trends and challenges. In: Goods and Services of Marine Bivalves. Springer International Publishing, pp. 7–26. https://doi.org/10.1007/978-3-319-96776-9_2.
- Willer, D., Aldridge, D.C., 2017. Microencapsulated diets to improve bivalve shellfish aquaculture. R. Soc. Open Sci. 4 (11). <https://doi.org/10.1098/rsos.171142>.
- Willer, D.F., Aldridge, D.C., 2019a. Microencapsulated diets to improve growth and survivorship in juvenile European flat oysters (*Ostrea edulis*). Aquaculture 505, 256–262. <https://doi.org/10.1016/j.aquaculture.2019.02.072>.
- Willer, D.F., Aldridge, D.C., 2019b. Microencapsulated diets to improve bivalve shellfish aquaculture for global food security. Glob. Food Sec. 23, 64–73. Elsevier B.V. <https://doi.org/10.1016/j.gfs.2019.04.007>.
- Willer, D.F., Aldridge, D.C., 2020a. Sustainable bivalve farming can deliver food security in the tropics. Nat. Food 1 (7), 384–388. <https://doi.org/10.1038/s43016-020-0116-8>.
- Willer, D.F., Aldridge, D.C., 2020b. Vitamin bullets. Microencapsulated feeds to fortify shellfish and tackle human nutrient deficiencies. Front. Nutr. 7. <https://doi.org/10.3389/fnut.2020.00102>.
- Willer, D.F., Furse, S., Aldridge, D.C., 2020. Microencapsulated algal feeds as a sustainable replacement diet for broodstock in commercial bivalve aquaculture. Sci. Rep. 10 (1). <https://doi.org/10.1038/s41598-020-69645-0>.
- Yang, C., Hao, R., Du, X., Wang, Q., Deng, Y., Su, R., 2019. Response to different dietary carbohydrate and protein levels of pearl oysters (*Pinctada fucata martensii*) as revealed by GC-TOF/MS-based metabolomics. Sci. Total Environ. 650, 2614–2623. <https://doi.org/10.1016/j.scitotenv.2018.10.023>.
- Yarnold, J., Karan, H., Oey, M., Hankamer, B., 2019. Microalgal aquafeeds as part of a circular bioeconomy. Trends Plant Sci. 24 (10), 959–970.
- Zhu, Y., Liao, K., Tian, J., Liu, Y., Xu, J., Liu, X., Zhang, L., Yan, X., 2022. Substitution of microalgae with microcapsule diet for two bivalve species, Manila clam *Ruditapes*

- philippinarum* and razor clam *Sinonovacula constricta*. Aquac. Rep. 22 (100991), 2022. <https://doi.org/10.1016/j.aqrep.2021.100991>.
- Zhukova, N.V., 1991. The pathway of the biosynthesis of non-methylene interrupted dienoic fatty acids in molluscs. Comp. Biochem. Physiol. B Comp. Biochem. 100 (4), 801–804.
- Zhukova, N.V., 2019. Fatty acids of marine mollusks: impact of diet, bacterial symbiosis and biosynthetic potential. Biomolecules 9. <https://doi.org/10.3390/biom9120857>. MDPI AG.