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Research Article

Growth and Physiological Performance in Growth Phenotypes of the Carpet Shell Clam (*Ruditapes decussatus*) Fed Diets of Variable Lipid/Carbohydrate Ratios

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Mixed suspensions of phytoplankton and yeast cells in different proportions were designed to achieve diets with a range of variation (0.6–2.2) in the ratio of lipid to carbohydrate while maintaining protein content constant. Juvenile specimens of the carpet shell clam (*Ruditapes decussatus*) from two segregated growth phenotypes (fast and slow growers) were food conditioned and the physiological components of the energy balance were determined with these different diet compositions in order to assess the combined effects of endogenous and nutritional factors on growth performance. Conditioning to lipid-rich diets increased growth rate relative to conditioning to carbohydrate-rich diets and resulted in higher scope for growth values in both growth groups. These dietary effects are mainly driven by differences in the absorption efficiency (AE) found between clams fed different food compositions, although the present results do not allow to ascertain whether the reduced AE recorded with the carbohydrate-rich diets results from reduced digestibility of yeasts cells due to structural restrictions or either reflects the digestive imbalance of lipids associated to higher production of metabolic fecal losses. Greater phenotypic plasticity was seen to enable the fast-growing clams fed a carbohydrate-rich diet to overcome the above digestive limitations through an overfeeding response; however, the resource to such kind of physiological mechanism appeared limited by the nutritional conditions (energetic status) prevailing during the conditioning phase.

1. Introduction

Biochemical composition of suspended food has been a frequent topic in growth and production studies in marine bivalves, particularly considering the specific benefits associated with the use of different microalgal species or species combinations [1–4], as well as regarding the potential for the use of nonphytoplanktonic nutritional supplements in an aquacultural context [5–11]. Much of the interest in testing the suitability of these supplements aims at the practical purpose of reducing the elevated costs of phytoplankton production required to sustain intensive phases of bivalve rearing in hatchery/nursery facilities, including broodstock conditioning (10%–30%), larvae rearing (10%–40%), and postset growth (50%–60%) to the appropriated seed size for transference to grow-out sites [9]. In accordance with the above figures, most researches in this field were performed on the spat of cultivated species and relied on the empirical assessment of the proportion of phytoplankton that, in terms of growth performance, can be effectively replaced by low-cost inert food items [12–14].

Regarding the major biochemical composition, two component ratios appear to be nutritionally important in the diet of marine bivalves: the protein/energy ratio, also referred inversely to the C/N index, and the lipid/carbohydrate ratio. According to the pivotal role played by proteins in the constitution of new tissues, many studies have reported a positive correlation between dietary protein content and growth rate in the early life stages of many bivalve species [15–18], including juveniles of clams of the genus *Ruditapes* [13, 19, 20], where negative impact on growth have been reported for diets presenting C/N indexes above 10.5 [21, 22]. On the other

hand, the specific nutritional interest in addressing the impact of lipid to carbohydrate ratio of the diet on rates of growth stems from the consideration that the energy reserves of phytoplankton, the main food component of bivalve diets in both natural or farming conditions, are in the form of lipids [23, 24], while marine bivalves store carbohydrates as a readily available reserve for maintenance (particularly under anaerobic conditions) as well as to sustain high-energy demanding processes such as gametogenesis [25–28]. Expected benefits of carbohydrate-rich food stuffs have been actually observed when comparing the impact of different microalgal diets on the growth performance of spat in oysters (*Crassostrea virginica*) [29] and clams (*R. decussatus*) [30].

Having this into account, some attempts have been made to substitute live microalgal diets with the less-expensive carbohydrate-rich foodstuffs, including different types of commercial wheat or corn flours [12–14, 18, 20, 31–34]. The experimental evidence concerning the effects of such supplements on the growth and biochemical profiles of body tissues is particularly abundant in spats and juveniles (seeds) of the carpet shell clam *R. decussatus*, where the use of mixed diets suggested a similar growth performance of clams feeding on pure microalgae suspension *Isochrysis galbana* (T-ISO) than when cornstarch [12, 35], cornmeal [12], or wheatgerm [20, 36] substituted up to 50% the phytoplankton dose. The addition of cornstarch in particular was seen to improve the growth of *R. decussatus* over the values recorded with monospecific microalgal diets [31].

On the other hand, because of their high-protein and carbohydrate contents and suitable particle size, yeast cells have interesting characteristics as the supplements of microalgae in aquaculture practice with bivalves [37, 38], although reduced digestibility might hindrance its utilization in some cases [10, 37]. In this respect, the highest success in replacing phytoplankton was found when carpet shell clam seeds were fed on a mix of microalgae species together with up to 80% of modified yeast suspensions [39]. In view of the extensive information available on the nutritional effects of formulated diets on growth along the early life stages in this clam species the present experiments were conducted in juvenile *R. decussatus* using mixtures of microalgae (*Rhodomonas lens*) and baker's yeast (*Saccharomyces cerevisiae*) combined in different proportions to produce a range in the proportion of lipids to carbohydrates in terms of gross biochemical composition of diets that otherwise remained constant in the protein content. These species of food particles were chosen on account of their similitude in terms of stability in the water column and cell size to prevent any confounding effect of differential retention based on particle size.

Beside nutritional conditions related to food ration and composition, one important factor in rearing management in hatcheries is the occurrence of large interindividual differences in growth performance that are widespread among bivalve species [40–46]. These differences that underlie size variability inside hatching cohorts have been reported to be persistent at least in the medium-term [42, 45, 47], and so are reputed as constitutive and of possible genetic origin (growth phenotypes). Because the knowledge of the interactions between this endogenous condition and nutritional factors might guide in the selection of lines for optimal production, the present experiments were conducted comparatively in two groups of assumedly constitutive fast and slow growers obtained by the segregation of extremes in a size distribution of spats belonging to a single age cohort.

In these experiments, the growth effects of changing nutritional features of the diet were approached in a double way: (1) by direct recording of size (length and live weight) increments from the onset to the end of the conditioning period; and (2) by the quantitative recording of physiological components of growth in experiments where the effects of dietary change on physiological parameters were assessed in both the chronic and acute responses and then integrated into an energy balance (scope for growth [SFG]). In addition to that SFG provides a reliable measurement of growth rate [22, 42, 47] benefits of methods of physiological energetics in analyzing nutritional effects relies on the possibility to identify which of the components of the chain of physiological processes connecting feeding and growth are more responsible for the observed effects as well as the eventual occurrence of compensatory responses to nutritional deficits.

In a preceding contribution [22], these methods of physiological energetics were applied to address the effects of feeding different growth phenotypes of the Manila clam on two diets that were isocaloric, but largely differed in protein/energy contents. Both feeding rate and absorption efficiency (AE) increased with the diet of high-N content, that improved (by ∼50%) both the SFG and actual growth, while higher phenotypic plasticity in the feeding response enabled fast growers to achieve a greater benefit from improved dietary quality (high-N diet) in comparison with the slow growers. In the present contribution, performed on fast- and slow-growing phenotypes of the carpet shell clam, we have pursued a similar approach to assess the effects on growth rate and energy balance of feeding spats with diets of variable composition where the differential factor in terms of biochemical composition was the ratio of carbohydrate to lipids, while protein content remained constant. Physiological behavior in response to both acute and chronic exposure to these different diets supplied at different rations were then analyzed in order to assess as to whether (a) feeding on diets of different biochemical compositions interacts with the growth condition to produce a range of growth rates; (b) the energy balances computed from physiological measurements might account for these growth differences; and (c) traits of physiological behavior observed in response to dietary changes are an exponent of possible mechanisms of compensation of nutritional limitations.

2. Materials and Methods

Spats ($n = 2{,}600$) of the carpet shell clam (*Ruditapes decussatus*) belonging to a single hatching cohort obtained through massive fertilization using conditioned brood stock, were supplied by the Centro de Investigacións Mariñas (CIMA, Ribadeo, Spain) and brought with ∼2 months of age to our facilities in the University of the Basque Country

Table 1: Composition, particle concentration (TPM and POM mg L^{-1}), and biochemical characteristics of experimental diets supplied.

Diet	Composition	Carbo-hydrates	Proteins	Lipids	N	C	H	TPM	POM	Energy content
D1	*R. lens*: 80% *S. cerevisiae*: 20%	15.47 (2.16)	50.33 (2.40)	34.20 (0.30)	14.45 (1.02)	68.93 (2.57)	16.62 (2.00)	1.09 (0.13)	1.01 (0.10)	26.29
D2	*R. lens*: 50% *S. cerevisiae*: 50%	24.03 (4.54)	49.09 (4.75)	26.87 (0.44)	14.39 (0.04)	65.90 (3.68)	19.72 (3.68)	1.15 (0.03)	1.08 (0.02)	23.12
D3	*R. lens*: 20% *S. cerevisiae*: 80%	32.60 (7.10)	47.85 (7.53)	19.55 (1.09)	13.99 (0.87)	67.76 (1.84)	18.25 (2.62)	1.16 (0.05)	1.07 (0.03)	19.96

Biochemical and elemental components are expressed as the percentage of each over the sum of the components or elements, respectively. Energy content (J mg^{-1}) was estimated following Platt and Irwin [54].

(UPV/EHU) (on January 19, 2017). Based on the size (length and live weight) distribution of these spats, two groups of clams were created by segregation of the larger (percentile 85) and smaller (percentile 30) clams that were characterized as fast- (F) and slow (S)-growing groups, respectively. Initial mean live weight representative of these groups of clams were 376.0 (71.6) and 179.1 (54.2) mg, respectively. Before the start of the experiments, selected groups of clams were monitored in the laboratory for 51 days, while fed a mixture of *Rhodomonas lens* and *Isochrysis galbana*, clone T-ISO (1 : 1 in terms of packed volume), with a double purpose: (a) to test the viability of the stock in future experiments, which rendered positive results since mortality rates were negligible (< 0.5%); and (b) to confirm that the assigned growth categories based on size segregation maintained their condition of fast and slow growers, respectively, under a common feeding regime in laboratory conditions.

2.1. Animal Maintenance and Diet Characteristics. Individuals from each F and S groups were randomly distributed into three different tanks where they were maintained under the same constant conditions of aeration, salinity (34‰), oxygen concentration (9.5 mg/L), and temperature (17°C). Along the conditioning period, each tank was supplied with a different composition of food (diets D1, D2, and D3) that was continuously dosed from concentrated stocks to achieve a particle concentration in the tanks of 2 mm^3 L^{-1} (approx. 20,000 cells mL^{-1}), kept constant by frequent checking and adjusting of the dosing rate using a Coulter Multisizer 3 (Beckman Coulter). Diet compositions were based on mixtures of cells of the microalgae *R. lens* and the yeast *Saccharomyces cerevisiae* in different proportions, as shown in Table 1. Microalgae were freshly collected from a lab culture, while the yeast derived from a commercial stock of dried pellets (baker's yeast Royal from Mondelez International) that were previously suspended in seawater. Yeast suspensions were tested for stability in preliminary assays, where neither changes in total matter nor fluctuations in cell diameter (measured in a Coulter Counter Multisizer 3) were appreciated within a 24 hr period. Owing to that, the stock suspensions were renewed every 24 hr or less. Although both species possess a median similar size (around 5 μm equivalent spherical diameter), percentages of mixtures were calculated based on packed particulate volumes (Coulter Counter Multisizer) rather than in terms of particle number. Mixed stocks of the diets were homogenized and maintained in suspension by magnetic stirrers inside beakers while dosed to the feeding tanks with a peristaltic pump. Tanks were cleaned from biodeposits and water changed on a daily basis.

Diet characterizations were carried out twice a week, using three replicates per sample, where different aliquots consisting of known volumes of water collected from the feeding tanks were filtered through glass fiber filters (GF/C) for different purposes. (a) *Weight of particulate matter*: water samples were filtered on preweighted filters, salts retained in the filters were then rinsed out with ammonium formate (0.9% w/v), and the filters were dried for 24–48 hr at 100°C to estimate dry weight. Ash weight was computed after combustion of the filters for 6 hr at 450°C. Total particulate matter (TPM, mg L^{-1}) and particulate inorganic matter (PIM, mg L^{-1}) were determined through the dry and ash weight, respectively, and particulate organic matter (POM, mg L^{-1}) was estimated as the difference between TPM and PIM. (b) *Elemental* (*CHN*) *analysis*: filters for elemental analysis were rinsed out with 50 mL of filtered seawater and immediately frozen at −20°C, lyophilized, and maintained at −20°C until being analyzed. Analysis occurred in the SGIker facility (UPV/EHU), by means of an Euro EA Elemental Analyzer (CHNS) from EuroVector, using acetanilide as standard. A subset of samples were calcined for 6 hr at 450°C and were subsequently measured in the elemental analyzer in order to subtract the inorganic C and N fractions.

Prior to the start of the experiments, diets were characterized to determine the proximate biochemical composition, as follows: samples of the stocks of diets (>1L) were centrifuged at 4°C and 3,700–3,800 rpm for 15 min, and the pellets were freeze-dried. Triplicate samples were then used for the independent extraction and quantification of carbohydrates, proteins, and total lipids, using colorimetric methods. Carbohydrates were extracted in trichloroacetic acid (5%) and quantified according to DuBois et al. [48] using dried glycogen from oysters as a standard. Proteins were extracted in NaOH (0.4 N) and quantified according to Lowry et al. [49] with a bovine serum albumin standard. Total lipids were pre-extracted in acetic acid [50], extracted twice in methanol:chloroform in proportions 2 : 1 and 1 : 2 [51, 52], and quantified according to Marsh and Weinstein [53] against a tripalmitin:phosphatidylcholine 1 : 1 standard.

As shown in Table 1, the differences in composition between diets were based on the proportion of microalgae to yeast. Most dietary parameters, including those for food ration (POM) and gross food composition (organic content:

POM/TPM or C:N ratio) were kept constant between diets, with the only important difference relying on biochemical composition and particularly in the carbohydrate/lipid ratio.

2.2. Experimental Design. Clams were maintained on the above diets for 30 days of food conditioning before conducting the physiological experimentation that was performed on the groups of clams conditioned to the extremes of diet composition (i.e., D1 and D3). During the experiments, each conditioning groups were fed diets D1 and D3, each dosed at two different rations: the high ration (H), that is coincident with the conditioning ration ($2\,mm^3\,L^{-1}$) and the low ration (L), that is half of the former ($1\,mm^3\,L^{-1}$).

2.3. Physiological Determinations and Scope for Growth. For each of the above experimental conditions, the physiological parameters of both fast- and slow-growing clams were separately determined in groups of 5–7 individuals since the small size of clams prevented from performing individual determinations. Reported values of these parameters were obtained as the mean of measurements performed with 5 of these groups ($n = 5$). Physiological measurements leading to the SFG determinations lasted four days for each food condition.

Clearance rate (*CR*, $L\,h^{-1}$) was measured by the flow-through chamber method [55]. Groups of clams were placed in a 125 mL flask, arranged in a bath at a constant temperature (17°C), with a constant supply of the diet, where flow rates were regulated independently in each flask in order to produce 15%–30% reduction of particle concentration. A flask without animals was used as the control chamber. A number of 12–16 measurements were considered sufficient to characterize the filtering activity and were distributed along the daytime (from 8 a.m. to 8 p.m.) to integrate possible fluctuations in the physiological response associated to tidal rhythms [56]. Individual CRs were computed according to the following expression:

$$CR(Lh^{-1}) = \frac{Ci - Co}{Ci} \times F, \tag{1}$$

where *Ci* and *Co* are the particle concentrations at the outlet of the control and experimental chambers, respectively; and *F* stands for the flow rate through the chamber.

Organic ingestion rate (*OIR*, $mg\,h^{-1}$) was computed as the product of CR and POM.

Absorption efficiency (*AE*, *decimal units*) was estimated by the method of Conover [57] from the organic contents of food and feces samples taken during CR measurements. Samples were filtered on GF/C filters and processed for TPM and POM following the same treatment described in Section 2.1. The organic content of diet (*F*) and feces (*E*) was estimated as the fraction of ash-free dry weight over the total dry weight of the sample and the AE was calculated as:

$$AE = \frac{F - E}{(1 - E) \times F}. \tag{2}$$

Absorption rate (*AR*, $mg\,h^{-1}$) of organic matter was estimated as the product of OIR and AE. Absorption rate in energy units ($J\,h^{-1}$) was obtained using the energy equivalents (26.29 and 19.96 $J\,mg^{-1}$ for diets 1 and 3, respectively), based on both biochemical and elemental composition [54].

Metabolic rate was indirectly assessed from the rate of oxygen consumption (VO_2, mL $O_2\,h^{-1}$). Groups of clams were placed in 150 mL sealed chambers filled with filtered seawater at constant temperature (17°C) for 4 hr. A luminescent dissolved oxygen probe connected to a Hatch HQ40d oximeter registered the decline in oxygen concentration in the chambers along the time. A chamber without animals was used to correct for changes in oxygen concentration not associated with the respiratory activity of animals. The rate of metabolic energy loss (R: $J\,h^{-1}$) was calculated using an energy equivalent or oxycaloric coefficient of 20.08 J mL O_2^{-1} [58].

Ammonia excretion rate (*VNH₄-N*, $\mu g\ NH_4\text{-}Nh^{-1}$) was determined from rates of ammonia production by animals in open flasks filled with 80 mL of filtered seawater (0.2 μm Millipore membrane) at constant temperature (17°C). Three subsamples per flask were extracted and the ammonia concentration of the water was determined according to the phenol-hypochlorite method [59]. Two flasks without animals were used as a control and processed in the same way as the former. Rates of ammonia excretion were converted to energy equivalents (U: $J\,h^{-1}$) by using a conversion factor of 24.85 $J\,mg^{-1}$ [60].

O:N index was calculated as the ratio in atomic equivalents of oxygen consumed and nitrogen excreted.

Scope for growth (*SFG*, $J\,h^{-1}$) was estimated according to the formula:

$$SFG = AR - (R + U). \tag{3}$$

All the rates were divided by the number of specimens inside each flask to express the individual rates. To correct for size variation among group treatments, all the physiological rates were standardized to a common dry weight of tissue (29.14 mg; obtained as the mean value of all individuals used in experiments), using the expression:

$$Y_{STD} = \left(\frac{W_{STD}}{W_{EXP}}\right)^b \times Y_{EXP}, \tag{4}$$

where Y_{STD} and Y_{EXP} represent, respectively, the standardized and experimental rate; W_{STD} and W_{EXP} are the standard and experimental weights of individuals; and b is the mass exponent scaling physiological rates to body size, according to own unpublished results: 0.609, 0.697, and 1.00, for CR, VO_2, and VNH_4, respectively.

2.4. Data Analysis. Analyses of conditioning effects on growth rate were conducted through two-way analysis of variance (ANOVA) (factors: conditioning diet and growth group). Acute effects of diets fed during the experiments on the physiological parameters and SFG were tested (separately for each acclimation condition) in the context of a three-way ANOVA (factors: diet composition, ration, and growth group). Analyses were performed after the data were tested for normality (Shapiro–Wilk) and homoscedasticity (Levene).

Table 2: Mean (SD) size parameters (L: length, wi: width, W: live weight) at the start and end of the conditioning period and growth rate (GR) in F and S clams conditioned to the three diets (D1, D2, and D3).

Condit.	G	Start of conditioning			End of conditioning			GR (mg d^{-1})	N
		L (mm)	Wi (mm)	W (mg)	L (mm)	Wi (mm)	W (mg)		
D1	F	14.41 (0.91)	9.51 (0.57)	449.97 (83.75)	14.74 (0.94)	9.98 (1.23)	484.48 (90.84)	1.4 (2.74)	122
	S	11.07 (0.89)	7.55 (0.74)	228.97 (76.59)	11.18 (0.98)	7.65 (0.82)	238.24 (86.01)	0.34 (2.49)	127
D2	F	13.65 (0.68)	9.01 (0.45)	374.8 (55.88)	13.78 (0.76)	9.1 (0.52)	399.71 (68.67)	1.02 (1.89)	147
	S	10.44 (0.55)	7.14 (0.5)	181.47 (49.07)	10.5 (0.65)	7.15 (0.58)	187.74 (55.29)	0.21 (1.47)	145
D3	F	14.57 (0.96)	9.57 (0.61)	457.23 (85.06)	14.63 (0.97)	9.61 (0.94)	471.12 (87.18)	0.57 (2.50)	122
	S	11.01 (0.91)	7.53 (0.69)	221.89 (71.72)	11.08 (0.91)	7.55 (0.7)	226.46 (71.17)	0.04 (1.94)	127

Table 3: Mean (SD) values of physiological parameters (CR: mL h^{-1}, AE: decimal units, AR: J h^{-1}, VO_2: μL h^{-1}, VNH_4-N: nL h^{-1}, and SFG: J h^{-1}) included in the energy balance of F and S clams conditioned to D1 and D3.

Diet	D1		D3		Statistical differences		
Growth group	F	S	F	S	D	G	D*G
CR	119.98 (37.6)	96.31 (16.11)	165.12 (69.85)	125.96 (20.51)	–	–	–
AE	0.79 (0.08)	0.89 (0.02)	0.38 (0.1)	0.5 (0.08)	***	**	–
AR	3.42 (0.95)	1.96 (0.35)	1.79 (0.56)	1.25 (0.21)	***	**	–
VO_2	29.92 (4.68)	34.38 (15.07)	28.1 (15.67)	10.34 (5.83)	*	–	*
VNH_4-N	425.87 (93.13)	477.41 (118.54)	286.6 (84.79)	211.69 (74.44)	***	–	–
SFG	2.81 (1)	1.26 (0.53)	1.22 (0.7)	1.04 (0.16)	**	*	*

Results of ANOVA analyses testing for significant effects (***$p<0.001$, **$p<0.01$, *$p<0.05$, "–" indicates n.s.) of diet (D), growth category (G) and interaction effects between them (D*G).

All statistical analysis and graphical elaboration of the figures were performed by means of the R software, version 3.5.1 [61].

3. Results

3.1. Growth Performance. Regarding the effects of diet conditioning, growth reached a maximum on the mixture containing the highest proportion of microalgae (80 : 20), followed by the intermediate diet (50 : 50), while growth was drastically reduced on the diet low in microalgae (20 : 80). Both factors (diet and growth condition) showed significant effects, where the conditioning effect ($F = 3.85$, $p = 0.02$) contributed to a lesser extent than the growth category ($F = 25.05$, $p < 0.001$), in spite of the high standard deviation in the comparison between F and S clams. Summarizing, the same trend regarding the negative effect of reducing the proportion of microalgae in the diet was observed for both growth groups, that maintained a consistent difference (approximately 5× between F and S clams) except for diet D3 where the growth rates of S clams were reduced by ∼10× with respect to F clams. In other words, feeding on a diet that limited growth rates also increased the differences between fast- and slow-growing clams.

3.2. Chronic Response. Physiological components of growth and SFG were compared for diet and growth group effects in the chronic response to assess the concordance between these indirect growth measurements and the actual measurements of growth rate shown in Table 2. Thus, comparisons included physiological measurements of each conditioning group (D1 and D3; note that physiological responses were only recorded for the extreme diets) while fed the diet used in conditioning (i.e., the composition D1 or D3 dosed at the high ration). To corroborate the main trends shown above (Table 2), the SFG also showed significant differences associated to both diet composition and growth category (Table 3), where D1 promoted higher SFG values than D3 and especially in F clams. These effects on SFG resulted from different physiological responses promoted by diets D1 and D3. For instance, net energy acquisition (absorption rate) and SFG were higher in clams conditioned to D1 due to the higher AE recorded with this diet, while F clams achieved higher SFG than S clams due to the increased rates of absorption, in spite of the higher AE values recorded in S clams (Table 3).

3.3. Acute Response. Main results concerning the effects on physiological parameters of acute exposure to D1 and D3 diets dosed at two rations on F and S clams are shown in Figure 1 for conditioning groups D1 and D3. Similarly, in order to simplify the interpretation of the analyses, significant effects of diet features on these physiological parameters were tested by means of a three-factor (diet composition, ration, and growth group) ANCOVA performed separately on both conditioning groups (Table 4). These responses were subsequently addressed comparatively between D1 and D3 conditioned clams.

CR (Figure 1(a)) showed significant differences in the response to exposure diets only in clams conditioned to D1 (Table 4), where a change from D1 to D3 promoted increases in their filtration activity (77% mean rise). Similarly, remarkable interindividual differences were displayed for D1 conditioned clams, F clams doubling the filtration rate of S clams. These effects of diet composition or growth group were

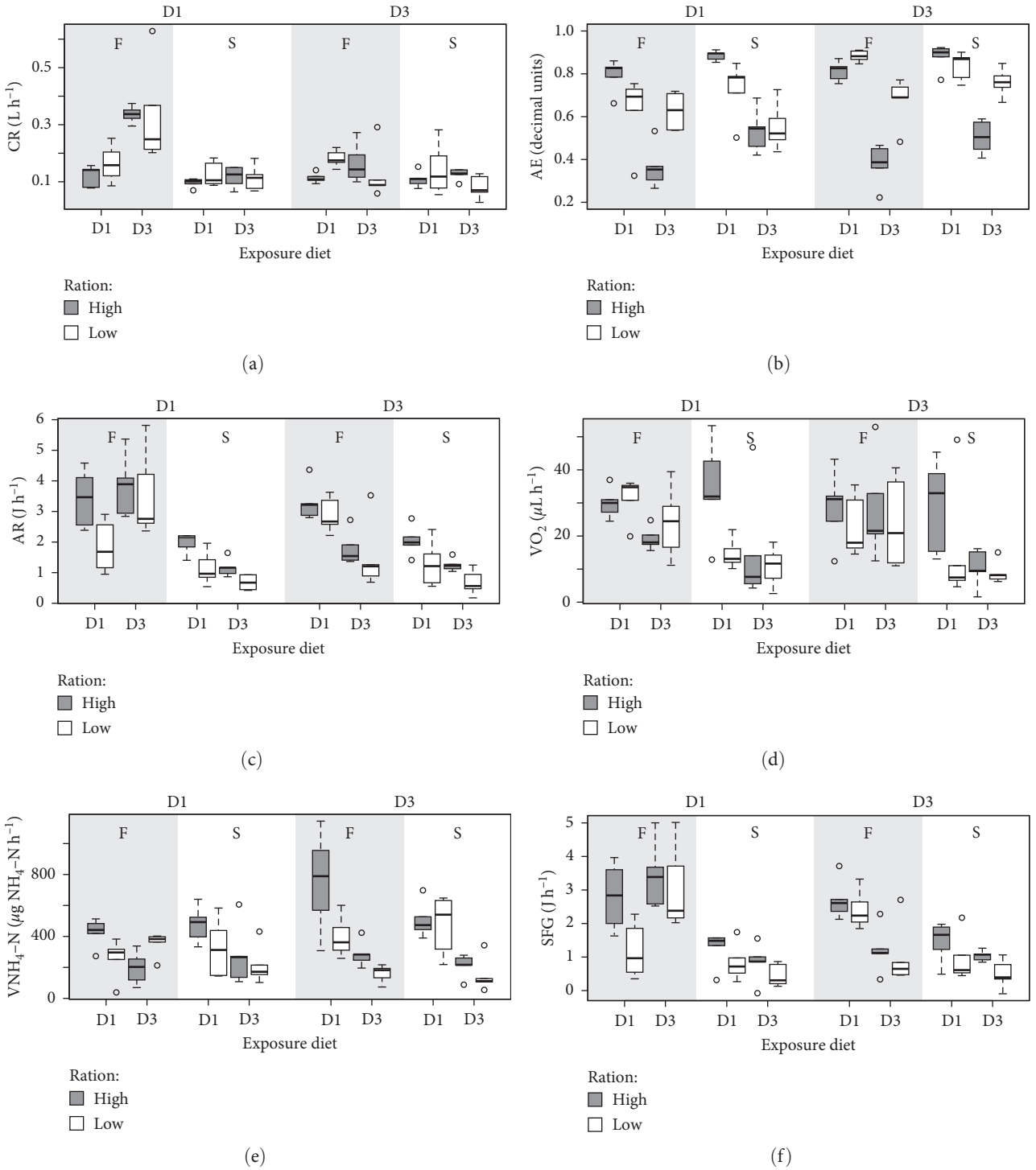

Figure 1: Mean values of physiological parameters in F (shaded area) and S (white area) clams fed diets D1 and D3. gray and white boxes stand for high and low rations, respectively. Results for specimens conditioned on diets D1 and D3 are shown in the left and right panels, respectively. (a) Clearance rate (CR); (b): absorption efficiency (AE); (c) absorption rate (AR); (d) rate of oxygen consumption (VO_2); (e) rate of ammonia excretion (VNH_4); (f) scope for growth (SFG). All rates are given as standardized values for a common size clam.

found to be not statistically significant in clams conditioned to D3, although trends indicate higher CRs in F clams (27% mean increase). Overall, ration effects were not significant due to the contrasting behavior found with exposure to diets D1 and D3: rising particle concentration promoted a decline in CR with D1 composition, while the opposite occurred for D3 (accounted for by the interaction term D*R, only significant for D3; Table 4).

The main effects on AE were associated to the composition of food supplied during the experiments (Figure 1(b)), irrespective of growth group or conditioning diet; thus, AE was significantly higher (46%) with diet D1 compared to D3

Table 4: Main ANOVA results for clearance rate (CR, $mL\,h^{-1}$), absorption efficiency (AE, decimal units), absorption rate (AR, $J\,h^{-1}$), metabolic rate (R, $J\,h^{-1}$), ammonia excretion rate (U, $J\,h^{-1}$), and scope for growth (SFG, $J\,h^{-1}$) for clams previously conditioned to D1 and D3, for comparisons among exposure diets (D), ration (R), and growth category (G).

Factor	CR	AE	AR	R	U	SFG
Conditioned to D1						
D	**F = 17.42**, $p<0.001$	**F = 47.044**, $p<0.001$	F = 0.659, $p = 0.423$, $p<0.001$	**F = 10.229**, $p = 0.003$	**F = 6.638**, $p = 0.015$	F = 2.576, $p = 0.118$
R	**F = 0.521**, $p = 0.476$	F = 0.101, $p = 0.753$	**F = 9.112**, $p = 0.005$	F = 2.085, $p = 0.158$	F = 1.74, $p = 0.197$	**F = 7.066**, $p = 0.012$
G	**F = 28.362**, $p<0.001$	**F = 4.322**, $p = 0.046$	**F = 55.008**, $p<0.001$	**F = 5.289**, $p = 0.028$	F = 0.134, $p = 0.717$	**F = 46.944**, $p<0.001$
D:R	**F = 0.767**, $p = 0.388$	**F = 19.126**, $p<0.001$	F = 2.444, $p = 0.128$	F = 2.082, $p = 0.159$	**F = 5.742**, $p = 0.023$	F = 1.438, $p = 0.239$
D:G	**F = 16.184**, $p<0.001$	F = 0.488, $p = 0.49$	**F = 10.551**, $p = 0.003$	F = 0.139, $p = 0.712$	F = 1.041, $p = 0.315$	**F = 9.93**, $p = 0.004$
R:G	**F = 0.019**, $p = 0.892$	F = 2.793, $p = 0.104$	F = 0.277, $p = 0.603$	**F = 5.947**, $p = 0.02$	F = 1.357, $p = 0.253$	F = 1.271, $p = 0.268$
D:R:G	**F = 0.024**, $p = 0.878$	F = 3.22, $p = 0.082$	F = 0.864, $p = 0.359$	F = 0.802, $p = 0.377$	F = 1.842, $p = 0.184$	F = 1.306, $p = 0.262$
Conditioned to D3						
D	F = 0.462, $p = 0.502$	**F = 135.754**, $p<0.001$	**F = 27.274**, $p<0.001$	F = 2.502, $p = 0.124$	**F = 42.64**, $p<0.001$	**F = 22.039**, $p<0.001$
R	F = 0.086, $p = 0.771$	**F = 38.272**, $p<0.001$	**F = 5.971**, $p = 0.02$	F = 2.356, $p = 0.135$	**F = 8.191**, $p = 0.007$	F = 3.543, $p = 0.069$
G	F = 2.977, $p = 0.094$	**F = 6.137**, $p = 0.019$	**F = 26.357**, $p<0.001$	**F = 6.302**, $p = 0.017$	F = 1.66, $p = 0.207$	**F = 18.213**, $p<0.001$
D:R	**F = 6.669**, $p = 0.015$	**F = 31.518**, $p<0.001$	F = 0.15, $p = 0.701$	F = 0.737, $p = 0.397$	F = 0.989, $p = 0.327$	F = 0.003, $p = 0.96$
D:G	F = 0.35, $p = 0.558$	**F = 4.355**, $p = 0.045$	F = 3.244, $p = 0.081$	F = 2.726, $p = 0.108$	F = 0.182, $p = 0.672$	**F = 6.265**, $p = 0.018$
R:G	F = 0.28, $p = 0.6$	F = 2.692, $p = 0.111$	F = 0.612, $p = 0.44$	F = 0.106, $p = 0.746$	F = 3.581, $p = 0.068$	F = 0.47, $p = 0.498$
D:R:G	F = 0.137, $p = 0.713$	F = 0.597, $p = 0.445$	F = 0.004, $p = 0.951$	F = 0.423, $p = 0.52$	F = 1.622, $p = 0.212$	F = 0.035, $p = 0.853$

The values highlighted in bold represent significant results ($p<0.05$).

(Table 4). Increasing ration produced opposite effects on the AE recorded with diets D1 (positive effect) and D3 (negative effect) as accounted for the interaction D*R (Table 4). Differences between growth groups achieved less significance, although S clams registered approximately 10% higher AEs than F clams.

Absorption rates (Figure 1(c)) reflected the positive effects of the ration in the form of a general increment associated to high food concentration (Table 4); although such effects were partly counterbalanced in the case of D3, due to the decline of AE with the high ration of this diet. Consistent differences in CR between growth groups also resulted in rates of absorption that were significantly higher (2–2.5 times) in F clams compared with S clams (Table 4). In summary, AR (Figure 1(c)) shows a general tendency to decrease with declining ration (H vs. L), food quality (D1 vs. D3), or growth performance (F vs. S); with the sole exception of F clams conditioned to D1 and fed the diet D3 where a strong overfeeding response was recorded.

Main differences in metabolic rates (Figure 1(d)) were found between growth groups, with higher rates recorded for F clams (Table 4); however, large intragroup variability reduced the significance of these effects. Moreover, rates of metabolism tended to decline in clams fed on D3 diet, although this effect was only significant following conditioning to the D1 diet (Table 4). No significant effects of ration were recorded except for S clams (R*G interaction) conditioned to D1 (Table 4).

Ammonia excretion rate (Figure 1(e)) constituted a minor component in the energy budget, and indeed only around 2% of metabolic energy expenditure corresponded to ammonia release. Yet, responses were consistent between conditioning groups as to the increased N excretion rates recorded with exposure to D1 compared with D3 (Table 4). Feeding a high ration also increased ammonia release, with different intensities in clams acclimated to D1 (20% increase) and D3 (50% increase). No significant differences in rates of excretion were detected between growth groups.

SFG (Figure 1(f)) largely reflected the behavior of absorption rates (Figure 1(c)), with the growth condition exerting highly significant effects in both conditioning groups (Table 4). On average, the values for F clams attained over twice the values of S clams (2.23 and 0.91 J h^{-1}, respectively). Moreover, the average values of SFG were higher in clams conditioned to D1 compared to D3; although not statistically tested, these differences were mainly associated to the behavior of fast growers (Figure 1(f)). Compared with feeding the D1 diet, there is a general decline in the SFG values recorded with the D3 diet, which is mainly due to the reduction in AE values observed with this last diet, especially when fed the high ration (Figure 1(f)). This effect of food composition was more intense in slow compared with fast-growing clams (as accounted for the significant interactions D*G; Table 4). In contrast with the general behavior of clams conditioned to the D3 diet, F clams conditioned to the D1 diet were found able to compensate the negative effects of reduced food quality by means of a positive feeding response, resulting in the absence of significant differences in SFG between D1 and D3 diets (Table 4).

4. Discussion

Actual measurements of growth and energy balance recorded in juvenile *Ruditapes decussatus* reflected a combination of effects associated to both endogenous and exogenous factors related to features of food suspensions. Overall, growth conditions improved with an increasing proportion of phytoplankton relative to yeasts in the diet that, in terms of biochemical composition, represented both a negative effect of increasing the carbohydrate to lipid ratio and a positive effect of dietary energy content. Indeed, physiological components of growth performed better in clams either acclimated or exposed to D1 diet relative to D3 diet and benefited more from the increase in ration with the high-quality diet. Similarly, actual growth determinations based on size increments during conditioning revealed a continuous decreasing trend from D1 to D3. Original differences in growth performance between F and S clams also increased from D1 to D3, which reflect a higher ability for F individuals to maintain a comparatively higher rate of growth than S specimens under poorer feeding conditions. Similar results were reported in the Manila clam (*R. philippinarum*) [22] where feeding on low-quality diets—represented in this case by low-N content—enhanced constitutive differences in growth rate between F and S clams.

Much of the above dietary effects, differentially expressed in fast and slow growers, rely on the feeding response, as exemplified in D1 conditioning by the increase in CRs observed in F clams when fed the D3 diet at both rations (Figure 1(a)). A similar, although much more intense overfeeding response was reported in this species when fed a carbohydrate-rich diet in comparison with a microalgal diet [31]. Hence, this increase can be interpreted to act as a compensatory mechanism for the general reduction in AE (Figure 1(b)), to be discussed later, and resulted in the regulation of absorption rates that reached similar values in both regimes of diet exposure (Figure 1(c)). Such compensatory feeding response was restricted to F individuals, revealing that an increased endogenous capacity for food collection would be a key feature underlying better growth performance. Traits of physiological behavior fitting the energy acquisition model [62] have been largely reported in the congeneric clam species *R. philippinarum* [22, 42, 47, 63] where a greater capacity for feeding and absorption characterizes fast-growing phenotypes. However, no such overfeeding response was observed in F clams conditioned with the growth limiting diet (D3), suggesting that energy restrictions during the conditioning period might, in addition to limit growth, also result in the virtual cancelation of physiological compensatory adjustments.

Digestive behavior of food ingested when feeding diets of different composition is virtually the most important physiological trait as regards to energy balance. The reduction of AE associated to food composition, from exposure diet D1 to D3, was highly significant in all conditions (Table 4), and this quality-dependent effect appeared reinforced in clams that had been conditioned to D3. For instance, the combined acute and chronic effects of decreasing food quality resulted

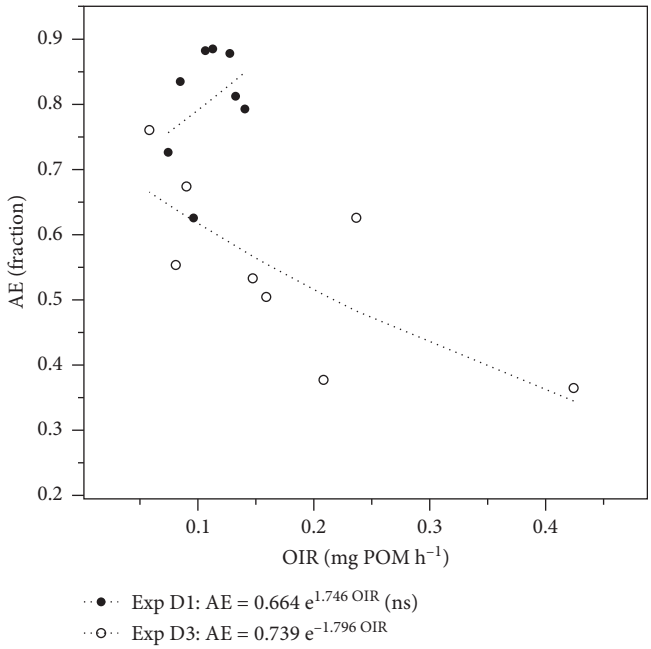

Figure 2: Relationship between AE and OIR for each exposure diet (D1: closed circles and D3: open circles). Fitted regressions were only significant for clams exposed to D3.

in an average decrease by ∼50% in the AE (Table 3). Rates of absorption—representing the net energy gain—and SFG values reflected the effects of food composition on digestive performance, concomitantly with the overall positive effects of ration and interindividual differences in feeding rates accounted for by the sequence of values shown in Figures 1(c) and 1(f), where F clams ranked above S clams and clams fed the D1 and high ration ranked above those fed the D3 and low ration (with the already discussed exception of F clams conditioned to D1 and fed D3).

Opportunities brought about by increasing food concentration (ration) as regards energy gain are, however, different between D1 and D3 since AE was virtually independent on ingestion rate with diet D1 found to sharply decline with increasing rates of ingestion of D3 (Figure 2), thus partly canceling out the benefits of feeding at high ration of this diet. In addition to the overall reduction in AE, strict reliance of this efficiency on gut transit time of food, which is implicit in this relationship for D3, has been identified in several bivalves as a trait characteristic of the gut processing of poorly digestible items such as phytodetritus, particularly of vascular plant origin [64–66], or low-quality phytoplankton [22]. Digestibility can be assumed to result from the substrate specificity of the digestive enzyme pool fitting the biochemical composition of food. Unlike the digestive processing of phytoplankton, it is uncertain as to whether enzymes in the gut of bivalves might be fully effective in the digestion of *S. cerevisiae* cells, that is the main component (80%) of D3 diet. Digestive breakdown of glycogen, the main energy reserve in yeasts (up to 40% in weight; [67, 68]), is very likely performed by α-amylase, abundantly present in both the digestive gland and crystalline style of bivalves [69–72], but it is much more problematic to ascertain the effectiveness of the cellulolytic complex, primarily designed for the cleavage of phytoplankton cell wall [73] against the polysaccharide composition of the yeast's cell wall, including different beta-glucans, mannans, and chitin [74]. In this respect, chemical and enzymatic treatment of yeast cells to partly remove the mannoprotein layer [75, 76], as well as the use of mutant variants that are deficient in mannan synthesis [77] have been reported to greatly increase the nutritional value baker's yeast, allowing the effective replacement (up to 80%) of phytoplankton by yeast in the cultivation of the spat of clams *R. decussatus* [39] and *Mercenaria mercenaria* [76].

Moreover, the benefits of phytoplankton in the diet of clams might not only rely on its better digestibility, but also on the provision of more appropriate nutrient balance. As the main structural components of tissues, proteins have been addressed as the key constituent in supporting growth [78–81], but all diets tested in this study had the same protein content. However, the increased proportion of carbohydrates at the expense of lipids in the diet with the greatest degree of substitution of microalgae by yeasts (e.g., D3; Table 1) might result in nutrient limitation under some conditions. Digestion in bivalves comprises a great amount of intracellular processes hold by the digestive gland that results in the breaking off and subsequent release of digestive cell apices in the form of membranous "fragmentation spherules" [82, 83] which are reputed to have a high lipid content [84]. This forms the bulk of metabolic fecal losses (MFL) [85] an organic component of endogenous origin voided with the feces that has been reported to constitute a very relevant although variable component in the digestive balance of bivalves: for instance, MFL were quantified in cockles (*Cerastoderma edule*) to represent between 12% in readily digestible diets like phytoplankton, and 26% of organic ingestion, in diets more refractory to digestion like vascular plant detritus [66]. Given the abundance of lipids in MFL composition, the above is consistent with the finding that the digestive balance of lipids was drastically reduced (even to negative values) under feeding conditions involving high MFL [70, 86]. Those conditions are very likely represented by diet D3 in the present case, where a high demand of dietary lipids to offset digestive constraints met in the form of MFL would occur concomitantly with the dietary restriction of this biochemical component. Such limitations, along with reduced enzymatic digestibility, would account for most of the restrictions on net energy gain found with this diet in comparison with diet D1.

Energy expenses represented only a minor component (∼20%) of energy balances, with over 90% of these expenses being accounted for by metabolic rates (R) and less than 10% by N excretion (U). Regarding the costs of foodstuff assimilation, the amount of energy respired per unit of energy absorbed (R/AR) was seen to keep a rather constant proportion across diets, but revealed a trend of change associated to growth condition ($F = 2.96$, $p = 0.09$), with F clams exhibiting lower unitary costs than S clams (0.23 (0.15) vs. 0.32 (0.28)). Although not statistically significant in this case,

due to large variances, the occurrence of this ∼30% difference between F and S clams is consistent with broadly reported evidence in bivalve species of higher unitary metabolic costs in constitutive slow growers compared with fast growers [40–42, 44, 47, 63, 87–91] fitting the metabolic efficiency model [62].

One important conclusion of the present study is that the growth performance of carpet shell clams (measured in terms of both growth rate and SFG) improved with the increasing proportion of lipids to carbohydrate in the diet, which corresponds in this case with the relative abundance of microalgae versus yeasts cells in the feeds mixture. Regarding physiological parameters, these dietary effects are mainly driven by differences in the AE found between clams fed the extreme food compositions. However, the present results do not allow to ascertain whether the reduced AE recorded with the diet D3 relative to D1 diet results from reduced digestibility *per se* of yeasts cells due to structural restrictions or either reflects the digestive imbalance of lipids associated to high production of MFL. Irrespective of food composition, growth performance was higher in F compared with S clams, with higher feeding rates and reduced metabolic costs of F clams accounting for these growth differences. Moreover, increased phenotypic plasticity in the form of an overfeeding response was seen to enable the fast-growing clams to overcome the above referred limitations in the digestive processing of D3 diets; however, the resource to such kind of physiological mechanism appeared limited by the nutritional conditions (energetic status) prevailing during the conditioning phase.

Data Availability

The data that support the findings of this study are available in Zenodo at: https://doi.org/10.5281/zenodo.7971828.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] M. A. Borowitzka, "Microalgae for aquaculture: opportunities and constraints," *Journal of Applied Phycology*, vol. 9, pp. 393–401, 1997.

[2] C. E. Epifanio, "Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria mercenaria* (L.)," *Aquaculture*, vol. 18, no. 1, pp. 1–12, 1979.

[3] A. E. Pronker, N. M. Nevejan, F. Peene, P. Geijsen, and P. Sorgeloos, "Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus 1758). part i. impact of different micro-algae mixtures on broodstock performance," *Aquaculture International*, vol. 16, pp. 297–307, 2008.

[4] Y. Ouagajjou, A. Aghzar, M. Ahensal, N. Berday, and M. Idhalla, "Behavioural pattern in changes to diets and food availability during broodstock conditioning of captive clam *Ruditapes decussates*," *E3S Web of Conferences*, vol. 337, Article ID 03002, 2022.

[5] C. J. Langdon and C. A. Siegfried, "Progress in the development of artificial diets for bivalve filter feeders," *Aquaculture*, vol. 39, no. 1–4, pp. 135–153, 1984.

[6] I. Laing, "The use of artificial diets in rearing bivalve spat," *Aquaculture*, vol. 65, no. 3-4, pp. 243–249, 1987.

[7] E. R. Urban Jr. and C. J. Langdon, "Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements," *Aquaculture*, vol. 38, no. 4, pp. 277–291, 1984.

[8] J. Knauer and P. C. Southgate, "A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture," *Reviews in Fisheries Science*, vol. 7, no. 3-4, pp. 241–280, 1999.

[9] P. Coutteau and P. Sorgeloos, "The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey," *Journal of Shellfish Research*, vol. 11, no. 2, pp. 467–476, 1992.

[10] P. Coutteau and P. Sorgeloos, "The use of artificial diets in the hatchery rearing of bivalve mollusks," *Meded.-Fac. Landbouwwet. Rijksuniv. Gent*, vol. 57, pp. 2111–2119, 1992.

[11] R. Robert and P. Trintignac, "Substitutes for live microalgae in mariculture: a review," *Aquatic Living Resources*, vol. 10, no. 5, pp. 315–327, 1997.

[12] A. Pérez-Camacho, M. Albentosa, M. J. Fernández-Reiriz, and U. Labarta, "Effect of microalgal and inert (cornmeal and cornstarch) diets on growth performance and biochemical composition of *Ruditapes decussatus* seed," *Aquaculture*, vol. 160, no. 1-2, pp. 89–102, 1998.

[13] M. Albentosa, A. Pérez-Camacho, M. J. Fernández-Reiriz, and U. Labarta, "Wheatgerm flour in diets for Manila clam, *Ruditapes philippinarum*, spat," *Aquaculture*, vol. 212, no. 1–4, pp. 335–345, 2002.

[14] N. Z. Mamat and A. C. Alfaro, "Evaluation of microalgal and formulated diets for the culture of the New Zealand pipi clam *Paphiesaustralis*," *International Aquatic Research*, vol. 6, Article ID 57, 2014.

[15] S. D. Utting, "A preliminary study on growth of *Crassostrea gigas* larvae and spat in relation to dietary protein," *Aquaculture*, vol. 56, no. 2, pp. 123–138, 1986.

[16] D. A. Kreeger and C. J. Langdon, "Effect of dietary protein content on growth of juvenile mussels, *Mytilus trossulus* (Gould 1850)." *The Biological BulletIn*, vol. 185, no. 1, pp. 123–139, 1993.

[17] I. Uriarte and A. Farías, "The effect of dietary protein content on growth and biochemical composition of Chilean scallop *Argopecten purpuratus* (L.) postlarvae and spat," *Aquaculture*, vol. 180, no. 1-2, pp. 119–127, 1999.

[18] A. N. Maeda-Martínez, P. E. Saucedo, J. M. Mazón-Suástegui, H. Acosta-Salmón, and Z. Romero-Meléndez, "Scope for growth of juvenile Cortez oyster, *Crassostrea corteziensis* fed isocaloric diets made up with microalgae and cereal flours,"

Latin American Journal of Aquatic Research, vol. 44, no. 4, pp. 845–849, 2016.

[19] R. W. Langton, K. C. Haines, and R. E. Lyon, "Ammonia-nitrogen production by the bivalve mollusc*Tapes japonica* and its recovery by the red seaweed*Hypnea musciformis* in a tropical mariculture system," *Helgoländer wissenschaftliche Meeresuntersuchungen*, vol. 30, pp. 217–229, 1977.

[20] M. Albentosa, M. J. Fernández-Reiriz, A. Pérez-Camacho, and U. Labarta, "Growth performance and biochemical composition of *Ruditapes decussatus* (L.) spat fed on microalgal and wheatgerm flour diets," *Journal of Experimental Marine Biology and Ecology*, vol. 232, no. 1, pp. 23–37, 1999.

[21] S. M. Gallager and R. Mann, "The effect of varying carbon/nitrogen ratio in the phytoplankter *Thalassiosira pseudonana* (3H) on its food value to the bivalve *Tapes japonica*," *Aquaculture*, vol. 26, no. 1-2, pp. 95–105, 1981.

[22] K. Arranz, I. Urrutxurtu, I. Ibarrola et al., "Physiological processes modulate acute and chronic responses to dietary protein/energy ratio fluctuations in individuals and families of Manila clam (*Ruditapes philippinarum*) selected for variable growth rates," *Aquaculture*, vol. 521, Article ID 735056, 2020.

[23] Y.-C. Chen, "The biomass and total lipid content and composition of twelve species of marine diatoms cultured under various environments," *Food Chemistry*, vol. 131, no. 1, pp. 211–219, 2012.

[24] J. N. C. Whyte, "Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves," *Aquaculture*, vol. 60, no. 3-4, pp. 231–241, 1987.

[25] P. G. Beninger and A. Lucas, "Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L.(Jeffreys) and *Tapes philippinarum* (Adams & Reeve)," *Journal of Experimental Marine Biology and Ecology*, vol. 79, no. 1, pp. 19–37, 1984.

[26] P. A. Gabbott and B. L. Bayne, "Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L," *Journal of the Marine Biological Association of the United Kingdom*, vol. 53, no. 2, pp. 269–286, 1973.

[27] E. Rodríguez-Moscoso and R. Arnaiz, "Gametogenesis and energy storage in a population of the grooved carpet-shell clam, *Tapes decussatus* (Linné, 1787), in northwest Spain," *Aquaculture*, vol. 162, no. 1-2, pp. 125–139, 1998.

[28] D. I. Zandee, J. H. Kluytmans, W. Zurburg, and H. Pieters, "Seasonal variations in biochemical composition of mytilus edulis with reference to energy metabolism and gametogenesis," *Netherlands Journal of Sea Research*, vol. 14, no. 1, pp. 1–29, 1980.

[29] A. R. Flaak and C. E. Epifanio, "Dietary protein levels and growth of the oyster *Crassostrea virginica*," *Marine Biology*, vol. 45, pp. 157–163, 1978.

[30] M. Albentosa, A. Pérez-Camacho, U. Labarta, and M. J. Fernández-Reiriz, "Evaluation of live microalgal diets for the seed culture of *Ruditapes decussatus* using physiological and biochemical parameters," *Aquaculture*, vol. 148, no. 1, pp. 11–23, 1996.

[31] M. Albentosa, M. J. Fernández-Reiriz, U. Labarta, and A. Pérez-Camacho, "Absorption of biochemical components and feeding behaviour with natural and carbohydrate-rich diets in *Ruditapes decussatus* and *Venerupis pullastra* clams," *Journal of Shellfish Research*, vol. 22, no. 1, pp. 443–449, 2003.

[32] M. J. Fernández-Reiriz, U. Labarta, M. Albentosa, and A. Pérez-Camacho, "Lipid composition of *Ruditapes philippinarum* spat: effect of ration and diet quality," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 144, no. 2, pp. 229–237, 2006.

[33] D. S. Haven, "Supplemental feeding of oysters with starch," *Chesapeake Science*, vol. 6, pp. 43–51, 1965.

[34] J. M. Mazón-Suástegui, K. M. Ruíz-Ruíz, A. Parres-Haro, and P. E. Saucedo, "Combined effects of diet and stocking density on growth and biochemical composition of spat of the Cortez oyster *Crassostrea corteziensis* at the hatchery," *Aquaculture*, vol. 284, no. 1–4, pp. 98–105, 2008.

[35] M. J. Fernández-Reiriz, U. Labarta, M. Albentosa, and A. Pérez-Camacho, "Lipid profile and growth of the clam spat, *Ruditapes decussatus* (L), fed with microalgal diets and cornstarch," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 124, no. 3, pp. 309–318, 1999.

[36] M. J. Fernández-Reiriz, U. Labarta, M. Albentosa, and A. Pérez-Camacho, "Effect of microalgal diets and commercial wheatgerm flours on the lipid profile of *Ruditapes decussatus* spat," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 119, no. 1, pp. 369–377, 1998.

[37] C. E. Epifanio, "Comparison of yeast and algal diets for bivalve molluscs," *Aquaculture*, vol. 16, no. 3, pp. 187–192, 1979.

[38] S. Tanyaros, C. Sujarit, N. Jansri, and W. Tarangkoon, "Baker's yeast as a substitute for microalgae in the hatchery rearing of larval and juvenile tropical oyster (*Crassostrea belcheri*, Sowerby 1871)," *Journal of Applied Aquaculture*, vol. 28, no. 1, pp. 35–46, 2016.

[39] M. Albentosa, E. Naessens, P. Léger, P. Coutteau, P. Lavens, and P. Sorgeloos, "Promising results in the seed culturing of the Manila clam *Tapes semidecussata* with a manipulated yeast product as a partial substitute for algae," *European Aquaculture Society*, vol. 89, pp. 7-8, 1989.

[40] B. L. Bayne, "Relations between variable rates of growth, metabolic costs and growth efficiencies in individual Sydney rock oysters (*Saccostrea commercialis*)," *Journal of Experimental Marine Biology and Ecology*, vol. 251, no. 2, pp. 185–203, 2000.

[41] B. L. Bayne, D. Hedgecock, D. McGoldrick, and R. Rees, "Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters [*Crassostrea gigas* (Thunberg)]," *Journal of Experimental Marine Biology and Ecology*, vol. 233, no. 1, pp. 115–130, 1999.

[42] D. Tamayo, I. Ibarrola, M. B. Urrutia, and E. Navarro, "The physiological basis for inter-individual growth variability in the spat of clams (*Ruditapes philippinarum*)," *Aquaculture*, vol. 321, no. 1-2, pp. 113–120, 2011.

[43] D. Prieto, I. Urrutxurtu, E. Navarro, M. B. Urrutia, and I. Ibarrola, "*Mytilus galloprovincialis* fast growing phenotypes under different restrictive feeding conditions: *Fast feeders* and *energy savers*," *Marine Environmental Research*, vol. 140, pp. 114–125, 2018.

[44] D. Tamayo, I. Ibarrola, I. Urrutxurtu, and E. Navarro, "Physiological basis of extreme growth rate differences in the spat of oyster (*Crassostrea gigas*)," *Marine Biology*, vol. 161, pp. 1627–1637, 2014.

[45] D. Prieto, D. Tamayo, I. Urrutxurtu, E. Navarro, I. Ibarrola, and M. B. Urrutia, "Nature more than nurture affects the growth rate of mussels," *Scientific reports*, vol. 10, Article ID 3539, 2020.

[46] X. Zhang, B. Ye, Z. Gu et al., "Comparison in growth, feeding, and metabolism between a fast-growing selective strain and a cultured population of pearl oyster (*Pinctada fucata martensii*)," *Frontiers in Marine Science*, vol. 8, Article ID 770702, 2021.

[47] D. Tamayo, I. Ibarrola, J. Cigarría, and E. Navarro, "The effect of food conditioning on feeding and growth responses to variable rations in fast and slow growing spat of the Manila clam (*Ruditapes philippinarum*)," *Journal of Experimental Marine Biology and Ecology*, vol. 471, pp. 92–103, 2015.

[48] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, no. 3, pp. 350–356, 1956.

[49] O. H. Lowry, N. J. Rosebrough, A. Lawis Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[50] F. C. Phillips and O. S. Privett, "Extraction and analysis of lipids from immature soybeans," *Lipids*, vol. 14, no. 11, pp. 949–952, 1979.

[51] E. G. Bligh and W. J. Dyer, "A rapid method of total lipid extraction and purification," *Canadian Journal of Biochemistry and Physiology*, vol. 37, no. 8, pp. 911–917, 1959.

[52] J. Folch, M. Lees, and G. H. Sloane Stanley, "A simple method for the isolation and purification of total lipides from animal tissues," *The Journal of Biological Chemistry*, vol. 226, no. 1, pp. 497–509, 1957.

[53] J. B. Marsh and D. B. Weinstein, "Simple charring method for determination of lipids," *Journal of Lipid Research*, vol. 7, no. 4, pp. 574–576, 1966.

[54] T. Platt and B. Irwin, "Caloric content of phytoplankton," *Limnology and Oceanography*, vol. 18, no. 2, pp. 306–310, 1973.

[55] D. J. Crisp, "Energy flow measurements," in *Methods for the Study of Marine Benthos*, N. A. Holme and A. D. Mc Inture, Eds., pp. 197–323, Blackwell, 1971.

[56] B. L. Bayne, A. J. S. Hawkins, and E. Navarro, "Feeding and digestion in suspension-feeding bivalve molluscs: the relevance of physiological compensations," *American Zoologist*, vol. 28, no. 1, pp. 147–159, 1988.

[57] R. J. Conover, "Assimilation of organic matter by zooplankton," *Limnology and Oceanography*, vol. 11, no. 3, pp. 338–345, 1966.

[58] E. Gnaiger, "Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power," *Journal of Experimental Zoology*, vol. 228, no. 3, pp. 471–490, 1983.

[59] L. Solórzano, "Determination of ammonia in natural waters by the phenolhypochlorite method 11 this research was fully supported by U.S. atomic energy commission contract no. ATS (11-1) GEN 10, P.A. 20." *Limnology and Oceanography*, vol. 14, no. 5, pp. 799–801, 1969.

[60] J. M. Elliott and W. Davison, "Energy equivalents of oxygen consumption in animal energetics," *Oecologia*, vol. 19, pp. 195–201, 1975.

[61] R Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, 2018.

[62] B. L. Bayne, "Physiological components of growth differences between individual oysters (*Crassostrea gigas*) and a comparison with *Saccostrea commercialis*," *Physiological and Biochemical Zoology*, vol. 72, no. 6, pp. 705–713, 1999.

[63] D. Tamayo, I. Ibarrola, and E. Navarro, "Thermal dependence of clearance and metabolic rates in slow-and fast-growing spats of manila clam *Ruditapes philippinarum*," *Journal of Comparative Physiology B*, vol. 183, pp. 893–904, 2013.

[64] U. Arambalza, I. Ibarrola, E. Navarro, and M. B. Urrutia, "Ingestion and absorption of particles derived from different macrophyta in the cockle *Cerastoderma edule*: effects of food ration," *Journal of Comparative Physiology B*, vol. 184, pp. 179–192, 2014.

[65] E. Navarro, S. Méndez, M. B. Urrutia, U. Arambalza, and I. Ibarrola, "Digestive selection underlies differential utilization of phytoplankton and sedimentary organics by infaunal bivalves: experiments with cockles (*Cerastoderma edule*) using cross-labelled mixed diets," *Marine Environmental Research*, vol. 120, pp. 111–121, 2016.

[66] E. Navarro, S. Méndez, I. Ibarrola, and M. B. Urrutia, "Comparative utilization of phytoplankton and vascular plant detritus by the cockle *Cerastoderma edule*: digestive responses during diet acclimation," *Aquatic Biology*, vol. 6, pp. 247–262, 2009.

[67] P. S. Deshpande, S. N. Sankh, and A. U. Arvindekar, "Study of two pools of glycogen in *Saccharomyces cerevisiae* and their role in fermentation performance," *Journal of the Institute of Brewing*, vol. 117, no. 1, pp. 113–119, 2011.

[68] S. H. Lillie and J. R. Pringle, "Reserve carbohydrate metabolism in Saccharomyces cerevisiae: responses to nutrient limitation," *Journal of Bacteriology*, vol. 143, no. 3, pp. 1384–1394, 1980.

[69] I. Ibarrola, E. Navarro, J. I. P. Iglesias, and M. B. Urrutia, "Time-course of digestive-enzyme acclimation in the cockle *Cerastoderma edule*," *Marine Biology*, vol. 135, pp. 47–56, 1999.

[70] I. Ibarrola, E. Navarro, and J. I. P. Iglesias, "Short-term adaptation of digestive processes in the cockle *Cerastoderma edule* exposed to different food quantity and quality," *Journal of Comparative Physiology B*, vol. 168, pp. 32–40, 1998.

[71] R. G. B. Reid, "The distribution of digestive tract enzymes in lamellibranchiate bivalves," *Comparative Biochemistry and Physiology*, vol. 24, no. 3, pp. 727–736, 1968.

[72] L. J. Seiderer, R. C. Newell, and P. A. Cook, "Quantitative significance of style enzymes from two marine mussels (*Choromytilus meridionalis* Krauss and *Perna perna* Linnaeus) in relation to diet," *Marine Biology Letters*, vol. 3, pp. 257–271, 1982.

[73] V. Brock, "*Crassostrea gigas* (Thunberg) hepatopancreas-cellulase kinetics and cellulolysis of living monocellular algae with cellulose walls," *Journal of Experimental Marine Biology and Ecology*, vol. 128, no. 2, pp. 157–164, 1989.

[74] B. Aguilar-Uscanga and J. M. François, "A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation," *Letters In Applied Microbiology*, vol. 37, no. 3, pp. 268–274, 2003.

[75] P. Coutteau, P. Lavens, and P. Sorgeloos, "Baker's yeast as a potential substitute for live algae in aquaculture diets: artemia as a case study," *Journal of the World Aquaculture Society*, vol. 21, no. 1, pp. 1–9, 1990.

[76] P. Coutteau, N. H. Hadley, J. J. Manzi, and P. Sorgeloos, "Effect of algal ration and substitution of algae by manipulated yeast diets on the growth of juvenile *Mercenaria mercenaria*," *Aquaculture*, vol. 120, no. 1-2, pp. 135–150, 1994.

[77] A. Loor, P. Bossier, and N. Nevejan, "Dietary substitution of microalgae with the *Saccharomyces cerevisiae* mutant, *Δmnn9*, for feeding Pacific oyster (*Crassostrea gigas*) juveniles," *Aquaculture*, vol. 534, Article ID 736253, 2021.

[78] B. L. Bayne, "Carbon and nitrogen relationships in the feeding and growth of the Pacific oyster, *Crassostrea gigas* (Thunberg)," *Journal of Experimental Marine Biology and Ecology*, vol. 374, no. 1, pp. 19–30, 2009.

[79] M. R. Brown, S. W. Jeffrey, J. K. Volkman, and G. A. Dunstan, "Nutritional properties of microalgae for mariculture," *Aquaculture*, vol. 151, no. 1–4, pp. 315–331, 1997.

[80] A. Grémare, J. M. Amouroux, F. Charles et al., "Temporal changes in the biochemical composition and nutritional value of the particulate organic matter available to surface deposit-feeders: a two year study," *Oceanologica Acta*, vol. 150, no. 1/3, pp. 195–206, 1997.
[81] A. J. S. Hawkins and B. L. Bayne, "Nutrition of marine mussels: factors influencing the relative utilizations of protein and energy," *Aquaculture*, vol. 94, no. 2-3, pp. 177–196, 1991.
[82] B. S. Morton, "Feeding and digestion in Bivalvia," in *The Mollusca: Physiology, Part 2*, A. S. M. Saleuddin and K. Wilbur, Eds., vol. 5, pp. 65–147, Academic Press, 1983.
[83] R. D. Purchon, "Digestion in filter feeding bivalves—a new concept," *Journal of Molluscan Studies*, vol. 39, no. 4, pp. 253–262, 1971.
[84] G. Owen, "Lysosomes, peroxisomes and bivalves," *Science Progress*, vol. 60, no. 239, pp. 299–318, 1972.
[85] A. J. S. Hawkins and B. L. Bayne, "Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements," *Marine Ecology Progress Series*, vol. 25, pp. 181–188, 1985.
[86] I. Ibarrola, J. I. P. Iglesias, and E. Navarro, "Differential absorption of biochemical components in the diet of the cockle *Cerastoderma edule*: enzymatic responses to variations in seston composition," *Canadian Journal of Zoology*, vol. 74, pp. 1887–1897, 1996.
[87] B. L. Bayne and A. J. S. Hawkins, "Protein metabolism, the costs of growth, and genomic heterozygosity: experiments with the mussel *Mytilus galloprovincialis* Lmk," *Physiological Zoology*, vol. 70, no. 4, pp. 391–402, 1997.
[88] M. J. Fernández-Reiriz, J. Irisarri, and U. Labarta, "Flexibility of physiological traits underlying inter-individual growth differences in intertidal and subtidal mussels *Mytilusgalloprovincialis*," *PLOS ONE*, vol. 11, no. 2, Article ID e0148245, 2016.
[89] A. J. S. Hawkins and A. J. Day, "The metabolic basis of genetic differences in growth efficiency among marine animals," *Journal of Experimental Marine Biology and Ecology*, vol. 203, no. 1, pp. 93–115, 1996.
[90] D. A. Pace, A. G. Marsh, P. K. Leong, A. J. Green, D. Hedgecock, and D. T. Manahan, "Physiological bases of genetically determined variation in growth of marine invertebrate larvae: a study of growth heterosis in the bivalve *Crassostrea gigas*," *Journal of Experimental Marine Biology and Ecology*, vol. 335, no. 2, pp. 188–209, 2006.
[91] D. Tamayo, K. Azpeitia, P. Markaide, E. Navarro, and I. Ibarrola, "Food regime modulates physiological processes underlying size differentiation in juvenile intertidal mussels *Mytilus galloprovincialis*," *Marine Biology*, vol. 163, Article ID 131, 2016.
[92] K. Arranz, *Stoichiometric characterization and physiological bases of differential growth in the congeneric clam species Ruditapes decussatus and R philippinarum*, PhD Thesis, University of the Basque Country (UPV/EHU), Bilbao, 2021.